Chapter 1

Introduction

The cell is the unit of function and structure of an organism. It is the complex system consisting of many components that provide both the structure as well as the functional ability to the cell. The complex working of the cell is attained through the interaction of different molecules (i.e. proteins, lipids, free radicals etc.) in the form of cascade (pathways). These pathways (both non-metabolic and metabolic) work together to cover all the basic life processes. There are number of uncovered problems at the molecular level that are not yet been solved. So it is the need of the time to get a system level understanding of complex molecular signalling pathways. The large and complicated network of molecules consists of modules (sub-networks) each performing certain specific biological function. These interactions of biomolecules in the form of network lead to the organization of the system by integrating various components to attain a new emergent biological function of cellular process. The signalling system on the other hand controls the overall scenario of cellular process thus, driving the set of proteins (or genes) through signalling molecules (proteins, free radicals, mRNA etc.) to carry out various functions and then establish a communication between neighboring cells.
1.1 p53 as important cellular regulator

p53, a 393 amino acid protein transcribed from Tp53 gene located on chromosome 17, is the most popular molecule in multicellular organisms like human controls the regulation of cell cycle [1]. p53 is an important hub controlling various cellular genes as well as protein/gene networks, which in turn are the part of cell cycle regulation, apoptosis, proliferation and growth mechanisms, etc. [2–4]. The p53 protein holds central point in signal transduction pathways involving a colossal number of genes that respond to diverse signals of stress and reduce the risk of mutation and circumstances that can steer to produce cancer or other pathological diseases [5]. Also, p53 delimited genes create a multitude of proteins that prevent DNA damage and create feedback loops to enhance the activity of p53 and communication to other signalling pathways (Figure 1.1). The resultant effect created from the several breakdowns in the tumor suppression pathways yield an abnor-

Figure 1.1: A schematic representation of p53 under different cellular stress and playing a important role in different regulatory pathways.
Chapter 1. Introduction

mality called cancer. On the other hand, how cell goes into p53 the response (e.g. cell cycle stress, apoptosis etc.) and communicate with adjacent cells to result in the growth of cancer, is still an open question. The enormous big amount of big data generated from the high throughput techniques of genomics, proteomics and transcriptomics developed in the past ten years had urged the arrival of the field of computational system biology. The models and tools are proposed on this enormous data to arrange them in a systematic way so as to predict the behaviours and patterns of biological systems which may correspond to various cellular and physiological states. Expression and regulation of p53 in gene regulatory network often involves both negative and positive feedback loops. Most of the mathematical modelling had represented these gene regulatory networks deterministically for predicting cellular mechanisms and behaviours. However, some stochasticity is an inherent property of the dynamics of real biological systems, modelling of biological systems under stochastic formalism and requires the in-depth understanding of the system and can cable to predict cellular mechanisms which are very mimic actual experimental situations. The representation of such complex dynamics involves the construction of molecular noise and preparation of proficient computational algorithms to tackle the complex dynamics of interacting cellular pathways and emergent properties.

1.1.1 p53 as a regulator of apoptosis

p53 is an important protein that controls the cellular homeostasis [1]. It is a nucleoprotein having a short half-life of approximately 4-6 hours. It plays the role of guardian in keeping the integrity of the cellular genome. In normal condition p53 is low in the cell, as it is found in an inactive state and also due to its short life span. While, in stressed condition p53 gets activated, either due to conformational change or through signalling mechanism, it gets stabilized owing
to the DNA damage [1, 6]. p53 was discovered more than three decades earlier, it is found as an important protein, so it is called by several names like “death star”, “gatekeeper”, “guardian of genome” [4, 7, 8].

p53 acts to assimilate various stress signals into a series of diverse reactions through various pathways. To maintain homeostasis, the tumor suppressor (p53 and family) regulates more than one process that can prevent proliferation [9]. p53 is most studied tumor suppressor protein that is activated under various stresses condition and mediates anti-proliferative processes through biochemical or reactions (see Figure 1.1). One of these diverse set of reactions or pathways is the regulation of apoptosis. p53 can be stimulated by a variety of signals like DNA damage, DNA repair, cellular senescence, spindle damage, oncogenic activation and apoptosis [10, 11] (Figure 1.2). As a result, interruption of p53 function stimulates checkpoint faults in cellular pathways, cellular immortalization, genomic insecurity and inappropriate survival, allowing the continued spread and growth of impaired cells [4]. Given that the profound anti-proliferation effect is produced when it gets inactivated, while on other hand supreme attention for p53 has been focused on its part in various types of cancer. Indeed, hyper-activation of p53 has been connected with a variety of degenerative diseases [12].

The considerable amount of research in p53 accounts for its ability to control

![Figure 1.2: p53 triggers apoptosis.](image)
apoptosis. The preliminary evidences were gathered from the work of Oren and co-workers who took p53-deficient myeloid leukemia cell line and reintroduced p53 [13]. Here, p53 induced apoptosis in cytokine dependent manner. In other study of p53 knockout experiment in mice, confirmed its involvement for radiation based apoptosis [14]. Moreover the role played by p53 biology in apoptosis has been emphasized by its conservation in Drosophila and C. elegans as both the orthologs are important components for damage surveillance [15].

Apoptosis proceeds in a p53-dependent manner through two different pathways extrinsic and intrinsic. However, the apoptotic machinery is run primarily by intrinsic pathways as the contribution of extrinsic pathways has been poorly understood [16]. Both these pathways get controlled at multiple levels, as for extrinsic regulation consists of surface receptors and associated cytoplasmic protein that set up the rheostat. While on the other hand intrinsic centers are present on mitochondria contain key apoptotic regulators with IAPs (Inhibitory Apoptotic Proteins). p53 modulates key points in both these apoptotic programs. Thus, this transcription factor (i.e. p53) targets different point (say levels) in apoptotic program which are not isolated sets, but a part of large coordination process. These co-ordinated set of process increase the probability for the forward propagation of apoptotic pathways once it is initiated. Moreover, p53 also has the ability to initiate cell cycle arrest, DNA repair, etc. which is driven by the strength and nature of the stimulus imparted and thus, making its decision contextual [17]. Notably, such a program is built on a variety of control levels (or points) integrating cellular environment and making p53 as a certain regulator of apoptosis.

1.1.2 Decision maker of the cell cycle fate

p53, in addition to its ability in regulating apoptosis can also control the fate of cell cycle. The fate decision depends upon the cell type or the originating tissue
for instance, $\gamma$-radiation in fibroblasts results in G1 cell cycle arrests while, on the other hand, in thymocytes it produces apoptosis [14, 18]. Although only cell type difference is not sufficient in explaining the different outcomes, as in fibroblasts the Myc oncoproteins go through p53-dependent apoptosis in reaction to $\gamma$-irradiation or another form of DNA damage. Similar to this process lymphoma cell undergo apoptosis due to chemotherapeutic drug. While if these cells have over expression of Bcl-2 then they go through p53-dependent senescence [19]. So, the response of p53 can be a consequence of resultant impact of both microenvironment and genetic setting. The result of p53 activation can also be swayed by the strength or the type of the activating stimulus. As for example, in fibroblasts p53 promote apoptosis; while Ras activated p53 promote senescence [20]. It is interesting that at both these instances, ARF is required to stabilize p53 by interfering with its negative regulator Mdm2 [21]. Moreover, p53 is itself a fundamentally different regulator depending on the activation through the stimulus or cell type resulting into the qualitative differences in the output signals. Interestingly, these different signals like UV or $\gamma$ radiations are able to induce different target genes for p53 within the same cell type [22]. Thus, distinct stimulus leads to distinct modifications (most primarily the post-translational phosphorylation) which in turn recruit different population of target transcriptional co-activators, and finally ending into different target genes for p53 and different cellular interactions. In simple words, a qualitative effect on p53 (i.e. phosphorylation) has a quantitative influence on its (p53) signalling mechanism.

Further, p53 pulse governs cell cycle fate through choice of selected target gene induction. According to Lahav and co-workers the pulsating response by p53 which is an important reaction in response to DNA damage [23]. In another study, Zhang and co-workers [24] observed the existence of variability in p53 pulses quantitatively and generally these pulses have a sigmoidal relationship with the
dosage of stress in the form of radiation. Thus this response in the pulse to p53 level enables the p53 network to frequently estimate signal of damage and poses a pretty consistent hold of cell fate decision. As an effective controller the p53 pulses are rapidly terminated to avoid apoptosis when the DNA damage is fixed to maintain the integrity of the genome.

1.1.3 Normal to cancerous phase transition

p53 is found, not only to control the cell cycle, but also many cellular activities. The cell cycle is an important event of the living organism which takes the decision for growth and development of an organism. An uncontrolled operation of the cell cycle leads to the cancerous stage (Figure 1.3). In normal condition, cell cycle is affected due to various stress which lead to the DNA damage [25]. If DNA damage happens then several important proteins are present in the cell that can trigger induction of the DNA repair proteins and mechanisms. p53 is the most important protein, which can induce DNA repair proteins. It acts as a gatekeeper of the cell cycle and it is generally activated because of DNA damage through a number of signalling molecules and cellular stimuli. This condition of the cellular ensemble leads to the stress cell [7]. Again, if the DNA damage is significantly large then p53 tries to repair back by several DNA repair proteins. Once the repair is successful stress cell revert to normal cell; however, if the DNA damage is irreparable the cell moves towards the cancerous or apoptotic stage. Several experimental studies suggest that mutation and loss of tumor suppressor protein such as p53 leads to the corrupt cell cycle and finally uncontrolled division of the cell known as a cancerous condition [26]. The analysis of the unsolved problem of different phases of p53 (shown in Figure 1.3) have been studied from computational point of view.
1.1.4 **p53 as stress manager in cell process**

p53 pathway is involves large number of genes which are being controlled and their products are linked to various signals of stress. The responses to these stress includes senescence, cell cycle arrest and apoptosis. In addition to this, p53 converse these stress signals to the neighboring cells using signalling pathways. The p53 signal transduction pathways involve a set of genes that respond to a range of stress signals (both intrinsic and extrinsic). These signals all crashes upon the homeostatic mechanism of the cell that pedals the reliability of DNA replication, cell-division and chromosome segregation [28] (Figure 1.1). There are several reasons (both physical and chemical) of DNA damage that includes

![Figure 1.3: (A) Different phases of cell: Transition of normal cell to stressed, cancerous, apoptotic cell. (B) Cytotoxic therapy: Adapted from Cheok, et al [27].](image-url)

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8
cross-linking, \( \gamma \) irradiation, de-purination of DNA and alklylation of bases. Each type of this damage (in DNA) is different and perceived by a dissimilar set of protein activities which undergoes the DNA damage in varied ways. There are many damage perceived by the cell, damage of each type can be reported by hubs i.e. p53 protein and related pathways [29,30]. This is observed that the main function of p53 is in those stressed networks, that have mutated p53 which is about 50 percent of all cancers [31]. While in apoptosis, cell cycle arrest and senescence forms the crucial resultant of the p53 decision pathway and of the other genes which give response to p53, that in turn defines many additional functions of p53 pathway [32]. The IGF-BP3, that binds to IGF-1 hormone, thus, prevent it from activating the growth in response to signal transduction pathway (includes IGF-1, IGF-1R, PI3K, PDK, AKT-1). In this case p53 is regulated negatively to control the cell growth and prevents cell division in nearby cells after the stress signal. From the pool of many questions, an significant question that remained unanswered, is extracting the output of p53 pathway. How a cell receives a stress signal and chooses which one of the three outputs namely apoptosis, cell cycle arrest or cellular senescence (Figure 1.4).

### 1.1.5 Somitogenesis: Wnt, Notch, FGF pathways

Somitogenesis is the multifaceted process of formation of somites (segments) in all vertebrate(s) body during early stages of developing embryo. During this process, the PSM (presomitic mesoderm) is gradually segmented into bilaterally symmetrical somites in both posterior and anterior direction. This is a periodic process with some periodicity and is believed to drive the formation of somites [33,34]. The segmentation clock that controls the dynamics of somite formation is encoded in so called clock genes. The clock genes Hes1 and Hes2 with Lunatic fringe (Lfng) in chicken, Hes1 and Hes7 in Mouse finally Her1 and Her7 in Zebrafish encode
somite formation in a posterior to anterior in a periodic manner [35, 36].

The dynamic expression profile in PSM is shared by the components of the Notch pathway [35, 36]. In addition to it the genes from Wnt and FGF pathways also take part in the dynamics of Somitogenesis [37]. The recent studies by Kroll and co-workers suggest their potential involvement and initiation of dynamics at posterior end of PSM [38]. In case of Notch signalling pathways the initiation and preservation of oscillations rely on negative-feedback loops driven by clock genes. The Notch is obligatory for synchronous oscillations of adjacent cells and is crucial in somite formation [39]. In addition to Notch, Wnt actively play an important role in safeguarding the correct periodicity of the oscillation [40]. Down regulation can be generally responsible in the halt of oscillation in the anterior PSM, the level of $\beta$-catenin in the nucleus is significantly decreased as compared to the rest of cell [41]. There is a perfect sign that Wnt, Notch and FGF pathways cross-talk among them to control the oscillations in somitogenesis [35].

1.2 Modelling p53 regulatory network

The p53 protein acts as an integrated central node (check point) in a signal transduction cellular network of a large number of genes and their respective products which respond to an extensive types of stress signals to minimize mutations and other errors that can lead to cancers or other pathologies [25]. Also, the p53 controlled genes produces proteins which transfer the stress signals to surrounding cells, in order to repair and prevent DNA damage so as to create feedback loop(s) that enhances or attenuates the activity of p53 and contact with other pathways for signal transductions. Cancer is a disease resulting from the breakdown of various checkpoints and tumor suppressing mechanisms in the transduction signalling pathways.

However, how a cell undergoes various dynamical p53 responses (e.g. apop-
tosis, senescence or cell cycle arrest) and communicates with its neighboring cells, resulting cancerous growth, still remain an open question (Figure 1.5). The huge amount of data which is generated by high-throughput technologies like genomic, proteomic and metabolic techniques developed within the last decade have motivated the emergence of computational systems biology to study this problem. Computational tools and models are proposed based on massive experimental data to process them systematically, to predict biological behaviours and patterns and to extract all possible information embedded in it. p53 often involve positive and negative feedback loops during regulation and expression scheme in its gene regulatory networks. Many of the genetic network are modeled mathematically, represents expression of genes and its regulation as a deterministic process. On the other hand, stochasticity due to noise is in fact an innate feature of dynamical processes which should be the subject of deep observation and analysis. The reason behind such dynamics is the involvement of the formulation for the correct representation of molecular noise, that is followed by the mathematical modelling to represent real cell processes. That also involves formulating a computationally efficient algorithms that is able to simulate and tackle the complexity in the

![Figure 1.4: The p53-Mdm2 autoregulatory feedback loop and Nutlin-3 in Mdm2 and inhibits the interaction of Mdm2 with p53 [42].](image)
involved dynamics for the set of various cellular pathways. Models and theories so far developed could not fully explain the mechanisms of the p53 protein signalling pathways and cellular communication in an ensemble of cancerous cells. To investigate important dynamical behaviour of p53 protein and then to study the molecular information processing within groups of coupling cells through signalling molecules or by direct contact or by hormone secretion to understand how the collective behaviour of these cells is organized. This study, using feasible and realistic model of p53 protein signalling pathways, may give rise to the important insights in the formation of cancer cells from a random population of normal cells and grow.

The large scale simulation of such systems will provide the model of cell expression and chemical reaction channel for each molecule, which are involved to express its effect. From experimental view these regulatory networks of p53 are evaluated by concentrations of product of molecular species (such as protein and gene). When proteins are synthesis from gene, the rate of formation is balanced by the rate of degradation. Thus the biological information and dynamics could be analyzed through mathematical modelling. By analyzing it deterministically and stochastically, the biological system which is then be integrated to have full cell model for important reaction network associated in a cell during certain disease or stress and also predict the effect on cell health.

The living cell is resembled to chemically reacting system. Various well defined signalling pathways allow the cell to receive the information, process and respond to information activities [43]. The living cells are inherently dynamic. Mathematical models of biological signalling pathways are appropriate tools that answer important unsolved biological questions and predictions. the widely used mathematical approach are modelling ordinary differential equations (ODEs) and Boolean modelling to answer these unsolved questions. There are two formalisms
for mathematical analysis the dynamics and temporal behaviour of homogeneous chemical system [44] viz. deterministic formalism and stochastic formalism.

### 1.2.1 p53-Mdm2 regulatory network

Mdm2 (Mouse double minute homolog 2) is an E3 ubiquitin-protein ligase discovered in mouse model protein. It acts in a manner of E3 ligase enzyme and allows degradation of p53 protein [45]. The phosphorylation of p53 decides the fate of p53-Mdm2 complex. It is been reported in many experimental studies that phosphorylation of p53 on multiple sites takes place in response to stress and DNA damage. These sites may sometimes involve the associated Mdm2 binding sites. Due to which, Mdm2 will no longer be associated with p53 protein and thus, p53 become free from Mdm2. Apart from interacting p53, Mdm2 interact with many other important proteins or molecular species like Rb, ARF, MTBP, Nitric oxide, PTEN, etc. The phosphorylation of Mdm2 is introduced by various kinases such as DNA-PK, ATM, AKT and Cdk. The studies have also suggested the phosphorylation of both Mdm2 and p53 with the same kinase [46]. It was found that p38 increases the p53 stability by phosphorylating at least two residues, on the other hand the reduction of stability is encountered in the case of Mdm2 phosphorylation [47].

The same case found with the phosphorylation driven by ATM on both p53 and Mdm2. The increase in nuclear import is accomplished in Mdm2 case where phosphorylation is done by AKT thus, effecting p53 activity and stability [48]. Many results from the experiment have reported the interaction of all non-Mdm2 regulators with p53 protein to be generally fitting within the proline rich domain of p53 and affect its stability. As, for instance, JNK phosphorylates p53 and enables it to be transcriptionally active, which is found to exist in the proline rich domain. In many studies it has been reported that Mdm2 concentration
level is found to be high in cancer cells [26]. Mdm2 protein contains several well preserved structural domains that include an N-terminal domain where p53 interaction takes place. An acidic domain with residues span from position 230 to 300, the site of phosphorylation which is very important for normal functioning of Mdm2. This central acidic domain plays a crucial role in trans-locating of Mdm2 from the nucleus to cytoplasm [46]. Zinc finger domain for Mdm2 protein is another conserved domain, that plays major role in conveying interaction of Mdm2 with the ribosomal proteins. The C-terminal RING domain residues from 430 to 480 of Mdm2 contains two zinc molecules. The proper folding of the RING domain is crucial for the activity of Mdm2.

![Diagram](image)

Figure 1.5: (A) p53 regulatory pathways and Nutlin-3a therapy [52]. (B) Nutlin concentration can drive the p53 dynamic to various oscillating states namely: stabilized or steady state, first damped oscillating state, sustain oscillation state, second damped oscillation state and apoptotic state.
domain requires binding of Zinc. Thus, this Mdm2 domain allows the attachment of ubiquitin to p53 which is important in regulating levels of p53 cells [4, 5].

The Network of p53 is generally switched off in normal conditions, while it switches on when there is a sense of any DNA damage or cellular stress [49]. Mdm2 is a negative regulator of p53 [50, 51]. Mdm2 interact with p53 through its zinc finger domain and act as E3 ligase enzyme, directly ubiquitinating p53 protein [4, 49]. Mdm2 could act on p53 in many isolated ways, as for example, increasing proteosomal degradation and promoting nuclear export, suppression of p53 acetylation etc [53]. As a results of these actions of Mdm2, the intracellular concentration level of p53 decreases i.e., p53 stabilizes within the cell. Since p53 is a transcription factor, it interacts directly to the Mdm2 gene and helps in its mRNA transcription. Mdm2mRNA is then translated into the Mdm2 protein. So the p53 protein positively regulates Mdm2 protein [54, 55]. The complex formed between p53 and Mdm2 is broken down due phosphorylation of p53 within the complex. Due to regulation of this binding, complex formation and phosphorylation, the population of both these protein (Mdm2 and p53) remain stable within the cell [56, 57]. p53 and Mdm2 regulatory network is very active and is generally driven by stress induced by number of inducer.

1.2.2 Incorporating stress inducing signalling molecules

(a) Nutlin

Nutlin is the analogs of cis-imidazoline. This is the small molecules that inhibit the interaction of p53 with Mdm2 (Figure 1.4, 1.5. Nutlin has three forms Nutlin-1, Nutlin-2 and Nutlin-3 [58] however, Nutlin-3 is most commonly used in anti-cancer studies [59]. Nutlin can only be used in tumors with aberrant Mdm2 and also the tumors harbouring wild-type p53 [58]. The small molecule antagonist of Mdm2 (i.e. Nutlin-3) is found to activate p53 pathways in neuroblastoma cells that have
wild p53. It has also been attributed to initiate a dose dependent response by inducing G1 cell cycle arrest regardless of MYCN amplification [60]. In the same study it has been seen that amplification of MYCN has no effect on the cytotoxic activity of Nutlin-3. Thus, Nutlin-3 is found to stabilize p53 by inhibiting p53/Mdm2 interaction. It also particularly initiates the expression of genes targeted by p53 that are present in neuroblastoma cells having wild-type p53 that eventually leads to G1 cell cycle arrest and cell death (apoptosis) [60]. In CLB-GA cells, Nutlin-3 promotes non cholinergic sympathetic neuronal differentiation, with an initial transient co-expression of sympathetic neuronal and neuroendocrine markers significant of the mixed neuronal/neuroendocrine phenotype in some maturing neuroblastoma tumors [61].

(b) Wnt

Wnt (wingless type) is a diffusible ligand protein. During the development in organism Wnt secreted proteins play(s) a critical role(s) in mediating cell to cell signalling. Wnt gene is defined by the encoded proteins having particular cysteine patterns, rather than by functional properties [62]. The canonical Wnt signalling pathway is featured with Wnt proteins binding to the trans-membrane receptors, composed of Frizzled proteins (FZF) and LRP5/6 co-receptors [63]. The events that follows the attachment of Wnt to receptors is critically dependent on the $\beta$-catenin, that transfer signal from plasma membrane to the nucleus. The Wnt signalling pathway is been presented as to control gene expression programs regulating cell fate and is frequently been deregulated in cancer. Wnt signalling is often gets activated by mutations in the oncogene responsible for various human cancers; the tumor suppressor genes, p53, $\beta$-catenin and Axin2. The Wnt signal is importantly required for differentiating melanocytes [64,65]. It is found that almost 30 percent of melanocyte cell have activated Wnt signalling, as can be
judged by the accumulation of $\beta$–catenin [66]. In mouse models, it has been seen that activated Wnt signalling promotes the metastasis of melanoma cells to lungs and lymph nodes [67]. Other studies suggest that the activated canonical Wnt signalling have an antagonistic effect on the proliferation and Metastasis, and in these stages the elevated level of Wnt signalling improves the survival of cases (patients) [68].

(c) Gsk-3β

Gsk-3 (Glycogen synthase kinase 3), being a threonine/serine kinase is a proficient in phosphorylating and inactivating the enzyme glycogen synthase [69, 70]. Apart from its role it plays in glycogen metabolism, it also acts to be as a downstream controlling switch, which controls the resultant of various signalling pathways started by types of stimuli [71, 72]. The Gsk-3 acts as a core regulator in various pathways, when get dysregulated get involved in the progress of diseases like Alzheimer’s disease, bipolar disorder and cancer. On account of being involved in many disease pathways it has always been a tempting target. On the other hand its involvement in many pathways at the same time poses a challenge of selectivity and also to get the access full spectrum of function. As, for example, it’s desired to be inhibited in one process (eg. preventing neuronal apoptosis) while, this inhibition may result in acceleration in hyperplasia [73]. There mammalian Gsk-3 is encoded in two isoforms Gsk-3a and Gsk-3b [74]. Both the isoforms have difference in molecular mass 51 kDa (Gsk-3a), 47kDa. Gsk-3a has a glycine-rich tail at the N-terminus that is not present in other isoform. Gsk-3 homologous are present in all the eukaryotes examined till date and also show good of similarity [75]. Gsk-3β is crucial in defining the development in the vertebrae embryonic axis. It is observed that the disruption in the gene Gsk-3b, results in lethality in embryo causing severe degeneration of liver degeneration as associated with the
TNFα toxicity. In addition to this Gsk-3β is found to regulate the function of NF-κB at transcription level [76].

(d) Nitric Oxide

Nitric oxide (NO) is an essential messenger molecule inside the cell. It is the key player in many regulatory functions of cell, which includes apoptosis, tumor development and cancer progression [77]. This oxide (i.e. NO) has a very short life in cellular matrix (∼1–10 seconds) and this bioactive molecule can trigger different physiological condition in a variety of cell types [78,79]. Synthesis of this oxide (i.e. NO) takes place owing to the action of various Nitric oxide synthase enzymes (often called NOS), such as neuronal (nNOS), inducible (iNOS) or endothelial (eNOS) [80,81]. NO plays different types of roles in different type of cells, NO initiates apoptosis in cells like macrophages, pancreatic β-cells, thymocytes etc, as in addition to these eosinophils, ovarian follicles, B-lymphocytes, embryonic motor neurons etc, are other cell in which apoptosis can be induced by NO. [82,83]. In a study of Marletta and co-workers, it is reported that Nitric oxide induces apoptotic signals in human lymphoblastoid cell by concealing tumor suppressor protein i.e. p53 protein [84]. Nitric oxide due to its smaller size and hydrophobic nature has the ability to readily diffuse through the plasma membrane and thus, for this reason can act as an excellent intercellular signalling [85,86]. As it diffuses easily it is also known to be an important coupling agent. However, the strength of coupling rest on the concentration of the molecule. In support of this many studies shows nitric oxide is considered to be a lead molecule giving solid coupling among a set of cells so that they can communicate efficiently [83,84]. Further, the high concentration of nitric oxide leads to the toxic effect in cells [78,87].
(e) Calcium

Calcium is a versatile molecule as it takes part in a number of metabolic networks such as muscles contraction, gene transcription, fertilization, liver metabolism, etc. [88]. This divalent cation is an important intracellular signalling molecule. Calcium signalling is directly relative to the calcium present inside the cell. There are roughly two sources of calcium, firstly from the extracellular environment by the way of diffusion and secondly from stores of calcium present in the ER (endoplasmic reticulum in cytoplasm) [89]. Calcium behaves differently in different tissues. Inside the cell, it right away interacts with enzyme PLC thus, sensitizing the IP2 which initiate the production of IP3. Due to the impact of IP3, the calcium induced calcium release (CICR) channel presence in endoplasmic reticulum releases calcium ions in turn increase its concentration [90]. Diffusion of calcium ions from one cell to another is implicitly controlled by membrane potential with the activation of ion current adapting Chlorine (Cl) and Na$^+/K^+$ channels. Many studies has revealed the relationship between the Ca$^{2+}$ diffusion through gap junction and passing information in the neighboring cells by increasing its concentration which activates the voltage gated ion channels. [88,91]. Sometime internal stores of calcium present in the ER is also liable for the diffusion of extracellular calcium inside the cell. Since the Ca$^{2+}$ it is very frequent that it can diffuse from one cell to another, it behaves as a good synchronizing molecule. This transfer of information between cells is considered as a means of information processing among cells and work in coordinating signals that involve various complex coupling mechanisms [92,93].

(f) p300

The name p300 is synonymcaly used for EP300 protein (E1A binding protein p300). It is a translation product of EP300 gene. The gene EP300 is present on
human chromosome number 22 (loci 13.3p). p300 protein has been shown to regulate a number of genes in different tissues throughout the body [94]. It interacts with numerous molecules such as STAT3/6, CEBPB, Mdm2, BCL3, PCNA, P53 and CDX2 etc. It is an intrinsic histone acetylase which acts as a transcriptional co-activating protein. p300 is inscribed with five domain, namely, the nuclear domain RID (receptor interaction domain), the KIX domain having CREB and MYB interaction domains, the IBiD (interferon response binding domain) and the cysteine/histidine regions (TAZ1 and TAZ2). Four domains of the p300 leaving RID, interacts tightly to the sequence spanning the TAD1 and TAD2 (transactivation domains 1 and 2) of p53 protein [95]. p300 is liable to drive key process like G-protein signalling, apoptosis, embryogenesis, cell cycle regulation etc. [94]. p300 acetylates p53 on interacting to its c-terminal and prevents the decay of p53 that allows its participation in various other reactions. So, the increase in the level of p300 will lead to increase in acetylated p53. Further it may become the leading cause of cell death, similar to cancer [95,96].

(g) HDAC1

HDAC1 (Histone Deacetylases 1), is a 482 amino acid enzyme and it works to remove acetyl group from the complex. It is known as a class I member and have a high sequence homology with yeast Rpd3. HDACs are commonly expressed in every mammalian cell. It can exist at both the places in the cell (nucleus and cytosol) and thus, can easily be translocated between the nucleus and cytosol [97]. It also acts as a transcriptional co-repressor with histone deacetylase activity and regulates a number of genes [98]. HDAC1 plays central role in many vital biological process like notch signalling pathway, embryonic digit morphogenesis, histone H3 deacetylation, histone H4 decetylation, nerve growth factor receptor signalling pathways, positive regulation of cell proliferation, positive regulation of receptor
biosynthetic process, blood coagulation, transcription initiation from RNA polymerase II promoter, transforming growth factor beta receptor signalling pathway etc [97]. HDAC1 directly interact with Mdm2 (protein) and forms binary (HDAC1-Mdm2) complex [96,99]. HDAC1-Mdm2 complex deacetylates the acetylated p53 leading to the elevation in the population of p53 that is now accessible to take part in other reactions and able to decay [99,100].

(h) MTBP

Mdm2 Binding Protein or MTBP is a cellular protein, containing 904 amino acids accounting to 102 kDa predicted molecular weight. It plays a critical role in various vital biological processes such as negative regulation of cell cycle arrest, cell proliferation and controlling the mitotic cell division. Studies have proved that the high concentration of MTBP is found in tumor cells. In a cell without stress (i.e. unstressed condition), the MTBP play the role of a co-factor for the regulation of p53 via Mdm2. MTBP decreases the chance of auto-ubiquitination by directly interacting with Mdm2. Increase in the level of MTBP leads to the stabilization of Mdm2 and achievement of steady state. Due to this enhancement of Mdm2 the level of p53 is also decreasing and thus, results decrease in the stability of p53. So, MTBP increases p53 ubiquitination as well as degradation in cancerous cells [101]. Thus, it should be noted that MTBP regulates the p53 indirectly by using the Mdm2 feedback mechanism. Also, the increase in the concentration of Mdm2 in many types of cancers can be related with increase in concentration level of MTBP [49]. So, the MTBP is a very important target molecule in cancer research.
1.2.3 Dynamic states of p53

Recent advances in the Microscopy have revealed the dynamical behaviour of many small signalling molecules in vitro [23, 102–106]. In some of the conditions the dynamical properties have been shown to control differentiation in cell [104, 106, 107]. These examples pose to regulation of much unexplored pathways. In recent studies of Purvis et al., 2012 [108], the normal and first damped oscillation state corresponds to the pulsating stage as achieved by them from gamma and UV radiation. While [108] has also achieved the sustained oscillation state (3rd stage in our stage) that corresponds to the complete cell cycle arrest (see Fig 1. in Purvis et al., 2012). Thus, pulsating stage (in our case stage 1 and 2) with increase in stress throws the cell into either repair back DNA or into the cell cycle arrest (or case stage 3). The dose given to the cell in terms of Nutlin-3, give the stability to the cellular p53 concentration and thus, giving time for the synthesis of AIP’s (anti apoptotic proteins) in the cell [109]. While on the other hand the increased level of (sustain oscillation stage 3) p53 by Nutlin-3a is associated with the increase p21 concentration that promotes cell cycle arrest simultaneously shooting then the level of Caspases-6 and 7 protein (apoptotic proteins) [110, 111]. Thus, the increase in the level of caspases-6 and 7 promote the arrival of apoptosis (in our case stage 5) [24]. The stage 4 corresponds to the intermediate stage (stage 4) in between arrest of cell cycle with increasing toxicity and apoptosis, that clearly captures the time lap for the synthesis of apoptotic proteins in p53 dependent manner [108, 110, 111]. The variations in the states of the dynamics of p53 are triggered by various stresses inducing signalling molecules like Nutlin, SMAR1, p300, Axin2, HDAC1 etc. When p53 regulatory network interacts with one of the signalling molecules, the network see that a signalling molecule as a sub-network, which involves a number of interactions and a number of complexes due to the interactions. So the variations in the p53 dynamics are due to the fluctuations in
the sub-network associated with the signalling molecule.

Variation in concentration level of these molecules triggers DNA damage which drives p53 dynamics to different distinct states: first steady state corresponding to nearly normal state, first damped oscillation state, sustain oscillation state corresponding to significantly strong stress condition, second damped oscillation state, second steady state corresponding to excess stress or apoptotic state.

**First steady state:** The significantly low amount of stress inducing signalling molecules allows p53 to maintain its normal state (stabilized state) in the system. The dynamics of p53 for low concentration of stress inducing signalling molecules give single spike due to sudden stress that stabilize the dynamics indicating normal nature of the system.

**First damped oscillation state:** As one increases the concentration of stress inducing signalling molecules in the system, signalling molecules starts active interaction with Mdm2 and other molecules forming various complexes followed by indirect interaction with p53. This indirect interaction of stress inducing signalling molecules and p53 imparts stress in p53 dynamics which starts exhibiting damped oscillation indicating the inducing oscillation for a certain interval of time, and then its dynamics become stabilized. The small stress is given to the system; the system initially will go to the exited and stress state, repairing back the changes of system and can come back to the usual condition.

**Sustain oscillation state:** For sufficiently excess concentration of stress inducing signalling molecules, the p53 dynamics become sustain oscillation state corresponding to significantly strongly activated or strong stress condition where amplitude of p53 of the corresponding sustain oscillation is maximum. This dynamical state may correlate to activated state of system where active interaction of the molecular species are involved.

**Second damped oscillation state:** Further increase in concentration of stress
inducing signalling molecules forcing the sustain oscillation of p53 to become damped oscillation whose decreased time interval in case of damped oscillation as stress is increased. It indicates that the excess of stress in p53 due to stress inducing signalling molecules may become toxic to the system that is reflected in p53 dynamics. In this state system triggers large stress, which cannot be repaired back and may probably go to apoptosis.

**Second steady state:** Excess stress due to stress inducing signalling molecules drives the dynamics to stabilized dynamical state, which may correspond to apoptosis or cell death.

### 1.2.4 Stress propagation in network

Living Cells respond to stress signals and process information using complex biochemical networks of interacting molecular species such as genes and proteins [112]. The complex cellular biochemical networks are regulated by various leading hubs of interconnected molecular component. Perturbation in chemical kinetics or molecular concentrations may affect various activities in cellular processes. At a distinct point of the biological network (input) a signal is detected and then is propagated to sub-network components (output). In information processing in such system, the living cell needs a high degree of sensitivity to the received (input) signal but a low sensitivity to random noise (fluctuations) in the transmitted signal. The fundamental issues of the system are namely, interference of noise in system dynamics, patterns of order driven by fluctuations [113], perturbation induced change of dynamical states [114], system’s adaptation to a change, and their significance in real biological systems. Cellular processes are generally driven by intrinsic and extrinsic fluctuations due to random interaction of intermolecular species in various cellular pathways and molecular cross-talks with the surrounding environments respectively [115, 116]. Stress in such processes, due to external influences,
abnormalities in the genetic regulations and failures of some molecular mechanisms, is propagated spatio-temporally throughout the network perturbing the pathways encountered along its path. However, the nature of stress propagation and management in the complex cellular processes is still an open question.

The stress signal propagators and receivers reveals the inheritance of fundamental properties of the stress signal from propagators to receivers, indicating that the component pathways (signal propagators and receivers) at each dynamical state of the system are strongly correlated. Hence, the responsibilities, beneficial and injuries due to stress signal are democratically distributed among the important candidate proteins and/or genes in the interacting pathways. However, depending on the nature of stress signal and magnitude, and stress receivers, the inherited properties are modified according to their needs and comfortability. Since the response of the stress propagated among the cross-talking pathways are received with excellent correlation via various mediators, the responsibilities of protecting the system as whole from any change due to external perturbations or failures in internal mechanisms might be democratically shared among these pathways.

For example, stress induced in Notch-Wnt-p53 cross-talk by stress inducing molecules (Nutlin and Axin2 in the following chapters), which are exhibited in stress p53 dynamics, drives the system at various dynamical states defined by different fractal laws, and the system switch to these dynamical states depending on the amount of stress induced. This stress system prefers to stay in active dynamical state which has a simple fractal rule subjected to the optimal fluctuations available due to active molecular interaction driven by stress. However, the system still associates a group of few hubs (assortive topology), but not in dependent manner (absence of these hubs do not cause system’s breakdown), for better signal processing and system regulation. Then this stress signal is propagated
throughout the pathways, and found to inherit all the properties of the propagator pathway to the receiver pathways may be with slight modifications in them. The excellent co-ordination in cross-talk helps the system to save it from one directional apoptosis (once the system falls in this phase, it can never come back to normal situation) by regulating available active molecular interaction. This regulating mechanism could be different depending on the type of stress induced in the system (Nutlin and Axin2 in our case).

1.3 Network analysis of time series data: visibility graph approach

Time series [117] analysis is a central topic in physics, physiology, medicine, biology, society and economics. It is a powerful method to characterize the data in biology, physiology, medicine and economics to understand their underlying dynamical origin. Living Cell is composed of complex network of various interacting molecular species to transfer and process information. The computational approaches of living cell [118] are responsible for various important cellular processes like signal transduction, cell-cycle regulation. Complex network theory gives us with a resent aspect and a powerful tool for understanding a complex system from the relations between the components in a global way. Network theory may be an effective tool for revealing embedded information in time series [119, 120].

Complex network theory [120] is a powerful tool in time series analysis, According to some specified mapping algorithm it is based on the transformation of a time series into a complex network. The properties of complex systems are inherited from their respective time series data. Systematic dealing with these time series data could provide inherent properties of the complex system. The conversion of time series to complex network allows us to characterize the underlying
dynamics of the time-series in terms of topology of the network.

1.3.1 Network assortivity: rich–hub formation

Network assortivity is a highly studied approach in terms of topological analysis for complex networks [121,122]. Network assortivity has been explained to quantify the capability in the complex networks where individual nodes connect with other nodes which are similar to themselves [121].

Hierarchically organized networks generally qualify most of the features of self-organization, namely, fractal behaviours of topological parameters, system level organization of modules and absence of central control mechanism (removal of hubs do not cause network breakdown) [123]. Consider \( c_N(k) \) neighborhood connectivity of nodes of degree \( k \).

\[
C_N(k) = \sum_q q P(q|k) \tag{1.1}
\]

The function value \( C_N(k) \) is expressed as \(-1 \leq C_N(k) \leq +1\), if the function \( C_N(k) \) is positive, the network is assortivity, it is quantify that lower degree nodes are, on average, connected to other nodes with lower degree and higher degree nodes are, on average, connected to other nodes with higher degree. Assortivity gives information about the dynamical behaviour of complex networks.

For examples, neighborhood connectivity in these networks constructed from the dynamical states of stress p53 driven by stress inducing molecules like Nutlin, Axin2, ROS, SMAR1 etc. follows, \( C_n(k) \sim k^\beta \) which is a power law of positive exponent. 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. 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. The fractal behaviour is the signature of importance of few hubs and their interaction.
exhibiting assortivity nature of the network topology [124,125]. Even though the few hubs do not have the capability of full control of the network, their significantly strong cross-talks can have the possibilities of regulating the network up to some extent. In such networks, there could be few hubs corresponding to few time states, which are responsible for signal processing and stress management.

This explained that in the time series corresponding to dynamical states of the system, interaction of few hubs (formation of rich-club) is probably essential for stress management and efficient signal propagation.

1.4 Multi-Fractal Detrended Fluctuation Analysis (MF-DFA) analysis of time series data

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]. Multifractal detrended fluctuation analysis (MF-DFA) is a technique used to determine fractal properties, and to detect important correlations in nonstationary time series [127]. 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 on self-organization [130], and their implications in real biological systems.

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],

1.4.1 Mandelbrot’s picture of fractal and multifractal nature

The classical definition of fractal in Mandelbrot’s picture [131], for a heterogeneous time series of window size $T(\kappa t)$, with scale factor $k$ and time sequence $t$, the multifractal function $D$ follows the following relationships,

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

(1.2)

Self-similar process satisfy the simpler scaling law, $D(\kappa) = \kappa^\phi$, where, $\phi$ is self-affinity index or scaling exponent of $T(t)$ [132]. Hence, time series data of a system’s dynamics generally have statistically independent multiple of such scaling behaviours exhibiting multifractal nature due to scale dependent broad probability distributions of the time series, and different long and short range correlations amplified and rectified by large and small fluctuations in the time series [126].

1.4.2 Singularity exponent: nature of time series data

The singularity exponent is a tool used in multifractal analysis in the complex systems to describe in the subset of points belonging to the group of points the fractal dimension that has the same Holder exponent ($Hq$). Initially, the singularity exponent gives a value for how ”fractal” an ensemble of points are in a function [133]. Usually in simplest form, the singularity spectrum $D(\alpha)$ as a function of $f(x)$ is defined as

$$D(\alpha) = D_F\{x, \alpha(x) = \alpha\}$$

(1.3)

where $\alpha(x)$ is the function taking care of Holder exponent, $\alpha(x)$ of $f(x)$ at the test point $x$ and $D_F\{\}$ is the Hausdorff dimension of a point set.
1.4.3 Origin of multifractal nature in time series

In systems where one exponent is not enough to describe it completely, the spectrum of exponents is required to describe its behaviour completely [134]. Its origin has been attributed to the convergence of mathematical convergence related to the central limit theorem, the main feature of this theorem is having a foci of convergence, which is the family of statistical distributions, called as the Tweedie exponent dispersion models [135] as well as the Tweedie geometric models [136]. The first convergence effect is responsible for monofractal sequences and the second convergence yields the changes in the fractal dimension of the monofractal sequences [137].

1.5 Measure of complexity: permutation entropy

A simple and robust way to compute the complexity of various time-series data be it theoretical, i.e. chaotic, noisy or empirical from the real world, has been introduced by Bandt & Pompe in 2002 [138], the permutation entropy. So far there are other entropy measures like Shannon entropy [139], Kolmogorov-Sinai entropy [140] etc., but they are either computationally expensive or neglects the temporal order of values in a time-series data [141].

1.5.1 Analysis of time series data using permutation entropy

In self-organized state one has $H \to 0$. The information carried in the time-series data has been accessed by correlating consecutive patterns of dimension $n$ to be represented in the form of permutation entropy $H_n$. Thus, it is an elementary way to understand the mechanism underlying a given process. The complexity of the dynamical states driven by stress can be measured by calculating permutation
entropy $H_n$. We can analyze the complexity in the time series data of the different distinct cellular stress, namely Stabilized, sustain oscillation and damped states. The high values in the $H_n$ generally indicate high complex and vice versa. The permutation enthalpy can characterize the activities in the system states and content of unpredictability.

1.6 System level organization in p53 regulatory network

Natural systems are complex due to internal organization of the functional components in the network [120,142]. The organization in such complex system can be well explained by network theory which is based on Graph theory where the system is described by a network of nodes (elements/objects/entities/molecular species) with specialized interaction among them [143] and structural dynamics can be studied by modelling the system using deterministic and stochastic descriptions [44,144]. Most of the biological networks fall in hierarchical network. The hierarchical network usually have two important controllers of the network properties, firstly emergence of functional modules to preserve the network properties and secondly interaction of hubs to interfere the modules and overall network [145]. Each module is composed of sub-modules [146], each sub-module are further composed of sub-sub-modules and so on [145,147]. This leads to the system level organization of the network which is an important working principle of most of the real complex networks [145,148]. p53 is the central hub of signalling network which communicate (interact) with a different signalling molecules in the cell [149]. The communication of p53 with a few signalling molecular species are already discuss above. p53 is a versatile molecule, can interact more than one signalling molecules at the same time. The better understanding of p53 signalling
network at system level is needed to find out described information of molecular interaction exists at molecular level [120].

1.6.1 Scale free and hierarchical network

**Scale free:** Scale free network which can be constructed based on the growth of network and preferential attachment (new node tend to connect with high degree nodes in the network) [151] qualifying most of the real networks available in nature (biological, food-web, ecological, social networks, etc.) [120] and it follows power law scaling behaviour in $P(k)$ where emergence of large number of connected hubs takes place [150, 151] which is more significant than in random network. According to Barabasi-Albert (BA) model of scale free network, formation of nodes ($n$) with $m$ edges are connected to the pre-existing set of nodes at each time step. Randomly, new edge(s) are not connect, however, preferential attachment $p_i$,

$$p_i = \frac{k_i}{\sum_i k_i}$$  \hspace{1cm} (1.4)

where $k_i$ is the degree of the node $i$. The scale free network is constructed based on growth process which includes a power-law $P(k) \sim k^{-\gamma}$, wherever $P(k)$ is degree distribution, $\gamma$ is the power-law exponent (degree exponent) that characterizes the topological properties of the network. Scale free networks with the power-law exponents vary $2 < \gamma < 3$ is observed in most biological and non-biological artificial network. For a linear growth process the power-law exponent is where as $\gamma = 3$, in case of non-linear preferential attachment the exponent $\gamma \leq 2$ [152, 153].

Scale-free networks permit the emergence of vital hubs, removal of those hubs causes network breakdown representing central control system. The characteristic average path length of Barabasi-Albert Scale free network is shorter than random small-world networks and characteristic average path length $L$ increase approximately logarithmically with $N$ network size that is a lot of exactly with double logarithmic correction issue, i.e., $L \sim \log \log N$. So increase in average path length
with network size is short in BA scale free network than random networks. Moreover, according to Barabasi-Albert model, network does not have basic modularity, therefore the clustering coefficient $C(k)$ is independent of degree $k$. According to BA model [151], scaling of average clustering coefficient $C(n) \sim n^{0.75}$ is system size-dependent and this is often stronger than clustering of the random network $C(n) \sim n^{-1}$ [120]. BA model networks are more robust than random node attack. To illustrate tumor inducing virus (TIV) acts as biological hackers that target the p53 protein interaction network is the hub and breakdown network [biological hackers and robustness of the p53 network].

**Hierarchical network:** The hierarchical network reveals the organization of small modules, with every module additional organized by sub-module into increasingly large module in hierarchical manner. Hierarchical network deals with sparse distribution of few hubs and extremely clustered modules [146] that have differing types, namely, hierarchical scale free network that follows scale freeness with large number of connected modules [146,154], fractal modular hierarchical network that follows fractal structure of modules and randomized hierarchical network that has random distribution of modules with possibility of central hub within the network [147]. The integration of the various modules and scale invariant distribution of degree under the scale-free network architecture, that is a very important step ahead to understand the approach for the system-level organization of complex network. One drawback was the comparatively terribly high clustering coefficient of these systems. Unlike scale-free networks, several networks behaves exceedingly in an different way: the mean clustering coefficient seems to be nearly invariant that is against variation in size, and therefore the node with high degree then less is the cohesiveness among its neighbors. Therefore, in the complex systems the low-degree nodes are from extremely clustered coefficient. The clusters are integrated into a system incorporating the higher degree nodes.
whose cohesiveness lessened with degree, however, still the general distribution of being scale-free degree is maintained. This causes the clustering coefficient that varies as a function of node degree. The hierarchical network, which have high clustering co-efficient and follows power law scaling behaviour usually have various levels of organization such as modular level then to sub-modular level in each module and so on and lastly motif level organization. There could be different types of motifs of functional significance. If the network has high clustering co-efficient the most probable motif type a network can have is triangular motif (one example is feed forward loop). Ravaz et al. [146] reported a model pictured the conceptual idea of hierarchical network, according to this model the small and highly clustered modules are integrated around a hub node with less number of connections among modules on the fringe. Similar organization rule is applied at every level. One module have been generated in three replicas, the external nodes of the above said replicated clusters have been connected to the older cluster through central node due to this a large 16-nodes modules come into existence. Then three replicas of thin module of 16-nodes are being generated among them 16 peripheral nodes are being connected with the old module through the central node, which gives a new 64-nodes module. Therefore, the properties (topological) of hierarchical network model replicate the characteristic of varied complex networks like World Wide Web, language networks, actor networks, protein-protein interaction network, metabolic networks etc. In the E. coli’s metabolic network, there is a robust correlation between the organization (hierarchical) of the metabolites and therefore the present biological and functional classification of organisms [146]. Later it had been said that the exponent $\alpha$ of the scaling law $C \sim k^{(\alpha)}$ varies as the fraction (i.e. $f$) of nodes that are newly linked to the central node in every level. The less the fraction is, that reflect the rise is in the exponent $\alpha$, and because of the fraction $f \to 1$ the exponent $\alpha \to 1$ [155]. A specific feature of
networks (hierarchical) is that the exposure of modules that may be functionally relevant, and additionally the existence of major hubs that sparsely distributed. The removal of major hubs does not cause the breakdown in the network and such networks carry important features of self-organized systems [156, 157]. Another necessary results of the model (hierarchical) is their stress on the significance of hub nodes in maintaining powerful communication among distributed modules, and therefore hubs in different hierarchal levels seems to have a crucial role that facilitates and integrate the information processing of the whole network.

1.6.2 Origin of system level organization in hierarchical network

System level organization of fundamental functional components of a hierarchical network at different levels is maintained to perform important specific tasks at those various levels in self-organized fashion of the components [146] and emergence of important regulators at local and global level referred to as hubs [120, 156] are some of the fundamental feature of most of the natural and artificial networks. This hierarchical organization of the network extends from how cells function [158], brain organization [159] and how proteins cross-talk at molecular level [160], evolution of species during prebiotic era [161, 162] to inter and intra cellular talks in tissue network [163]. Traditionally, biology has been based on the central idea that life processes are hierarchically organized and indicates that it is this structure which
controls the system’s dynamics. Surprisingly, we lack an objective manner to assess how real this hierarchical organization is, even if we are given different levels and their interactions in the hierarchy [164]. One of the topological properties of network is functional organization of it via various fundamental functional units known as network motifs [165] and their roles in building up the network organization/reorganization [156, 166] that led the network complex in nature [167]. Motifs in a network may be of many types [165], and every network motif performs a well-defined function within the network [168] and most motifs in the network overlap to process information among them [156]. Clustering of motifs (similar types or different) by overlapping structural and functional modules of various topologies, clustering modules form super-modules which cross-talk among them and so on to organize the whole network [154, 165].

1.6.3 Topological properties of hierarchical network

The properties (topological) of the network that are hierarchical network model reflects properties of various networks that are complex like World Wide Web, language networks, actor networks, protein-protein interaction network, metabolic networks etc. The network behaviour and properties of it can be characterized using topological properties of the constructed networks. In such networks, the topological parameters like probability of degree distribution \( p(k) \), neighborhood connectivity \( C_n(k) \) and clustering co-efficient \( C(k) \) follow power law behaviour as a function of degree \( k \). The power law behaviours in these topological properties are confirmed and varied by applying a standard fitting technique to test power law distribution which was proposed by Clauset et al. [169]. The probability degree distribution with \( k \) follow power, \( p(k) \sim k^{-\gamma} \), where \( \gamma \) is the degree exponent which characterizes topological properties of the network. For hierarchical feature the degree of exponent around \( \gamma \approx 2.26 \) using mean-field theory [151]. High
average clustering coefficient is maintained in this network type, \( \approx 0.6 \) clustering coefficient scale as \( C(k) \sim k^{-1} \) where degree of nodes is shown as \( k \). Finally, the mean clustering co-efficient is found almost invariant against the size of the network. The neighborhood connectivity \( C_N(k) \sim k^\beta \), where it \( \beta \) is positive power, \( \beta \) indicate the evidence of assortivity in the network which shows the importance of few hubs forming a cluster (rich-club formation). If \( \beta \) is negative, then there is no evidence of rich-club formation, the power law behaviours of these topological parameters are the signatures of Hierarchical features [125].

Centrality measurements, namely, betweenness \( C_B \), closeness \( C_C \), and eigenvector \( C_E \) centralities, which give the nature of the information processing and to identify most influencing nodes in the network, again follow power low behaviours as a function of \( k \).

\[
\begin{bmatrix}
C_B \\
C_C \\
C_E
\end{bmatrix} \sim \begin{bmatrix}
k^x \\
k^n \\
k^\delta
\end{bmatrix}
\] (1.5)

The increase in these centrality values as a function of the degree \( k \) indicate that hubs are most influencing nodes, and take significant roles in information processing in the network. Even though the absence of these hubs do not cause network breakdown, they and their interaction among them have better responsibilities in regulating the network.

### 1.7 Self-organization in time series data

One of the most important issues in complex network is the dynamical behaviour of network subjected to fluctuations in the number of nodes in due course of time. The reason is due to the fact that in and out diffusion of nodes in the network can induce local perturbations in the network and large local perturbations in turn cause global impact in the network [170]. These perturbations affect the stability
of the network which is a universal phenomenon taking place in most of the real networks [148]. The question is how do networks in general self-organized subject to these perturbations to maintain their overall network properties.

### 1.7.1 Definition of self-organization

Self-organization is the process by which a complex system become systematically organized with proper functioning of its components in spatio-temporal system evolution. It leads to spontaneous emergent properties [171], the global pattern of complex system is formed by local and internal interactions of its components without taking reference to an external template [172]. These local internal interactions generally perform feedback loop, to confers robustness on complex system. Self-organized systems generally are of wider breaking symmetry and nonlinearity from stochastic perturbation (fluctuation) [173]. Further self-organization is generally distinguished from self-assembly because self-organized patterns believe on input of continuous energy to be maintained. There are several instances of self-organization shown in biological system such as macromolecular complex formation during the construction of cytoskeletal structures [174–176]. Arabidopsis thaliana shoot apical meristem [177], Dictyostelium discoideum has migratory slug and the formation of fruiting body [178], embryonic cell re-aggregates, gene regulatory systems etc.

### 1.7.2 Fractal nature as signature of self-organization

The power law nature in topological parameters of complex networks is the emergence of the fractal nature of the networks which can be taken as the signature of self-organization. The power law scaling behaviours of topological parameters are the Hierarchical features [146] in these networks exhibiting fractal/multifractal nature in their structures [179,180]. Fractals have special properties in which
the properties of the system are self-similar independent of any scale, which reveals that every part of the system is a reduced-size template of the whole. This nature could be a self-organization in the system and implies that properties in the system are invariant across spatial and temporal scales. Natural systems are generally self-organized. However, the inter-relation of local and global dynamics and how stability is maintained in overcoming these perturbations are still open questions. One of the most important features of hierarchical complex network is the ability of it to self-organize the network to maintain its stability display all local and global perturbations. Perturbation in a network is caused in various ways such as in and out diffusion of nodes, local edge fluctuation in the network etc. to cause change in the local stability [170]. If the local perturbation is large then it can cause not only the local stability, but also the global stability of the network that may attack overall topological properties of the network. However, if the perturbation is removed from it the network self-organized itself to come back to its normal functioning (which happen in most of the natural networks). If the change is beneficial to the system, then the system adapts to the change. Further the roles of the network motifs as safeguards to maintain the stability of the network are still not known fully.

1.7.3 Fractal nature in time series data

The physiological time series which are subjected to spatio-temporal perturbations (fluctuation) of the many processes such as blood pressure [181], heart rate [182,183], and fetal breathing, etc. are found to exhibit fractal/multifractal nature [184,185]. Such systems try to maintain fractal patterns, when they are driven by uncorrelated perturbation with characteristic time series. The fluctuations that are satisfied from the scaling relationship of the power law could have different correlated properties rising from the complex and nonlinear dynamical
systems instead of those rising from environmental perturbations [186]. Therefore, it is important to find out the fractal dimension of the biological time series data to get the understanding of the generated mechanism as well as measuring the correlation of the components for emerging properties [187]. The analysis of the time series data at different dynamical states of biological pathways from topological properties of networks constructed from these time series, could highlight the emergence of fractal nature of the networks as an indicator of self-organization. The dynamics of the system governed by a time series, $T(t)$ which follow Mandelbrot’s multifractal behaviour [179] throughout the time series, can be defined as,

$$\frac{T(\kappa t)}{T(t)} \approx F(\kappa); \quad F(\kappa_1, \kappa_2, \ldots, \kappa_n) = F_1(\kappa_1)F_2(\kappa_2)\ldots F_n(\kappa_n),$$

$$\forall t, 0 < \kappa, \kappa_1, \kappa_2, \ldots, \kappa_n < 1 \quad (1.6)$$

where, the fractal function $F_i(\kappa_i)$ follows simple power law $F_i(\kappa k_i) \sim k^\psi$ with self-affinity index $\psi$.

### 1.8 Overview of the chapters

This thesis consists of ten chapters. The first chapter gives an introduction of the thesis. The second chapter presents the methodology used in this study in formulating and analyzing the constructed models. The third chapter presents the proposed model of p53-Wnt regulatory network. The fourth chapter presents the second proposed model of SMAR1 induced p53 regulatory network. The fifth chapter present third proposed model of Notch–Wnt–p53 regulatory biochemical network. The sixth chapter presents the third proposed model of p53-Mdm2-miR-125b regulatory network. The seventh chapter presents the identification of fundamental key regulators in ovarian cancer network. The eight chapter presents the Identification of inference genes in breast cancer network. The ninth chapter
presents the identification of fundamental key regulators in breast cancer network. Finally, the last chapter gives conclusions, contributions and future research aspects.