# Structural Bioinformatics Protocol to Modulate the Activity of Human Telomerase (hTERT) For the Age Reversal Outcomes

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# Dedication

This study is dedicated to my parents, and to my brother *Hamza Ahmed,* who has been the source of constant inspiration and gave me the strength when I was about to give up, who continuously provided emotional, spiritual, and financial support.

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# List of Abbreviations

| | |
|---|---|
| **hTERT** | Human telomerase reverse transcriptase enzyme |
| **P53** | Cellular tumor antigen p53 |
| **DDR** | DNA damage response |
| **MDS** | Molecular dynamic simulations |
| **BRN** | Biological regulatory network |
| **PDB** | Protein databank |
| **RMSD** | Root mean square deviation |
| **FFT** | Fast Fourier transforms |
| **SASP** | Senescence-associated secretary phenotype |
| **ROS** | Reactive oxygen species |
| **ncRNA** | Non-coding RNAs |
| **RAP1** | Repressor/activator protein 1 |
| **LTL** | Leucocytes telomere length |
| **TERRA** | Telomeric repeat-containing RNA |
| **dilncRNA** | Telomeric damage-induced long ncRNAs |
| **PHF20** | PHD finger protein |
| **ATM** | Ataxia-Telangiectasia mutated |
| **P300** | Histone Acetyl-transferase p300 |
| **RND1 GTpase** | Rho-related GTP-binding protein RHO6 |

# Abstract

Cell division causes telomere shortening, which contributes to the aging process. The telomeres are regulated by telomerase enzyme however its activity is limited naturally. Therefore, activation of telomere through telomerase (hTERT) may reduce the shortening of telomere which reverse or slow down aging. Various studies have highlighted that the hyper activation of hTERT can cause cell proliferation or cancer. P53 is a gene responsible for cell apoptosis but mutations in it also cause cancer or uncontrolled cell proliferation. Therefore, a normal range of activity is required for both hTERT and p53 to keep cell proliferation and apoptosis in control and to limit or reverse the effects of aging. A Biological Regulatory Network was constructed that proves the role of hTERT mediated cell proliferation and p53 mediated cell apoptosis in cell fate determination. Also the docking and MD simulations of hTERT and its regulators were performed and interaction profiles were identified as important for future design of artificial activators. In this study of p53 and its activators, the protein docking is done with its regulators which determined the binding patterns of p53 activators like Phf20 and p300. Molecular Dynamic Simulation validated the stability of docked complexes through RMSD, RMSF and Radius of Gyration. Additionally, the binding site residues of p53 Arg 158, Arg 267 showed hydrogen bonding in Phf20-p53 complex before and after simulation whereas the interacting residue Glu-224 showed the stable hydrogen bonding before and after simulation in p300-p53 complex. Asp-208, Arg-158 formed the salt bridges in Phf20-p53 complex and Glu-224 and Asp-186 showed salt bridges in p300-p53 complex. Thus the interaction profiles of Phf20-p53 complex and p300-p53 complex is identified as important for the future design of artificial activators of p53 or to classify the peptides or monoclonal antibodies of p53 as activator or non-activator of p53 by different machine learning models on the basis of evaluation by these binding patterns.

# Chapter 1

# Introduction

# Chapter 1

# 1. Introduction

## 1.1 Overview of Aging

Aging has been a universal human phenomenon since conception. Most scientists believe old age starts in life's fourth decade and ends with biological death. Aging in humans is an individualized and complex process that affects people on psychological, biological, and social levels [1]. The gradual loss of physiological health, leading to impaired function and increased mortality risk, is known as aging. It is a multi-factorial process that drives many severe diseases like Alzheimer's, cardiovascular disorders, cancer, diabetes, and neurodegenerative diseases [2]. It is not only about the lifespan but also about the quality of life on Earth. According to a 2015 UN assessment on the world population aging, in the next 35 years, the number of persons 60 and older globally is expected to more than double, reaching about 2.1 billion people. As the world's population ages, it is essential to keep people healthy in old age [3].


**Figure 1.1 Aging and certain diseases caused by it, like dementia, cancer, and diabetes.**

It is a complex, irreversible physiological process that develops gradually over time. Human aging is a universal phenomenon that ends with death only. The final stage of the aging process is old age, in which there is a reduction in body functions & overall health [2]. Aging is the leading cause of age-related diseases, and this concept came under discussion in the middle of the 20th century [4]. Aging is the decline in physical function and several physiological processes, which increases the chance of developing several diseases and disorders [5]. The genetic code of organisms is

considered to be the underlying etiological pathological mechanism of aging, along with other biological and psychosocial factors [1]. The highlighted factors of biological aging include lack of physical activity, poor nutrition, the psychomotor load, acute and long-term medical conditions, and psychosocial changes in the surrounding environment, like loneliness or isolation [6]. Each multi-cellular biological organism can develop and preserve its identity for a limited period using the energy from the sun. They then age as degradation of the body overtakes synthesis [7]. Aging is the deterioration of the physiological functions and accumulation of the senescent cells necessary for survival and fertility [8]. Senescent cells accumulate in organisms and are thought to contribute to aging and physiological dysfunction [9]. Over time, the accumulation of senescent cells develops a SASP (senescence-associated secretory phenotype), producing a chronic inflammatory microenvironment that speeds the process of aging [10]. Aging is the amalgamation of genetic, biochemical, and specific environmental processes that combine, interact, and overlap on several levels. Scientists proposed two theories of aging, "the programmed theories of aging and the damage theories of aging," behind mechanisms underlying the cause, effect, and what keeps aging aside. More than 300 theories were once put forth to explain the occurrence [11].

## 1.2 Programmed theories of aging

The programmed theories reflect that aging is an inherent part of biology. The cells are programmed naturally to decay and deteriorate in their function over time. All living cells have a finite lifespan [11-13].

### Genetic Theory

Aging, according to genetic theory, is caused by the switching of ‘on’ and ‘off’ specific genes in the organism [14, 15].

### Endocrine Theory

Aging is linked with hormone levels; according to this theory, control processes like blood sugar regulation decline and cellular processes deregulation with age.

### Immunological Theory

According to this theory, the immune system of organisms is designed to be at its maximum during adolescence, and it declines over time with age, eventually increasing susceptibility to illness [11].

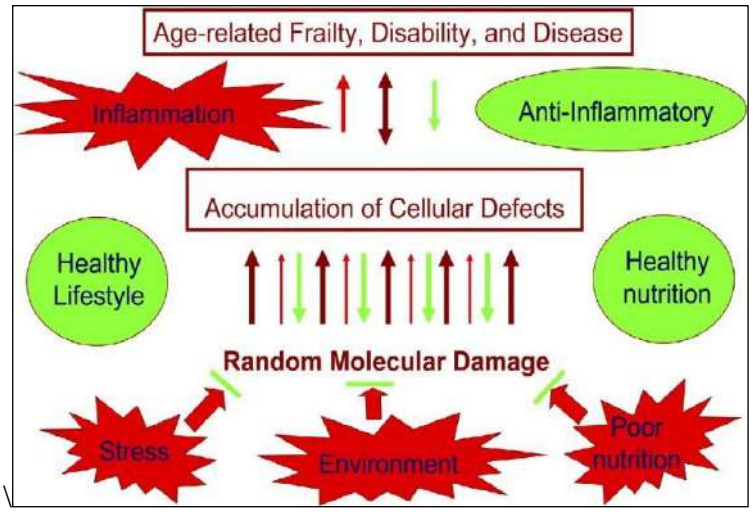



**Figure 1.2 Different theories of aging and factors that cause aging rapidly.**

## 1.3 Damaged theories of aging

According to damage theories of aging, the damage is caused by environmental factors. The main drivers of aging are wear and tear [11-13].

### Wear and Tear Theory

Cells and tissues do not live forever; with continued use, the cells and tissues wear out over time.

### Rate of Living Theory

This theory describes that an organism's metabolism rate determines the human lifespan, and faster metabolism results in a shorter lifespan.

### Cross-linking Theory

The accumulation of cross-linked proteins is responsible for aging, and their accumulation also damages cells and tissues.

### Free radical Theory

Aging is caused by free-radical exposure in an environment that damages DNA, proteins, and lipids. Environmental exposures include the smoke of cigarettes, pollution, UV UV rays, etc.

### Genome Instability Theory

This theory proposes that aging results from damaged cell DNA, specifically mitochondrial DNA.

**Information Theory**

In the book *Lifespan by* Dr. David Sinclair proposed; *"Why We Age and Why We Don't have to"*, aging is labeled as information loss. Damaged cells lose the information encoding their genetic identity. Accumulation of the damage deteriorates the tissue and organ function, which results in aging [16].

## 1.4 Types of Aging

There are multiple types of aging: like Cellular Aging [17], Hormonal Aging [18], Accumulative Damage, and The Metabolic Aging [19]

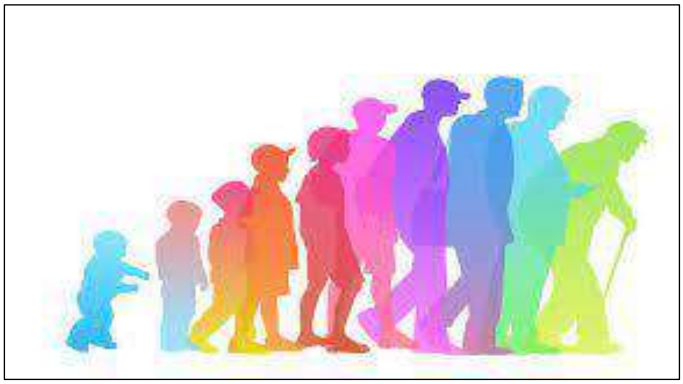


**Figure 1.3 Different stages of life & aging.**

Cellular senescence is experienced in diploid cells [20], a condition of cell cycle arrest that shortens the proliferative life of cells. This phenomenon was initially described in the 1960s by Hay Flick and Moorhead [21], who noticed that human diploid fibroblasts in culture could only undergo cell divisions before their growth was stopped. This biological clock is known as the "Hay flick limit" [22].

## 1.5 Hayflick's Limit

The capability of the proliferation of normal human somatic cells is rigorously limited, and when it is reached, senescence sets in. Senescence, which is found in telomeric or non-telomeric areas of the genome, is thought to be a reaction to significant and irreversible DNA damage [23]. The primary factor causing this harm is oxidative stress, which is intensifying as a result of

mitochondrial dysfunction [24]. Senescent cells increase in tissues with an increase in age, and this is the reason for multiple chronic diseases, including cancer, in the elderly [25]. Hay flick limit, which occurs by the progressive shortening of telomeres during each cell division, is a natural reaction to stop genomic instability and, consequently, the buildup of DNA damage. The replicative senescence term is currently used to describe this process [26]. The direct correlation between organismal aging and cellular senescence is known as Hay Flick's connection [25]. The "Hayflick limit" or replicative senescence was named after Hayflick and Moorhead's 1961 discovery that human fetal cells had a restricted capacity for replication, which was 50 to 60 doublings [27]. The 3 best-known Hay flick factors are: Telomere length shortening, DNA damage accumulation, and depression of the INK4a or ARF locus [28]

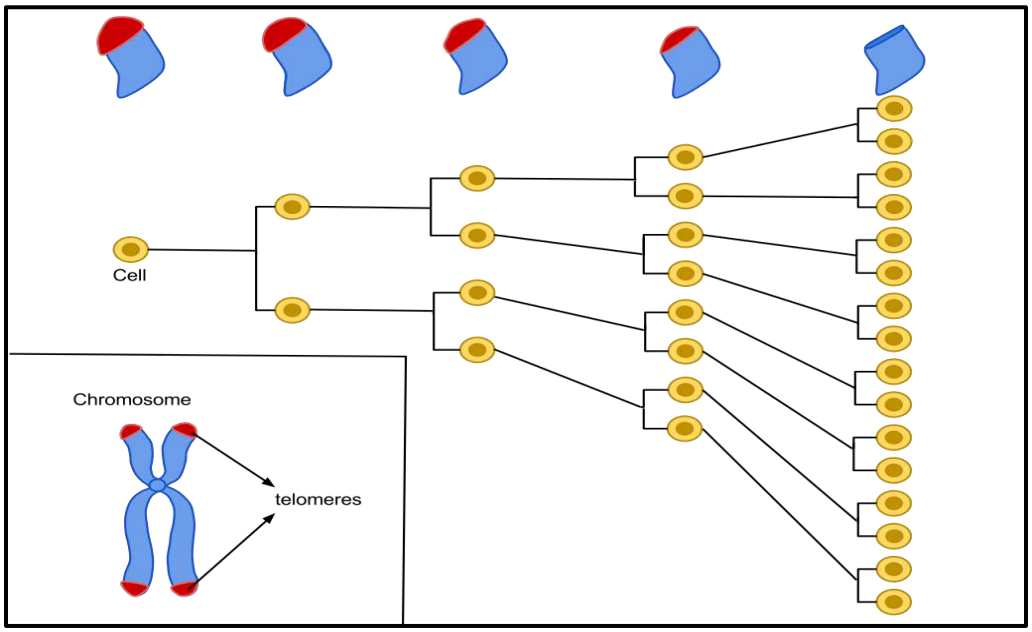



**Figure 1.4 Hayflick's theory of aging states that each cell undergoes a limited number of cell divisions, and when it is reached, senescence sets in.**

## 1.6 Gerontology

Gerontology, the study of aging, is focused on understanding and managing every element that contributes to the impermanence of individual existence. Any living entity that reaches adulthood experiences the process of aging. Gerontology can be summed up as the study of the three

characteristics of longevity, aging, and death from an evolutionary and individual ontogenetic perspective. The length of an organism's life is its longevity. Aging is the gradual change of an organism that raises the risk of diseases, infirmity, and mortality. These aging-related characteristics are referred to as senescence [9]. Cognitive frailty is coined by the “International Academy of Nutrition and Aging and the International Association of Gerontology and Geriatrics” and in longitudinal studies, gerontology is the risk for poor quality of life, aging, dementia, disability, and ultimately death [29]. The two actuarial functions that describe the ability of a population to survive are the survivorship curve and the age-specific death rate, also known as the Gompertz function. The relationship between these actuarial functions and variables like aging traits, constitutional vigor, physical factors, food, and exposure to pathogens is complicated. However, as indicators of aging, there is no alternative for them [9].

## 1.7 Hallmarks of Aging

The senescent phenotype is characteristic of each species [30]. The noticeable known causes of aging are genomic instability [31], epigenetic alterations [32], loss of proteostasis [33], disabled macroautophagy [34], deregulated nutrient-sensing, mitochondrial dysfunction [35], cellular senescence [36], stem cell exhaustion, altered intercellular, communication, chronic inflammation [37] and dysbiosis, And telomere attrition [38]

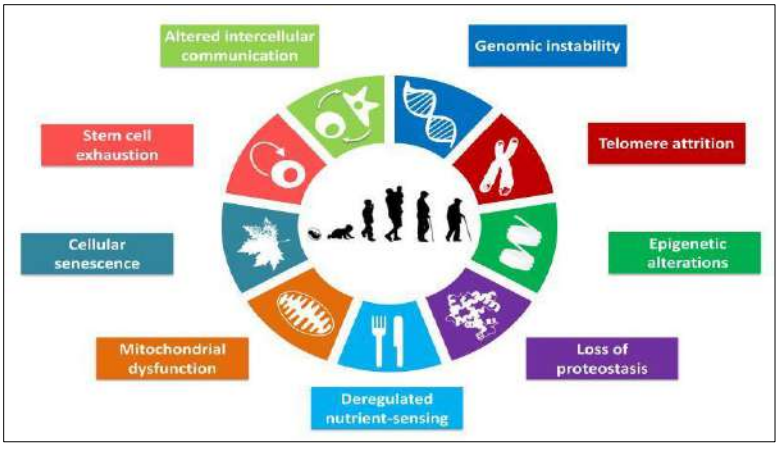



**Figure 1.5 Aging and its major hallmarks including stem cell exhaustion, mitochondrial dysfunction, cellular senescence, telomere shortening, etc.**

### (A) Oxidative Damage

The leading theoretical cause of aging is our metabolism. Theoretically, aging is the byproduct of normal mammalian metabolism, and no mutations are required [39]. ROS or Reactive oxygen species are created by organelle mitochondria when they fail to degrade 2-3% of the oxygen atoms they take in. The ROS contains superoxide ion, the hydroxyl radical, and hydrogen peroxide. Proteins, nucleic acids, and cell membranes are oxidized and damaged by ROS [40].

### (B) Genetic Instability

One of the earliest theories for the general senescent phenotype is the wear-and-tear theory of aging [41]. As one ages, little traumas to the body accumulate. Point mutations increase, and the efficiency of the enzymes declines. Furthermore, the cell would produce many faulty proteins if a mutation occurred in the protein synthesis machinery [42]. The mutation rate would be expected to increase if mutations occurred in the DNA-synthesizing enzymes. 2 scientists, Murray and Holliday, first discovered dysfunctional DNA polymerases in senescent cells in 1981. Additionally, DNA repair may have a role in delaying senescence, as evidenced by the extended lifespan of species whose cells include more effective DNA repair enzymes. Also, genetic flaws in DNA repair enzymes can cause premature aging diseases in humans [40].

### (C) Epigenetic Alterations

Changes in patterns of DNA methylation, improper histone post-translational modifications, divergent chromatin remodeling, and deregulated non-coding RNA are epigenetic changes [43] that contribute to aging. These regulatory changes alter the expression of genes and other cellular processes, which results in age-related pathologies like cancer [38].

### (D) Loss of Proteostasis

Damaged homeostasis of proteins is linked to aging and related illnesses, including cataracts, Alzheimer's, Parkinson's disease, and ALS (amyotrophic lateral sclerosis). This leads to the accumulation of misfolded, oxidized, or ubiquitinylated proteins, which frequently aggregate into intracellular inclusion bodies or extracellular amyloid plaques [38].

### (E) Cellular Senescence

Cellular senescence is the permanent arrest of the cell cycle. The changes in cell morphology, physiology, chromatin organization, gene expression, and alterations in the secretome. Cellular senescence is the response of acute or chronic damage like telomere shortening, ROS, and DNA replication stress. The senescent cells accumulate in human tissues and start affecting fibroblasts, endothelial cells, and other cell types, contributing to aging and its associated deterioration [44].

## 1.8 Telomere

Telomeres are repeated DNA sequences present at the ends of chromosomes [8]. The complex structures telomeres TTAGGG are found at the ends of human chromosomes. Previous research demonstrated the vital function of telomeres in maintaining the cohesion and integrity of chromosomes [45]. Its length and 3D structure preserve the function of the telomere. At the end of each chromosome, telomeres are made up of millions of repeating DNA base pairs but do not express any known proteins. The sequence is 5'-(TTAGGG) n-3' telomeric repeat in humans and several other species. The 5' end chain is shorter, and the single-stranded G-rich segment of the telomere is present; the lateral portion of the telomere does not finish in a double strand of DNA [46]. Shelterin is a complex protein made up of 6 proteins that are localized at telomeres, specialized regions at the chromosome end. Its primary function is to protect the G-rich nucleotide (TTAGGG) repeats at the terminal regions of chromosomes. Interactions between telomere shelterin proteins and interstitial telomeric sequences (ITS) are recent research areas into the role that 3D telomere looping plays in cellular aging and genome stability. Shelterin a complex of six proteins, namely **POT1** (protection of telomere protein 1), **RAP1** (repressor/activator protein 1), **TIN2** (TRF1-interacting protein 2), **TPP1** (TIN2- and POT1-interacting protein), and **TRF1** and **TRF2** (telomeric repeat binding factors 1 and 2), collaboratively functions to ensure the stability of telomeres [47].

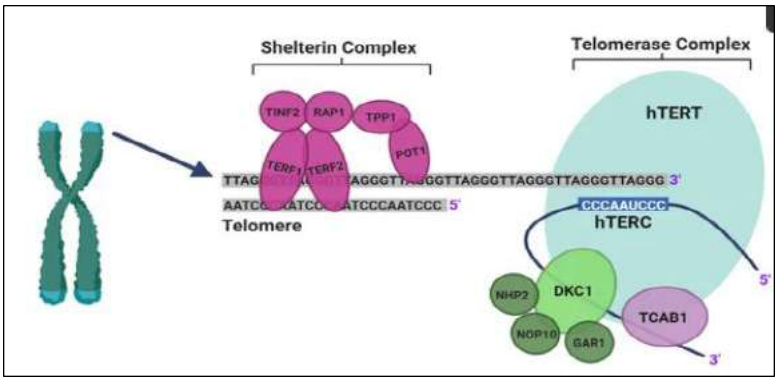



**Figure 1.6 The structure of telomere in complex with shelterin protein and its six protein components, and telomerase complex with its two components hTERT and hTERC.**

Every time a cell divides, the telomeres become shorter [2]. The telomere length differs widely and is linked with age and organism atherosclerotic burden. The telomere length varies with tissue type because of proliferation rate; there is an association between telomeres in different tissues and PBL (peripheral blood leucocytes). Thus, the length of the leucocyte telomere has been considered a main marker of telomere length in the body [46]. Loss of telomere is also correlated with the process of aging in vivo.[48] Early studies investigated the association of leucocyte telomere length (LTL) shortening with coronary artery disease (CAD) progression. In 2010 and 2021, 2 studies were conducted, which discovered that leucocyte telomere length with stable CAD (Coronary artery disease) was shorter in patients than in healthy individuals of the same age [46]. The present study assessed the role of telomere length by PCR (real-time). They discovered that the increased incidence of Gastric Cancer was substantially linked with telomere shortening. In comparison to healthy controls, Gastric Cancer sufferers' relative telomere length was shorter [49]. Telomeres are not replicated by DNA polymerase; if they aren't maintained by telomerase, they will shorten at every cell division. Each time a cell divides, telomerase attaches a telomere to the chromosome [40]. Usually, DNA replication starts with pairing RNA primer to the strand in the opposite direction and provides the free 3′-OH needed for DNA polymerases to start synthesis. The RNA primers are removed and replaced by DNA. At replicating chromosome 3′ ends, removing RNA primer would result in losing 20 nucleotides for each DNA replication. This loss of 20 DNA nucleotides gradually leads to cellular senescence [50]. Before entering the cell cycle, every typical human diploid cell has 46 chromosomes and 92 telomeres [51]. The telomere shortening acts as a "clock," which eventually prevents the cells from further dividing. Mammal

somatic cells usually lack telomerase. Human fibroblasts replicate only for a limited number of times in culture, and their telomeres shorten as a result. These cells can continue dividing if modified to express telomerase [40].

In the 1930s, McClinton and Muller identified the unique structure at the end of chromosomes in Zea mays and Drosophila melanogaster, known as telomeres, crucial for chromosome end fusion prevention. Muller named the structure telomere from the Greek word telo, meaning "end," and meros, meaning part, hence end-part [27]. Aging and cellular senescence are associated with telomere shortening and damage. The telomere dysfunction is linked with several human disorders during normal aging [9]. One theory of aging describes that animal genes contain a specific "program" that determines their life duration. This theory of aging focuses on the number of repeats in a telomere sequence that determines the maximum life span of a cell; each time a cell divides, multiple telomere repeats are lost [48]. Telomeres are the nucleoprotein complexes found at the ends of chromosomes. Telomere structures are made up of two parts: (i) specific DNA repeat sequences (5′ TTAGGGn) and (ii) the shelterin complex, made up of 6 proteins that regulate telomere metabolism and preserve its structure [52]. The genomic regions at the end of chromosomes are known as telomeres. The telomeric repeats TTAGGG that make the telomeric DNA in vertebrates are bound by a group of proteins that regulate their biological activities and prevent them from being identified as DNA damage which can initiate DNA damage response (DDR) [9].

Standard DNA polymerases usually cannot adequately copy DNA in the absence of telomerase; therefore, chromosomes with shorter telomeres are generated [9]. The transcripts of telomeric repeats play significant roles in end protection of chromosomes and maintenance of genome stability [53]. When telomeres reach a critical length, they lose the ability to bind sufficient amounts of telomere-capping proteins, and they are then recognized as exposed ends of DNA. This causes DNA Damage Response pathways to be activated, which causes the p21 and p16 cell cycle inhibitors to be induced, stopping the growth and proliferation of cells. However, these tiny telomeres still contain enough telomere-binding proteins to prevent fusions, inhibit DNA repair, and provide a persistent DNA damage signal that enforces a long-term DNA damage-induced proliferative halt. This causes cellular senescence, a leading factor in aging and age-related chronic illnesses [9]. Telomere damage or DNA damage at the ends of chromosomes in the cells has a role

in aging and age-related disorders [15]. Telomere length is a potential cellular marker for biological aging [54]. The telomeric Repeat-containing RNA and telomeric damage-induced long ncRNAs play significant roles in age-related pathways [53]. Telomeres stop chromosomal DNA base pair loss during the subsequent cell divisions. Telomere length gradually shortens until it is too short for the cell to divide further, resulting in cellular senescence. The incapacity of DNA polymerase to replicate is known as the "end replication problem." Telomerase is an enzyme with a protein reverse transcriptase catalytic unit that meets the end replication problem by lengthening the telomere [54].

## 1.9 Telomerase

Telomerase is a DNA polymerase enzyme that extends the three ′ ends of DNA chromosomes by synthesizing multiple telomeric repeats [50]. Telomerase is a ribonucleoprotein enzyme that synthesizes telomeric DNA to combat the shortening of telomere length [55]. The two subunits of the enzyme telomerase are (a) TERC, “the telomerase RNA component, which plays the role of RNA template,” and (b) hTERT, “the catalytic element, human telomerase reverse transcriptase.” The function of hTERT is to catalyze the RNA template-based formation of new telomeric repeats. This enzyme's expression and activity are seen during fetal development [56]. In most normal cells, telomerase expression and activity are also severely restricted [52].

Telomerase, reverse transcriptase (hTERT) extends short telomeres to a specific length; TERT catalyzes the process using its template of RNA components, and telomerase completes the ends of DNA chromosomes using an internal RNA template [46]. Telomerase activity is essential for tissue regeneration and malignant infiltration [57]. Generating stem, germline, and highly proliferative cells requires telomerase activity. Premature aging occurs in mouse models due to a lack of telomerase RNA. Studies have demonstrated that excessive hTERT expression leads to immortalization in healthy primary fibroblast cells [2]. The telomerase enzyme is responsible for maintaining these nucleoprotein caps. The primary human fibroblast studies provided evidence for the significance of optimal activity of telomerase and telomere length maintenance for both replicative potentials in the culture and aging in organisms [45]. The primary subunit of telomerase, hTERT, is controlled at numerous levels in terms of expression and activity. The

alternate splicing of hTERT's pre-mRNA controls how it is regulated. The literature has described more than 20 splice hTERT variants, but only full-length hTERT is catalytically active. Most of the total hTERT mRNA is made up of two splice variants. The RTD (reverse transcriptase domain) portion in the hTERT protein disappears, and its catalytic activity is lost because of the 36 nucleotide deletion in exon 6 of the αlpha variant. The deletion of 182 nucleotides from exons 7 and 8 of the βeta variant shifts the reading frame, which causes an early stop codon on exon ten, and the production of a shortened version of the hTERT protein occurs. This spliced version of hTERT functions as a dominant antagonist [58]. Telomere shortening can be reversed by a highly activated telomerase enzyme in proliferating cells like male germ cells, lymphocytes, and cancer cells [59]. Telomerase uses its unique TERT (telomerase reverse transcriptase) and telomerase RNA (TER), which have the template that drives repeat synthesis, to synthesize copies of the telomere repeat, thereby extending the three ′ end of telomere [50].

Nguyen et al. investigated the hTERT gene's methylation. They discovered that methylation patterns help forecast how cells react when given all-trans-retinoic acid. ATRA (All-Trans Retinoic Acid) causes suppression of hTERT in promyelocytic leukemia cells (acute), which leads to differentiation or even death, as is well known in the literature. Based on the methylation of the hTERT gene, the authors proposed expanding the use of ATRA to treat additional malignancies [60]. According to a study by Lupatov and Yarygin, stem cells have unique telomerase regulatory characteristics. The proliferation potential of stem cells can be changed by the temporal activation of telomerase [58]. Activation of telomerase by some natural molecules is considered as an anti-aging modulator that can play a role in treating diseases related to aging [61]. The enzyme telomerase was discovered by Blackburn and Greider in 1985. This enzyme is capable of adding DNA repeat sequences to chromosomal ends and causing telomere lengthening [27]. Since most adult somatic cells in humans lack enzyme telomerase, their proliferation contributes to the progressive telomere shortening with aging, which eventually results in aging and death [59]. In 2012 a scientist Jesus et al, tested the effects of a telomerase gene therapy in 2 groups of mice, by treating them with an adeno-associated virus (AAV), as a tumor suppressor, expressing mouse TERT that resulted in an increase in median lifespan by 24% in young mice and by 13% in old mice. Also, results proved that telomerase gene therapy-treated mice did not develop cancer at all [62].

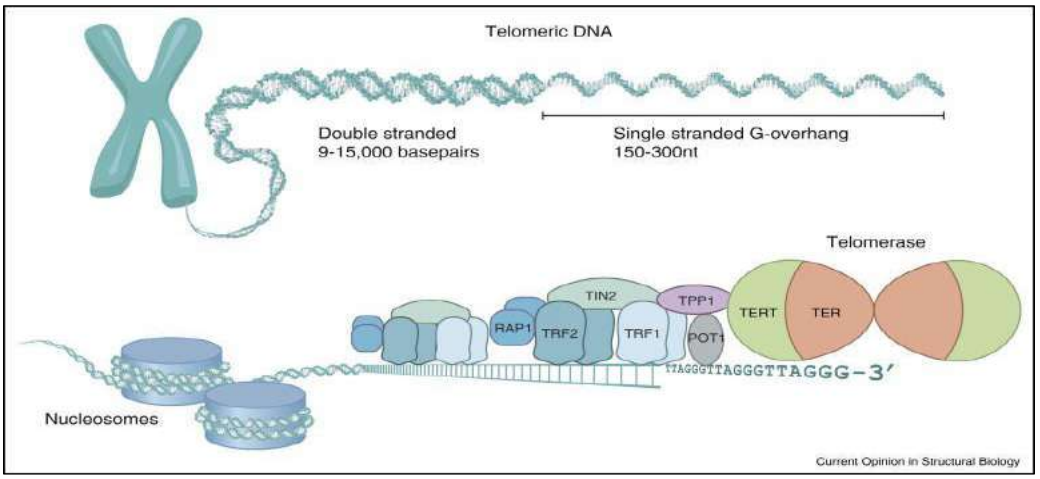



**Figure 1.7 Telomerase & the binding proteins at the end of each chromosome**

## 1.10 Telomere Shortening

Telomeres reach a critical length after a specific number of divisions. When they malfunction, the natural chromosomal ends are exposed to the DDR machinery [63]. In cells, the accumulation of short and dysfunctional telomeres due to aging causes stem cell instability and tissue degeneration due to replicative senescence [2]. In peripheral blood leukocytes, telomeres shortening has been linked to increased mortality rates in human population studies [64]. Several inherited degenerative diseases have been studied, suggesting the significance of telomeres in regulating a healthy human lifespan. For instance, mutations in the catalytic TERT, or TERC, are now known to be present in patients with ADDC (autosomal dominant dyskeratosis congenital) [45]. Telomere shortening, which results from increased oxidative stress and cell division, causes cellular senescence [65].

An article by Maugeri et al. relates the effect of pregnancy on fetal telomere length. The length of fetal telomeres is shorter when a pregnant woman is overweight or under-weight than women of an average weight when the pregnancy occurs. This finding emphasizes the significance of pregnancy for the telomere biology and the child's overall health. The length of telomere and genomic instability of fibroblasts from individuals with idiopathic pulmonary fibrosis were investigated in the paper [58]. Cells enter a dormant state when their telomeres get too short. At the M1 stage, DNA damage signaling, and senescence begin, and together, they offer a crucial

defense mechanism that stops the formation of an oncogenic state. Yet, there are circumstances where cells, instead of senescing, bypass cell cycle checkpoints provided by p16INK4a, TP53, and Rb and enter another state, the crisis state (M2). Chromosome end fusion, genomic instability, and cell apoptosis are all brought on by short telomeres. Ultimately, this results in cell death [66].

## 1.11 P53

The p53 is a sequence-specific DNA-binding protein that regulates transcription. Two N-terminal trans-activation domains are followed by a core DNA binding domain, another conserved proline-rich region, and a C-terminus that codes for the protein's nuclear localization and an oligomerization domain for transcriptional activity [67]. The p53 is 393 amino acids long protein and contains transcription factor structural domains. In human cells, p53 is inactivated by its negative regulatory mdm2 or hdm2 and its low level. When DNA damage is sensed, p53 rises, binds to regulatory sites in the damaged DNA, and starts producing proteins that stop cell division until the damage is repaired. If DNA damage is not correctable, p53 initiates programmed cell death, known as apoptosis, that removes the damage permanently. The protein p53 is crucial in cell cycle arrest, senescence, and differentiation. P53 is known as the Guardian protein that destroys abnormal or damaged cells. P53 is a remarkably potent growth inhibitor of cells, causing cell cycle arrest, depending on the cell type and environment [68]. Inactivated p53, either due to mutation or deletion, is present in 50% of adult malignancies, whereas the remaining 50% have wild-type p53 function. Hence, activating the p53 could be an intriguing therapeutic strategy for treating human malignancies [69]. Protein p53 is a transcription factor that activates numerous genes necessary to maintain genetic stability. The p53 gene is the most mutated in all human tumors. A 1/3 of these p53 mutations are structural, meaning mutants' protein conformations are mutated and altered [70].

The discovery of p53 occurred during extensive research into tumor viruses. It was observed that p53, a host protein with a molecular weight of 53 kD, formed a complex with 40 giant T antigen simian virus in cells that had undergone viral transformation. Initially categorized as an oncogene, recent investigations have elucidated that the wild-type p53 encoded by the TP53 gene exerts inhibitory effects on cellular growth and oncogenic transformation in in-vitro cell culture systems

[67]. In response to DNA damage, the tumor suppressor p53 protein plays a critical function in regulating cellular proliferation. A limited lifespan characterizes the p53 protein, and a range of mechanisms, like transcriptional and translational regulation and posttranslational modifications, regulate its functionality. Furthermore, it has been observed that p53 undergoes fast ubiquitination mediated by MDM2, leading to its subsequent destruction through the ubiquitin-proteasome system. Nevertheless, when DNA damage occurs, the stabilization of p53 is facilitated by the signaling pathway responsible for DNA damage repair. The p53 protein is activated by some Post Translational Modifications, including phosphorylation by the phosphatidylinositol kinase-related kinases mutated ATM on serine 15, and ATM and Rad3-related (ATR), or by the CHK2 at the N-terminal transactivation domain on serine-20, which stops the interaction between MDM2 and p53, causing the p53 stabilization and accumulation [70]. The most potential function of p53 in the transcriptional regulation of hTERT was initially suggested by evidence demonstrating a negative association between telomerase activity and the level of p53. This role has since been verified. The overexpression of p53 in SiHa cervical cancer cells was examined through results to decide whether the p53-mediated hTERT suppression loss contributed to the activation of telomerase found during transformation. It was determined that the suppression of transcription of hTERT was considerably influenced by two binding sites of p53 at 1954 and 1317 base pairs upstream of the ATG [62].

Geno toxins constantly attack P53 and aging the genome. The development of cancer is facilitated by the mutagenic effects resulting from DNA damage. Also, the use of radiation and chemotherapy initiates DNA damage, which subsequently triggers cellular processes such as apoptosis or senescence because of the DDR. Recently, the role of DNA damage as a contributing component to the aging phenomenon has been recognized. There is a relationship between DNA damage and aging, as well as the risk of developing age-related illness, and congenital progeroid syndromes exemplify this. These syndromes arise from mutations in pathways like p53, which are responsible for maintaining genome stability [71]. The pathway of P53 is the primary regulator of at least three hallmarks of aging, including cellular senescence, mitochondrial dysfunction, and genomic instability. The tumor suppressor p53 can facilitate injured cells' survival, repair, and elimination in response to diverse stress signals. These mechanisms hold significant implications for the aging of organisms. The mouse model studies have provided significant insights into the fundamental importance and intricate nature of the p53 network in the progression of aging. The decline in p53

function has been associated with increased longevity in normally aged mice. It is postulated that the observed aging phenotypes in these animals are attributable to two potential mechanisms: (1) the presence of consistently active p53 resulting from deregulation of MDM2 (chronic stress) or (2) alterations in p53 signaling outcomes [26]. P53 also regulates multiple hallmarks of aging, like genomic instability, cellular senescence, mitochondrial dysfunction, and certain altered metabolic pathways [10].

## 1.12 What has already been done

To increase the cell replication process? Normal cell proliferation and apoptosis are very significant. This project aimed to activate hTERT in the normal range by co-targeting p53 and elucidating their interaction profiles. For this purpose, a knowledge-driven BRN was constructed in yED software and simulated in Jimena software. The results show that SP1 had a dual role in activating hTERT and p53. SP1 was the primary activation node for both responses, but it cannot be considered the primary target because of its presence in many other pathways. This BRN highlighted the interactions of oncogenes and tumor suppressor genes with hTERT and p53 in cell cycle progression. The results of simulations demonstrated that SP1 activates both p53 and hTERT, and hTERT inhibits ROS, but p53 activates NFkB, and ROS further activates NFkB through phosphorylated IKB. NFKB initiates c-Myc transcription, and c-Myc binds on the TERT promoter region to the E-box and thus activates hTERT. The simulation of SP1 at different concentrations in Jimena was observed, and the simulation where SP1 was set as 0.5 (moderately active) shows promising results where both responses were activated in the balanced and normal range. These results aligned with the objective of this project, which was to do a dynamic simulation of the hTERT pathway to determine its concentration gradient in standard human cell modulation of telomeres and also the modulation of p53 along hTERT for regulation of normal cell proliferation and apoptosis through dynamic modeling.

Another objective was to elucidate the interaction pattern of hTERT with its positive regulators. The protein-protein docking of hTERT with its positive regulators was performed in ClusPro. The four positive regulators, c-Myc, STAT3, HIF1, and c-Jun, were selected through a literature review, and their structures were obtained from PDB. The docking results showed a robust binding

pattern between complexes, and interactions were viewed through PDBsum online. Based on availability and interactions, the top 2 complexes, c-Myc-hTERT and STAT3-hTERT, were selected for Molecular Dynamic Simulations, and it was found that both docked complexes achieved a stable RMSD between 5 and 6 A. The Glu-209, Ser-227, Ala-228, Arg-293, and Val-299 residues of hTERT were found commonly interacting in both c-Myc-hTERT and STAT3-hTERT complexes before and after simulation, making them the most significant residues of hTERT. The interaction profiles of both complexes can be used for future design of artificial activators of hTERT.

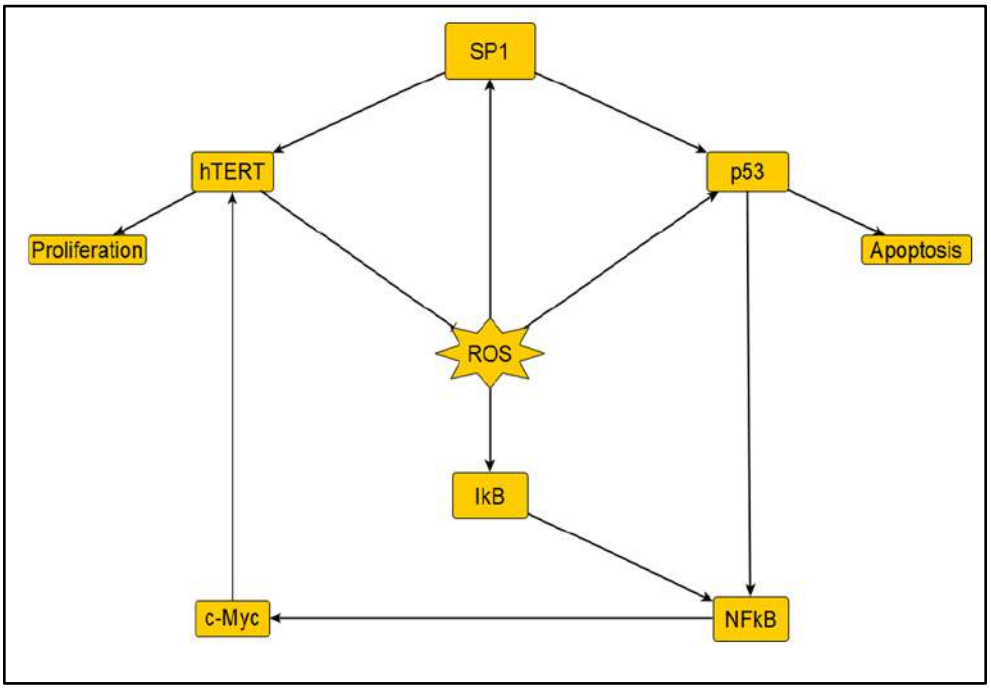



**Figure 1.8 Biological regulatory network (BRN) of hTERT and p53 in a normal human cell. The hTERT is essential in cell proliferation, and p53 plays a crucial role in cell apoptosis.**

## 1.13 Problem Statement

Telomere shortening with every cell division without the enzymatic activity of telomerase results in cellular aging. Progression of telomere shortening leads to senescence apoptosis in human cells,

affecting health and lifespan. Therefore, telomerase activity must be enhanced momentarily to reduce or reverse cellular aging.

## 1.14 Proposed Solution

Drug-induced age reversing by an increased but normal spike in hTERT and p53 activity has been indicated as a promising anti-aging therapy**.**

## 1.15 Objectives

- Co-targeting of hTERT and p53 by regulators for the telomeres-mediated age-reversing process.
- To elucidate the interaction of positive regulators of hTERT and p53 for the design/virtual screening of new biological/chemical entities against the age reversal process.

# Chapter 2

# Literature Review

# Chapter 2

# 2. Literature Review

## 2.1 Aging

Aging, according to Peter Medawar's hypothesis from 1952, is caused by a decrease in the force of natural selection following reproduction. The discovery that caloric restriction increased lifespan in mice and rats in 1939 was a critical first step in studying aging. This discovery was replicated in other species, including primates, and that was the first to show that the aging process is, therefore, a sign of future genetic research. Notably, dietary restriction enhanced maximum lifespan and slowed the onset of age-related illnesses. These findings gave rise to the hypothesis that longevity was linked to a slower rate of aging and a longer life expectancy [4]. One major hallmark of aging is cellular senescence, the fine walking line between Life and Death [72]. Multiple shreds of evidence linked cellular senescence with age-related human diseases like cardiovascular disease, neurodegenerative disorders, atherosclerosis, diabetes, pulmonary and renal, and liver failure [73]. Many genes have been found to influence aging. Hutchinson-Gilford progeria syndrome causes children to age quickly and typically pass away at the age of 12 from heart failure. It has resorbed bone mass, hair loss, thin skin with age spots, and arteriosclerosis as symptoms. A dominant mutant gene brings it on. Mutations in the klotho gene in mice result in a condition similar to progeria [20]. Sirtuin 2 is involved in lifespan extension associated with calorie restriction, mostly in lower organisms [28].

## 2.2 P53

Salavati Pour et al., 2022 investigate the effects of platelet-derived microparticles (PMPs) on the proliferation & growth of Mesenchymal stem cells through expression of hTERT, c-MYC, p16, p53, and p21, the aging and cell longevity genes. The PCR data indicated that PMPs were able to remarkably up-regulate hTERT and c-MYC gene expression while down-regulating the expression of p16, p21, and p53 genes, proving PMPs are a safe and compelling candidate for prolonging the lifespan [74]. The consequences of NPM1 removal on hematopoietic stem cells and HSCs aging and their role in myelodysplastic syndrome were investigated. An Npm1 deleted mouse model discloses that its loss can cause the premature aging of hematopoietic stem cells. The findings

indicate that the loss of NPM1 results in the accelerated aging of hematopoietic stem cells and heightened inflammation within the microenvironment of cells. All these factors contribute to the pathogenesis of myelodysplastic syndromes. Also, the loss of the p53 gene worsens the development of MDS, indicating complex interactions between NPM1, p53, HSC aging, and MDS pathogenesis [75]. The p53 pathway is responsible for preserving the stability of the genome and the balance inside cells by activating mechanisms that prevent tumor formation, including halting the cell cycle, inducing cellular senescence, and facilitating the repair of DNA damage [76]. The p53 protein can induce apoptosis and Ferro ptosis to remove damaged cells[10]. When a tumor suppressor p53 is inactivated due to mutation, the cell proliferation checkpoints lose their function. In response to DD, the p53 tumor suppressor gene is known to have a crucial function in regulating cellular proliferation. Typically, the p53 protein exhibits a relatively short lifespan, with its functionality regulated by various mechanisms, including transcriptional red translational regulations and post-translational modifications [71]. The aging process has been found to have a major impact on multiple aspects of p53, including its transcriptional activity, its ability to induce apoptosis in a p53-dependent manner, and its overall efficiency in responding to cellular stress. The decline in functional activity of p53 with age has been linked to high oxidative stress and a decrease in autophagy, the inhibitory impact of NFκB on p53 function, and the promotion of inflammatory responses by NFκB. To address the issue of energy insufficiency, AMPK (AMP-activated protein kinase) triggers p53 activation through phosphorylation at Ser-15, resulting in the cell cycle halt. Also, when AMPKα2 is artificially activated, it promotes the transcription of the p53 gene and facilitates its phosphorylation at Ser-46, ultimately resulting in programmed cell death. Hence, the activation of p53 incurs a trade-off since cells having active p53 endure either cellular senescence or apoptosis [77].

The level of p53 is undetectable in those mammalian cells unstressed by the processes of continuous ubiquitination and subsequent degradation by the Proteasome [28]. The p53 is recognized for its importance in inhibiting cancer progression, thereby making it an essential factor in the suppression of aging and the promotion of longevity. The utilization of p53-deficient mice as a model for investigating the involvement of p53 in the aging process is limited due to their premature mortality resulting from malignancies. Nevertheless, rapid aging has been observed in two lines of mice that exhibit full-length p53 in conjunction with the p53 C-terminal segment. Conversely, the null mutation in p66Shc, linked to a deficiency in the p53 and p21 stress response,

resulted in a notable 30% extension of the organism's lifespan. It was found that the process of aging in mice was delayed, and age-related damages were reduced by the activation of the Arf/p53 pathway [77]. Cancer progression is an age-related disorder. The relationship between cancer and aging is becoming increasingly understood in terms of molecular connections. The process of aging is linked to various molecular and cellular occurrences that impact the development of cancer and its subsequent progression. In response to multiple forms of stress, the senescence at the cellular level is characterized by an irreversible end of the cell cycle. There were mutations in 2 alleles of the p53 gene in the tumors of mice, in vitro cultivated cell lines of humans, and DNA extracted from colon malignancies of humans. All mentioned findings establish p53 as a tumor suppressor gene. Also, p53 influences aging processes and longevity [28].

The study observed a decline in p53 activity in aging mice, which is linked with an increase in the occurrence of tumor/malignancies and life duration reduction. The transgenic mice that exhibited p53 overexpression together with p19 ARF were not only cancer-resistant but also exhibited deceleration in the aging process. Nevertheless, transgenic mice exhibiting the expression of some mutant's p53 or naturally present isoforms had consistently heightened p53 activity, leading to exceptional resistance to cancer and the manifestation of many indications of accelerated aging. The impact of p53 on aging is intricate due to the complexity of cell cycle arrest, cellular growth senescence, and programmed cell death or apoptosis [28].

## 2.3 P53 and MMD2 (mouse double minute 2)

The protein p53 undergoes fast ubiquitination facilitated by MDM2, resulting in its subsequent destruction through the ubiquitin-proteasome system (UPS). Nevertheless, when DNA damage is present, the stabilization of p53 occurs through the activation of DNA damage response (DDR) signaling. One example is the activation of p53 through various post-translational modifications (PTMs). Phosphorylation at serine-15 by the phosphatidylinositol kinase-related kinases, specifically ataxia-telangiectasia mutated (ATM) and ATM and Rad3-related (ATR), or at serine-20 by the checkpoint kinase (CHK2) at the N-terminal transactivation domain, disrupts the inhibitory interaction between MDM2 and p53. Consequently, p53 undergoes stabilization and accumulation. The DNA binding activity of p53 can be augmented or adjusted through

phosphorylation and acetylation processes occurring at both the DNA-binding domain and the C-terminal regulatory region. These modifications play a role in regulating the many cell fate determinations. There exist a numerous regulatory response that determine the quality of the p53 DNA damage response (DDR) [71].

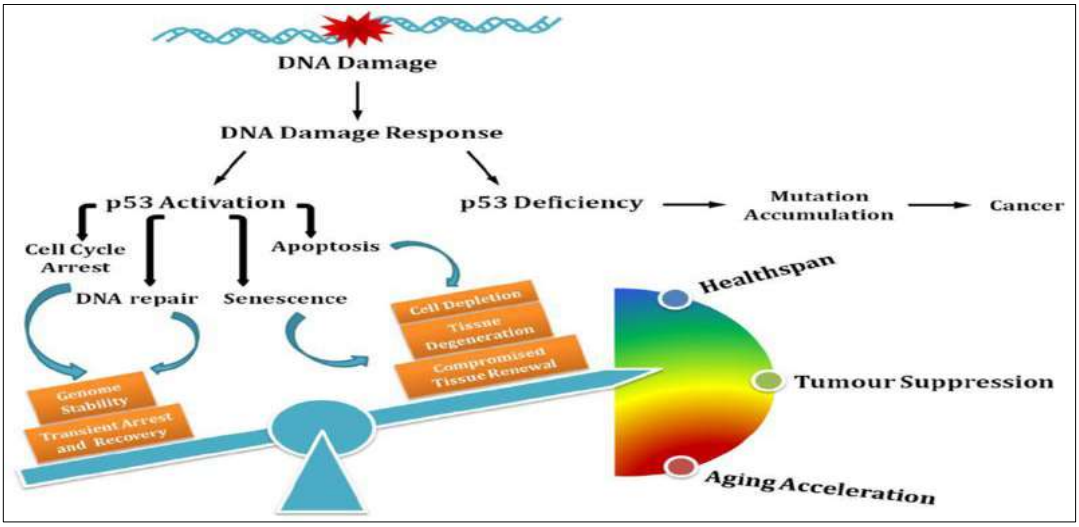



**Figure 2.1 Influence on the development of cancer & the aging process by p53-mediated cell fate decision.**

The p53 serves as a transcriptional regulator for a wide range of target genes, with some being regulated by initial levels of p53, while many others require increased levels of p53. Various internal and external stresses typically induce these raised levels. MDM2 has been extensively established as the primary p53 negative regulator. MDM2, an E3 ubiquitin ligase, possesses the capability to attenuate p53 functionality through 3 distinct mechanisms:

- Poly-Ubiquitylation, which facilitates the targeting of p53 for degradation by the proteasome;
- Mono-ubiquitylation, leading to the exportation of p53 from the nucleus;
- And direct interaction with p53, resulting in the inhibition of transactivation of critical target genes. While there is a well-established association between the p53–MDM2 axis and the aging process, the specific mechanism behind this relationship remains unclear [10].

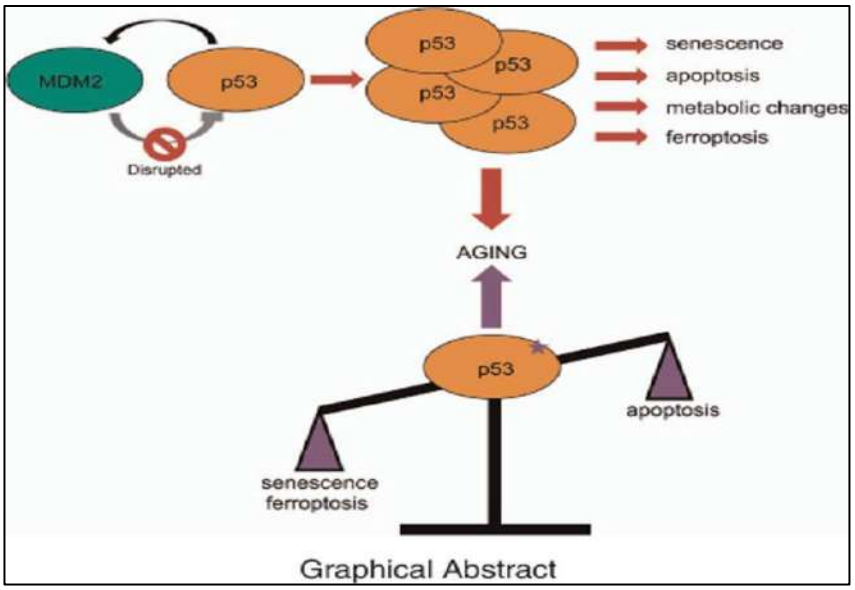



**Figure 2.2 Ubiquitylation of p53**

## 2.4 P53 Activation

The administration of the p53 pathway has been a primary objective for both scholarly researchers and pharmaceutical enterprises since p53 was identified as the "guardian of the genome." [78]. The p53 plays a crucial role in preserving the stability of the genome by halting the cell cycle and promoting programmed cell death, known as apoptosis, as a means of eliminating cells that have incurred damage in response to stress. Several nucleolar proteins have been shown to have a role in stabilizing the p53 protein by disrupting the link between Mdm2 and p53 during times of cellular stress. However, additional methods via which nucleolar proteins activate p53 still have yet to be identified [79]. A paper in 2002 found that HDM2 can facilitate the deacetylation of p53 by recruiting a complex that includes HDAC1. Consequently, inhibiting HDAC1 could enhance the stability and functionality of p53 [78]. The p53 utilizes the cGAS or STING natural innate immune system pathway to implement both cell-intrinsic and cell-extrinsic tumor suppressor functions [80].

Several significant findings were reported to investigate the activation of p53 in the context of aging. Firstly, despite skeletal muscle being largely post-mitotic, a study revealed increased mRNA levels of genes associated with regulating the cell cycle. Specifically, the key regulators of

cell cycle arrest followed by DNA damage, p21 and GADD45a, displayed substantial increases in expression in aged muscle, and this pattern was consistent with prior observations in humans, monkeys, and mice. Moreover, the elevated expression of p21 and GADD45a is typically controlled by the p53 pathway. As a result, it systematically examined differentially expressed genes between young and old muscles to identify other transcripts associated with p53 activity. It was found that transcripts activated by p53, or known to interact with p53, showed a significant increase with age, while genes known to stop p53 activity displayed reduced expression in older muscles.

The influence of caloric restriction on p53-related genes was studied. Caloric restriction appeared to have a preventive effect on these genes, as it reversed a substantial 87% portion of genes exhibiting age-related changes in expression. This suggests that Caloric Restriction plays a regulatory role in p53-related gene activity in the context of aging. To validate these findings, the study conducted RT-PCR to confirm the age-associated changes in the expression of p53-related genes. Additionally, protein analysis revealed a significant increase in p21 and GADD45a in the skeletal muscle of older mice, with some reversal observed in CR-treated mice. However, quantifying p53 protein levels proved challenging due to low signal intensity in Western blot analysis.

Furthermore, the study explored the relationship between oxidative stress and the expression of p53-related genes. This was done by comparing transcript levels in mice with mutations related to oxidative stress. The findings suggested that oxidative damage did not consistently trigger the expression of these genes, possibly due to different types of DNA damage in mutant mice. Overall, the research provided evidence of age-related changes in the expression of genes related to p53 activity in skeletal muscle. These findings suggest a potential role for p53 in the aging process and indicate the important influence of caloric restriction on regulating these genes [81]. Another study reveals that activation of p53, a critical tumor suppressor protein, is triggered by various stresses and signals at cellular levels. One of the most prominent triggers for p53 activation is the presence of damage in DNA. Many types of DNA damage, such as double-strand breaks, UV radiation-induced damage, or chemical-induced lesions, can initiate the flow of events that lead to p53 activation. This process involves the activation of various kinases such as ATM (ataxia telangiectasia mutated) and ATR (ATM- and Rad3-related), which phosphorylate and stabilize

p53, preventing its degradation and allowing it to perform its crucial functions in DNA repair, cell cycle arrest, and programmed cell death.

Moreover, high levels of reactive oxygen species can cause oxidative stress, leading to cellular damage and potentially triggering p53 activation. The ROS accumulation-induced DNA damage and other cellular alterations can serve as signals for the activation of p53, prompting its involvement in orchestrating the cellular response to oxidative stress. Additionally, low oxygen levels or hypoxia can activate p53 as well. Hypoxia-inducible factor 1-alpha (HIF-1α) is a key regulator of the cellular response to hypoxia and can cause the transcription of p53, leading to apoptosis or cell cycle arrest depending on p53t in response to the hypoxic environment.

Furthermore, activating certain oncogenes, such as Ras, Myc, or E2F1, can induce cellular stress and trigger the activation of p53. Oncogene-induced stress can lead to DNA damage or replication stress, thereby signaling the activation of p53. This serves as a protective mechanism to prevent aberrant cell proliferation and the development of tumors. Additionally, progressive telomere shortening, often associated with cellular aging, can activate a DDR, leading to the p53 activation. Understanding the diverse range of signals that activate p53 is crucial in comprehending its pivotal role as a central tumor suppressor protein and an important regulator of cellular homeostasis. The activation of p53 serves as a critical defense mechanism, orchestrating various cellular responses to maintain the stability of the genome and prevent the progression of damaged cells into malignancy [82]. The p53 regulators used in this research are discussed further.

## 2.5 RND1_Human

The Rho-related GTP-binding protein Rho6 is 232 amino acid proteins. In mammals, Rnd proteins constitute a subfamily of Rho GTPases represented by Rnd1, Rnd2 and Rnd3. The Rho family of GTPases is categorized within the Ras superfamily of small GTPases. In humans, this family comprises 20 members that are further categorized into eight subfamilies based on their sequence homology. These subfamilies include RhoA, Rac, Cdc42, RhoU/V, RhoD/F, RhoH, RhoBTB, and Rnd. A notable and prevalent characteristic of Rho GTPase protein is its ability to interact with nucleotides and undergo a dynamic transition between an inactive state bound to GDP (OFF) and an active state bound to GTP (ON) [83]. RND1 is recently discovered as a novel regulator of p53

and a positive activator of the p53 signaling pathway [84]. Rnds expressions are regulated at the transcriptional levels or post-transcriptional levels. Activation of RNDs is facilitated by post-translational modifications and interactions with other proteins [83]. A primary role of the RND family, including RND1 GTPase, is to monitor and regulate the morphology and cytoskeletal organization [84]. The deletion of Rnd1 triggers a strong activation of Ras, leading to uncontrolled proliferation and epithelial-mesenchymal transition in the mammary epithelium. These are associated with the activation of the Raf-ERK cascade. Similarly to mammary epithelial cells that exhibit overexpression of mutant Ras, mammary epithelial cells that experienced a depletion of Rnd1 went through senescence unless they were driven to overexpress c-Myc or had a loss of function in p53. The simultaneous existence of these collaborating oncogenic changes, coupled with the inactivation of Rnd1, resulted in neoplastic conversion and epithelial-mesenchymal transition (EMT). The results suggest that the suppression of Ras by Rnd1 inhibits uncontrolled cell proliferation and the disturbance of cell adhesion and polarity in mammary epithelial tissue [85]. In human beings, the RND1 gene is located on chromosome 12 [83].

The RND1 expression levels in various human normal tissues and tumor tissues were investigated using the Human Protein Atlas database. RND1 was present in human liver, lung, and brain tissues, and its down-regulation in various tumors was observed. RND1 expression in gliomas was searched from TCGA, and it was found that RND1 messenger RNA level was down-regulated in glioblastoma multiforme tissue as compared to normal brain tissue. The expression of RND1 in clinical glioblastoma multiform (GBM) tissues was further examined through the utilization of reverse transcription polymerase chain reaction (RT-PCR) tests, focusing on evaluating its correlation with patient prognosis. The samples were categorized into two categories, high RND1 and low RND1 expression groups, based on the mRNA level of RND1. Following the Kaplan-Meier analysis, it was observed that patients in the elevated RND1 expression group had a significantly high survival rate. Therefore, it may be inferred that RND1 had a more favorable prognosis in individuals diagnosed with glioblastoma multiforme (GBM) and functioned as a gene that inhibits tumor growth in glioma [84]. The expression of Rnd1 in mouse models suppresses both spontaneous and experimental lung colonization of metastasis. The findings from genomic investigations have provided evidence that RND1 inactivation in human breast cancer is primarily caused by gene loss, accompanied by epigenetic silencing or, less frequently, point mutation [85]. The RND1 Gtpase expression up-regulation stops the degradation of p53 by de-ubiquitination and

activates the p53-SLC7A11 axis in glioblastoma multiforme. RND1 increased the p53 expression without affecting p53 mRNA levels, proposing that it regulates p53 post-transcriptionally [84].

## 2.6 ATM (Ataxia Telangiectasia-Mutated)

The ataxia-telangiectasia mutated (ATM) protein serves as a major regulator in the cellular response to DNA damage, regulating the activation of many downstream pathways associated with cell cycle checkpoints, DNA damage repair, transcriptional regulation, immunological response, central nervous system development, and metabolism. The pleiotropic neurodegenerative disorder known as ataxia-telangiectasia (A-T) is observed in humans when there is a loss of ATM function. This condition is characterized by several symptoms, including immunodeficiency, the propensity to malignancy, accelerated aging, and insulin-resistant diabetes [86]. ATM is a kinase protein of 3056 amino acids activated by autophosphorylation upon DNA double-strand breaks caused by errors during replication, byproducts of metabolism, chemotherapy, etc [87]. The protein kinase ATM was observed to phosphorylate the tumor suppressor protein p53 in an in vitro setting, explicitly targeting a solitary amino acid residue, serine-15. This residue is known to undergo phosphorylation in vivo as a reaction to DNA damage. The observed activity experienced a significant improvement shortly after administering a radiomimetic medication to the cells, but the overall quantity of ATM remained constant. The augmentation of ATM's protein kinase activity can trigger a range of responses in response to damage [88].

The condition known as ataxia-telangiectasia (A-T) arises due to the lack of ATM catalytic activity; ATM is a kinase protein that holds significant importance in several cellular processes such as the response to DNA damage, cellular metabolism, redox regulation, and homeostasis of mitochondria, as well as control of the cell cycle. Ataxia-telangiectasia is a multifaceted condition primarily distinguished by the gradual deterioration of the cerebellum, compromised immune system, heightened sensitivity to radiation, instability of the genome, and increased susceptibility to cancer. The significance of A-T's premature aging in driving this disease's progression is widely acknowledged, making A-T a compelling model for investigating the mechanisms underlying the aging process [89].

## 2.7 Plant homeodomain finger protein 20 (PHF20)

PHF20 comprises 1012 amino acids and belongs to the PHF family. It is comprised of two conserved Tudor domains and one plant homeodomain (PHD) [90]. PHF20 is a constituent of the MOF-NSL lysine acetyltransferase complex, which plays a crucial role in acetylating histone H4 and non-histone proteins like p53. This complex involves transcriptional regulation, and the DNA damage response mediated by Ataxia telangiectasia mutated [91]. PHF20 is a highly effective transcriptional activator that exhibits binding affinity towards methylation lysine residues on the histone tail. PHF20 exhibits higher expression levels in multiple cancerous tissues when compared to adjacent normal tissues, encompassing advanced small-cell lung malignancies and advanced adenocarcinomas. In addition, it is noteworthy that PHF20 has a significant upregulation in primary human glioma samples [92]. PHF20 expression plays an important role in cancer development and progression. Also, high PHF20 expression was associated with the TGF-β signaling pathway, Wnt signaling pathway, and adherens junction [93]. The acetylation of p53 is facilitated by the multidomain protein PHF20. Using biochemical, biophysical, and cellular methodologies, it has been discovered that PHF20 functions as a direct regulator of the p53 protein. The Tudor domain present in PHF20 has recognition capabilities towards p53 that have undergone dimethylation at either Lys370 or Lys382. Furthermore, it has been observed that a homodimer configuration of this Tudor domain displays an increased affinity for p53 at two dimethylated sites, suggesting a multivalent interaction. The presence of PHF20 is associated with enhancing p53 stability and activation through the reduction of Mdm2-mediated p53 ubiquitylation and its degradation. The involvement of phf20 in upregulating p53 protein, followed by DNA damage, has been observed. Furthermore, introducing PHF20 into several cell lines results in discernible phenotypic alterations characteristic of p53 activation [94].

## 2.8 P300

P300 is a 2414 amino acid protein. The transcriptional coactivator p300 is essential in regulating eukaryotic gene expression, principally operating as a histone acetyltransferase. Additionally, it plays a significant role in the acetylation process of several nonhistone proteins, with p53 being the most notable example. The recruitment of p300 to p53 plays a crucial role in regulating genes

dependent on p53. Recent findings indicate a growing body of data supporting the idea that p300 undergoes a conformational change when interacting with the tetrameric form of p53. This conformational change leads to an increase in the acetylation activity of p300. p300 is a modular protein with several distinct and well-defined domains [95]. Histone acetylation, a post-translational modification of histones, is facilitated by histone acetyltransferases (HATs) and is crucial in various physiological mechanisms. The EP300/CBP HAT domain has lately garnered attention as a promising target for tumor treatment [96]. The E3 ubiquitin ligase MDM2 effectively maintains low levels of p53 in cells that are not under stress. Following the occurrence of DNA lesions, the DDR kinases ATM, ATR, Chk1, and Chk2 engage in the phosphorylation of p53. This process disrupts the connection between p53 protein and Mdm2, thereby facilitating p53 accumulation. The phosphorylated activation of the p53 protein is subsequently achieved through acetylation, with the critical lysine-acetyl-transferase responsible being p300/CBP. This enzyme acts explicitly on several residues of p53, including lysine-382 (K382). The acetylation of this particular position engages in a competitive relationship with ubiquitination, hence facilitating the stabilization and activation of p53 [97]. P300 has a significant role in moderating the transcriptional activity of p53 [95]. The p53 acetylation by acetylated p300 can result in its transcriptional activity simulation and subsequent initiation of apoptosis dependent on p53 [97].

## 2.9 Previously known therapies/agents for age reversal:

In the past 20 years, few genetic pathways that control the aging process and lifespan have been characterized. Many anti-aging drugs are being developed, including caloric restriction mimics, autophagy inducers, putative cell regeneration enhancers, DNA methyl transferase, and histone deacetylase inhibitors as regulators of gene activity. In 2003, the mTOR pathway was discovered as an aging regulator in nematodes Caenorhabditis elegans and fruit flies Drosophila melanogaster. Rapamycin was first proposed as a potential anti-aging therapy [98]. Stem cells can slow down aging and combat age-related diseases. Another therapy called stem cell rejuvenation is also used to treat the effects of aging naturally. The patient receives an IV injection of many young stem cells. By lowering inflammation, controlling the immune system, and encouraging tissue repair and regeneration, they replace old cells and help the body function more effectively. Numerous studies have linked dietary supplements to telomere length, telomerase activity, and oxidative

stress. It has also been identified that taking multiple antioxidants as natural products is more effective than taking just one antioxidant [98].

Inhibition of the mTOR signaling pathway prolongs lifespan, while the long-term activation of mTOR prolongs age-dependent conditions. The longevity of model species like fruit flies and worms is extended by mTORC1 signaling pathway mutations or upstream inhibitor activation [99]. The increase in NAD levels has been shown to extend the lifespan of mice. NAD+ supplements are safe in humans and increase NAD+-related metabolites, but the influence on cellular energy-sensing pathways and aging itself has not shown precise results [100]. The senolytic medications dasatinib and quercetin (D + Q) had favorable effects on pulmonary and physical function in investigations of rats with idiopathic pulmonary fibrosis. Trials with senolytics have shown promising systemic results in subjects with idiopathic pulmonary fibrosis, diabetes, and kidney dysfunction, but Senolytics cause further inflammation in already inflamed tissues due to immunosenescence. Other potential side effects are delayed skin healing and unwanted targeting of proliferating cells [101]. An important feature of potential aging drugs is that they must be without side effects. The clinical trials targeting aging in humans have shown promising but limited results on biomarkers so far.

## 2.10 Some already known therapies and medicines for anti-aging

The **Table 2.1** contains some already known medicines and therapies for anti-aging or age reversing [73, 100, 102, 103].

**Table 2.1 some already-known medicines for age-reversal or anti-aging**

| **Medicines/Therapies** | **Purpose** | **Limitations** |
|---|---|---|
| Antioxidants | Neutralize harmful radicals. They contribute to aging. e.g., Vitamin C/E, Selenium | Mixed results for anti-aging purposes. A high dose is harmful. |
| Hormone replacement therapy | Replace hormones that decline with age and improve menopause & bone density. | Risk of heart disease, stroke, and breast cancer. |
| Stem Cell Therapy | Using stem cells to replace or repair damaged cells. | Limited effectiveness, expensive |
| Metformin | Treat type II diabetes. Improve insulin sensitivity & increase lifespan in animals. | Effects in humans are not apparent, such as gastrointestinal upset & lactic acidosis. |
| Rapamycin | Has Potential anti-aging effects and Improves immune function. | Gastrointestinal problems, high blood sugar, Limited research, costly |
| NAD+ (nicotinamide adenine dinucleotide) supplements | Cellular energy production, DNA repair, Neuroprotection | Nausea, diarrhea, headaches, Limited research, expensive |
| Alpelisib | Treat breast cancer, improve cellular function, and extend lifespan in an animal model. | Nausea, diarrhea, affected blood sugar levels, and infections. Limited research |

# Chapter 3

# Methodology

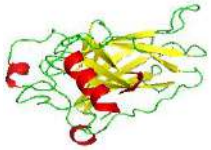
# Chapter 3

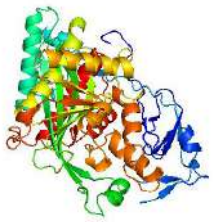
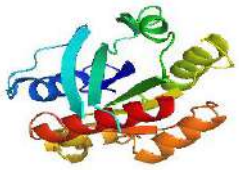
# 3. Methodology

## 3.1 Collection of data

The p53 activators were selected through an extensive literature search, and the data of positive regulators of P53 was made publicly available. The 3D structures of activators including PHD finger protein 20 (Tudor 2 Domain), Ataxia-Telangiectasia Mutated (ATM), Histone Acetyltransferase p300 (HAT Domain), and Rho-related GTP-binding protein RHO6 (RND1 GTpase) were retrieved from Research Collaboratory for Structural Bioinformatics Protein Databank Database (RCSB-PDB). The desired structures were selected based on BLAST results.

**Table 3.1 The PDB IDs, resolutions, and structures of p53 and its positive regulators.**

| Regulator | PDB ID | Resolution | Structure | Reference |
|---|---|---|---|---|
| P53 | 7XZZ | 4.07 Å |  | [104] |
| PHF20 | 3P8D | 2 Å |  | [94] |
| ATM | 6K9K | 7.82 Å |  | [86] |

| | | | | |
|---|---|---|---|---|
| P300 | 8GZC | 2.00 Å |  | [96] |
| RND1 | 2CLS | |  | 'The Crystal Structure of the Human Rnd1 GTPase in the Active GTP Bound State' (Yet to be published) |

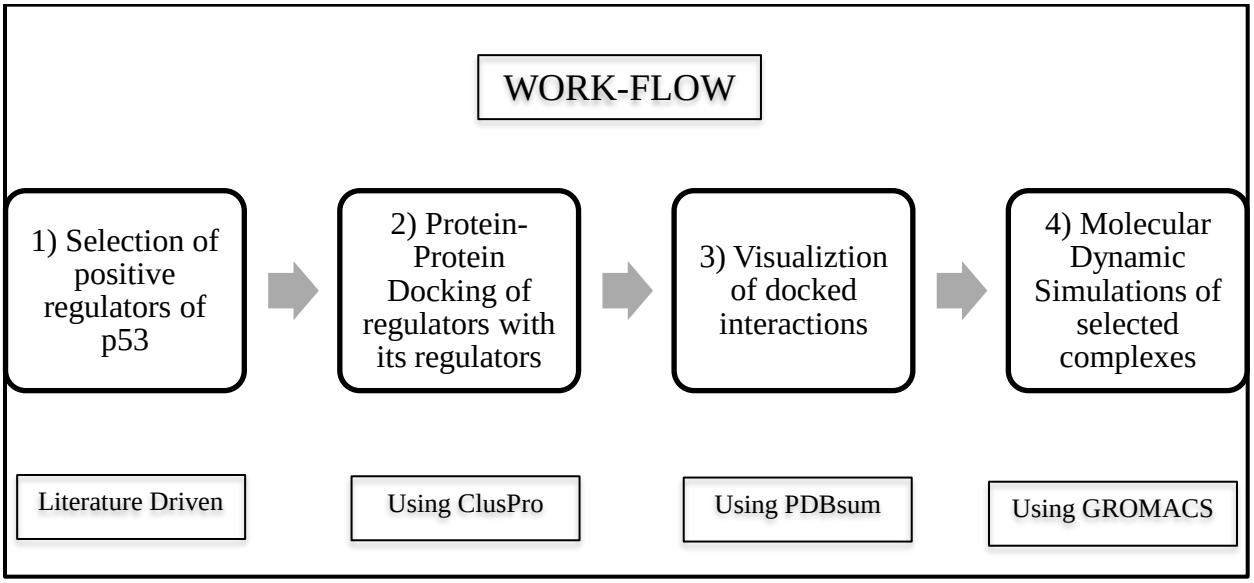



**Figure 3.1 Schematic diagram representing the methodology used in this study.**

## 3.2 Molecular Docking

The study of protein-protein interactions holds significant importance in comprehending cellular function and organization. Two molecular docking methods are classified: direct and template-

based [105]. The rigid protein docking was performed to investigate the molecular interactions between p53 and its regulators. For this purpose, automated online software "ClusPro" was used.

ClusPro is a web-based server for the direct docking of two interacting proteins. It was introduced in 2004 [106]. The user interface of ClusPro is characterized by its simplicity, as it simply requires the user to provide two protein structures in .pdb format that are known to interact, along with a valid email account [107]. The server executes three computational processes in the following manner: The proposed methodology involves three main steps.

- Rigid body docking is performed by sampling many conformations using PIPER, a docking program based on the Fast Fourier Transform (FFT) correlation approach.
- A clustering technique based on root-mean-square deviation (RMSD) is applied to the 1000 lowest energy structures generated. This clustering process aims to identify the most significant clusters, which are considered to represent the most probable models of the complex.
- The selected structures undergo refinement through energy minimization.

By default, ClusPro generates four distinct sets of docked structures using scoring algorithms as (i) balanced, (ii) electrostatic-favored, (iii) hydrophobic-favored, and (iv) van der Waals + electrostatics [105].

We selected the top-ranked docking structures with the lowest energies for all the activators of p53.

## 3.3 Visualization and Analysis of Docked Complexes

The selected docked files were analyzed to investigate the interactions between the residues of both interacting proteins within the complex. An online web server PDBsum was used to determine the information about the interface area and interacting residues between docked complexes [108]. The Protein Data Bank (PDB) was established in 1995 at University College London (UCL) to create a comprehensive visual repository of proteins and their complexes [108]. This server summarizes the information about bonded and non-bonded contacts, hydrogen bonding, salt

bridges, and the interacting residues between the two proteins of a docked complex. Visualization of all the interacting residues was done by MOE and PyMol [108].

## 3.4 MD Simulations of Docked Complexes

The molecular dynamic simulations were performed on PHF20-P53 and p300 HAT-p53 to check their stability. The software GROMACS version 2018 (5.0) was used to evaluate the stability of the docked complexes' binding residues and hydrogen bonds.[109] The structure of the docked protein-protein complex was constructed using the all-atom force field Charmm27. Solvation was carried out in a cubic box using the SPC216 water model, and the results were neutralized with Na+ and Cl- addition. Initially, energy minimization was completed using the steepest descent minimization algorithm for 50000 steps and a tolerance of 1000J/Å to eliminate the steric clashes of the complexes. Under constant temperature and pressure conditions, the equilibrium of minimum energy was reached for pressure of default value 1000ps. The Berendsen thermostat and barostat were used to maintain 300K temperatures and 1 atm pressure for the duration of the Molecular dynamic simulation sessions. Direct electrostatic interactions with a cut-off value of 1 nm were calculated using the fast smooth Particle- Mesh Ewald (PME) summation step. Various MD runs up to 100ns duration were investigated.

# Chapter 4

# Results

# Chapter 4

# 4. Results

## 4.1 Molecular Docking

The interaction analysis of p53 with its naturally occurring regulators or activator proteins, including 'PHF20 Tudor 2 domain, p300 HAT domain, ATM, RND1-Gtpase' was performed using the docking program ClusPro. For this purpose, the structure of p53 and its activators were extracted from the PDB database, and the p53 N-chain (94-291) was used. Due to the unavailability of the whole 3D structure of some activators, the specified structures and chains, according to the literature, were selected for docking. For phf20 chain A or Tudor 2 domain (85-137) was selected, p300 Hat domain chain A (1162-1664), ATM chain M (1-263), and RND1 Gtpase chain A (1-198) were used.

From the docking results of all protein-protein complexes, the top-ranked 0 clusters based on the highest number of poses and the least cluster center score were selected. For each protein-protein complex, the number of generated conformations and cluster center score is shown in **Table 4.1.**

**Table 4.1 ClusPro generated the number of conformations and cluster center scores of the ranked model from all docked complexes.**

| **Docked complexes** | **No. of poses** | **Cluster Centre Score** |
|---|---|---|
| Phf20-p53 | 244 | -748.7Kcal/mol |
| P300-p53 | 75 | -643.2Kcal/mol |
| RND1-p53 | 65 | -630Kcal/mol |
| ATM-p53 | 67 | -851.3Kcal/mol |

## 4.2 Interactions of the Docked Complexes

### (i) PHF20-P53 Complex:

For phf20-p53 complex, in **Figure 4.1**, 22 residues of p53 and 16 residues of phf20 showed total of 170 bonds among them. Out of these bonds were 15 hydrogen bonds, two salt bridges, and 153 non-bonded contacts. The residues Val 97, Ser 99, Lys 101, Tyr 103, Arg 158, Asp 208, Gly 262, and Arg 267 were involved in H bonding, as shown in the figure. All the residues of p53 that interacted with phf20 residues were from the DNA binding domain of p53 from residue no 95-267.

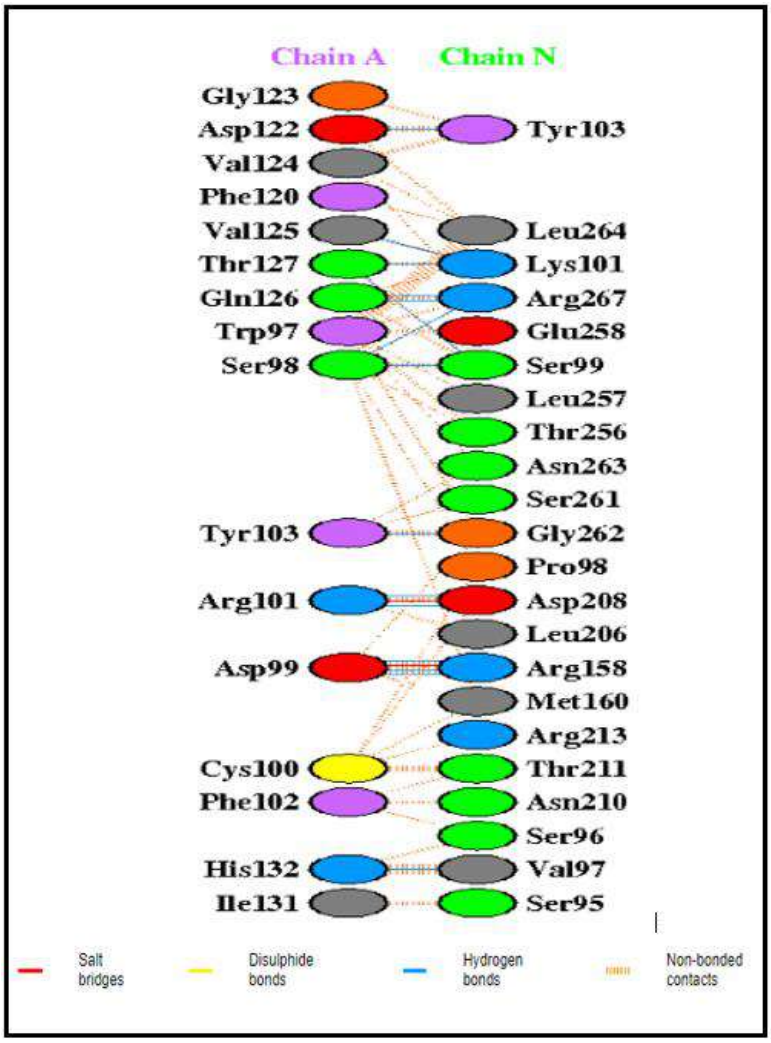



**Figure 4.1 Phf20-p53 pdbsum of interacting residues.**

### (ii) P300-P53 Complex

For the complex p300-p53, the 19 residues of p53 and 20 residues of p300 interacted with each other and made a total of 145 bonds shown in **Figure 4.2**. Among them, 8 were hydrogen bonds, 3 were salt bridges, and 134 were non-bonded contacts. Residues involved in H bonding are Gly 187, Gly 199, Leu 201, Pro 222, Glu 224, and Thr 231 of p53. All these bonds were formed between residue no: 187-233, the region of the DNA binding domain of p53.

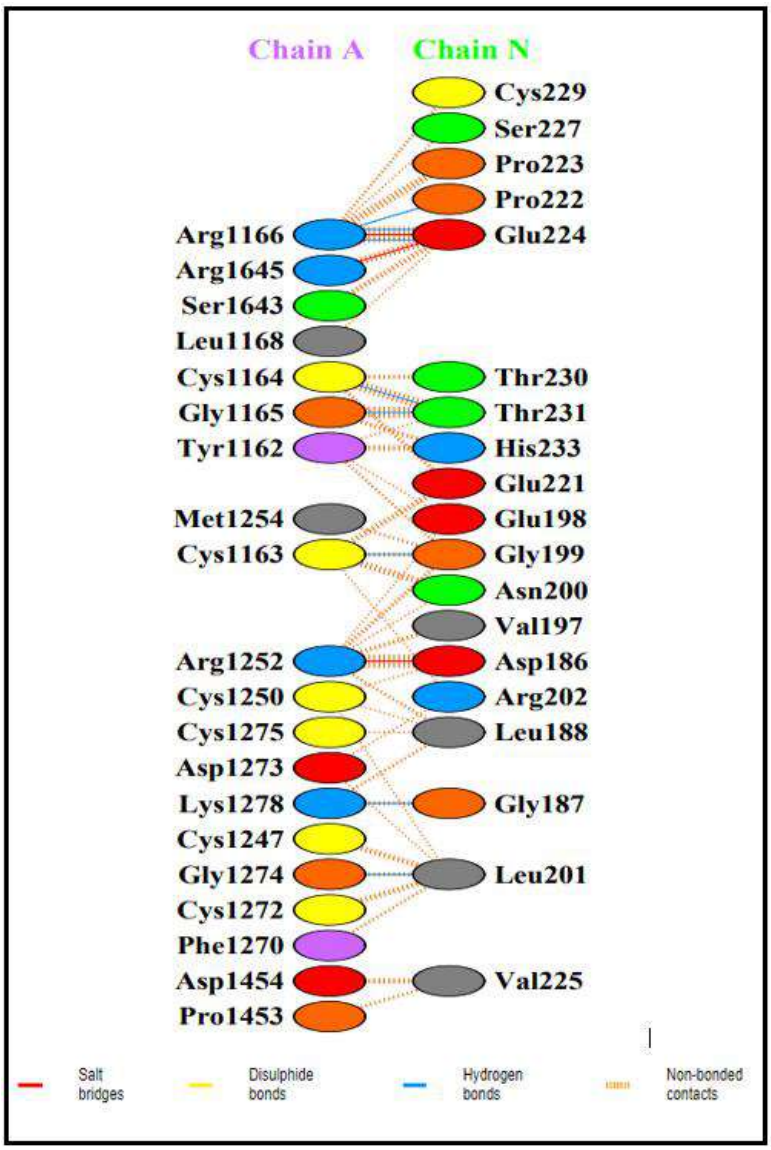



**Figure 4.2 P300-P53 complex pdbsum of interacting residues.**

### (iii) RND1-P53 Complex

18 residues of p53 and 19 residues of RND1 interacted to form 141 total bonds between RND1-P53 complex. Out of 141 bonds, there were 13 hydrogen bonds, three salt bridges, and 125 non-bonded contacts. Residues from 121 to 276 of p53 from the DNA binding domain were involved in all interactions. Hydrogen bonds were formed by Ser 121, Thr 123, Lys 139, His 178, Arg 181, Ser 183, Asn 239, Ser 241, and Arg 280 shown in **Figure 4.3.**

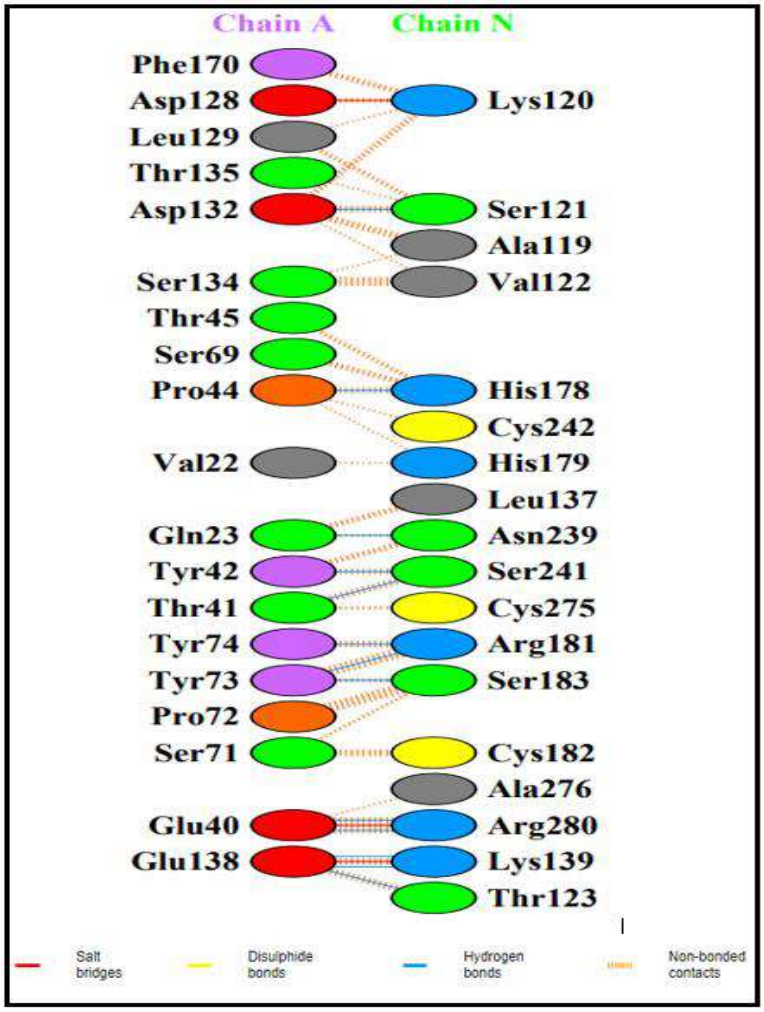



**Figure 4.3 RND1-P53 complex from pdbsum showing the bonds, types of bonds, and interacting residues.**

### (iv) ATM-P53 Complex

The complex ATM-P53 showed only two salt bridge interactions. The residues Glu 204 and Arg 202 from the region of the DNA binding domain were involved. No hydrogen bond is formed in this complex, as shown in **Figure 4.4.**

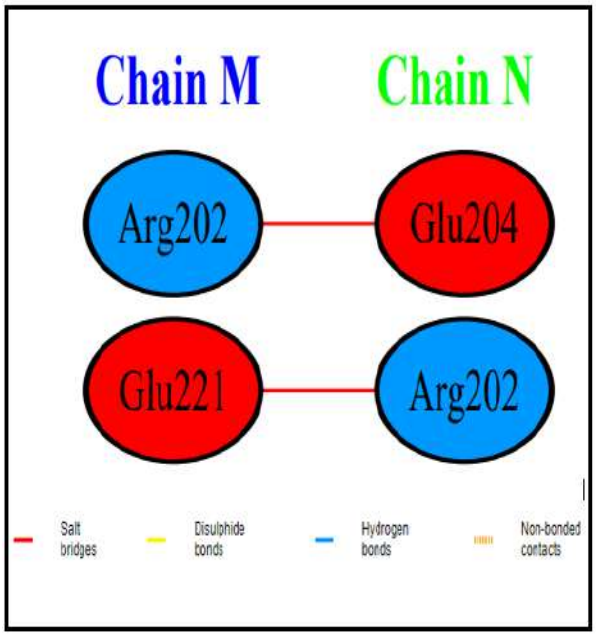



**Figure 4.4 ATM-P53 complex generated through pdbsum showing that only two salt bridges were formed.**

## 4.3 Molecular Dynamic Simulation of Docked Complex

The stability of docked complexes of p53 and its activators were checked through molecular dynamic simulation at average human body temperature and pressure.

The complex Phf20-p53 RMSD plot in **Figure 4.5** shows that the docked complex was stable till 80ns with almost 0.3nm RMSD, and the complex showed minimal fluctuations around 0.3 to 0.35 on 80 to 100ns. Root Mean Structure Deviation measures how much structure deviates from its original.

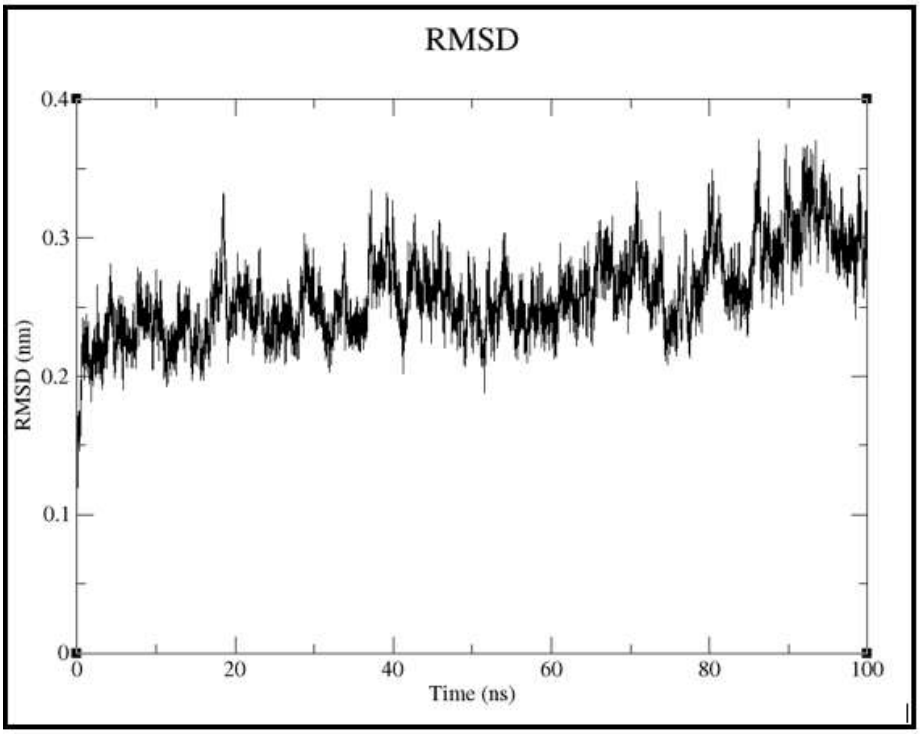



**Figure 4.5 MD simulation of Phf20-p53 complex for 100 ns, which depicts the lowest RMSD at 0 ns and 0.3-0.3.5 at 100 ns.**

The structure of the docked complex of phf20-p53 before and after simulations was almost the same, as shown in **Figure 4.6**.

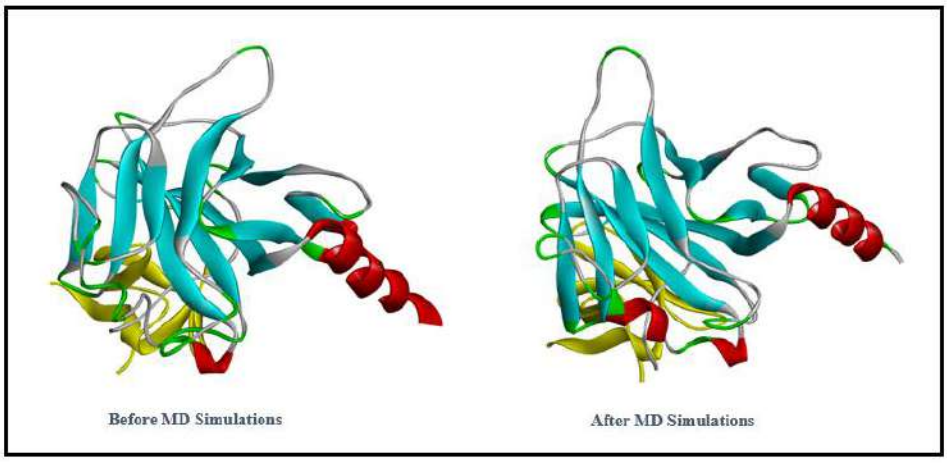



**Figure 4.6 The structure of phf20-p53 complex before and after simulations**

**Table 4.2** shows the binding site residues of p53 before and after simulations, and it has been identified that the PHF20-P53 complex has hydrogen bonds formed by ARG-158 and ARG-267 were common in the complex before and after Molecular Dynamic simulation structures. This indicates that interactions identified after the docking protocol were stable enough to be used as artificial activators in the future.

**Table 4.2 Phf20-p53 complex Hydrogen bonding residues and binding interactions at 0 ns and 100 ns**

| BEFORE SIMULATION | | | | AFTER SIMULATION | | | |
|---|---|---|---|---|---|---|---|
| **RESIDUES** | **Receptor protein atom** | **Ligand protein atom** | **H-bond Distance** | **RESIDUES** | **Receptor protein atom** | **Ligand protein atom** | **H-bond Distance** |
| Val-97 | O | ND1 | 3.O6 | ARG-158 | NH1 | OD2 | 2.71 |
| SER-99 | O | OG | 2.69 | ARG-158 | NH2 | OD1 | 2.90 |
| SER-99 | OG | O | 2.85 | ARG-267 | NH1 | OG | 2.94 |
| LYS-101 | NZ | O | 2.64 | | | | |
| LYS-101 | NZ | OG1 | 2.64 | | | | |
| TYR-103 | OH | OD2 | 2.82 | | | | |
| ARG-158 | NH1 | O | 2.80 | | | | |
| ARG-158 | NH1 | OD1 | 2.74 | | | | |
| ARG-158 | NH2 | OD1 | 2.76 | | | | |
| ASP-208 | OD1 | NH1 | 2.75 | | | | |
| ASP-208 | OD1 | NH2 | 2.67 | | | | |
| GLY-262 | O | OH | 2.71 | | | | |
| ARG-267 | NH1 | OG | 2.70 | | | | |
| ARG-267 | NH1 | OE1 | 2.70 | | | | |
| ARG-267 | NH2 | OE1 | 2.73 | | | | |

The RMSF plot as in **Figure 4.7** depicts the residues that are most fluctuating from the mean structure. The residues Asn-111 and Ser-137 from the phf20 chain, Ser-185, Val-225, and Meth-

243 from the p53 chain are the most fluctuating from the docked complex mean structure. But all of these fluctuating residues are located in the loops and are not involved in any interaction, so these will not affect the binding pattern of the complex. This might be the reason for their fluctuation, as these residues lie in the structure loop.

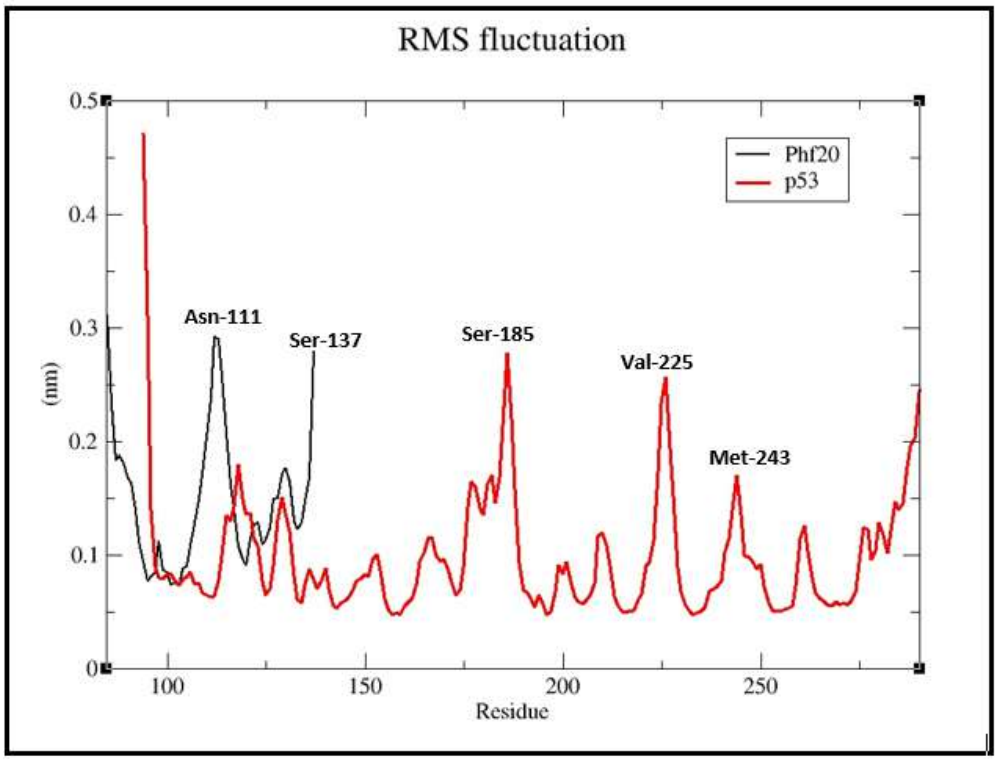



**Figure 4.7 Aspirin, Serine of phf20 and Serine, Valine and Methionine of p53 are the most fluctuating residues in the phf20-p53 complex.**

The last plot for MD simulation of phf20-p53 complex is of Radius of Gyration. The radius of gyration tells us about the compactness of the complex. The graph in **Figure 4.8** shows stability at 1.9nm throughout the simulation till 100ns.

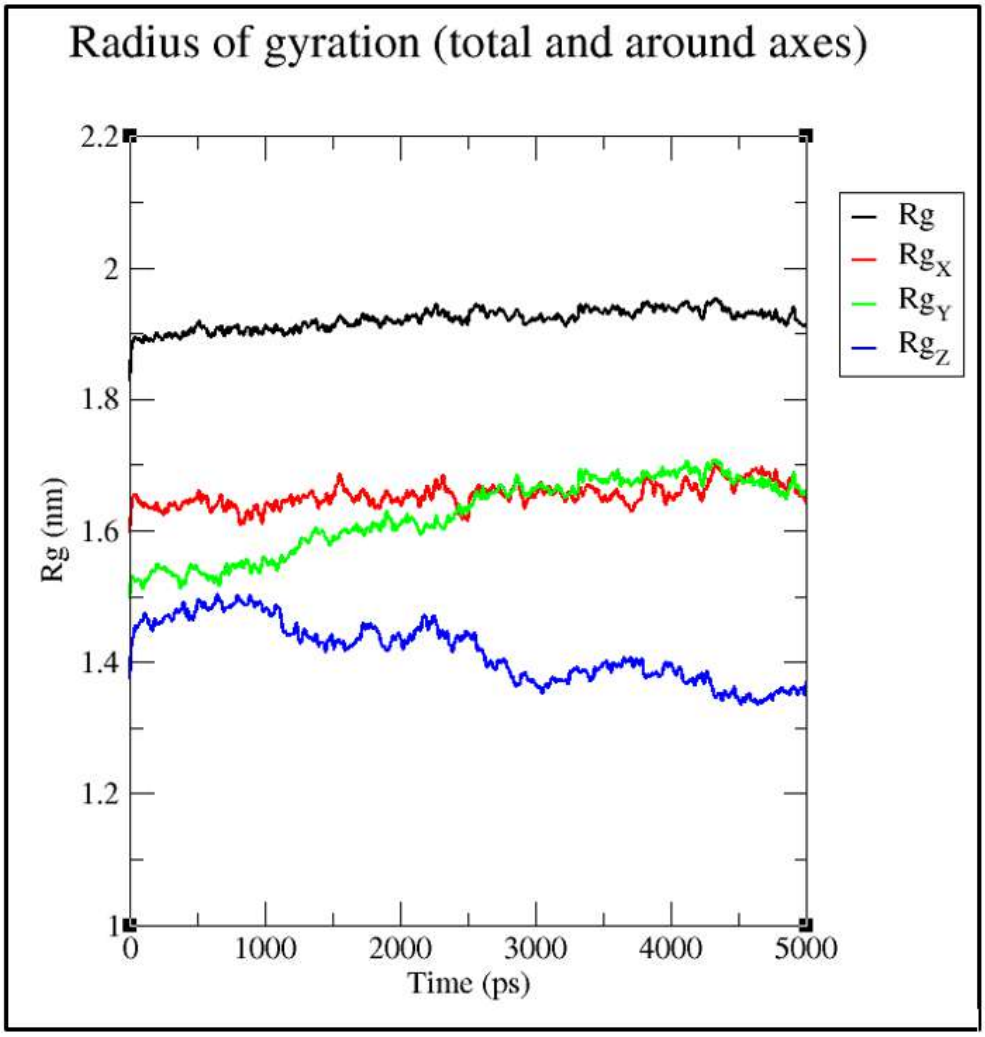



**Figure 4.8 Radius of Gyration at 1.9 nm shows that the phf20-p53 complex is stable and compact.**

In the case of other complex P300-P53, the RMSD graph in **Figure 4.9** shows that complex RMSD rapidly increases initially but then becomes stable after 20ns with RMSD of 0.5nm till 90ns and then again fluctuates a little around 0.6nm at 100ns. The low RMSD values (below 0.6nm) indicate a stable protein structure. However little fluctuations over time suggest that the protein complex is

still under some dynamic motion. Overall, the graphs showed that the protein complex structure is stable till 100ns.

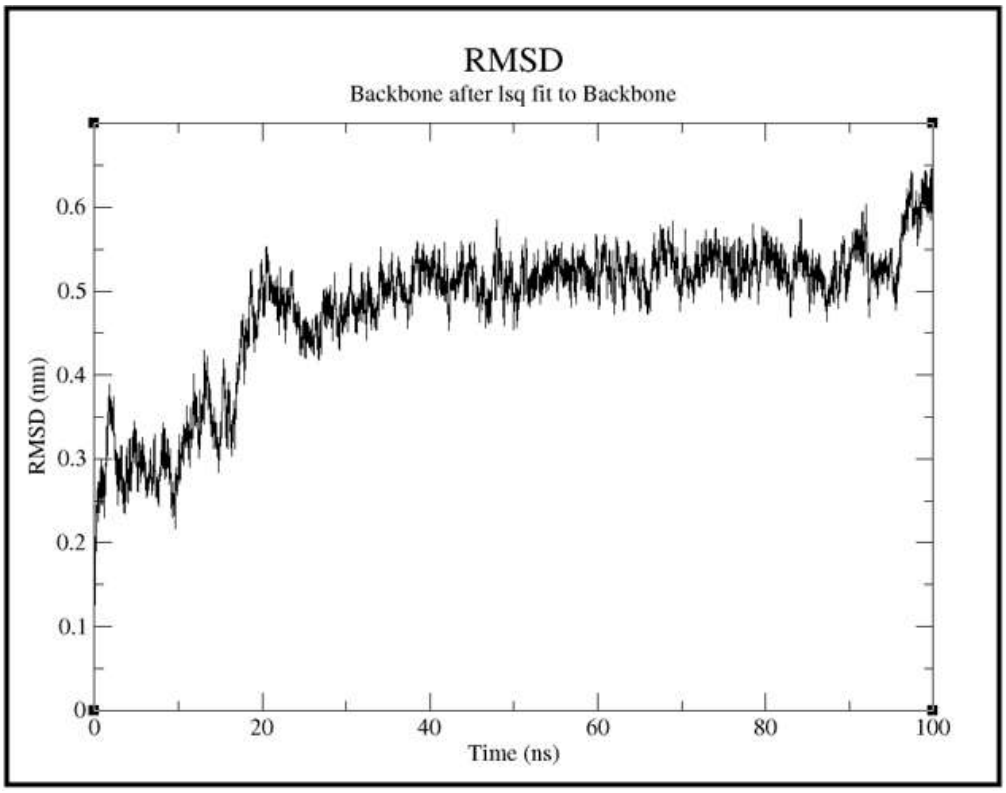



**Figure 4.9 Molecular Dynamic simulation of p300-p53 complex for 100ns which depicts lowest RMSD at 0ns and 0.5-0.6 at 100ns**

The binding site of the p300-p53 complex was not much changed during the 100ns simulation, and the structure of the docked complex before and after MD was almost identical, as shown in **Figure 4.10**

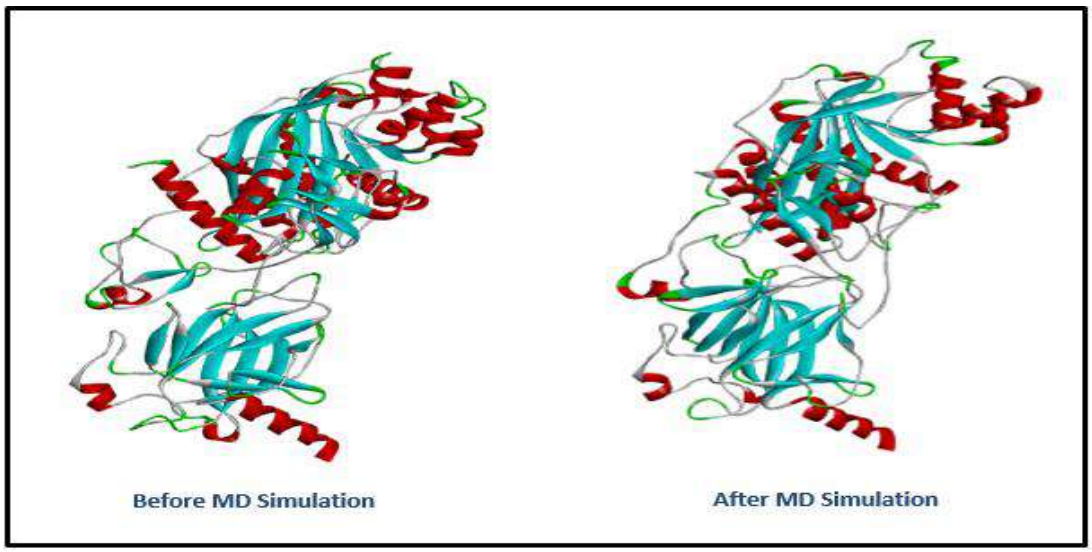



**Figure 4.10 The structure of the docked complex of p300-p53 before and after molecular dynamic simulation.**

**Table 4.3** shows the binding residues of p53 before and after simulations, and there are eight hydrogen bonds in the structure before and after simulation. Also, it can be seen that the p300-p53 complex has 2 hydrogen bonds formed by Glu-224 were found common in the before and after MD structures. This indicates that these interactions identified after the docking protocol were stable enough.

**Table 4.3 P300-P53 docked complex hydrogen bonding residues and binding interactions at 0ns and 100ns**

| Before simulation | | | | After Simulation | | | |
|---|---|---|---|---|---|---|---|
| **Residues** | **Receptor Protein atom** | **Ligand Protein atom** | **H-bond Distance** | **Residues** | **Receptor Protein atom** | **Ligand Protein atom** | **H-bond Distance** |
| Gly-187 | O | NZ | 2.53 | Asp-186 | OD2 | NE | 2.64 |
| Gly-199 | O | N | 2.70 | Ag-202 | NE | OD1 | 2.63 |
| Leu-201 | O | N | 2.84 | Arg-202 | NH2 | OD1 | 2.63 |
| Pro-222 | O | NH2 | 3.31 | Glu-204 | OE2 | NZ | 2.95 |
| Glu-224 | OE1 | NH1 | 2.73 | Glu-221 | OE2 | NH2 | 2.65 |
| Glu-224 | OE2 | NH2 | 2.81 | Glu-224 | OE1 | NH2 | 2.71 |
| Thr-231 | N | O | 2.98 | Glu-224 | OE2 | OG | 2.71 |
| Thr-231 | OG1 | O | 2.81 | Glu-224 | OE2 | NE | 2.67 |

In **Figure 4.11**, the RMSF graph shows the residues that are most fluctuating from their mean structure in both chains of the docked protein complex. The red lines indicate the p53 chain and the black line indicates p300 chain. The residues Ser-94 and Arg-290 from the p53 chain and Glu-1211, Glu-1229, and Gln-1663 from the p300 chain are the most fluctuating ones. But all of these residues are located in the loop region and are not involved in binding so these will not affect the binding pattern of complex as well.

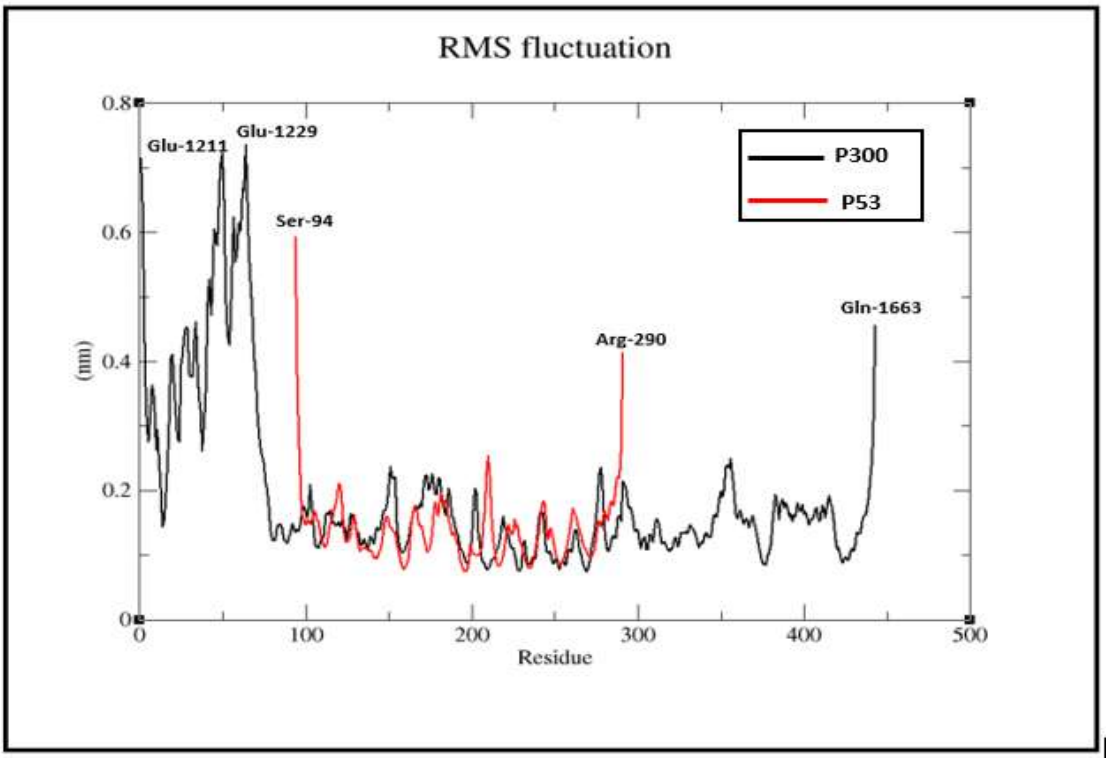



**Figure 4.11 Glu-1211, 1229 & Gln-1663 of p300 and Ser-92 & Arg-290 of p53 are the most fluctuating residues in the p300-p53 complex.**

The radius of gyration for the p300-p53 complex was stable at 3.2nm throughout the simulation time, as shown in **Figure 4.12,** which shows the compactness of the complex.

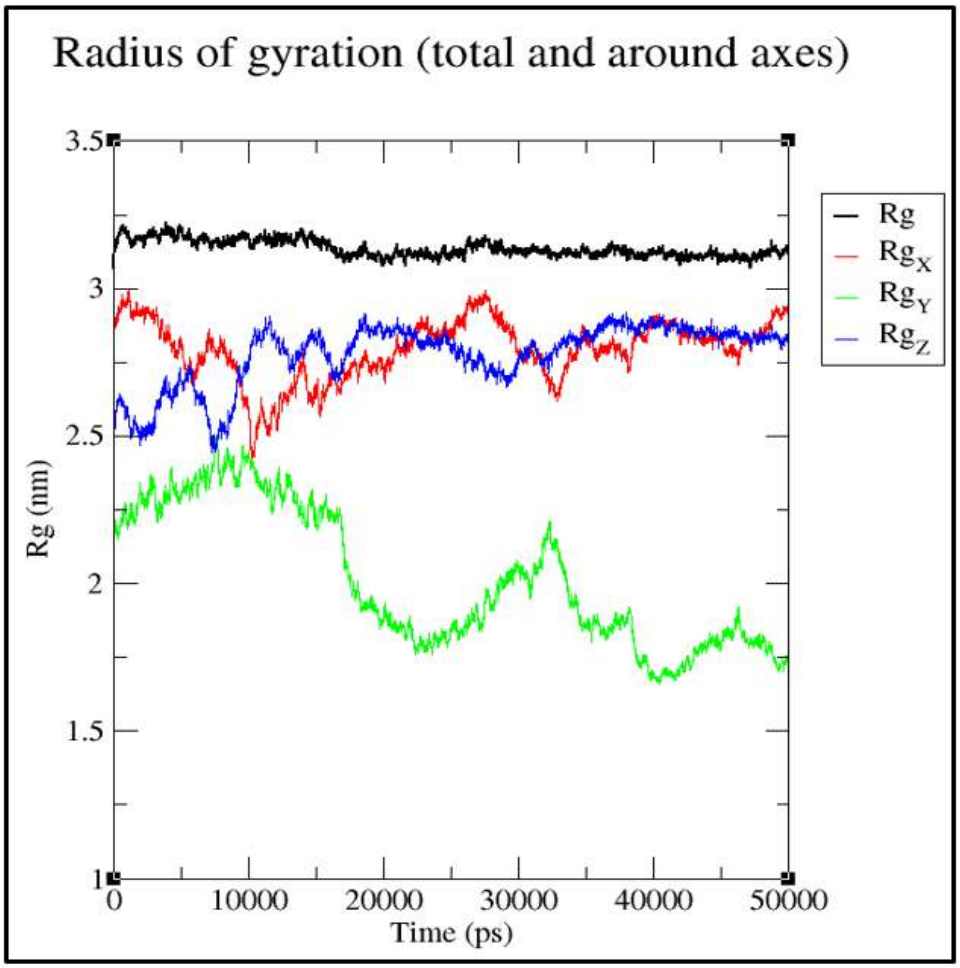



**Figure 4.12 Radius of Gyration at 3.2 nm shows that the p300-p53 complex is compacted throughout the simulation.**

Based on **Table 4.2** and **Table 4.3** (the interaction profiles of the activators of p53), new artificial activators of p53 can be designed in the future to reverse or slow down the aging process. Furthermore, we also have data on the interaction profile of hTERT with its positive activators, as they were already explored in one previous thesis.

Later on, different machine-learning models can be built to evaluate these interaction profiles with the interaction profiles of unknown peptides of monoclonal antibodies of hTERT and p53 to classify them as activators or non-activators.

## 4.4 Discussion

The p53 is the guardian protein located in the nucleus of each cell. A tumor suppressor gene controls cell apoptosis and regulates cell division. P53 binds directly to DNA. On the other hand, hTERT is a cell-proliferating protein that helps delay aging. Therefore, in normal limits, both p53 and hTERT are significant targets for slowing aging. This study is designed to activate the hTERT in the normal range along with p53 and elucidate both proteins' interaction profiles with their positive regulators. For this purpose, the interaction profile of hTERT and its activators was already built by another researcher in the first half of the designated research study.

Specifically, in this research, the protein p53 is targeted with its positive regulators to build an interaction profile for future design of artificial activators. The data about positive activators of p53 were obtained from a literature study, and their structures were retrieved from the Protein Data Bank. The positive regulators of p53 are the Phf20 tudor two domain, P300 Hat domain, RND1 GTPase domain, and ATM. All these activators were docked with p53 individually in ClusPro software. The protein-protein docking showed several interactions between p53 and its activators that showed multiple strong binding patterns between them.

Furthermore, these regulators were ranked based on early availability and interactions with p53, and the top two docked protein-protein complexes were selected for further analysis. These were Phf20-p53 and p300-p53 docked complexes.

The molecular dynamic simulations of docked complexes were performed in GROMACS. The MD analysis of the PHF20-P53 complex was performed, and it was found that it achieves stable RMSD between 2 and 3.5 Å. Simulation Analysis helps in the identification of meaningful interactions under normal human body temperature and pressure for a specific period. Only the most stable bonds are sustained during MD simulation due to continuous exertion force. The stability of the docked complex depends on receptor protein stability. The binding cavity did not change after MD, but binding residues within the same cavity changed. The change in binding residues was due to the energy minimization step during MD simulation, which breaks old bonds and makes new bonds to achieve the most stable conformation. Hydrogen bonds at Arg-158 and Arg-267 were the same before and after the simulation.

The MD simulation of the P300-P53 complex showed stable RMSD at 0.5nm, and the protein structure was stable till 100ns.

Moreover, two hydrogen bonds at Glu-224 were common in the complex before and after simulation, indicating them to be highly stable interactions after the docking protocol. Arg-158, Arg-267, and Glu-224 of p53 were found to be common before and after simulation, representing them as highly significant residues of p53. The interaction profiles of both complexes phf20-p53 and p300-p53 can be used in the future to design artificial activators of p53.

# Chapter 5

## 5. Conclusion

To extend the average human health span and lifespan, scientists worldwide are looking for ways to progress in regulating longevity and slowing down aging. Aging is about the life duration and the quality of life we spend. At the end of each chromosome, the protective caps of Telomeres, which are responsible for normal cell proliferation and aging phenomena, are present. The telomere length decides the number of divisions each cell can undergo. Each time the cell divides, the length of telomere shortens, and telomere shortening leads to age-related cognitive decline and risk of diseases, including cardiovascular diseases, diabetes, Alzheimer's, and neurological disorders. Identifying aging and its related pathways is a way to regulate longevity and develop anti-aging therapies. This project highlights hTERT and p53 signaling pathways in modulating the aging process.

Telomere hTERT is a cell proliferating unit, and p53 is the cell apoptosis protein. The balance between proliferation and apoptosis is crucial for healthy cell function and preventing aging. Because uncontrolled growth can lead to cancer development, so for this purpose, a knowledge-driven BRN was constructed at the start of this project and is simulated to interpret the effects of hTERT and p53 regulators in cell proliferation and apoptosis to reverse the aging and prevent cancer risk. The hTERT was studied already with its naturally occurring biological molecules c-Myc, STAT3, HIF-1, and c-Jun to influence the telomere length through simulation of hTERT expression, and results suggested that telomerase activators could be a promising anti-aging agent in the future. In this specific study, the p53 is docked, and MD simulations were performed with its naturally occurring biological regulators. The biological regulators of p53 are PHF20, P300, ATM, and RND1 GTPase. The docking protocol is performed with all four regulators, and Molecular Dynamic Simulations were performed on two complexes, only phf20-p53 and p300-p53 complexes. Arg-158, Arg-267, and Glu-224 are found interacting in docking and after MD simulations. Therefore, these are of indeed significance in the p53 interaction profile. However, for designing synthetic activator of p53, one similar set of interactions at the DNA binding site was not achieved which means targeting the first activator in the sequential binding. The phf20 activator binds first with p53 in DNA binding domain region as supported by literature and then

followed by p300 and others activators. Moreover, in the future, interaction analysis can help design artificial regulators of hTERT and p53.

By further expansion of this research in the future by activating hTERT and p53, may lead to the discovery of mechanisms and substances that target telomerase length and pave the way for increasing human lifespan without the risk of cancer.

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