Observer Based Intervention Design for Breast Cancer Network

by

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TO MY PARENTS AND BELOVED FAMILY

CERTIFICATE OF APPROVAL

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DECLARATION

I declared that this is my original work report, except otherwise acknowledge in references and text where this work is not submitted in any form for any type of certificate or degree at any institution or university for tertiary education and will not be submitted in future for any certificate or degree to this or any other university or institution.

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ABSTRACT

Modeling of genetic regulatory networks and complex biological signaling pathways is marred by the fact that the values of the model parameters vary in a wide range. Such modeling is used in control theory for development of intervention design for complex biological signaling pathways which helpful in drugs discovery and prevention of disease. Boolean control networks easily explained the dynamics of cellular pathways. Based on that approach, the authors have proposed observer based Controller/Intervention design for the growth factor signaling pathway which is responsible for the over expression of an enzyme FASN which increase the production of De-novo fatty acid one of the major cause of breast cancer.

Boolean control network is used to model the growth factor signaling pathway. Observability is checked and Luenberger-like observer is used for state estimation of signaling pathway. An observer based intervention design is carried out to find the drugs for inhibited pathway. The selected drugs are used as a control input and by simulation the state of each drugs is identified for inhibited growth factor signaling pathway.

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LIST OF ACRONYMS

BN	Boolean Network
BCN	Boolean Control Network
STP	Semi Tensor Product
FASN	Fatty Acid Synthesis
HER2	Human Epidermal Receptor 2
FDA	Food and Drugs Administration
PI3K	Phospho Inositide 3-Kinase
mTORc1	Mammalian Target of Rapamycin Complex1
pSREBP1c	Precusor Sterol Regulatory Element Binding Protein-1c

LIST OF SYMBOLS

Symbol	Description
$\neg$	Negation
$\vee$	Disjunction
$\wedge$	Conjunction
$\rightarrow$	Implication
$\leftrightarrow$	Equivalence
$\otimes$	Tensor Product
$\ltimes$	Semi Tensor Product
α	Least Common Multiple(LCM)
I_n	Identity matrix of Size n
ϕ_n	Power Reducing Matrix of Size n

Chapter 1

INTRODUCTION

The Human Genome Project (HGP) gave birth to a new field of biology called systems biology. In systems biology the behavior and relationship of genes, proteins and cells is investigated. Genes and protein form cellular networks and signaling pathways needed for body functions. In cellular networks and cellular pathways these genes and proteins are logically connected. The state of a gene or protein is determined by the state of neighbor genes and proteins.

1.1 Boolean Networks and Cellular pathways

Computational modeling of cellular pathways and Genetic Regulatory Networks (GRN) is an important problem in systems biology. In turn it gives the structure and dynamical properties of cellular pathways which leads to the development of intervention strategies for prevention and control of disease like cancer [1]. The description of chemical reactions, complexity of model and estimation of parameter for such cellular pathways make its modeling difficult. Boolean control networks play an important role in modeling such cellular pathways because it has a basic property of quantized level. Boolean network gives two value to a state or gene ON and OFF, a gene or node may be ON that means 1 or OFF that means 0.

Boolean networks is first introduced by Kauffman [2] for genetic circuits. It was found that genes act like switches and they can turn ON and OFF one another. Such behavior of genetic regulatory networks can be best modeled by using BCNs.

Definition 1: A Boolean network is a set of nodes, $x_1, x_2, \ldots, x_n$ which interact with each other and at each time $t = 0, 1, 2, 3, \ldots$ the nodes have only one value which may be 1 or 0. Boolean networks are represented by network graph or connectivity graph.

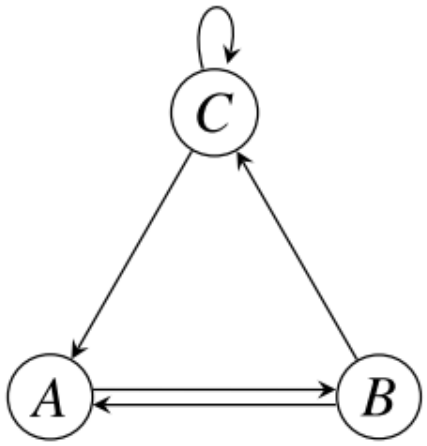

FIGURE 1.1: Network Graph of Boolean Network

Definition 2: A network graph or connectivity graph consists of nodes and edges, $\Sigma = \{N, E\}$. The set of nodes $N = \{x_1, x_2, x_3, \ldots, x_n\}$ and edges $E \subset \{x_1, x_2, x_3, \ldots, x_n\} \times \{x_1, x_2, x_3, \ldots, x_n\}$ form the Boolean network. There is an edge from one node to another node which shows that this node is effected by another node. The state of any node at time t is denoted by $x_i(t)$ and state at time $t+1$ is given by:

$$x_i(t+1) \quad = \quad f_i(x_{i1}, x_{i2}, \ldots, x_{in})$$

Figure 1.1 shows a simple Boolean network consists set of nodes $N = \{A, B, C\}$ and edges $E = \{(A,B), (B,A), (B,C), (C,A), (C,C)\}$.

1.1.1 Boolean Network for Breast Cancer

Obesity is a major cause of breast cancer which arises from the mutation of growth factor signaling pathway. Growth factor signaling pathway consists of network nodes (proteins, genes) and external inputs which effects the pathway. Boolean control network

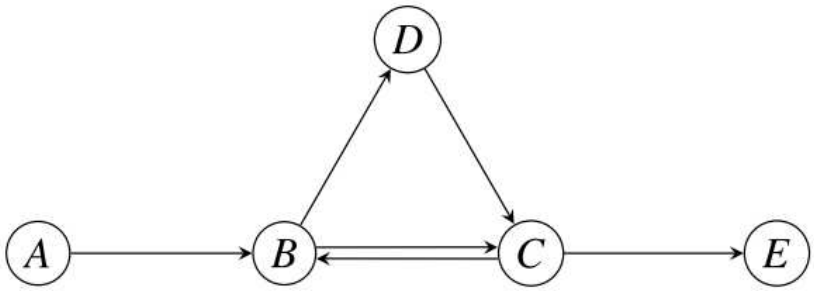

FIGURE 1.2: Network Graph of Boolean Control Network

is used to model such pathway due to its binary characteristic which easily explains the network nodes (protein, genes). These nodes are connected with each other through logical function.

Figure 1.2 consists of three network nodes $\{B,C,D\}$, one input node $\{A\}$ and one output node $\{E\}$. All the input, network and output nodes are connected through some logical functions. The input nodes may be in the form of boolean sequence or it may satisfy some logical rules.

1.1.2 Semi-tensor Product (STP)

Recently semi tensor product (STP) is a new matrix approach used for solving the BCNs [3]. Here we present some notation regarding to STP.

(i) $D = \{T,F\}$ = $\{1,0\}$

(ii) $D^n = D_1 \times D_2 \times ....D_n$

(iii) In matrix form 1 is True (T), 0 is False (F)and denoted as:

$$T = \begin{bmatrix} 1 \\ 0 \end{bmatrix}$$

$$F = \begin{bmatrix} 0 \\ 1 \end{bmatrix}$$

(iv) δ_k^i is the i^{th} columns of identity matrix I_k.

(v) $\triangle_n$:The set of $\{\delta_n^1, ...\delta_n^n\}$

Assume $\triangle = \triangle_2 = \{\delta_2^1, \delta_2^2\}$

(vi) Vector form of logical value can be represented as :

$\delta_2^1 = \begin{bmatrix} 1 \\ 0 \end{bmatrix}$

$\delta_2^2 = \begin{bmatrix} 0 \\ 1 \end{bmatrix}$

Definition1:Let $A \in R^{m\times n}$ and $B \in R^{p\times q}$ are two matrix. $A \ltimes B$ is called the semi tensor product of matrix A and B. It can be calculated as :

$A \ltimes B = (A \otimes I_{\frac{\alpha}{n}})(B \otimes I_{\frac{\alpha}{p}})$

where $\alpha = lcm(n, p)$ is the least common multiple (lcm) of (n,p) and $\otimes$ is the tensor product or knonecker product of matrices which is define as:

$A \otimes B = \begin{bmatrix} a_{11}B & \dots & a_{1n}B \\ \vdots & \dots & \vdots \\ a_{m1}B & \dots & a_{mn}B \end{bmatrix}$ Where $A \otimes B \in R^{mp\times nq}$

if $n = p$ than $A \ltimes B = AB$ which shows that STP is a special case of matrix multiplication. In this report all multiplications used are Semi Tensor Product (STP).

Example 1

Consider $A = \begin{bmatrix} 1 & 0 & 0 & 0 \\ 0 & 1 & 1 & 1 \end{bmatrix}$ and $B = \begin{bmatrix} 1 \\ 0 \end{bmatrix}$ where no of columns of A is n=4, no of rows of B is p=2 and lcm is $\alpha = 4$

$A \ltimes B = (A \otimes I_{\alpha/n})(B \otimes I_{\alpha/p})$

$A \ltimes B = (A \otimes I_{4/4})(B \otimes I_{4/2})$

$A \ltimes B = (A \otimes I_1)(B \otimes I_2)$

$$A \ltimes B = \begin{bmatrix} 1 & 0 & 0 & 0 \\ 0 & 1 & 1 & 1 \end{bmatrix} \begin{bmatrix} 1 & 0 \\ 0 & 1 \\ 0 & 0 \\ 0 & 0 \end{bmatrix}$$

$$A \ltimes B = \begin{bmatrix} 1 & 0 \\ 0 & 1 \end{bmatrix}$$

1.2 Basic Logical operator

There are some basic logical operators used in Boolean Control Network. These logical operators with their structure matrices are listed below:

Negation

$$M_n = M_\neg = \begin{bmatrix} 1 & 0 \\ 0 & 1 \end{bmatrix} = \delta_2[1\ 2]$$

Disjunction

$$M_d = M_\vee = \begin{bmatrix} 1 & 1 & 1 & 0 \\ 0 & 0 & 0 & 1 \end{bmatrix} = \delta_2[1\ 1\ 1\ 2]$$

Conjunction

$$M_c = M_\wedge = \begin{bmatrix} 1 & 0 & 0 & 0 \\ 0 & 1 & 1 & 1 \end{bmatrix} = \delta_2[1\ 2\ 2\ 2]$$

Implication

$$M_i = M_\rightarrow = \begin{bmatrix} 1 & 0 & 1 & 1 \\ 0 & 1 & 0 & 0 \end{bmatrix} = \delta_2[1\ 2\ 1\ 1]$$

Equivalence

$$M_e = M_\leftrightarrow = \begin{bmatrix} 1 & 0 & 0 & 1 \\ 0 & 1 & 1 & 0 \end{bmatrix} = \delta_2[1\ 2\ 2\ 1]$$

1.3 Network Transition Matrix

In Boolean control networks all nodes (network nodes, input nodes and output nodes) are connected with basic logical operator (NOT, AND, OR). The network transition matrix can be formed by using STP among the structure matrices of logical operator. The network transition matrix $L \in L_{m\times n}$ can be written as:

$L = [\delta_m^{i_1}\ \delta_m^{i_2}\ \ldots\ \delta_m^{i_n}]$

For more compactness it can be written as:

$L = \delta_m[i_1\ i_2\ \ldots\ i_n]$

1.3.1 Dynamics of Boolean Control Network

A BCN with n network nodes m input nodes and p output nodes can be written as:

$$\begin{aligned}
x_1(t+1) &= l_1(x_1,\ x_2....x_n,\ u_1....u_m)\\
x_2(t+2) &= l_2(x_1,\ x_2....x_n,\ u_1....u_m)\\
&\vdots\\
x_n(t+1) &= l_n(x_1,\ x_2....x_n,\ u_1....u_m) \qquad (1.1)\\
y_1 &= h_1(x_1,\ x_2....x_n)\\
y_2 &= h_2(x_1,\ x_2....x_n)\\
&\vdots\\
y_p &= h_p(x_1,\ x_2....x_n) \qquad (1.2)
\end{aligned}$$

Here

$x_i(t)\ \varepsilon\{1,0\},\ i = 1,2,\ldots,n$ are network nodes

$u_i(t)\ \varepsilon\{1,0\},\ i = 1,2,\ldots,m$ are input nodes

$x_i(t)\ \varepsilon\{1,0\},\ i = 1,2,\ldots,p$ are output nodes

$l_1, l_2, \ldots, l_n$ are logical functions.

Using a new matrix product called semi tensor product equactions 1.1 and 1.2 can be written as:

$$\begin{aligned} X(t+1) &= L \ltimes X(t) \ltimes U(t) \\ Y(t) &= H \ltimes X(t) \end{aligned} \tag{1.3}$$

where $X \in \triangle_{2^n}$, $U \in \triangle_{2^m}$, and $Y \in \triangle_{2^p}$ are network nodes, input nodes and output nodes respectively. $L \in R_{2^n \times 2^{n+m}}$ and $H \in R_{2^p \times 2^n}$ are state transition matrix and output transition matrix.

1.3.2 Swap Matrix

A swap matrix is $mn \times mn$ matrix $W_{[m,n]}$ defined as: The columns and rows of swap matrix are labeled by double index (I,J) and (i,j). Then rows will be: $I_d(I,J;n,m) = \{(1,J)(2,J)(3,J),\ldots,(m,J), J = 1,2,3,\ldots,n\}$and the columns are arranged as: $I_d(i,j;m,n) = \{(i,1)(i,2)(i,3),\ldots,(i,n), i = 1,2,3,\ldots,m\}$ then

$$W_{(IJ),(ij)} = \delta_{i,j}^{I,J} = \begin{bmatrix} 1 & I = i \& J = j \\ 0 & Otherwise \end{bmatrix}$$

Let m=3, n=2 then the swap matrix will be

$$W_{3,2} = \begin{bmatrix} 1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 \end{bmatrix} = \delta_6[1\ 4\ 2\ 5\ 3\ 6]$$

If $A \in R^m$ and $B \in R^n$ are two columns vector then

$W_{[m,n]} \ltimes A \ltimes B = B \ltimes A$

$W_{[n,m]} \ltimes B \ltimes A = A \ltimes B$

This property of swap matrix is used to interchange the nodes of Boolean control network.

1.3.3 Power Reducing Matrix

The Power Reducing Matrix is defined as:

$$M_r = \begin{bmatrix} 1 & 0 \\ 0 & 0 \\ 0 & 0 \\ 0 & 1 \end{bmatrix} = \delta_4[1\ 4]$$

If $A \in \triangle_2$ is a node of Boolean control network then $A^2 = M_r A$. Power reducing matrix is used for reducing the order of a node.

1.3.4 Transition Matrix

The transition matrix of Boolean control network is calculated from structure matrices of logical operator by using STP with swap matrix and power reducing matrix. The method of calculating the transition matrix of BCNs can be explained with example.

Example 2

$A(t+1) = B(t) \wedge C(t)$

$B(t+1) = \neg A(t)$

$C(t+1) = B(t) \vee C(t)$

The structure matrices for logical operator can be written as:

$A(t+1) = McB(t)C(t)$

$B(t+1) = MnA(t)$

$C(t+1) = MdB(t)C(t)$

Let $x(t) = A(t)B(t)C(t)$ then

$x(t+1) = A(t+1)B(t+1)C(t+1)$

Putting the values

$x(t+1) = McB(t)C(t)MnA(t)MdB(t)C(t)$

$= Mc \ltimes (I_4 \otimes Mn) \ltimes B \ltimes C \ltimes A \ltimes Md \ltimes B \ltimes C$

$= Mc \ltimes (I_4 \otimes Mn) \ltimes (I_8 \otimes Md) \ltimes B \ltimes C \ltimes A \ltimes B \ltimes C$

$= Mc \ltimes (I_4 \otimes Mn) \ltimes (I_8 \otimes Md) \ltimes W_{[2,4]} \ltimes A \ltimes B \ltimes C \ltimes B \ltimes C$

$= Mc \ltimes (I_4 \otimes Mn) \ltimes (I_8 \otimes Md) \ltimes W_{[2,4]} \ltimes A \ltimes B \ltimes W_{[2]} \ltimes B \ltimes C^2$

$= Mc \ltimes (I_4 \otimes Mn) \ltimes (I_8 \otimes Md) \ltimes W_{[2,4]} \ltimes (I_4 \otimes W_{[2]}) \ltimes A \ltimes B^2 \ltimes C^2$

$= Mc \ltimes (I_4 \otimes Mn) \ltimes (I_8 \otimes Md) \ltimes W_{[2,4]} \ltimes (I_4 \otimes W_{[2]}) \ltimes A \ltimes Mr \ltimes B \ltimes Mr \ltimes C$

$= Mc \ltimes (I_4 \otimes Mn) \ltimes (I_8 \otimes Md) \ltimes W_{[2,4]} \ltimes (I_4 \otimes W_{[2]}) \ltimes (I_2 \otimes Mr) \ltimes A \ltimes B \ltimes Mr \ltimes C$

$= Mc \ltimes (I_4 \otimes Mn) \ltimes (I_8 \otimes Md) \ltimes W_{[2,4]} \ltimes (I_4 \otimes W_{[2]}) \ltimes (I_2 \otimes Mr) \ltimes (I_4 \otimes Mr)ABC$

$x(t+1) = L \ltimes A(t)B(t)C(t)$

Where $L = Mc \ltimes (I_4 \otimes Mn) \ltimes (I_8 \otimes Md) \ltimes W_{[2,4]} \ltimes (I_4 \otimes W_{[2]}) \ltimes (I_2 \otimes Mr) \ltimes (I_4 \otimes Mr)$

Putting the values of logical matrices, use the swap matrix and power reducing matrix we obtain

$L = \delta_8[3\ 3\ 7\ 7\ 8\ 1\ 5\ 5\ 6]$

Chapter 2

LITERATURE REVIEW

The main problems associated with Boolean control networks is its controllability and observability. By studying literature of Boolean control networks, it shows that it starts from the work of Akutsu in 2007 [4]. He proposed that one of the major goal of system biology is to develop the control strategies for complex biological systems. Development of control laws not only important for theoretical work but also give practical results. Such control laws not only useful for system based drugs discovery but also useful for prevention of diseases. In order to develop control laws for such cellular systems and complex biological systems we need mathematical model. Boolean networks very easily express the behavior of such complex system because each node have any two possible value 1 or 0. Boolean model only shows that a node or gene is ON or OFF and it cannot show the detailed behavior of complex biological system.

In 2009 D.Cheng [3] use a mathematical technique called semi tensor product (STP) of matrices for BCNs and gives the condition for observability and controllability of BCNs. He suggested that STP is the best technique for solving the Boolean control networks. After the work of D.cheng the field of observability and controllability of Boolean control network has been started. Later on important results come up regarding to the observability and controllibility of BCN.

2.1 Observability of Boolean Control Network:

The observability of BCNs first proposed by D.Cheng in 2009 [3]. He gives the equivalent condition for the observability of BCNs. He determined that input sequence gives the states of system. In 2014 K.Zhang [5] summarize the different results and categorize the observability of Boolean control networks into four types based on its dependance

on initial state and/or inputs. Here we present the four types of observability with its mathematical interpretation.

1. *Definition* 1 : A Boolean control network with system transition matrix L and output transition matrix H is said to be observable if for initial state x_0 there exist at least a BC input sequence $\{u_1, u_2, u_3, \ldots\}$ such that the initial state x_0 can be determined by the output $\{y_1, y_2, y_3, \ldots\}$ sequence [3],, .This definition is concluded by theorem (26) in [6]. Assume that if the system is controllable than it is observable if C has all distinct columns. where
$C = \begin{bmatrix} \mathrm{H} \\ HLu_1 \\ HLu_2 \\ HLu_3 \\ \vdots \end{bmatrix}$, $u_1, u_2, u_3 \ldots$ are control input sequence.
We first assign an initial state x_0 and we observe Hx_0. Than using the control input we find $HLu_1, HLu_2, HLu_3 \ldots$. For same starting point we obtain linear independent rows set.

2. *Definition* 2 : [7][8] A Boolean control network is said to be observable if there is an control input sequence $\{u_{(1)}, u_{(2)}, u_{(3)} \ldots\}$ such that the initial state x_0 can be distinguished by the output $\{y_{(0)}, y_{(1)}, y_{(3)}, \ldots\}$. Definition 2 gives the distinguishablity of states that two different states $x_1, x_2 \in L_{2^n}$ are distinguishable if for any positive integer N and control input sequence $u = \{u_{(1)}, u_{(2)}, u_{(3)}, \ldots\}$ the output will be $y\{k; x_1, u\} \neq y\{k; x_2, u\}$ while the two states are said to be indistinguishable if $y\{N; x_1, u\} = y\{N; x_2, u\}$. This type of observability can be tested by the algorithm defined in [7]. Although the first definition gives the distinguishability of states but it is more simple than the second definition so first definition is mostly used.

3. *Definition* 3 : [9] This observability are dependent on control input and defines two types of control input and on the basis of control input the observability condition are given. A Boolean control network is said to be observable if for the control input $u = \{u_{(0)}, u_{(1)}, \ldots\}$ sequence the initial state x_0 gives the output $\{y_{(0)}, y_{(1)}, \ldots\}$. The control sequence will be of two types it will be a Boolean sequence like $u = \delta_m^i$ or it will be a control sequence which satisfy certain logical rules.

   $u(k+1) = h(u_1,\ u_2, \ldots u_m)$

   Suppose the control input is the free Boolean sequence and N is the smallest positive integer than according to theorem 2 [7] the Boolean control network will be observable if $O_N(\lambda)$ has all distinct column while

   $O_N(\lambda) = \sum_{k=0}^{N} \lambda^k [P_k \otimes 1_{2^{(N-k)m}}]$

   If the control input satisfy logical rules than from theorem 3 [9] the BCN is said to be observable if smallest positive integer N $M_N(\lambda)$ has at least one distinct column while

   $M_N(\lambda) = \sum_{k=0}^{N} \lambda^k Q_k$

4. *Definition* 4 : [10] This type of observability use the state input trajectories that for any two initial state $x_1 = \delta_{2^n}^i$ and $x_2 = \delta_{2^n}^j$ where $i \neq j$ and for any control input sequence $u : \{0, 1, 2, \ldots\} \in L_{2^m}$ the corresponding output sequence will be $\{y_1(t_0), y_1(t_0+1)\ldots\} \neq \{y_2(t_0), y_2(t_0+1)\ldots\}$. This definition gives a sufficient condition for the observability of BCN as it define that the states will be uniquely determine which show its distinguishability and by giving the different output for different state input trajectories it show that the states for given control input sequence will be reconstructible so in [10] theorem 3 split this definition into two parts.

   Distinguishability of states:

   For two different states $x_1, x_2 \in L_{2^n}$ and for any input sequence $u \in L_{2^m}$ the state input trajectories $(x_1, u) \neq (x_2, u)$ will satisfy $L \ltimes u \ltimes x_1 = L \ltimes u \ltimes x_2$ such that

$H \ltimes x_1 \neq H \ltimes x_2$. This shows that the two states are distinguishable and gives the output.

Reconstructibility of states:

Reconstructibility define that how to uniquely determine the final state with the knowledge of input/output trajectories $(y(t), u(t) \;\; t = 0, 1 \ldots)$. For the pair of input and output trajectories.

$((x_1, u_1), \ldots (x_k, u_k)) \neq ((\bar{x}_1, u_k), \ldots (\bar{x}_1, u_k))$

the corresponding output will be

$(Hx_1, \ldots, Hx_k) \neq (H\bar{x}_1, \ldots, H\bar{x}_k)$

The four types of observability are compared in [11] which shows that if the the Boolean control network is observable in the sense of definition 4, then it will be observable in the sense of definition 1,2 and 3. So definition 4, gives the condition for the observability of BCNs.

2.2 State Observers for Boolean Control Network:

In literature different types of state observers are presented for Boolean control network. Before starting to discuss the different types of observer designing for Boolean control network we first define the state observer for Boolean control networks .

Definition : A state observer receives the Boolean control network input and output as an input and estimate the current state of Boolean control network as an output. There are mainly three types of state observers which is used for Boolean control network.

1. *Shift Register Observer*

   As discussed in the definition of state observer that the BCNs input and output estimate the current state, but if we see the reconstructibility of state it shows that the state will be reconstructible or will be estimated if the input and output trajectories are admissible for the current state.The input and output will be admissible

if for an initial state x_0 the input $u(t)$ generate the output $y(t)$ at time t.
The use of shift register observer will be initialize, first an initial state will be generated by using the input $u(t)$ and output $y(t)$ admissible trajectories. Thus the initial state will be $z(r) = y(o)u(o)\ldots y(r)u(r)$. Then we proceed as :

$$\begin{aligned} \hat{x_s}(t) &= \hat{H}z(t) \\ z(t+1) &= (I^T_{2^{m+p}} \otimes I_{2^{m+p}})W_{[2^m+p,2^{(r+1)(m+p)}]}y(t+1)u(t+1)z(t), t = r, r+1, \ldots \end{aligned} \tag{2.1}$$

Where r is the minimal reconstructibility index which satisfied the reconstructibility of state and can be found by using reconstructibility matrix.

$$\Omega_j = \phi_n^T (I_{2^n} \otimes H^T) L(\Theta_{j-1} \otimes I_{2^{m+p}})$$
$$j = 1, 2, 3 \ldots r$$

ϕ_n is the power reducing matrix. $\hat{x_s}(t)$ is the estimate of BCNs state $x(t)$ and $y(t+1), u(t+1)$ gives the admissible input and output trajectories at time $(t+1)$. The matrix $\hat{H}$ can be find from input and output trajectories $u(t), y(t)$ where $t = t_0, t_0+1 \ldots t_0+r$ which is:

$$\hat{H} = \Omega_r \otimes I^T_{2^m} \tag{2.2}$$

Ω_r can be find from the reconstructibility matrix Ω_j if $j = 1, 2 \ldots r$. The columns of $\hat{H}$ have no non-zero entry and it can be replaced by $\delta^1_{2^n}$. The shift register observer with $\hat{H}$ can gives the exact estimate of the state $X(t) = \hat{X_s}(t)$ for $t \geq r$. The shift-register observer was first proposed in[10] but it only use the mapping of admissible vector and it has no observability matrix. The shift-register observer

is than modified in [11] which gives the transition matrix with its proof. So this is the modified shift register observer which is mostly used for Boolean control network.

2. *Luenberger – Like Observer*:

   Luenberger-like observer uses the same concept of observability that the input and output trajectories $u(t), y(t), t \in Z^+$ estimate the current state $\hat{x}(t)$. First the Luenberger-like observer is initialized with zero estimate such as [12]:

   $\hat{x}(0) = H^T y(0)$

   If there is an older estimate of the state is available or simply we say that the initial state is known, than the state can be estimate based on previous state and the knowledge of the input.

$$\hat{x}_s(t) = L \ltimes u(t-1) \ltimes \hat{x}_s(t-1)$$

Where $\hat{x}_s(t-1)$ is the older estimate. Now combining the initial state with input and output trajectories which can update the state by using the older state estimate.

$$\begin{aligned} \hat{x}_s(t) &= \phi_n^T \hat{x}_s(t-1) u(t-1) H^T y(t) \\ \hat{x}_s(t) &= \phi_n^T (I_{2^n} \otimes H^T) L \hat{x}_s(t-1) u(t-1) y(t) \end{aligned} \tag{2.3}$$

The Luenberger like observer provide the same state estimate as shift register observer. The Luenberger like observer is used to compare shift register observer in [11] with its proof which shows that Luenberger like observer is more simple, gives small size observability matrix, require less computational effort as compare to shift register observer.

As Shift-register observer generates high dimension observability matrix so need a lot

of memory and extra computational effort like the matrix $\hat{H}$ provide $R^{(2^n \times 2^{(r+1)(m+p)})}$ dimension which is multiplied with vector $z(t)$ of dimension $R^{2(r+1)(m+p)\times 1}$ for every current state while Luenberger-like observer require less computational effort such as providing $(2^n \times 2^{n+p+m})$ dimensional matrix and give the correct estimate of $\hat{x}(t)$ only using $\hat{x}(t-1)$ with input and output trajectories. That's why we preferred the Luenberger-like observer for BCNs.

Multiple-state observer is another observer used in [10] which estimates several states of BCNs but not gives the whole estimate of states for BCNs. This observer was defined with comparison to shift register observer but not good as compare to Luenberger-like observer so here we omit the multiple states observer.

2.3 State Feedback Stabilization

First Akutsu used the external input for Boolean control network and show that the external input can derive the states of Boolean control network to the desired states. After that D.Cheng [6] and Rui Li [13] gives the state feedback stabilization. They presented a general method for finding the globally stabilizing state feedback controllers for BCN by using the STP of matrices. A BCN is said to be globally stabilizable to a fixed point or attractor $\hat{X}$ if there exist a control sequence $u = \{u_{(0)}, u_{(1)}, u_{(2)}, \ldots\}$ such that $X(k; X_0; u) = \hat{X}$. Using the algebraic representation of BCN the feedback can be

find of the form.

$$\begin{aligned} x_1(k+1) &= l_1 \ltimes x(k) \ltimes u(k) \\ x_2(k+2) &= l_2 \ltimes x(k) \ltimes u(k) \\ &\vdots \\ x_n(k+1) &= l_n \ltimes x(k) \ltimes u(k) \\ u_1(k) &= K_1 x(k) \\ u_2(k) &= K_2 x(k) \\ &\vdots \\ u_m(k) &= K_m x(k) \end{aligned} \tag{2.4}$$

Using the STP of matrices they can be simplified to its discrete time dynamical system as :

$$\begin{aligned} x(k+1) &= L \ltimes x(k) \ltimes u(k) \\ u(k) &= K \ltimes x(k) \end{aligned} \tag{2.5}$$

Where K is the state feedback matrix and $u(k)$ is the solution of $X(k;X_0;u) = \hat{X}$ which satisfy the condition of global stabilization.

Chapter 3

FASN PATHWAY

Breast cancer the most prevalent type of cancer mostly occurred in the women worldwide. According to world cancer report 22.9% of breast cancer comprises in all types of cancer with 1.4 million cases increase annually. Breast cancer primarily effect women of 50 aged or older which shows a clear relationship between menopause and breast cancer. Menopause change the hormonal status and provide a favorable environment for the development of breast cancer [14].

Obesity has been considered the second cancer risk factor after tobacco. Statistics shows that people who reside in certain areas and have more energy dense diet consumption are at higher risk for breast cancer occurrence. De novo fatty acid synthesis is the metabolic pathway which synthesize fatty acid from nonlipid precursors. De novo fatty acid used by mammals for production of triglyceride for energy storage. An increase in De novo fatty acid synthesis produced higher amount of fats which leads to obesity. Obesity is not only the main contributor of breast cancer but also effects many other pathways. This shows a clear relationship between De novo fatty acid synthesis, obesity and breast cancer.

3.1 FASN as a Target for Cancer Therapy

FASN is a key enzyme which accelerate the formation of De novo fatty acid synthesis, that's why FASN is a critical target for drug discovery regarding cancer. Fatty acid can be obtained both from external source like from dietary food or from internal source which are known as De novo fatty acid synthesis. There is a difference between cancer cells and normal cells. The fundamental difference is its bioenergatic metabolism. Cancer cells not only use gylcolysis as energy source but also use the energy from high rate De novo fatty acid synthesis in order to sustain their high proliferation rates. While

the De novo fatty acid synthesis is independent of the dietary food. The high rate of De-novo fatty acid synthesis is a major source of energy for cancer proliferation and progression.

3.1.1 Highly expressed FASN

FASN is highly expressed in obese patients and cancerous cells. The inhibition of De novo fatty acid synthesis is related to the expression of FASN that's why FASN is a target for discovery of drugs and for the apoptosis of cancerous cells. The expression of FASN in normal cells and cancerous show that the inhibition of FASN expression effectively reduce the cancer cells growth and induced apoptosis. The inhibition of FASN reduce the formation of extra fat and stop the energy supply for growth of cell membrane. In short the inhibition of FASN inhibit the De novo fatty acid synthesis and as a result stop the replication of cancer cells.

3.1.2 Structure of FASN

FASN is the key enzyme for De-novo fatty acid synthesis. It bi-directionally catalyze two metabolism one is Glucose metabolism and the other one is lipid metabolism. In Glucose metabolism pathway it catalyze the Malonyl-CoA and in lipid metabolism it catalyze Acetyl-CoA to form the 16-carbon long fatty acid palmitate [14].

Palmitate is the first fatty acid from which other form of fatty acid can be formed. So the amount of palmitate formation can be controlled by the FASN enzyme. There are two major class of FASN. One class of FASN which found in bacteria and plants is called type II. The other is type I which is found in humans and mammals. The major difference between type I and type II are its functional domains. There are seven functional domains in both type I and type II. But in type II all functional domains are independent and form multifunctional system while in type I all domains form a single bond. A person with balanced diet form a little amount of de-novo fatty acid because it get lipid from balanced diet. In such, FASN is controlled at low level. There are three basic function of FASN: energy storage , synthesis of fats and lactation. In

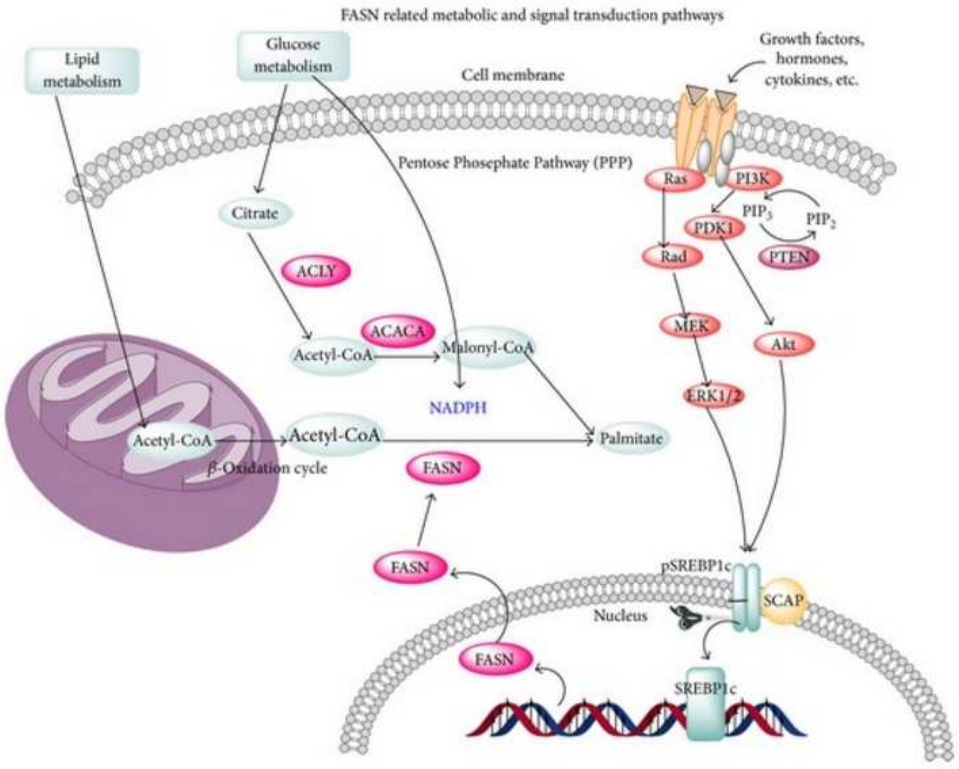

FIGURE 3.1: Role of FASN in Breast Cancer

lipogenic tissues FASN form fats from saturated carbon and stored as triglycerides while in lactating female FASN produced medium chain fatty acid and enables the baby to digest milk. Any abnormalities in FASN expression effect the FASN function.

3.1.3 FASN Signaling Pathway

The over expression of FASN accelerate the rate of De novo formation which leads to the construction of extra cell membrane and production of extra energy by beta-oxidation for cancerous cells. Their are various mechanisms which is responsible for the over expression of FASN which is still unclear but the main pathway which is related to FASN over expression is the growth factor pathway.

The growth factor receptor activate their signaling pathway downstream by activating PI3K-Akt and ERKI. FASN expression is regulated by other receptor like EGFR,HER2

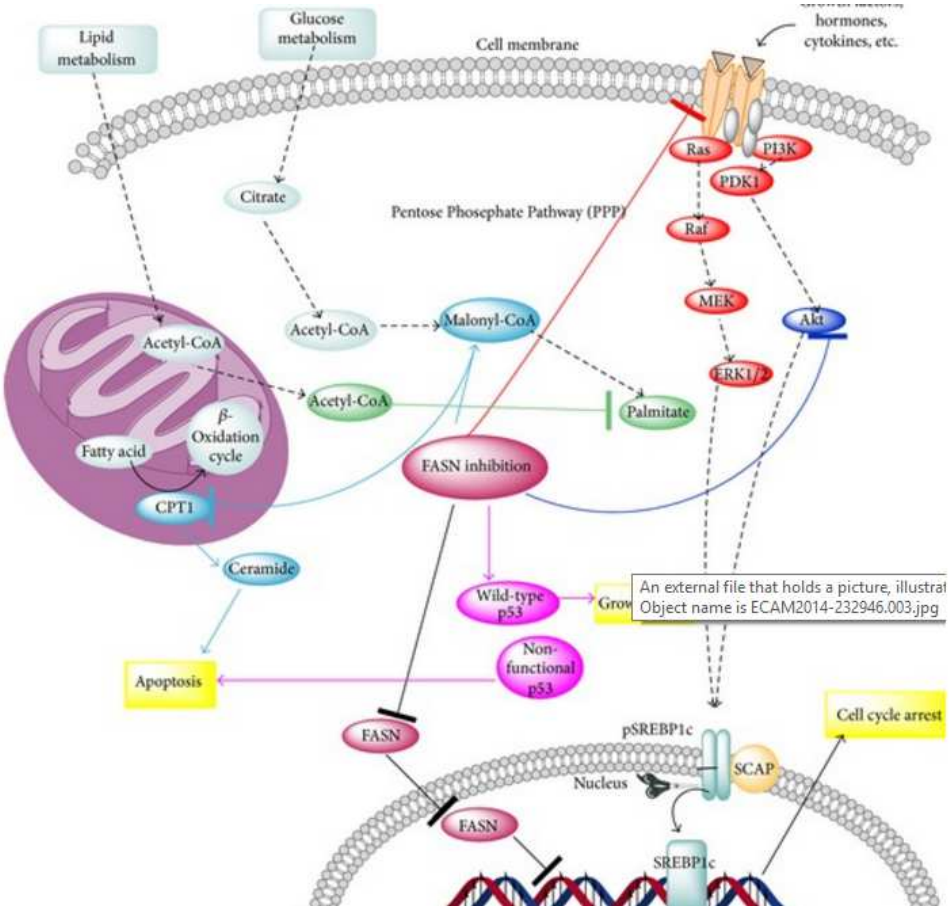

FIGURE 3.2: FASN signaling Pathway

through PI3K signaling pathway HER2 regulate FASN expression. There is a bidirectional mechanism between FASN and HER2. Over expression of HER2 produces drug tolerance for cancerous cells. HER2 and FASN are directly related as shown from the pathway and it is shown that in cancerous cells both are over expressed. Over expression of HER2 increase the FASN expression and inhibiting HER2 by drugs or any other inhibitor leads to inhibition of FASN. The growth factor signaling pathway with FASN and HER2 are shown in the figure 3.2.

The figure 3.2 show the FASN inhibition growth factor signaling pathway. HER2 (Human epidermal receptor) initialize the pathway and it is related to FASN directly and through indirect pathway. It also shows that FASN act as a feedback to HER2 and Akt so any abnormalities produce in pathway disturb the whole pathway and the expression

of FASN. For normal functionality of FASN growth factor inhibited pathway is taken.

3.2 Boolean Network for FASN Pathway

Boolean Networks works on two states either ON 1 or OFF 0. It shows that a gene or node can be expressed or not, or it is active or inactive. It also shows that it is below or above the threshold concentration if it is above the threshold it will be ON or 1 and if it is below the threshold it will be 0. The future value of a node or gene can be determined by the neighbor node by using the boolean logical rules. In FASN signaling pathway all nodes are logically connected and it has either 1 or 0 state. The state of any node depend on its neighbor nodes with its logical relationship. Then using STP on logical matrices give the logical transition matrix for the whole pathway which only comprises 1 and 0.

3.2.1 Growth Factor Pathway

The FASN over expression directly effect the production of De-novo fatty acid and over expression of FASN can be controlled by growth factor signaling pathway. In growth factor signaling pathway different external input are involved but the effective one is the HER2 (Human Epidermal Receptor) which directly and indirectly effect the FASN expression.

Figure 3.3 shows the growth factor signaling pathway HER2 , SPOT14, USP2a , environmental stress are external agents which effect the growth factor signaling pathway. RKTs is another growth factor epidermal receptor which works combine with HER2 but HER2 is effective. SPOT14 , USP2a are lipogenesis-related nuclear protein which can be controlled by genes. The abnormalities which effect the pathway are mainly concerned FASN and HER2 so other external agents are consider to be normal. PI3K AKT mTORc1 pSREBP1c are the internal nodes which may also directly or indirectly effect

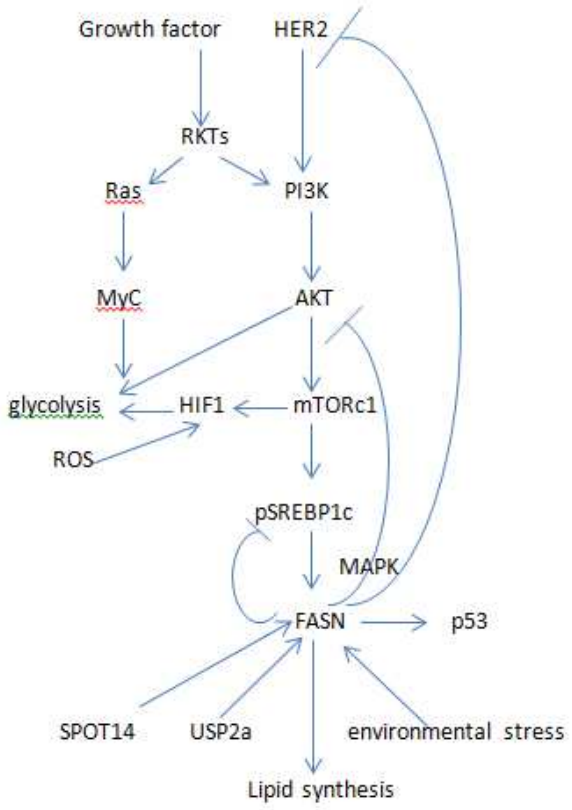

FIGURE 3.3: Growth Factor Signaling Pathway

the FASN expression. pSREBP1c is the transcription factor which bind with the promoter region of FASN for its activation. AKT activation also regulate FASN expression and promote the cell death for FASN inhibition.

3.2.2 Boolean Model

The dynamical Boolean model of growth factor signaling pathway can be formulated by using the Boolean rule [15][16]. These rules are based on the logical interaction of signaling pathway nodes. If a node is directly regulate another node than the future value of this node depend on the past value that node. If the node inhibited the another node than the future value of this node will be the negation of this, like if a node A is inhibited by another node B than $A(k+1) = \neg B$. If there is a node which depend

on more than one node than its future value can be decided by checking the regulation or inhibition of this node. If both the node regulate than AND will be used and can be written as $A(k+1) = B \wedge C$. If one regulate and other inhibit than OR will be used $A(k+1) = B \vee C$. A, B and C are three nodes. Using these simple rules the dynamical Boolean model is formulated for growth factor signaling pathway which is

$$\begin{aligned} HER2(t+1) &= \neg FASN(t) \\ PI3K(t+1) &= HER2(t) \rightarrow PI3K(t) \\ AKT(t+1) &= FASN(t) \wedge PI3K(t) \\ mTORc1(t+1) &= AKT(t) \rightarrow mTORc1(t) \\ pSREBP1c(t+1) &= FASN(t) \wedge mTORc1(t) \\ FASN(t+1) &= pSREBP1c(t) \rightarrow FASN(t) \end{aligned} \tag{3.1}$$

Equation 3.1 gives the dynamical Boolean model for growth factor signaling pathway. From figure 3.3 the Boolean model is derived. As HER2 is directly related to FASN so the activation or deactivation of HER2 is specified by FASN. PI3K is related to HER2. AKT is identified by PI3K with inhibition of FASN, AKT implies to mTORc1, pSREBP1c shows same character like AKT and implies to FASN.

Chapter 4

OBSERVER DESIGN

Boolean control networks gain great attention due to its simple modeling for complex cellular pathways. Such modeling used for the development of intervention design which helps in drugs discovery and prevention of disease. The intervention design or state feedback controller design for signaling pathways need information of internal states. In such control problem, state observer provides the information of internal states.

Three types of state observer used for Boolean Control Networks states estimation, shift register observer, Luenberger-like observer and multiple state observer. The first two gives some useful application for Boolean Control Networks while the shift register observer gives more computational effort for lager networks so the Luenberger-like observer is used for the states estimation of complex cellular pathways.

4.1 Boolean Control Network Model of FASN Pathway

The Boolean model of FASN signaling pathways is given in 3.1. The external agents are not considered here because the FASN or HER2 over expression occur due to the pathways internal mutation. If the pathway is disturbed, it effects the FASN or HER2 expression than external inputs are used for pathway to inhibit the over expression of FASN. Suggested drugs are used as control inputs for the inhibition of pathway.

4.1.1 Control Input for FASN Pathway

If the network nodes of FASN signaling pathway is mutant then the pathway is disturbed which leads to the FASN over expression. For such network nodes drugs are used as control inputs.

The drugs used in the treatment of several genes, involved in the breast cancer are obtained through Gene Cards database. Gene Cards database gives genomic, proteomic, transcriptomic, genetic and functional information on all the known and predicted human genes, along with the information of drug compounds and chemicals used to treat those genes and the details of their pathways. From lists of drug compounds only those drugs are selected, which are approved by FDA. The FDA approval for drugs is confirmed by the reported literature and reports of clinical trials. Side effects of all the drugs are collected from drugs.com. It is an online pharmaceutical encyclopedia contains information of drugs for consumers and healthcare professionals.

Three types of side effects are mentioned in two categories in the drugs.com i-e Major side effect and Minor side effects, both categories contain more common, rare and less common side effects. Only more common from major side effects are selected. For FASN signaling pathway the drugs are given here which used as a control input for signaling pathway for inhibition of nodes or FASN expression [17][18][19][20].

$u = [Tratuzumab \;\; Miltefosine1 \;\; Miltefosine2 \;\; Miconazole \;\; Orlistat \;\; Inhibitor]$

4.1.2 Dynamics of FASN Pathway

The drugs is used as control inputs to turn OFF the pathway so any inhibitor or drugs u_i can inhibit the node P if it interact in the following pattern [21]:

$P(t+1) = \neg u_i(t) \wedge P(t)$

Now considering the drugs with the suggested interaction with nodes the Boolean control network model is presented as: Figure 4.1 shows the Boolean control network model for FASN signaling Pathway where six control input and six network nodes interact to form the FASN signaling pathway. Using the Boolean networks rules 4.1 can

Drugs	Status	Role	Side effects	Mechanism of action
Trastuzumab	Approved/investigational	antagonist/ Biomarker/Inhibitor	Dizzeness,Fever or chill, Headache, Vomiting,Shortness of breath,skin rash,muscle aches	HER2 antagonist,HER2 inhibitor, Epidermal growth factor receptor inhibitor
Miltefosine	Approved	Inhibitor	Abdominal or stomach pain,swelling of the face,arms,legs and lower feet,chill or fever,itching or rash,dizzeness, unusual bleeding and weakness	PI3K/AKT inhibitor
Miconazole	Approved/investigational	antagonist/pore blocker	Body aches or pain,Cough, difficulty with breathing,fever or chills,loss of voice,headache,pale skin,sneezing	Inhibitor
pSREBP1c	-	-	-	-
Orlistat	Approved/investigational	Inhibitor,Target	Pain in joints,loss of appetite, pain and swelling of joints,tense,hot skin,unusual weakness	Inhibitor

TABLE 4.1: Selected drugs for FASN Pathway Nodes

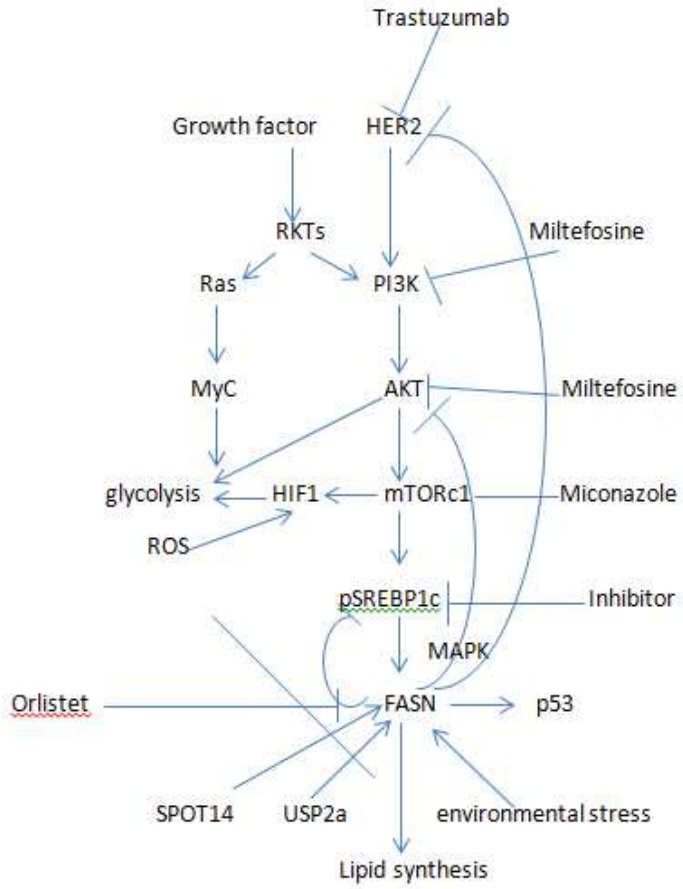

FIGURE 4.1: BCN Model for FASN Pathway

be written in its logical form as:

$$
\begin{aligned}
HER2(k+1) &= \neg Trastuzumab \wedge FASN(k) \\
PI3K(k+1) &= \neg Miltefosine1 \wedge HER2(k) \rightarrow PI3K(k) \\
AKT(k+1) &= \neg Miltefosine2 \wedge FASN(k) \wedge PI3K(n) \\
mTORc1(k+1) &= \neg Miconazole \wedge AKT(k) \rightarrow mTORc1(k) \\
pSREBP1c(k+1) &= \neg Inhibitor \wedge FASN(k) \wedge mTORc1(k) \\
FASN(k+1) &= \neg Orlistet \wedge pSREBP1c(k) \rightarrow FASN(k) \\
y_1(k) &= HER2(k) \\
y_2(k) &= FASN(k)
\end{aligned} \tag{4.19}
$$

1. **Networks nodes**: The FASN signaling pathway consist of 6 network nodes. Here number of nodes n is 6 which denoted as:
   $x = [HER2 \;\; PI3K \;\; AKT \;\; mTORc1 \;\; pSREBP1c \;\; FASN]$

2. **Input nodes**: The drugs used as inputs to network nodes which is 6 network inputs. Here number of nodes m is 6 denoted as:
   $u = [Tratuzumab \;\; Miltefosine1 \;\; Miltefosine2 \;\; Miconazole \;\; Orlistat \;\; Inhibitor]$

3. **Output nodes**: Two nodes FASN and HER2 is taken as output so number of output p is 2 which is denoted as:
   $y = [HER2 \;\; FASN]$

Using the logical matrices of logical operator 4.1 can be simplified as:

$HER2(k+1) = Mn\ Mc\ Trastuzumab\ FASN(k)$

$PI3K(k+1) = Mn\ Mc\ Miltefosine1\ MI\ HER2(k)\ PI3K(k)$

$AKT(k+1) = Mn\ Mc\ Miltefosine2\ Mc\ FASN(k)\ PI3K(k)$

$mTORc1(k+1) = Mn\ Mc\ Miconazole\ MI\ AKT(k)\ mTORc1(k)$

$pSREBP1c(k+1) = Mn\ Mc\ Inhibitor\ Mc\ FASN(k)\ mTORc1(k)$

$FASN(k+1) = Mn\ Mc\ Orlistet\ MI\ pSREBP1c(k)\ FASN(k)$

$X(k+1) = Mn\ Mc\ Trastuzumab\ FASN(k)\ Mn\ Mc\ Miltefosine1\ MI\ HER2(k)\ PI3K(k)\ Mn\ Mc\ Miltefosine2\ Mc\ FASN(k)\ PI3K(k)\ Mn\ Mc\ Miconazole\ MI\ AKT(k)\ mTORc1(k)\ Mn\ Mc\ Inhibitor\ Mc\ FASN(k)\ mTORc1(k)\ Mn\ Mc\ Orlistet\ MI\ pSREBP1c(k)\ FASN(k)$

$Y(k) = HER2(k)\ FASN(k)$

$$\begin{aligned} x(k+1) &= L \ltimes x(k) \ltimes u(k) \\ y(k) &= H \ltimes x(k) \end{aligned} \tag{4.2}$$

where $x(k) \in \triangle_{2^n}$, $u(k) \in \triangle_{2^m}$, $y(k) \in \triangle_{2^p}$, $L \in R^{2^n \times 2^{n+m}}$ and $H \in R^{2^p \times 2^n}$. For 4.1 $L \in L_{2^6 \times 2^{12}}$ and $H \in L_{2^2 \times 2^6}$ can be calculated as:

$L \in L^{64\times 2096} = [64\ 64\ 4\ 64\ 64\ 64\ 64\ \ldots]$

$H \in L^{4\times 64} = [1\ 3\ 1\ 3\ 1\ 3\ 1\ \ldots]$

L and H are network transition matrix and output transition matrix respectively.

4.2 Observability of FASN Pathway

The observability of FASN pathway are tested here using definition 4 which gives two condition for observability of Boolean control network.

1. There are two distinct states $x_1, x_2 \in L_{2^n}$ and control input $u \in L_{2^m}$ such that
   $L \ltimes u \ltimes \ltimes x_1 = L \ltimes u \ltimes \ltimes x_2$
   $H \ltimes x_1 \neq H \ltimes x_2$
   Let $x_1 = \delta_{64}^1, x_2 = \delta_{64}^2$ and $u = \delta_{64}^1$ then using STP
   $L \ltimes u \ltimes \ltimes x_1 = L \ltimes u \ltimes \ltimes x_2 = \delta_{64}^{64}$ and
   $H \ltimes x_1 = \delta_4^1$ and $H \ltimes x_2 = \delta_4^3$ which shows that
   $H \ltimes x_1 \neq H \ltimes x_2$

2. There are two different input states trajectories $((x_1,u)(x_2,u)) \neq ((x_2,u)(x_1,u))$.
   The corresponding output trajectories will be different.
   $((\delta_{64}^1, \delta_{64}^1), (\delta_{64}^2, \delta_{64}^1)) \neq ((\delta_{64}^2, \delta_{64}^1), (\delta_{64}^1, \delta_{64}^1))$ implies that
   $(\delta_4^1, \delta_4^3) \neq (\delta_4^3, \delta_4^1)$

The two conditions of observability are satisfied for FASN signaling pathway which shows that the states of FASN pathway can be estimated using the Boolean control networks states observers.

4.3 State Observer

The states of observable Boolean control networks can be estimated using Boolean networks state observer. There are three different types of state observer used for states

estimation, Luenberger-like observer, shift register observer, multiple state observer. Here we use Luenberger-like observer because it has the following advantages over other types of observer [11]:

1. Lower memory: Luenberger-like observer needed a lower memory for state estimation it produce $2^n \times 2^{n+p+m}$ size matrix which is much smaller than shift register observer which produce $2^n \times 2^{(r+1)(p+m)}$ size matrix.

2. Lower computation: Estimation only required $\hat{x}_s(t-1) \in \triangle_{2^n}, y(t) \in \triangle_{2^p}$ and $u(t-1) \in \triangle_{2^m}$.

3. Precise estimation: Luenberger like observer gives the precise estimation of states only using inputs and outputs trajectories with no needs of $r+1$.

The Luenberger like observer with initial state estimate $\hat{x}_0 = H^T y_0$ is described as:
$\hat{x}_s(t) = \phi_n^T \ltimes (I_{2^n} \otimes H^T) \ltimes L \ltimes \hat{x}(t-1) \ltimes u(t-1) \ltimes y(t)$
It provide the same states estimate as shift register observer or multiple state observer which is shown in [11].

4.3.1 State Observer for FASN Pathway

The Luenberger like observer is used to estimate the states of FASN signaling pathway 4.1.

1. **Observability matrix**

   First the observability matrix O is generated for 4.1.

   $O = \phi_n^T (I_{2^n} \otimes H^T) L$

   $O = \phi_6^T (I_{64} \otimes H^T) L$

   $\phi_6 = [\delta_{64}^1 \otimes \delta_{64}^1 \;\; \delta_{64}^2 \otimes \delta_{64}^2 \ldots \delta_{64}^{64} \otimes \delta_{64}^{64}]$ so

   $O \in R^{64 \times 16384} = [64 \;\; 64 \;\; 64 \;\; 64 \;\; 64 \ldots 5 \;\; 44 \;\; 1 \;\; 43]$

2. **Initial estimate**

   $\hat{x}_0 = H^T y_0$ is the initial estimate at $(t = 0)$ where H^T is the transpose of the output transition matrix and $y_0 = \delta_4^i$ where $i = 1, 2, 3, \ldots, 64$

3. **Control Input**

   The control input $u(t) \in \delta_{2^m}^i$ where $i = 0, 2, 3 \ldots, 63$ is randomly choosen as control input for observer. The control input $u(t) = u_1(t) \ltimes u_2(t) \ltimes u_3(t) \ltimes u_4(t) \ltimes u_5(t) \ltimes u_6(t)$ gives all possible control input for $t = 0, 2, 3 \ldots, 64$. Thus for observer we used all possible control input to estimate the states for a given initial condition.

4. **State estimation**

   The observability matrix previous control input with initialize state estimate are used by the observer to estimate the current state as:
   $\hat{x}_s(t) = \phi_n^T \ltimes (I_{2^n} \otimes H^T) \ltimes L \ltimes \hat{x}s(t-1) \ltimes u(t-1) \ltimes y(t)$

5. **Simulation**

   The above steps are executed and simulation results are given in figure 4.2. The state $x(t)$ and $\hat{x}_s(t)$ are generated for control input $u(t) \in \delta_{2^m}^i$ where $i = 1, 2, 3 \ldots, 64$.

   X denoted by $\circ$ are states of FASN pathway X_s are the estimated states denoted by $\times$.

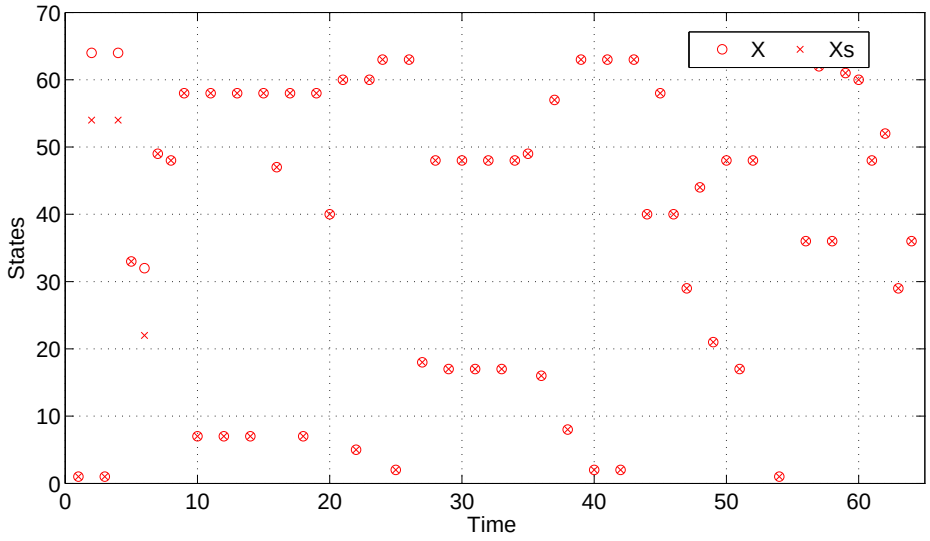

FIGURE 4.2: Observer Design for FASN Pathway

Chapter 5

INTERVENTION DESIGN

One of the main objective of Boolean Control Networks is to develop the control strategies for complex biological signaling pathways. The control input take any one of the values 0 or 1. 0 indicates that a particular intervention or control input is not given at that time while 1 indicates that the intervention is applied at that time or control input is applied. The development of control theory for Boolean Control Network enable us that manipulate biological networks by the application of external inputs. Such external input brings the biological networks into a desirable state such as healthy one.

5.1 State Feedback Controller

A general approach for finding a state feedback controller for BCNs is still unknown. But using the algebraic representation of BCNs in term of STP enable us to find a globally stabilizing state feedback controllers [13] for Boolean Control Networks.

5.1.1 Global Stabilizing Controller

A BCN with n network nodes m input nodes can be described as:

$$\begin{aligned} x_1(t+1) &= l_1(x_1,\, x_2....x_n,\, u_1....u_m) \\ x_2(t+2) &= l_2(x_1,\, x_2....x_n,\, u_1....u_m) \\ &\vdots \\ x_n(t+1) &= l_n(x_1,\, x_2....x_n,\, u_1....u_m) \end{aligned} \tag{5.1}$$

where $l_1, l_2, \ldots l_n$ are Boolean function $X = [x_1 \ltimes x_2 \ltimes \ldots \ltimes x_n]$ and $U = [u_1 \ltimes u_2 \ltimes \ldots \ltimes u_m]$ are network nodes and input nodes respectively.

Definition: A Boolean control network in equation 5.1 is said to be globally stabilizable to $X^* = \{1,0\}^n$ if for every $X_0 = \{1,0\}^n$ there exist a control sequence $U = \{1,2,3\ldots\} = \{1,0\}^m$ such that $X(t;X_0;U) = X^*$. Then the feedback law will be in form of:

$$\begin{aligned} u_1(t) &= K_1(x_1,\ x_2 \ldots . x_n) \\ u_2(t) &= K_2(x_1,\ x_2 \ldots . x_n) \\ &\vdots \\ u_m(t) &= K_m(x_1,\ x_2 \ldots . x_n) \end{aligned} \tag{5.2}$$

where $K_1, K_2, \ldots K_m$ are Boolean function that globally stabilize the Boolean control network 5.1 to X^*. Using STP 5.2 and 5.1 can be simplified as:

$X(t+1) = L \ltimes X(t) \ltimes U(t)$

$U(t) = K \ltimes X(t)$

where $L \in R^{2^n \times 2^{m+n}}$ and $K \in R^{2^n \times 2^m}$. K is called the state feedback matrix.

5.1.2 State Feedback Matrix

The state feedback matrix can be found using the following steps:

1. First the network transition matrix can be found using STP.
   $X(t+1) = L \ltimes X(t) \ltimes U(t)$
2. The reference attractor X^* can be found which will be in the form of:
   $\delta_{2^n}(r) = [\delta_2^1(i) \ltimes \delta_2^2(i) \ltimes \ldots \ltimes \delta_2^n(i)]$

where $i \in \{1,0\}$ and n is number of network nodes.

3. The reference attractor gives $r \in \delta_{2^n}^{j}$ where $j = \{1,2,3,\ldots 2^n\}$. Use the value of j to find the p_i and q_i by using the equation:
   $\alpha_{(p-1)2^n+q} = r$ where $L = \delta_{2^n}[\alpha_1, \alpha_2, \ldots, \alpha_{2^{m+n}}]$

4. The set of q_i is taken is $E_1(r)$ then find the remaining q_i which are taken as $E_2(r)$ if
   $\triangle_{2^n} = E_1(r) \bigcup E_2(r)$
   This prove the stability of reference attractor.

5. Now use theorem 2 of [13] for $i = \{1,2,\ldots,2^n\}$ find p_i using
   $\alpha_{(p-1)2^m+i} = r$
   The indices of this equation gives p_i.

6. The desired state feedback matrix will be:
   $K = \delta_{2^m}[p_1 \;\; p_2 \;\; \ldots p_{2^n}]$
   This feedback matrix stabilize the Boolean control network 5.1 to x^*.

7. The structure matrices of Boolean function can be found as :
   $K_i = S_i K$ for $i = \{1,2,\ldots,m\}$ where
   $S_i = 1_{2^{i-1}} \otimes I_2 \otimes 1_{2^{m-i}}$ for each $i = \{1,2,\ldots,2^m\}$.
   K_i can be converted into its logical form which gives the intervention.
   $U_i = K_i X$

5.2 Intervention Design for FASN Pathway

In FASN signaling pathway drugs are used as control inputs which change the state of nodes to desired state. By using the intervention design find the state of drugs for a healthy pathway. State observer estimate the states of pathway for intervention design.

The algebraic representation for FASN signaling pathway 4.1 is given as:

$$\begin{aligned}
HER2(k+1) &= \neg Trastuzumab \wedge FASN(k) \\
PI3K(k+1) &= \neg Miltefosine1 \wedge HER2(k) \rightarrow PI3K(k) \\
AKT(k+1) &= \neg Miltefosine2 \wedge FASN(k) \wedge PI3K(n) \\
mTORc1(k+1) &= \neg Miconazole \wedge AKT(k) \rightarrow mTORc1(k) \\
pSREBP1c(k+1) &= \neg Inhibitor \wedge FASN(k) \wedge mTORc1(k) \\
FASN(k+1) &= \neg Orlistet \wedge pSREBP1c(k) \rightarrow FASN(k)
\end{aligned} \tag{5.3}$$

1. First using STP 5.3 can be simplified as: $X(t+1) = L \ltimes X(t) \ltimes U(t)$

   $X = HER2 \ltimes PI3K \ltimes AKT \times mTORc1 \ltimes pSREBP1c \ltimes FASN$

   $U = Tratuzumab \ltimes Miltefosine1 \ltimes Miltefosine2 \ltimes Miconazole \ltimes Orlistat \ltimes Inhibitor$

   $L = [64\ 64\ 64\ 64\ 64\ 64\ 64\ \ldots\ 43\ 44\ 43\ 42]$

2. The reference attractor or fixed point for 5.3 is chosen as:

   $\delta_{64}(r) = [\delta_2(2) \ltimes \delta_2(1) \ltimes \delta_2(2) \ltimes \delta_2(1) \ltimes \delta_2(2) \ltimes \delta_2(2)]$

   This gives $r = 44$ so the attractor will be δ_{64}^{44}.

3. For $r = 44$ find the indices of r from L which is:

   $In(r) = [1281\ 1282\ 1283\ 1284\ 1289\ \ldots\ 4043\ 4094]$

   For $In(r)$ find p_i and q_i using:

   $\alpha_{(p-1)2^n+q} = r$

   which gives

   $p = [21, 21, 21, 21, \ldots, 64, 63, 64]$

   $E_1(r) = q = [1, 2, 3, 4, 9, \ldots, 62, 64, 62]$

4. Find $E_2(r)$ which consist of the remaining q_i for indices find in L using

   $\alpha_{(p-1)2^n+q} = r$

$E_2(r) = [5,6,7,8,17,18,19,20,\ldots,53,54,55,56]$

if $D = E_1(r) \bigcup E_2(r)$ where $D = \{1,2,3,4,5 \ldots 62,63,64\}$ which shows that 5.3 is globally stabalizibale to X^*.

5. Find the p_i for the indices of $In(r)$ using

   $\alpha_{(p-1)2^m+i} = r$ where $i = \{1,2,3,\ldots,64\}$

   $p = [21,21,21,21,69,59,56\ldots,21,21,22]$

6. So the state feedback matrix will be

   $K = [21,21,21,21,69,59,56\ldots,21,21,22]$ where

   $K_i = S_i K$ for $i = \{1,2,3,6,5,6\}$ so

   $K_1 \in R^{2\times 64} = [1,1,1,1,1,\ldots 1,1,1,1]$

   $K_2 \in R^{2\times 64} = [2,2,2,2,2,\ldots 1,1,1,1]$

   $K_3 \in R^{2\times 64} = [1,1,1,1,2,\ldots 1,1,1,1]$

   $K_4 \in R^{2\times 64} = [2,2,2,2,1,\ldots 2,2,2,2]$

   $K_5 \in R^{2\times 64} = [1,1,1,1,2,\ldots 1,1,1,1]$

   $K_6 \in R^{2\times 64} = [1,1,1,1,2,\ldots 1,1,1,2]$

7. Find the control input using feedback law:

   $U(t) = KX(t)$

   $U(t) = K\hat{x}_s(t)$ where

   $\hat{x}_s(t) = \{ObsHER2 \ltimes ObsPI3K \ltimes ObsAKT \times ObsmTORc1 \ltimes ObspSREBP1c \ltimes ObsFASN\}$

   $U(t) = K\{ObsHER2 \ltimes ObsPI3K \ltimes ObsAKT \times ObsmTORc1 \ltimes ObspSREBP1c \ltimes ObsFASN\}$

   $\hat{x}_s(t)$ can be find from state observer. So

   $u_1 = Trastuzumab = K_1\hat{x}_s(t) = \delta_2^1$

   $u_2 = Miltefosine1 = K_2\hat{x}_s(t) = \delta_2^2$

   $u_3 = Miltefosine2 = K_3\hat{x}_s(t) = \delta_2^1$

   $u_4 = Miconazole = K_4\hat{x}_s(t) = \delta_2^2$

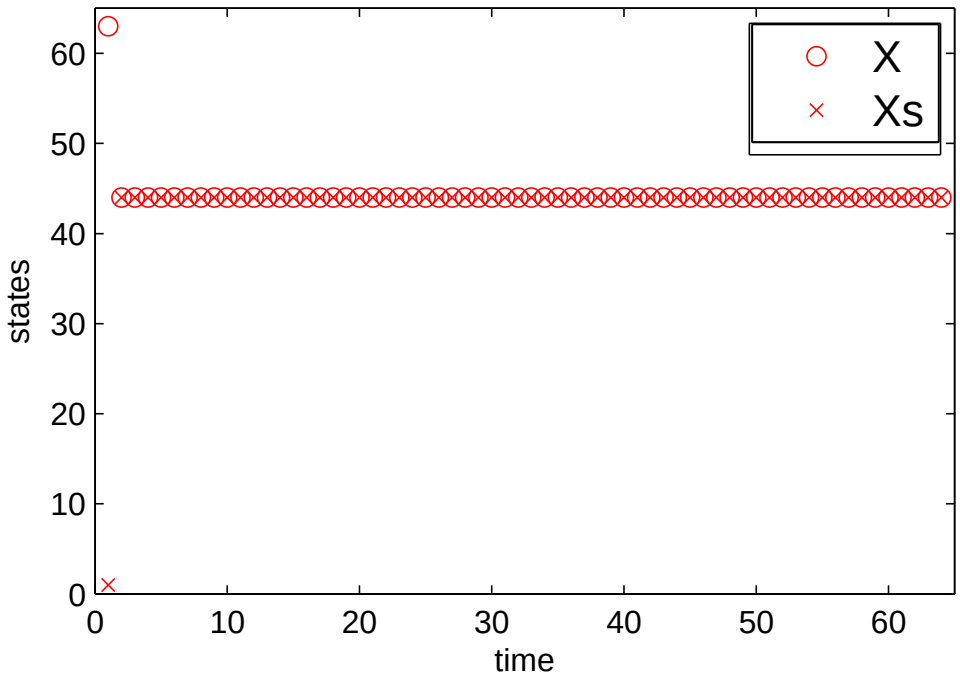

FIGURE 5.1: Intervention Design for FASN Pathway

$$u_5 = Inhibitor = K_5\hat{x}_s(t) = \delta_2^1$$
$$u_6 = Orlistat = K_6\hat{x}_s(t) = \delta_2^1$$

Figure 5.1 show the intervention design that using control input, drugs given in figure 5.2 will stabilize the FASN pathway to the global attractor 5.3 for all initial states. $X(t;X_0;U) = X^*$.

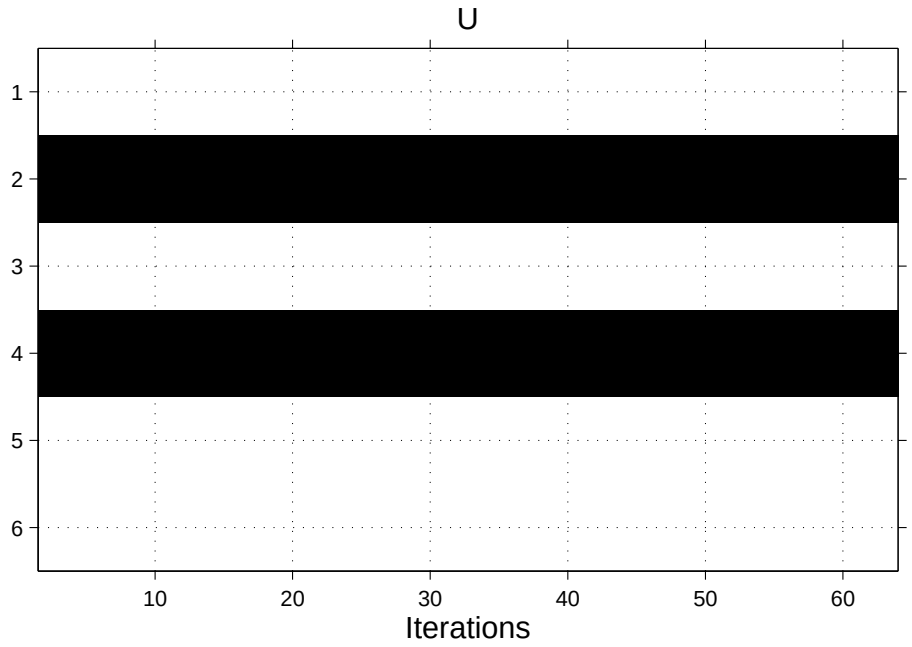

FIGURE 5.2: Selected Drugs

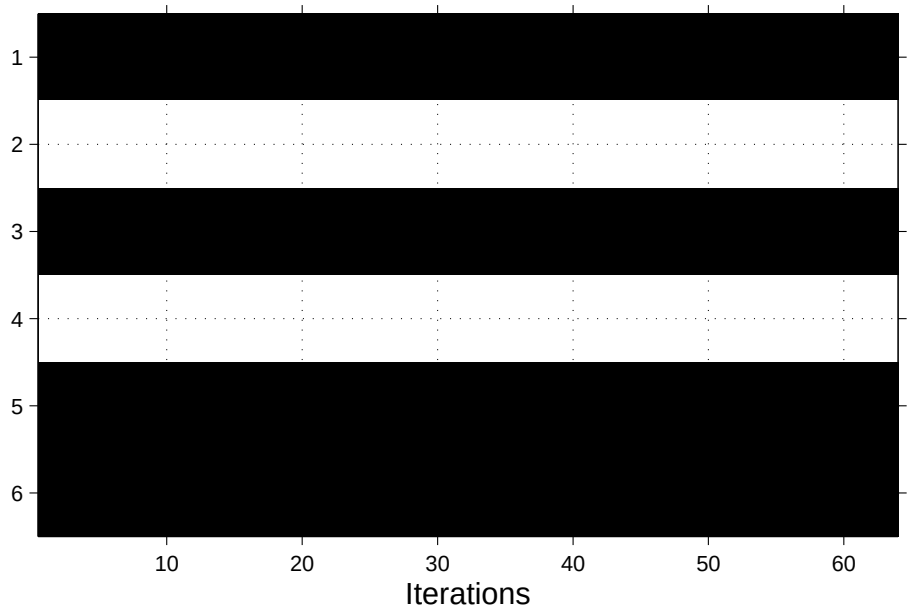

FIGURE 5.3: Global Attractor

Chapter 6

CONCLUSIONS AND FUTURE WORK

6.1 Conclusions

It has been shown that growth factor signaling pathway is responsible for the over expression of FASN which is the cause of breast cancer. All the nodes of pathway are logically connected, using the Boolean rules the mathematical model for FASN pathway is formulated. Selected drugs for each nodes are identified and use as control input to form the Boolean control network model for intervention design.

The observability of such Boolean control network model can be checked. The state observer, Luenberger-like observer with its transition matrix are formulated for FASN pathway than the states of model are estimated using input state trajectories.

The intervention/controller design for signaling pathway need the state vector estimation which can be obtained by Luenberger-like observer. A global attractor is find using the transition matrix of the model which gives the state feedback matrix. The state feedback matrix and the estimated states of observer for the global attractor gives the states of each drugs for inhibited signaling pathway.

6.2 Future Work

The work carried out in this thesis can be extended in several ways. A benchmark has been laid down which can be extended to find intervention design for several pathways. There are many other signaling pathways, which are involved in cancer and other disease so using this procedure intervention design can be simulated for different pathways. When the number of nodes increases in cellular pathways the size of transition matrix increases which produces much complexity that need to be solved so large networks can be modeled by using Boolean Networks.

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