Chapter 4

Controlling mechanism of apoptosis by SMAR1 via p53

4.1 Introduction

Proper coping up with data obtained as time series may offer complicated inherent properties of the system, how the system is interacting with the environment, and other systems close to it. Following the classical definition of fractal in given by Mandelbrot’s picture [131], the self-similarity dimension $D$ of a time series having window size of $T(\kappa t)$, with scale factor $\kappa$ and time sequence $t$ follow the scaling law [132],

$$T(\kappa t) = \kappa^D T(t); \quad w = \frac{T(\kappa t)}{T(t)}; \quad w = \kappa^D$$  \hspace{1cm} (4.1)

Complex time series usually has multiple of such scaling behaviours exhibiting multifractal nature owing to scale dependent broad chance distributions of the time series, and varied range of long and short correlations revised by small and large variation in the time series [126]. Since natural systems are complex as they reflect self-organization in their dynamics, we will find ourself eligible to address this
fundamental issues of self-organization by analyzing their sophisticated time series data, for example hypothesis of noise favoured self-organization [128], patterns of order through fluctuations in dynamical systems [129], random perturbation induced change of dynamical states [114], concept of absence of centralized controls in self-organization [130], and their implications in real biological systems.

Found over three decades prior, p53 protein is still a vital and basic molecule to study to investigate new understanding in cell functional association. Various experimental studies have been done on p53 to see how it manages different cell capacities, however the framework level association of these useful pathways in normal, stress and cancerous cells are still to be examined to comprehend the part of p53 in different cell states at key level. On the fundamental level p53 is composed of 393 amino acids [11] and it has a very short half-life of 15–30 minutes [245]. p53 is a multifunctional protein and takes part in various influential processes of cell such as cellular differentiation, maintaining genome integrity, apoptosis, etc. [5]. It is likewise an extremely sensitive molecule which is for the most part enacted because of a few sorts of cell stresses, to be specific DNA damage, interaction with various signalling molecules such as nitric oxide (NO), reactive oxygen synthase (ROS), calcium concentration, and so forth. For instant, in the case of DNA damage when a cell comes under stress the inactivated form of ATM kinase get activated and this inactivated ATM send the spoiled information to p53 [25]. Consequently, phosphorylation of p53 takes place, and the cell is activated into several states to help in repairing the DNA damage, otherwise chooses apoptosis [246, 247]. One of the most vital negative regulators of p53 is Mdm2 [5, 248], and p53 interfaces with Mdm2 through feedback mechanism to keep up with the normal state by concealing such cellular stresses [95]. Further, p53 also acts as transcription factor for various important signalling molecules which participate in several important cellular networks and pathways.
p300, acetylates p53 at its c-terminal to avoid p53-Mdm2 complex formation, and this activity results in the concealing of p53 degradation [242, 249]. It additionally interacts with Mdm2 to form the p300-Mdm2 binary complex as a result of which the level of Mdm2 is decreased in the system [216, 250, 251].

On the other hand, HDAC1 undo the effect of p300 as it deacetylates the acetylated form of p53. This action of deacetylation of p53 by HDAC1 is indirect and which triggers the enhancement of Mdm2 by HDAC1 [99]. Although, the deacetylated form of p53 is very much prone to vulnerability and comes easily in contact with Mdm2, which leads to the degradation of p53 [99, 252].

SMAR1, a target of p53 gene, is a very adaptable molecule as reported by various experimental studies [253–255], and it can interact with p53,Mdm2 and p300 molecules as well with different proclivity [254]. It is been registered that that it enhances the transcriptional activity of p53 and stability of p53 [253, 254]. Moreover, it also shows a negative impact on both Mdm2 and p300 [254]. On DNA damage SMAR1 is being communicated to be active in a p53-dependent manner [255]. It is also been said that the interaction of SMAR1 with p53 helps in stabilizing the p53 in the nucleus by uprooting its negative regulator Mdm2 [256]. Further, p53 has been appeared to be deacetylated by its association with Mdm2 through the recruitment of HDAC1 [99]. SMAR1 can also associated with and deacetylate p53 by selecting HDAC1 [257]. In any case, knockdown of HDAC1 only partially means to rescue p53 acetylation that directly suggests that SMAR1 employees supplemental mechanisms to regulate p53 acetylation [258]. Hence, SMAR1 can be considered as a vital nuclear matrix binding transcription factor which work as a repressor by selecting HDAC1 [259].

Since SMAR1 advances p53 deacetylation by connecting HDAC1 [250] and hinders p300 expression [260], it targets p53 control regulation depending upon the strength of the stress signal given into the system. Couple of exploratory
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reports demonstrate that SMAR1 prompts anti-apoptotic signal of mild DNA damage [254], nonetheless, it supports the cell to go into apoptosis for strong stress signal [260, 261]. But the clear and complex function of SMAR1 relying upon the measure of DNA damage in managing apoptosis still should be examined to comprehend fundamental components of SMAR1, and its part in directing apoptosis. The investigation of the part of SMAR1 in p53 control may open up another comprehension of the regulatory system, stress administration in DNA damage, checking apoptosis and exchanging in malignancy stage.

4.2 p53–SMAR1 complex regulatory biochemical network

4.2.1 Regulatory network model description

In normal cell, p53 is at its minimum concentration condition of due to feedback regulation mechanism established between p53 and Mdm2 [248]. The details of

Figure 4.1: The schematic diagram of SMAR1 driven p53 regulatory network
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reactions and molecular species involved in the model (Figure 4.1) are given in Table 4.1 and Table 4.2 respectively. In the process (Figure 4.1), p53 communicates with Mdm2 gene leading to the transcription of Mdm2 mRNA followed by Mdm2 translation of protein. Since, properties of Mdm2 protein is reported as E3 ubiquitin ligase, it targets p53 protein, which leads to the degradation of p53 protein. Thus, it is clear that the p53 and Mdm2 proteins are attached to each other through an auto-regulatory negative feedback loop which keep up the low cellular p53 level (reaction 7 in Table 4.2). When a stress is experienced by the cell, the inactive ATM (ATM\textsubscript{I}) changes to into activated form ATM\textsubscript{A}, and acts as a catalyst to phosphorylate p53. Then dephosphorylation of p53\textsubscript{P} to p53 allows to increase p53 population. However, in the absence of stress, ATM\textsubscript{A} will change back to ATM\textsubscript{I} to maintain normal state. p300 recruits p53-p300 complex which is followed by acetylation of p53 and subsequent deacetylation of p53 leads to increase in the production of p53. In addition to it, p300 can also communicates with Mdm2 to form complex as Mdm2-p300, and when this association communicates with p53 it degrade itself as well as suppressing p53 [95, 249]. Then again, p53-Mdm2 can associate with p300 to form a triplet p53-Mdm2-p300, following this dissociation of this triplet complex to p53-p300 complex and Mdm2 (i.e. activating Mdm2). The key role of HDAC1 is to deacetylate the acetylated p53 through intermediate formation of Mdm2-HDAC1 complex to recruit p53 [250]. SMAR1 an important DNA binding protein phosphorylate p53 [262, 263], but helps in selecting p53 from p53\textsubscript{P}-p300 complex in its presence [250]. It can interact with Mdm2 directly to create Mdm2-SMAR1 complex [250], or indirectly in the process of phosphorylation of p53 from p53-Mdm2-SMAR1 complex [250]. This Mdm2-SMAR1 complex has an affinity to interact with HDAC1 to form Mdm2-SMAR1-HDAC1 complex. The SMAR1 play a critical role on p300 that is, to degrade p300 in its presence and interact with p53 via p53-p300 complex in the dephosphorylating process of
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p53. The workflow of the study of the model system is given in Figure 4.2.

![Figure 4.2: Workflow of the methods we implemented in the analysis of the model system](image)

Table 4.1: Molecular species and their initial concentration of p53–SMAR1 model

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Species Name</th>
<th>Description</th>
<th>Notation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>p53</td>
<td>Unbounded p53 protein</td>
<td>x1</td>
</tr>
<tr>
<td>2.</td>
<td>Mdm2</td>
<td>Unbounded Mdm2 protein</td>
<td>x2</td>
</tr>
<tr>
<td>3.</td>
<td>Mdm2 mRNA</td>
<td>Mdm2 messenger mRNA</td>
<td>x3</td>
</tr>
<tr>
<td>4.</td>
<td>p53-Mdm2</td>
<td>Mdm2 with p53 complex</td>
<td>x4</td>
</tr>
<tr>
<td>5.</td>
<td>ATM$_I$</td>
<td>Inactivated ATM protein</td>
<td>x5</td>
</tr>
<tr>
<td>6.</td>
<td>ATM$_A$</td>
<td>Activated ATM protein</td>
<td>x6</td>
</tr>
<tr>
<td>7.</td>
<td>p53$_P$</td>
<td>Phosphorylated p53 protein</td>
<td>x7</td>
</tr>
<tr>
<td>8.</td>
<td>p300</td>
<td>Unbounded p300 protein</td>
<td>x8</td>
</tr>
<tr>
<td>9.</td>
<td>p53$_P$-p300</td>
<td>Phosphorylated p53 and p300 complex</td>
<td>x9</td>
</tr>
<tr>
<td>10.</td>
<td>p53$_A$</td>
<td>Acetylated p53 protein</td>
<td>x10</td>
</tr>
<tr>
<td>11.</td>
<td>HDAC1</td>
<td>Unbounded HDAC1 protein</td>
<td>x11</td>
</tr>
<tr>
<td>12.</td>
<td>Mdm2-SMAR1-HDAC1</td>
<td>Mdm2, SMAR1 and HDAC1 complex</td>
<td>x12</td>
</tr>
<tr>
<td>13.</td>
<td>p53-Mdm2-p300</td>
<td>Mdm2, p53 and p300 complex</td>
<td>x13</td>
</tr>
<tr>
<td>14.</td>
<td>Mdm2-p300</td>
<td>Mdm2 and p300 complex</td>
<td>x14</td>
</tr>
<tr>
<td>15.</td>
<td>SMAR1</td>
<td>Unbounded SMAR1 protein</td>
<td>x15</td>
</tr>
<tr>
<td>16.</td>
<td>Mdm2-SMAR1</td>
<td>Mdm2 and SMAR1 complex</td>
<td>x16</td>
</tr>
<tr>
<td>17.</td>
<td>p53-Mdm2-SMAR1</td>
<td>p53, Mdm2 and SMAR1 complex</td>
<td>x17</td>
</tr>
<tr>
<td>18.</td>
<td>Mdm2-HDAC1</td>
<td>Mdm2 and HDAC1 complex</td>
<td>x18</td>
</tr>
</tbody>
</table>
4.2.2 Mathematical model of the network

The p53-SMAR1 regulatory network is modeled by a set of variables \( \{X_i\}_{i=1,2,...,N} \) with \( N = 18 \) (18 molecular species given in Table 4.1) which undergo thirty five reaction channels (\( M = 35 \) given in Table 4.2). The state vector \( \vec{X} \) at any instant of time ‘\( t \)’ is given by \( \vec{X}(t) = (X_1, \ldots, X_N)^T \), where the variables in the vector are populations of the molecular species listed in Table 4.1. The deterministic (classical) equations constructed from the reactions in the network (Figure 4.1) are given. The coupled set of non-linear ODEs can be solved numerically using the standard 4\(^{th}\) order Runge–Kutta algorithm of numerical integration [188] to study dynamical behaviour of the system.

where \( \{k_i\} \) and \( \{x_i\} \), for \( i = 1, 2, \ldots, N \), represent the sets of rate constants of the reactions (Table 4.2) and concentration of the variables corresponding to the molecular species \( x_i = X_i/V \), where \( V \) is system size (Table 4.1), respectively.

\[
\frac{dx_1}{dt} = -k_1x_1x_{14} + k_6 - k_8x_1x_2 + k_9x_4 - k_{12}x_1x_6 + k_{13}x_7 + k_{17}x_{10}x_{12} - k_{29}x_1x_{15} + k_{33}x_{11}x_{18} + k_{34}x_9x_{15} \tag{4.2}
\]

\[
\frac{dx_2}{dt} = k_2x_3 - k_5x_2 + k_7x_4 - k_8x_1x_2 + k_9x_4 - k_{18}x_2x_{15} - k_{20}x_2x_8 + k_{21}x_{13} - k_{32}x_2x_{11} \tag{4.3}
\]

\[
\frac{dx_3}{dt} = k_3x_1 - k_4x_3 \tag{4.4}
\]

\[
\frac{dx_4}{dt} = -k_7x_4 + k_8x_1x_2 - k_9x_4 - k_{19}x_4x_8 - k_{30}x_4x_{15} \tag{4.5}
\]

\[
\frac{dx_5}{dt} = -k_{10}x_5 + k_{11}x_6 \tag{4.6}
\]

\[
\frac{dx_6}{dt} = k_{10}x_5 - k_{11}x_6 - k_{12}x_1x_6 \tag{4.7}
\]

\[
\frac{dx_7}{dt} = k_{12}x_1x_6 - k_{13}x_7 - k_{15}x_7x_8 + k_{29}x_1x_{15} + k_{31}x_{17} \tag{4.8}
\]

\[
\frac{dx_8}{dt} = -k_{14}x_8 - k_{15}x_8x_7 - k_{19}x_4x_8 - k_{20}x_2x_8 + k_{21}x_{13} + k_{23} - k_{35}x_8x_{15} \tag{4.9}
\]
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\[
\begin{align*}
\frac{dx_9}{dt} &= k_{15}x_8x_7 - k_{16}x_9 - k_{34}x_9x_{15} \\
\frac{dx_{10}}{dt} &= k_{16}x_9 - k_{17}x_{10}x_{12} - k_{33}x_{10}x_{18} \\
\frac{dx_{11}}{dt} &= -k_{25}x_{11}x_{16} - k_{22}x_{11} + k_{24} - k_{32}x_2x_{11} \\
\frac{dx_{12}}{dt} &= -k_{17}x_{10}x_{12} + x_{25}x_{11}x_{16} \\
\frac{dx_{13}}{dt} &= k_{19}x_4x_8 - k_{21}x_{13} \\
\frac{dx_{14}}{dt} &= -k_{1}x_1x_{14} + k_{20}x_2x_8 \\
\frac{dx_{15}}{dt} &= -k_{18}x_2x_{15} + k_{26} - k_{27}x_{15} - k_{29}x_1x_{15} \\
\frac{dx_{16}}{dt} &= k_{18}x_2x_{15} - k_{25}x_{11}x_{16} - k_{28}x_{16} + k_{31}x_{17} \\
\frac{dx_{17}}{dt} &= k_{30}x_4x_{15} - k_{31}x_{17} \\
\frac{dx_{18}}{dt} &= k_{32}x_2x_{11} - k_{33}x_{11}x_{18}
\end{align*}
\]  

(4.10) – (4.19)

4.2.3 Quasi–steady–state approximation

The system of reactions listed in Table 4.2 can be approximately divided into two types of elementary reactions, namely, fast and slow reactions within the formalism of quasi–steady–state approximation [264]. Hence the variables in the state vector \( \vec{x} \) can be divided into fast and slow vectors. Since the dynamics of fast variables reach equilibrium much faster than the slow variables dynamics, one can approximately reduce the dynamics of state variables to the dynamics of the slow variables only [264]. This reduced system of ODEs can be solved analytically to understand the approximate behaviour of the state variables (see chapter 2 section 2.3 for detail) The system of reactions (Table 4.2), from which the ODEs (4.2)–(4.19) were constructed, can be approximately divided into two types of elementary reactions, namely, fast and slow reactions [264]. The variables in the
### Table 4.2: List of chemical reaction, kinetic law and their rate constant of p53–SMAR1 model

<table>
<thead>
<tr>
<th>S.No</th>
<th>Reaction</th>
<th>Name of process</th>
<th>Kinetic Law</th>
<th>Rate Constant</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$x_1 + x_{14} \xrightarrow{k_1} \phi$</td>
<td>p53 degradation</td>
<td>$k_1(x_1)(x_{14})$</td>
<td>$8.25 \times 10^{-4}$sec$^{-1}$</td>
<td>[95, 249]</td>
</tr>
<tr>
<td>2</td>
<td>$x_3 \xrightarrow{k_2} x_3 + x_2$</td>
<td>Mdm2 creation</td>
<td>$k_2(x_3)$</td>
<td>$4.95 \times 10^{-4}$sec$^{-1}$</td>
<td>[25]</td>
</tr>
<tr>
<td>3</td>
<td>$x_1 \xrightarrow{k_3} x_1 + x_3$</td>
<td>Mdm2 mRNA creation</td>
<td>$k_3(x_1)$</td>
<td>$1.0 \times 10^{-4}$sec$^{-1}$</td>
<td>[25]</td>
</tr>
<tr>
<td>4</td>
<td>$x_3 \xrightarrow{k_4} \phi$</td>
<td>Mdm2 mRNA degradation</td>
<td>$k_4(x_3)$</td>
<td>$1.0 \times 10^{-4}$sec$^{-1}$</td>
<td>[25]</td>
</tr>
<tr>
<td>5</td>
<td>$x_2 \xrightarrow{k_5} \phi$</td>
<td>Mdm2 degradation</td>
<td>$k_5(x_2)$</td>
<td>$4.33 \times 10^{-4}$sec$^{-1}$</td>
<td>[25]</td>
</tr>
<tr>
<td>6</td>
<td>$\phi \xrightarrow{k_6} x_1$</td>
<td>p53 synthesis</td>
<td>$k_6$</td>
<td>$0.078$sec$^{-1}$</td>
<td>[25]</td>
</tr>
<tr>
<td>7</td>
<td>$x_4 \xrightarrow{k_7} x_2$</td>
<td>p53-Mdm2 degradation</td>
<td>$k_7(x_4)$</td>
<td>$8.25 \times 10^{-4}$sec$^{-1}$</td>
<td>[265, 266]</td>
</tr>
<tr>
<td>8</td>
<td>$x_1 + x_2 \xrightarrow{k_8} x_4$</td>
<td>p53-Mdm2 synthesis</td>
<td>$k_8(x_1)(x_2)$</td>
<td>$11.55 \times 10^{-4}$sec$^{-1}$</td>
<td>[25]</td>
</tr>
<tr>
<td>9</td>
<td>$x_4 \xrightarrow{k_9} x_1 + x_2$</td>
<td>p53-Mdm2 dissociation</td>
<td>$k_9(x_4)$</td>
<td>$11.55 \times 10^{-6}$sec$^{-1}$</td>
<td>[25]</td>
</tr>
<tr>
<td>10</td>
<td>$x_5 \xrightarrow{k_{10}} x_6$</td>
<td>ATM activation</td>
<td>$k_{10}(x_5)$</td>
<td>$1.0 \times 10^{-4}$sec$^{-1}$</td>
<td>[266, 267]</td>
</tr>
<tr>
<td>11</td>
<td>$x_6 \xrightarrow{k_{11}} x_5$</td>
<td>ATM deactivation</td>
<td>$k_{11}(x_6)$</td>
<td>$5.0 \times 10^{-4}$sec$^{-1}$</td>
<td>[266, 267]</td>
</tr>
<tr>
<td>12</td>
<td>$x_1 + x_6 \xrightarrow{k_{12}} x_6 + x_7$</td>
<td>Phosphorylation of p53</td>
<td>$k_{12}(x_1)(x_6)$</td>
<td>$5.0 \times 10^{-4}$sec$^{-1}$</td>
<td>[266]</td>
</tr>
<tr>
<td>13</td>
<td>$x_7 \xrightarrow{k_{13}} x_1$</td>
<td>Dephosphorylation of p53</td>
<td>$k_{13}(x_7)$</td>
<td>$5.0 \times 10^{-1}$sec$^{-1}$</td>
<td>[266, 267]</td>
</tr>
<tr>
<td>14</td>
<td>$x_8 \xrightarrow{k_{14}} \phi$</td>
<td>p300 degradation</td>
<td>$k_{14}(x_8)$</td>
<td>$1.0 \times 10^{-4}$sec$^{-1}$</td>
<td>[262, 263]</td>
</tr>
<tr>
<td>15</td>
<td>$x_7 + x_8 \xrightarrow{k_{15}} x_9$</td>
<td>p53-p300 formation</td>
<td>$k_{15}(x_7)(x_8)$</td>
<td>$1.0 \times 10^{-4}$sec$^{-1}$</td>
<td>[216]</td>
</tr>
<tr>
<td>16</td>
<td>$x_9 \xrightarrow{k_{16}} x_{10}$</td>
<td>Acetylation of p53</td>
<td>$k_{16}(x_9)$</td>
<td>$1.0 \times 10^{-4}$sec$^{-1}$</td>
<td>[242, 250]</td>
</tr>
<tr>
<td>17</td>
<td>$x_{10} + x_{12} \xrightarrow{k_{17}} x_{11}$</td>
<td>Deacetylation of p53</td>
<td>$k_{17}(x_{10})(x_{12})$</td>
<td>$1.0 \times 10^{-5}$sec$^{-1}$</td>
<td>[250]</td>
</tr>
<tr>
<td>18</td>
<td>$x_2 + x_{15} \xrightarrow{k_{18}} x_{16}$</td>
<td>Creation of Mdm2-SMAR1</td>
<td>$k_{18}(x_2)(x_{15})$</td>
<td>$2.0 \times 10^{-4}$sec$^{-1}$</td>
<td>[250]</td>
</tr>
<tr>
<td>19</td>
<td>$x_4 + x_3 \xrightarrow{k_{19}} x_{13}$</td>
<td>Creation of p53-Mdm2-p300</td>
<td>$k_{19}(x_4)(x_3)$</td>
<td>$5.0 \times 10^{-4}$sec$^{-1}$</td>
<td>[216]</td>
</tr>
<tr>
<td>20</td>
<td>$x_2 + x_8 \xrightarrow{k_{20}} x_{14}$</td>
<td>Formation of Mdm2-p300</td>
<td>$k_{20}(x_2)(x_8)$</td>
<td>$5.0 \times 10^{-4}$sec$^{-1}$</td>
<td>[25, 95]</td>
</tr>
<tr>
<td>21</td>
<td>$x_{13} \xrightarrow{k_{21}} x_2 + x_9$</td>
<td>Dissociation of p53-Mdm2-p300</td>
<td>$k_{21}(x_{13})$</td>
<td>$1.0 \times 10^{-4}$sec$^{-1}$</td>
<td>[216, 265]</td>
</tr>
<tr>
<td>22</td>
<td>$x_{11} \xrightarrow{k_{22}} \phi$</td>
<td>Degradation of HDAC1</td>
<td>$k_{22}(x_{11})$</td>
<td>$1.0 \times 10^{-4}$sec$^{-1}$</td>
<td>[250]</td>
</tr>
<tr>
<td>23</td>
<td>$\phi \xrightarrow{k_{23}} x_8$</td>
<td>p300 synthesis</td>
<td>$k_{23}(x_{p300})$</td>
<td>$0.1$sec$^{-1}$</td>
<td>[262, 263]</td>
</tr>
<tr>
<td>24</td>
<td>$\phi \xrightarrow{k_{24}} x_{11}$</td>
<td>HDAC1 synthesis</td>
<td>$k_{24}(x_{HDAC1})$</td>
<td>$2.0 \times 10^{-4}$sec$^{-1}$</td>
<td>[250]</td>
</tr>
<tr>
<td>25</td>
<td>$x_{11} + x_{16} \xrightarrow{k_{25}} x_{12}$</td>
<td>Synthesis of Mdm2-SMAR1-HDAC1</td>
<td>$k_{25}(x_{11})(x_{16})$</td>
<td>$1.0 \times 10^{-4}$sec$^{-1}$</td>
<td>[250]</td>
</tr>
</tbody>
</table>
state vector $\vec{x}$ can be divided into fast and slow vectors given by

$$\vec{x}^s = \begin{bmatrix} x_1 \\ x_2 \\ x_8 \\ x_{11} \\ x_{15} \end{bmatrix}; \quad \vec{x}^f = \begin{bmatrix} y_1 \\ y_2 \\ y_3 \\ y_4 \end{bmatrix}; \quad \vec{x} = \begin{bmatrix} \vec{x}^s \\ \vec{x}^f \end{bmatrix}; \quad y_1 = \begin{bmatrix} x_3 \\ x_4 \\ x_5 \\ x_6 \\ x_7 \end{bmatrix}; \quad y_2 = \begin{bmatrix} x_9 \\ x_{10} \end{bmatrix}; \quad y_3 = \begin{bmatrix} x_{12} \\ x_{13} \end{bmatrix}; \quad y_4 = \begin{bmatrix} x_{14} \\ x_{18} \end{bmatrix}$$

The fast variables are normally corresponding to complex molecular species. Generally, formation of complex molecular species due to fast reactions is followed by fast decay of these complexes, the dynamics of the fast variables reaches steady state much quickly as compared to the dynamics of slow variables [200, 268]. We then use Henri–Michaelis–Menten–Briggs–Haldane approximation to assume that the time evolution of fast state vector $\vec{x}^f$ reaches equilibrium state defined by $\vec{x}^{eq}$ much faster as compared to the time evolution of slow state vector $\vec{x}^s$ [200, 268].

### Table 4.2 Continue......

<table>
<thead>
<tr>
<th>S.No</th>
<th>Reaction</th>
<th>Name of process</th>
<th>Kinetic Law</th>
<th>Rate Constant</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>26</td>
<td>$\phi \xrightarrow{k_{26}} x_{15}$</td>
<td>SMAR1 synthesis</td>
<td>$k_{26}$</td>
<td>$0.08\text{sec}^{-1}$</td>
<td>[262,263]</td>
</tr>
<tr>
<td>27</td>
<td>$x_{15} \xrightarrow{k_{27}} \phi$</td>
<td>Degradation of SMAR1</td>
<td>$k_{27}$</td>
<td>$1.0 \times 10^{-4}\text{sec}^{-1}$</td>
<td>[250]</td>
</tr>
<tr>
<td>28</td>
<td>$x_{16} \xrightarrow{k_{28}} \phi$</td>
<td>Degradation of Mdm2-SMAR1 complex</td>
<td>$k_{28}(x_{16})$</td>
<td>$2.0 \times 10^{-4}\text{sec}^{-1}$</td>
<td>[250]</td>
</tr>
<tr>
<td>29</td>
<td>$x_1 + x_{15} \xrightarrow{k_{29}} x_7$</td>
<td>Phosphorylation of p53</td>
<td>$k_{29}(x_1)(x_{15})$</td>
<td>$1.0 \times 10^{-4}\text{sec}^{-1}$</td>
<td>[262,263]</td>
</tr>
<tr>
<td>30</td>
<td>$x_4 + x_{15} \xrightarrow{k_{30}} x_{17}$</td>
<td>Synthesis of p53-Mdm2-SMAR1 complex</td>
<td>$k_{30}(x_4)(x_{15})$</td>
<td>$1.0 \times 10^{-3}\text{sec}^{-1}$</td>
<td>[250]</td>
</tr>
<tr>
<td>31</td>
<td>$x_7 \xrightarrow{k_{31}} x_7 + x_{16}$</td>
<td>Phosphorylation of p53</td>
<td>$k_{31}(x_{17})$</td>
<td>$1.0 \times 10^{-3}\text{sec}^{-1}$</td>
<td>[250]</td>
</tr>
<tr>
<td>32</td>
<td>$x_2 + x_{11} \xrightarrow{k_{32}} x_{18}$</td>
<td>Mdm2-HDAC1 complex synthesis</td>
<td>$k_{32}(x_2)(x_{11})$</td>
<td>$2.0 \times 10^{-3}\text{sec}^{-1}$</td>
<td>[250]</td>
</tr>
<tr>
<td>33</td>
<td>$x_{18} + x_{10} \xrightarrow{k_{33}} x_1$</td>
<td>Synthesis of p53</td>
<td>$k_{33}(x_{18})(x_{10})$</td>
<td>$5.0\text{sec}^{-1}$</td>
<td>[262,263]</td>
</tr>
<tr>
<td>34</td>
<td>$x_9 + x_{15} \xrightarrow{k_{34}} x_1 + x_{15}$</td>
<td>Interaction of p53-p300 complex with SMAR1</td>
<td>$k_{34}(x_9)(x_{15})$</td>
<td>$1.0 \times 10^{-4}\text{sec}^{-1}$</td>
<td>[250]</td>
</tr>
<tr>
<td>35</td>
<td>$x_8 + x_{15} \xrightarrow{k_{35}} x_{15}$</td>
<td>Degradation of p300 by SMAR1</td>
<td>$k_{35}(x_8)(x_{15})$</td>
<td>$5.0 \times 10^{-1}\text{sec}^{-1}$</td>
<td>[262,263]</td>
</tr>
</tbody>
</table>
Applying this approximation, we can reach the following steady state for fast
variables,
\[ \frac{d\vec{x}^f}{dt} \approx 0; \quad \vec{x}^* = \begin{bmatrix} y_1^* \\ y_2^* \\ y_3^* \\ y_4^* \end{bmatrix}; \quad y_2^* = \begin{bmatrix} x_3^* \\ x_4^* \end{bmatrix}; \quad y_2^* = \begin{bmatrix} x_9^* \\ x_{10}^* \end{bmatrix}; \quad y_5^* = \begin{bmatrix} x_{12}^* \\ x_{13}^* \end{bmatrix}; \quad y_4^* = \begin{bmatrix} x_{16}^* \\ x_{17}^* \end{bmatrix} \tag{4.21} \]
such that the dynamics of the system for sufficiently large time is governed by the
dynamics of the slow variables given by
\[ \frac{d\vec{x}}{dt} = \frac{d}{dt} \begin{bmatrix} \vec{x}^s \\ \vec{x}^f \end{bmatrix} \approx \frac{d\vec{x}^s}{dt} = \frac{d}{dt} \begin{bmatrix} x_1 \\ x_2 \\ x_8 \\ x_{11} \\ x_{15} \end{bmatrix} \tag{4.22} \]

The approximate solution of the complex model can be obtained from this reduced
model using quasi–steady–state approximation.

### 4.2.4 Multifractal DFA approach

We then simulate the complete system model system, consisting of eighteen dif-
ferential equations (4.2)–(4.19) using standard 4th order Runge–Kutta algorithm
of numerical integration [188], to understand the dynamical behaviour of the state
variables of the system. We have implemented few techniques of time series anal-
ysis to investigate the dynamical properties of the state variables of the model
system (details can be obtained in chapter 2 section 2.5). To understand multi-
fractal properties and to detect important correlations of the non-stationary simu-
lated time series data of the state variables, we implemented Multifractal detrended
fluctuation analysis (MF-DFA) technique and its algorithm [127] (see chapter 2
section 2.5 for details). Fractal parameters, namely, Hurst exponent \((H)\), generalized dimension \((D)\) etc are calculated numerically using method adopted by Kantelhardt \textit{et al.} [126] to characterize the properties of the system.

### 4.2.5 Visibility graph of time series

Another technique, known as \textit{visibility graph}, which maps a time series to a network [201], where each observation in time series is translated to a node and an edge between any two nodes is introduced when the following visibility condition is satisfied, is used to understand the properties of the system by analyzing the topological properties of the constructed networks from the time series of the system. Since the properties of the time series are inherited to the corresponding network, the studies of this network provide useful information which can’t be observed in traditional time series data. The details of the method are provided in chapter 2 section 2.4.

### 4.2.6 Permutation entropy: a measure of complexity

The complex information contained in a system is inherited in the time series of the constituting variables of the system, and can be measured by calculating \textit{permutation entropy} of the time series [138,208] (See chapter 2 section 2.6). This technique can able to highlight the nature of complexity introduced in the system due to complicated inter-molecular interaction in the system and perturbation induced by the surrounding environmental (diffusion of molecules from other pathways and surrounding cells, temperature fluctuations and other related factors) fluctuations.
4.3 Results and discussion

4.3.1 Complexity in transition of p53 states

The sensitive and nuclear matrix SMAR1 interacts directly with Mdm2, and indirectly with p53 (Figure 4.1, Table 4.2) followed by the formation of various complexes. In this model, we focus on the study of the dynamical states of p53, which correspond to various cellular states, triggered by various signalling molecules, namely, SMAR1, p300 and HDAC1, respectively. For fixed concentration levels of p300 and HDAC1 (fixing their creation rates \( k_{p300} = 0.1 \) and \( k_{HDAC1} = 0.01 \)), variation in concentration level of SMAR1 (its creation rate \( k_{SMAR1} \)) drives p53 dynamics to three distinct states: first steady state corresponding to nearly normal state \( (k_{SMAR1} < 0.0001) \), sustain oscillation state corresponding to significantly strong stress condition \( (k_{SMAR1} \rightarrow [0.06-0.28]) \) and second steady state corresponding to excess stress or apoptotic state \( (k_{SMAR1} > 0.31) \) (Figure 4.3 panel A). Significantly high concentration level of SMAR1 trigger DNA damage to activate p53 exhibited in p53 dynamics, and its excess concentration drives the system to apoptosis [254]. Two mixed states (mixture of damped oscillation for certain time interval and steady state after that time interval in the single time series) are also observed for two different ranges of \( k_{SMAR1} \), i.e. \( [0.0001–0.005] \) and \( [0.06–0.28] \) respectively. The cellular states exhibited in p53 dynamics driven by SMAR1 can also be obtained modulated by HDAC1 keeping concentrations of SMAR1 and p300 constant as supported by earlier reports [262,269–271]. Similar behaviour with these three states is also found for the case of p300-induced p53 dynamics, keeping concentrations of SMAR1 and HDAC1 fixed. Reveals that this signalling molecule has also the tendency to induce apoptosis in the system [219,250].

The permutation entropies \( H_{p53} \) of the p53 dynamics driven by SMAR1 are
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Figure 4.3: The diverse dynamical states of p53 dynamics driven by SMAR1. (A) Time series dynamical states of p53. (B) Permutation entropy spectrum of three states. (C) Multifractal analysis: plot of Hurst exponent ($H_q$), multifractal generalized dimension ($D_q$) as a function of parameter $q$. (D) Amplitudes of p53($A_{p53}$ of the dynamical states as a function of $k_{SMAR1}$). (E) Phase diagram in the parameter space ($k_{HDAC1}$, $k_{SMAR1}$) and ($k_{p300}$, $k_{SMAR1}$) and their respective average amplitudes. (F) Network constructed from the dynamical states using visibility graph approach. (G) Topological properties of the networks corresponding to various dynamical states: The 1st, 2nd, 3rd, 4th, 5th and 6th columns are for $P^{p53}(k)$ vs k, $C^{p53}(k)$ vs k, $C_N^{p53}(k)$ vs k, $C_B^{p53}(k)$ vs k, $C_C^{p53}(k)$ vs k, $C_E^{p53}(k)$ vs k respectively.
calculated to understand the complexity of the cellular states reflected from perturbation induced by SMAR1 to the model network (for embedded dimension $r = 3$ and window size $w_s = 512$). The results (Figure 4.3, panel B) show that for nearly normal state ($k_{SMAR1} = 0.0001$) the values of $H_{p53}$ is low, with large gaps among nearly periodic curves which consist of large number of near zero points. These low values of $H_{p53}$ indicate more ordered state of the system. The increased values of $k_{SMAR1}$ ($k_{SMAR1} = 0.001, 0.01, 0.04$), attribute large values of $H_{p53}$ with decrease in the gap between neighboring curves, showing significant increase in $H_{p53}$ points as compared to the nearly normal state. This indicates that the increase in stress induced by SMAR1 in the system disturbs the ordered state forcing the system to a more complex state. The second stabilized state (with excess SMAR1 concentration in the system corresponding to $k_{SMAR1} = 0.3$) or apoptotic state has maximum $H_{p53}$ value showing most disordered state of the system. Similar behaviour is found for the case of Mdm2 case also [272].

### 4.3.2 Multifractal signature in p53 driven by SMAR1

The behaviour of Hurst exponent $H_q$ (define in chapter 2) as a function of $q$ for different p53 time series driven by SMAR1 (Figure 4.3 panels A) show $q$ dependence in the negative $q$ regime (Figure 4.3 panel C). $H_q$ becomes $q$ independent for $q > -0.6$ and $q < -3$ but depends on the time series at different states. Hence the multifractality in these time series are mainly due to long range correlations in the system. The long range correlation of a time series $x_i$ can be defined by the power law decay of correlation function $C(r) = \langle x_i x_{i+r} \rangle \sim r^{-\phi}$ for large scales of ‘$r$’ with ‘$\phi = 2 - 2H_q$’, or power law decay of power spectra of the time series, $S(f) \sim f^{-b}$, with frequency ‘$f$’ and ‘$b = 2H_q - 1$’, and short range correlation is given by, $C(r) \sim r^{-\phi}$, with $\phi \geq 1$ [126]. Multifractal range (range of $q$ within which $H_q$ depends on $q$) is maximum for active (sustain oscillation) state of p53
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with minimum average $H_q = 1.05 \pm 0.012$ indicating p53 behaviour in this state is due to large fluctuations established mainly by long range correlations due to stress induced by SMAR1. The multifractal range for p53 dynamics at nearly normal state (smallest value of $k_{SMAR1}$ in Figure 4.3 panel A) is reduced significantly with maximum average $H_q = 1.27 \pm 0.011$ value showing significant reduction of fluctuations to maintain towards equilibrium. However, even though this multifractal range is also reduced for near apoptotic state (largest value of $k_{SMAR1}$ in Figure 4.3 panel A) of p53, $H_q = 1.16 \pm 0.013$ is significantly reduced as compared to nearly normal state. This indicates that the dynamics of p53 is disturbed by large fluctuations triggered by excess stress or apoptotic signal. Similar behaviours of p53 at different states are also found in $D_q$ versus $q$ plots which is also reflected in $D_q$ as a function of $H_q$ plots (Figure 4.3 panel C).

Similar behaviours of $H_q$ and $D_q$ obtained in p53 dynamics are also found in Mdm2 dynamics for respective states corresponding to the values of $k_{SMAR1}$ (Figure 4.4 panel C).

4.3.3 Amplitude death: a signature of apoptosis

The amplitude of p53 oscillatory dynamics due to fluctuations induced by changes in the network (for example, changes in concentration of SMAR1, p300 and HDAC1) refers to the amount of stress induced in its dynamics. The amount of stress imparted in the system allows active interaction of p53 with the respective fluctuated molecular species directly or indirectly, and once the stress is removed, the active interaction stays for sometime with damped oscillation and then comes back to the normal state where the amplitude becomes zero (amplitude death) (Figure 4.3 panel D and Figure 4.4 panel D). This time for active interaction increases as the amount of stress is increased, and becomes infinite for a certain range of the value of stress parameter ($k_{SMAR1}$, $k_{p300}$, $k_{HDAC1}$, etc.) which is the
case of sustain oscillation. For excess value of stress parameter than this range, the transition from sustain to damped oscillation states takes place. Further, excess values of stress parameter force the dynamics to amplitude death scenario again (Figure 4.3 panel D and Figure 4.4 panel D). This transition of various oscillating states as a function of stress parameter gives corresponding signatures of the state of the system. Apoptotic state of p53 dynamics shows amplitude death scenario of p53 as a function of $k_{SMAR1}$ for different values of $k_{HDAC1}$ for fixed $k_{p300}$ and $k_{p300}$ for fixed $k_{HDAC1}$ (Figure 4.3 panel D). The non-zero p53 amplitude ($A_{p53}$) involves two states, namely damped state (mixture of stress then coming back to normal after removing of stress or go to apoptosis), and sustain oscillation state (we took long time series of 500 hours, i.e. 5 days duration after removing transients). The range of active state (non-zero $A_{p53}$) changes as a function of $k_{HDAC1}$ and $k_{p300}$.

4.3.4 Phase diagram driven by SMAR1

Study of transition of dynamical states of p53 driven by complicated concentration levels of stress inducers in the system may highlight stress management at different cellular phases in the system. The calculated critical value of $k_{SMAR1}$, $k_{cSMAR1}$, at which the amplitude of p53 is zero, and larger than this value the system goes to apoptosis, corresponds to a value of $k_{HDAC1}$ for each $k_{cSMAR1}$ (Figure 4.3 panel E). The phase diagram in the parameter space ($k_{HDAC1}, k_{cSMAR1}$) shows the distinct demarcation of stress and apoptotic states (Figure 4.3 E, left panel). This indicates that even for large value of $k_{SMAR1}$ which drives the system to apoptotic state, one can vary $k_{HDAC1}$ so that the range of stress state be broaden such that the system can be pulled back to normal state once the stress is removed.

The average value of mid-value of amplitude ($A_{p53}^{av}$) of p53 dynamics in sustain oscillation regime for ten ensembles with different initial conditions modulated by HDAC1 as a function of $k_{SMAR1}$ shows monotonous decrease of $A_{p53}^{av}$ as $k_{SMAR1}$
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Figure 4.4: (A) The diverse dynamical states of Mdm2 dynamics driven by SMAR1. (A) Time series dynamical states of Mdm2. (B) Permutation entropy spectrum of three states. (C) Multifractal analysis: plot of Hurst exponent \( H_q \), multifractal generalized dimension \( D_q \) as a function of parameter q. (D) Amplitudes of p53 \( A_{Mdm2} \) of the dynamical states as a function of \( k_{SMAR1} \). (E) Network constructed from the dynamical states using visibility graph approach. (G) Topological properties of the networks corresponding to various dynamical states: The 1st, 2nd, 3rd, 4th, 5th and 6th columns are for \( P_{Mdm2}^k \) vs k, \( C_{Mdm2}^k \) vs k, \( C_{N_{Mdm2}}^k \) vs k, \( C_{B_{Mdm2}}^k \) vs k, \( C_{C_{Mdm2}}^k \) vs k, \( C_{E_{Mdm2}}^k \) vs k respectively.
increases and will reach amplitude death for sufficiently large value of $k_{SMAR1}$ (Figure 4.3, E, lower left panel) [269, 273].

Similar study of the impact of $k_{SMAR1}$ on p53 dynamics in the presence of another stress inducer p300 via $k_{p300}$ shows different scenario. The phase diagram in the parameter space ($k_{p300}, k_{SMAR1}^c$) indicates two distinct scenarios, first, $k_{SMAR1}^c$ increases as $k_{p300}$ increases up to a maximum value, and secondly $k_{SMAR1}^c$ decreases as $k_{p300}$ increases (Figure 4.3, E upper right panel). In the first case, for any critical value of $k_{SMAR1}^c$, one can extend the range of stress regime by increasing the concentration of p300 (increasing the value of $k_{p300}$) in the system and save the system from apoptosis after removing the stress. In the second case, the range of stress can be increased for any value of $k_{SMAR1}^c$ by decreasing the concentration of p300 to save the system from apoptosis state.

The value of $A_{p53}^{av}$ modulated by p300 decays slowly as a function of $k_{SMAR1}$ (exponential decay) as compared to the case of HDAC1 (Figure 4.3, E lower right panel). The amplitude death scenario can be seen in this case also but with slow variation.

### 4.3.5 Perturbation in self-organization in stress p53

Multifractal properties of p53 dynamics at various dynamical states of the system show fractal nature which are signatures of self-organization at these states [130]. The networks constructed from the p53 dynamics in Figure 4.3 panel A using visibility graph procedure (provided in chapter 2 section 2.4) show complicated features of modular organization (Figure 4.3 panel F). The probability of degree distribution ($P(k)$) for all these states follows power law, $P_{p53}(k) \sim k^{-\gamma}$ with $\gamma$ in the range [2.47-4.06] (Figure 4.3, panel G first column). The decrease in $\gamma$ values due to increase in stress in the system ($\gamma = 2.47$ for p53 sustain oscillation state) shows the comfortable hierarchical organization of the system within an
optimal fluctuations or perturbations due to stress in the system. Increasing and decreasing in stress allow increase in $\gamma$ indicating the importance of hubs in the system. This signature of hierarchical organization of the network is supported by power law dependence of clustering co-efficient, $C_{p53}^{\alpha}(k) \sim k^{-\alpha}$ with $\alpha$ in the range $[0.998–1.002]$ (Figure 4.3, panel G second column), and neighborhood connectivity $C_{N}^{\beta}(k) \sim k^\beta$ with $\beta$ in the range $[0.08–0.2]$ (Figure 4.3, panel G third column).

The positive power in neighborhood connectivity shows the evidence of assortivity in the network, which indicates the importance of the few hubs forming a cluster (rich-club formation) in controlling the p53 dynamical states.

The betweenness centrality, which is another measure of topological properties of the visibility network of p53 dynamics, again follows power law, $C_B(k) \sim k^\epsilon$ with a range of $\epsilon$ [0.19-0.98] (Figure 4.3, panel G fourth column). This indicates that the SMAR1 induced p53 concentration at any instant of time is organized to optimize the information flow in the time series to enable to have effective signal flow. Similarly, other centrality measures, namely, closeness and eigenvector centralities also follow similar fractal nature, $C_C(k) \sim k^\eta$ and $C_E(k) \sim k^\delta$ (Figure 4.3, panel G fifth and sixth columns). This indicates that the concentrations of p53 in the time series which correspond to larger hubs are spreaders or receivers of the information flow in the p53 dynamical state. Similar fractal properties are found in Mdm2 dynamical states corresponding to various $k_{SMAR1}$ values taken in Figure 4.3.

Since nearly normal state, damped, sustain and apoptotic states of p53 are affected by stress, which induce fluctuations (large $H_q$ values) and long range correlations ($H_q$ dependence on negative $q$ values), the evidence of significant change in the self-organization with assortivity in the network constructed from p53 dynamics. Surprisingly, moderate stress (optimal value) favours large fluctuations and longer multifractality in the p53 dynamics.
4.3.6 Regulation of apoptosis

Taming stress imparted in a system by stress-induced parameters are important to save the system from apoptosis. Even though $k_{\text{SMAR1}}$ drives the p53 dynamics to apoptosis (Figure 4.5, left panel), this apoptotic state can be regulated by HDAC1 interaction to save the cell fate from apoptosis [269, 273]. Within an optimal strength of stress induced by SMAR1, the increase in this stress via interaction with HDAC1 allows the normal state of the system to stay longer for large range of HDAC1 concentration levels (large range of $k_{\text{HDAC1}}$ values) blocking from apoptosis. Since HDAC1 is also one of the stress inducer in the system, increase in $k_{\text{HDAC1}}$ allows the system to move to damped and sustain (active state with $A_{p53}$ nonzero) oscillating states. If we calculate the ranges $k_{\text{HDAC1}}$ for normal state (Figure 4.5 middle panel, magenta points) and minimum apoptotic state (Figure 4.5 middle panel, blue points) for each $k_{\text{SMAR1}}$ value. The phase diagram clearly shows the normal, stress and apoptotic regimes in p53 dynamical states. This shows that in the normal phase, SMAR1, which acts as a stress inducer, deacetylates p53 in the presence of HDAC1 blocking the system from apoptosis, and SMAR1 acts as anti-apoptotic signal supported by various experimental studies on SMAR1 [254, 260]. Excess stress allows SMAR1 not to interact with p53 any longer (SMAR1 becomes apoptotic signal), and the system goes to apoptosis [254].

The boundaries of anti-apoptotic (repairable stressed DNA) and apoptotic (unrepairable DNA damage) regulated by SMAR1 in the presence of HDAC1 is given by,

$$k_{\text{HDAC1}} = \frac{\lambda k_{\text{SMAR1}}}{ak_{\text{SMAR1}} + b} \text{ (anti - apoptotic);} \quad (4.23)$$

$$k_{\text{HDAC1}} = \Lambda \exp[ck_{\text{SMAR1}}] \text{ (apoptotic)} \quad (4.24)$$

where, $\lambda$, $a$, $b$, $c$ and $\Lambda$ are constants. This indicates that if the reactions involving $k_{\text{SMAR1}}$ and $k_{\text{HDAC1}}$ are monitored systematically in the model, one can always
have the chance to regulate apoptosis.

### 4.3.7 Approximate solution of the model

The fast state vector reaches the steady state quickly and can be taken as constant as compared to slow state variables (equations (4.21) and (4.22)). From equation (4.19) and equation (4.21), one can reach \( x_2 = \frac{k_{33} x_{*18}}{k_{32}} \) showing the direct dependence of \( x_2 \) on \( x_{*18} \), i.e. the steady state of HDAC1–Mdm2 complex. Similarly, from equations (4.4) and (4.21) we get \( x_1 = \frac{k_4 x_{*3}}{k_{3}} \) indicating direct proportional to the steady state of Mdm2 mRNA complex. Putting these equations to equation

![Figure 4.5: Phase diagram of dynamical states driven by SMAR1. (A) Plot of \( A_{p53} \) as a functions of \( k_{HDAC1} \) driven by SMAR1(\( k_{SMAR1} \)) (B) Regulation of apoptosis: Phase diagram in the parameter space (\( k_{SMAR1}, k_{HDAC1} \)) showing normal, stress and apoptosis with interaction mechanisms(indicating by arrows) (C) SMAR1 driven phase diagram: Plot of \( \Delta k_{HDAC1}^* \) as a function of \( k_{SMAR1} \).](image-url)
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(4.16) and using equation (4.21), we have the following equation,

\[ \frac{dx_{15}}{dt} + Ux_{15} = k_{26}, \]  

(4.25)

where \( U = k_{27} + \frac{k_{23}k_{32}}{k_{33}}x_{18}^* + \frac{k_{23}k_{33}}{k_{32}}x_{3}^* + k_{30}x_{4}^* \) is a constant within quasi-steady-state approximation. The solution of equation (4.25) is given by,

\[ x_{15}(t) = \frac{k_{26}}{U} \left[ (1 - e^{-Ut}) + x_{15}(0)e^{-Ut} \right], \]  

(4.26)

where \( x_{15}(0) \) is the initial concentration of \( x_{15} \) at \( t = 0 \). The solution (4.26) shows that the rate of increase of \( x_{15} \) (SMAR1) in the system is restricted by the steady-state values of \( x_{3}^*, x_{4}^* \) and \( x_{18}^* \) via \( U \); and time. The asymptotic value of \( x_{15} \) as \( t \to \infty \) is found to be \( x_{15} \approx \frac{k_{26}}{U} \) reaching a steady state. For small values of time \( t' \), keeping up to linear terms in the expansion \( e^{xt} \sim 1 + xt + O(t^2) \), we get \( x_{15}(t) \approx [k_{26} - Ux_{15}(0)]t \) which shows the minimal sufficient condition for \( x_{15} \) creation is \( k_{26} > Ux_{15}(0) \).

The equations (4.12), (4.21), (4.22) and \( x_2 = \frac{k_{23}k_{33}}{k_{32}}x_{18}^* \) can be used to get the following ODE of variable \( x_{11} \),

\[ \frac{dx_{11}}{dt} + Vx_{11} = k_{24}, \]  

(4.27)

where \( V = k_{22} + k_{25}x_{16}^* + k_{33}x_{18}^* \) is a constant. Then the solution of the ODE (4.27) is given by

\[ x_{11}(t) = \frac{k_{24}}{V} \left( 1 - e^{-Vt} \right) + x_{11}(0)e^{-Vt}, \]  

(4.28)

where \( x_{11}(0) \) is the initial value of \( x_{11} \) at \( t = 0 \). The asymptotic value of \( x_{11} \) at \( t \to \infty \) is given by \( x_{11} \approx \frac{k_{24}}{V} \) which is the steady state. At a small time limit where exponential expansion is approximated up to linear terms, we obtain \( x_{11}(t) \sim [k_{24} - Vx_{11}(0)]t \). The minimal sufficient condition for the formation of \( x_{11} \) is \( k_{24} > Vx_{11}(0) \).

Similarly, using equations (4.9), (4.22) and (4.21) we can reach the following ODE,
\[
\frac{dx_8}{dt} + x_8 \left[ W + k_{35} \left( \frac{k_{26}}{U} + We^{-Ut} \right) \right] = k_{23} + k_{13}k_{21}, \tag{4.29}
\]

where \( W = k_{14} + k_{15}x_7^* + k_{19}x_4^* + \frac{k_{20}k_{33}}{k_{32}} x_{18}^* \) is a constant. The solution of this ODE can be obtained by taking \( \int \rightarrow \int_0^\infty \) which is also true for positive values of \( x_8 \), and is given by,

\[
x_8(t) = \left[ C_1 - G \left( \frac{U}{W} \right)^{H/U} \Gamma \left( \frac{H}{U} \right) \right] e^{-Ht + \frac{W}{U} e^{-Ut}}, \tag{4.30}
\]

where \( G = \frac{k_{23} + k_{21}x_8^*}{U} \) and \( H = W + \frac{k_{26}k_{35}}{U} \) are constants. The constant \( C_1 \) can be obtained by applying initial condition, i.e. \( t = 0 \). Putting back the expression for \( C_1 \) into equation (4.30), we get,

\[
x_8(t) = x_8(0)e^{-\frac{W}{U} e^{-Ht + \frac{W}{U} e^{-Ut}}}. \tag{4.31}
\]

It is observed that for large value of \( t \), the term \( Ht \) dominates \( e^{-Ut} \), and therefore we have \( x_8(t) \propto e^{-Ht} \). However, for small \( t \), we have \( x_8(t) \sim x_8(0)e^{-W/U \left[ 1 - (H + W)t \right]} \), which indicates that the minimal existence of \( x_8 \) will have the condition \((H + W)t < 1\).

Now, to get the solution for \( x_2 \), the equations (4.13) and (4.17) using (4.21) are added, and the result is substituted in equation (4.3). The simplified ODE of \( x_2 \) is given by

\[
\frac{dx_2}{dt} + x_2 \left[ R + \frac{S}{e^{Vt}} \right] = D, \tag{4.32}
\]

where \( R = k_5 + \frac{k_{24}k_{32}}{V} \), \( S = k_{32} \left( x_{11} - \frac{k_{24}}{V} \right) \) and \( D = k_2x_3^* + k_7x_4^* + k_{21}x_{13}^* + k_{31}x_{17}^* - \frac{k_{17}x_8^*}{k_3}x_3^* - k_{17}x_{10}^*x_{12}^* - k_{28}x_{16}^* - \frac{k_{28}k_{31}}{k_3}x_3^* \) are constants. The solution of the equation (4.32) is given by,
\[ x_2(t) = \left[ C_2 - \frac{D}{S} \left( \frac{V}{S} \right)^{R-1} \Gamma \left( \frac{R}{V} \right) \right] e^{-Rt + \frac{S}{V}e^{-Vt}}, \]  

(4.33)

where \( C_2 \) is a constant which can be obtained from the initial condition \( t = 0 \).

Then putting back the expression for \( C_2 \) into the equation (4.33), we get,

\[ x_2(t) = x_2(0)e^{-\frac{S}{V}e^{-Rt + \frac{S}{V}e^{-Vt}}} \]  

(4.34)

The large ‘t’ limit in the equation (4.34) shows that \( x_2(t) \sim x_2(0)e^{-S/V e^{-Rt}} \), which shows that \( x_2(t) \propto e^{-Rt} \). However, it further indicates that \( \lim_{t \to \infty} x_2(t) = 0 \). Small ‘t’ approximation to the equation (4.34) leads to the expression \( x_2(t) \sim x_2(0)e^{-S/V [1 - (R + S)t]} \), which shows that the minimal condition for the existence of \( x_2 \) is \( 1 > (R + S)t \).

Similarly, proceeding the same way as above, from equations (4.2), (4.8), (4.9) and (steady state) we obtain the following ODE for \( x_1 \),

\[ \frac{dx_1}{dt} + Fx_1 = G + Pe^{-Vt}, \]  

(4.35)

where \( F = k_1x_{14}^* + \frac{k_8k_{13}k_{33}}{k_{32}}, \quad G = k_6 + k_9x_4^* + k_{13}x_7^* + k_{17}x_{10}x_{12}^* + k_{31}x_{17}^* - k_{13}x_7^* - k_{16}x_9^* + \frac{k_{14}k_{14}k_{33}}{k_{32}}, \) and \( P = k_{33}k_{18} \) are constants. The solution of the equation (4.35) is given by,

\[ x_1(t) = \frac{G}{F} (1 - e^{Ft}) + \frac{P}{F - V} (e^{-Vt} - e^{-Ft}) + x_1(0)e^{-Ft}. \]  

(4.36)

Now, the small ‘t’ approximation allows to simplify equation (4.36) to obtain \( x_1(t) \sim x_1(0) + t[P - G - Fx_1(0)]. \) The minimal condition for \( x_1 \) existence in the system is given by \( x_1(0) > t[G + Fx_1(0) - P] \). However, in the large ‘t’ approximation, we have \( x_1(t) = \frac{G}{F} (1 - e^{Ft}), \) and for non-negative values of \( x_1 \) the condition is \( e^{Ft} < 1 \). However, we have \( \lim_{t \to \infty} x_1(t) = -\infty. \)
4.4 Conclusion

p53 is multifunctional, and controls various cellular functions. Stress induced in a cellular system is generally achieved via DNA damage, and is reflected in the p53 dynamics. Depending on the amount of stress induced in the system, p53 dynamics is triggered at various dynamical states which correspond to different cellular states. SMAR1 is one of the most sensitive stress inducers, which interacts directly with Mdm2, but indirectly with p53 and via p53. It regulates p53 dynamics to interfere various cellular activities in a very different way. For mild stress, it, in the presence of HDAC1, behaves as anti-apoptotic signal by deacetylating p53 and blocks the cell from moving to apoptosis [254, 260]. However, for excess stress, it does not interact with p53 and acts as apoptotic regulator, helping the cell to go to apoptosis [260].

The behaviours of SMAR1 induced p53 dynamics at various dynamical states are inherited from complicated molecular interaction in various pathways of the cellular networks, and show multifractal nature. These multifractal nature of the p53 dynamics is mainly due to multi-scale fluctuations and long-range correlations in the cellular system. The range of multifractality is favoured by large fluctuations in the dynamics imparted by optimal stress in the system (dynamical state corresponding to sustain oscillation) [128]. The fractal properties embedded in the multifractal p53 dynamics can be observed in the networks constructed from this time series, and are signatures of self-organization in the system. However, the stress imparted in the system perturbs the self-organization, and importance of few hubs come into existence showing assortivity in the networks. These hubs in the p53 dynamics take up important roles in the signal propagation in the system which are reflected in various centrality measurements of the networks.

Understanding the regulation of apoptosis by SMAR1 via p53 in the presence of HDAC1 or p300 or other signalling molecules could one of the most important
issues clinical investigation. One reason could be modification of cellular fate by modulating biochemical reactions which involve these signalling molecules. The connection between the p53 time series at a particular dynamical state and the network constructed from this time series could give important timing/timings (identified hubs in the corresponding network) at which signal processing is optimized in stress management at that state. Further, the question how SMAR1 trigger stress and cancer phase transition depending on the stress imparted in the system is still an open question. The investigation of the roles of SMAR1 and other important signalling molecules in regulating cancer network and dynamics is also needed to understand basic regulators of target genes in different types of cancer. Experimental and theoretical investigations in this direction are needed because this study will open up a new understanding in the disease dynamics caused by these signalling molecules, their preventive measures, and cancer engineering. Further, even though the experimental report suggests that SMAR1 delays tumor progression by direct activation and interaction of p53 [274], the dynamics will be rather complicated in cancerous cells as compared to regulation in normal cells. Further, SMAR1 plays central role in coordinating p53 in human breast cancer [275], but mechanism is rather complicated. Mutant p53 directly interacts with DNA via SMAR1 and other binding factors [276]. So far our model is concerned, we have not incorporated the case of p53 regulation by SMAR1 in cancer systems, where, we expect different properties of dynamical states and other related phase transition. We hope that it could be important problem to address these issues with proper justification with experimental situations.