

T.R.
GEBZE TECHNICAL UNIVERSITY
GRADUATE SCHOOL

**COMPUTATIONAL ANALYSIS OF HUMAN TELOMERASE
PROTEIN AND THE EFFECTS OF TCAB1 MUTATIONS ON
COMPLEXATION**

KAMER NİSA BAZ

**A THESIS OF MASTER OF SCIENCE
DEPARTMENT OF BIOTECHNOLOGY**

ADVISOR: ASSOC. PROF. MEHMET ÖZBİL

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LİSANSÜSTÜ EĞİTİM ENSTİTÜSÜ

İNSAN TELOMERAZ PROTEİNİNİN HESAPLAMALI
ANALİZİ VE TCAB1 MUTASYONLARININ
KOMPLEKSLEŞME ÜZERİNDEKİ ETKİLERİ

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YÜKSEK LİSANS TEZİ
BİYOTEKNOLOJİ ANABİLİM DALI

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*To all the dearly
departed, who have left a
void in my heart...*

ABSTRACT

In linear DNA molecules, repetitive sequences that protect the ends of chromosomes from damage are called telomeres. The ribonucleoprotein that extends these telomere sequences, which decrease with each cell division, is named telomerase. Telomerase facilitates the transmission of genetic information across generations without degradation. In somatic cells, the activity of this enzyme is either tightly controlled through stringent mechanisms or suppressed. Insufficient or excessive functioning of the telomerase enzyme can lead to serious diseases. One such disease, Dyskeratosis Congenita, which arises from underactivity, significantly affects various aspects of health, from nail structure to lung health. Until recently, computational analyses of the mutations causing this disease could not be performed due to the absence of the telomerase structure. Following the acquisition of the telomerase structure in 2021, studies in this thesis have been conducted to address this gap. Within these studies, simulations of the wild-type and G435R TCAB1, R398W TCAB1, H376Y TCAB1, F164L TCAB1, H376Y/G435R TCAB1 and F164L/R398W mutant structures of the telomerase enzyme's H/ACA lobe have been performed. The TCAB1 protein and the H/ACA RNP lobe were obtained using the structure with PDB ID 7bgb. Subsequently, the mutant RNP complexes derived from these structures were subjected to molecular dynamics simulations at 300 °K and physiological pH using GROMACS 2021.6, and the resulting data were analyzed. Accordingly, it has been determined that the mutations in the TCAB1 protein affect the stability of the structure and influence its interaction with surrounding proteins. Consequently, it has been determined that the naturally occurring double mutations F164L/R398W and H376Y/G435R significantly impact the interactions of the TCAB1 protein with its surrounding environment. Moreover, these mutations have been found to disrupt the interaction with the RNA molecule in both cases.

Keywords: Telomerase, Telomer, Dyskeratosis Congenita, TCAB1

ÖZET

Lineer DNA moleküllerinde, kromozom uçlarının zarar görmesini önleyen tekrarlayan dizilimlere telomer adı verilir. Her hücre bölünmesiyle azalan bu telomer dizilimlerini uzatan ribonükleoprotein, telomeraz olarak adlandırılır. Telomeraz, genetik bilginin nesiller boyu bozulmadan aktarılmasını kolaylaştırır. Somatik hücrelerde bu enzimin aktivitesi ya sıkı mekanizmalarla kontrol edilir ya da baskılanır. Telomeraz enziminin yetersiz veya aşırı işlev görmesi ciddi hastalıklara yol açabilir. Bu hastalıklardan biri olan ve enzim aktivitesinin azalması sonucu ortaya çıkan Displastik Congenita, tırnak yapısından akciğer sağlığına kadar çeşitli sağlık yönlerini önemli ölçüde etkiler. Yakın zamana kadar, bu hastalığa neden olan mutasyonların hesaplamalı analizleri, telomeraz yapısının bulunmaması nedeniyle gerçekleştirilememiştir. 2021'de telomeraz yapısının elde edilmesinin ardından, bu tez kapsamında bu boşluk ele alınmıştır. Bu çalışmalarda, telomeraz enziminin H/ACA lobunun vahşi tip ve G435R TCAB1, R398W TCAB1, H376Y TCAB1, F164L TCAB1, H376Y/G435R TCAB1 ve F164L/R398W mutant yapılarının simülasyonları yapılmıştır. TCAB1 proteini ve H/ACA RNP lobu, 7bgb PDB ID'li yapı kullanılarak elde edilmiştir. Elde edilen mutant RNP kompleksleri, GROMACS 2021.6 kullanılarak 300 °K sıcaklıkta ve fizyolojik pH altında moleküler dinamik simülasyonlarına tabi tutulmuş ve sonuç verileri analiz edilmiştir. Buna göre, TCAB1 proteinindeki mutasyonların yapısal stabiliteyi etkilediği ve çevresindeki proteinlerle etkileşimini değiştirdiği belirlenmiştir. Sonuç olarak, doğal olarak meydana gelen F164L/R398W ve H376Y/G435R çift mutasyonlarının TCAB1 proteininin çevresiyle olan etkileşimlerini önemli ölçüde etkilediği tespit edilmiştir. Ayrıca, bu mutasyonların her iki durumda da RNA molekülü ile olan etkileşimi bozduğu bulunmuştur.

Anahtar Kelimeler: Telomeraz, Telomer, Dyskeratosis Congenita, TCAB1

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LIST OF ABBREVIATIONS

DNA	: Deoxyribo Nucleic Acid
RNA	: Ribo Nucleic Acid
TERT	: Telomerase Reverse Transcriptase
TERC	: Telomerase RNA Component
TCAB1	: Telomerase Cajal Body Protein 1
NHP2	: Nucleolar Protein 2
GAR1	: Glycine-Arginine Rich 1
NOP10	: Nucleolar Protein 10
snRNA	: Small Nucleolar RNA
DC	: Dyskeratosis Congenita
WT	: Wild Type
RMSD	: Root Mean Square Deviation
RMSF	: Root Mean Square Fluctuation
Rg	: Radius of Gyration
SASA	: Solvent Accessible Surface Area
nm	: Nanometre
ns	: Nanosecond

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1. INTRODUCTION

1.1. Importance of DNA Replication and the End Replication Problem

Cell division is crucial for the continuity of life, facilitating the growth and development of an organism. Alongside cell division, the equal distribution of vital genetic material, deoxyribonucleic acid (DNA), as cells divide is also essential. This equal distribution is achieved through DNA replication. From prokaryotes to eukaryotes, all organisms duplicate their genetic material before cell division and subsequently distribute this material equally to new daughter cells. DNA sequences are preserved and duplicated with great accuracy in every cell. The mutation rate, which is about one nucleotide alteration every 10^{10} nucleotides during DNA replication, is similar in organisms as diverse as bacteria and humans. Due to its exceptional precision, the sequence of the human genome, which consists of about 3.2×10^9 nucleotide pairs, remains unaltered or undergoes minimal changes with each division of a typical human cell. This enables the majority of humans to transmit precise hereditary information passed down from one generation to another through genetic codes while simultaneously preventing the occurrence of somatic cell alterations that result in illnesses (Bruce et al., 2015, **Molecular Biology of the Cell, 6th Edition, Chapter 5, Kunkel & Bebenek, 2000**).

1.2. DNA Replication Mechanisms

Through the replication process of DNA within a cell, each of the two original DNA strands acts as a template for the synthesis of a whole new strand. The reproduction DNA double helix is considered to be "semiconservative" since each daughter cell gets a new DNA double helix that consists of one original strand and one newly synthesized strand (Bell & Dutta, 2002; Kunkel & Bebenek, 2000).

Ensuring the complete and precise replication of DNA is crucial for preserving the genetic integrity of all living creatures. In eukaryotic cells, the initiation of this event occurs at numerous chromosomal elements known as origins of replication, which can range from hundreds to thousands in number. These sequences guide the process of assembling multiprotein complexes, which ultimately result in the formation of two

replication forks (because of its Y-shaped structure) at each origin. The sequences necessary for the initiation of DNA replication vary greatly among various eukaryotic organisms. The origin recognition complex is a complex composed of six subunits that serve as the initiator, responsible for selecting the locations where replication begins in eukaryotic origins of replication **(Bell & Dutta, 2002)**.

At first, it appeared that the most straightforward process of DNA replication involved the uninterrupted elongation of both new strands, one nucleotide at a time, at the replication fork as it traverses from one end of a DNA molecule to the opposite end. However, due to the antiparallel arrangement of the two DNA strands in the DNA double helix, this process would necessitate one daughter strand to synthesize in the 5' to 3' direction and the other in the 3' to 5' direction. A replication fork necessitates the presence of two different DNA polymerase enzymes. Although this hypothesis may seem appealing, it is important to note that DNA polymerases at replication forks can only synthesize in the 5' to 3' direction **(Berdis, 2009)**.

The mechanism of DNA strand elongation in the 3' to 5' direction was initially proposed based on the findings of a study conducted in the late 1960s. Scientists introduced a highly radioactive substance called ³H-thymidine to bacteria that were undergoing cell division. This was done for a brief period, allowing just the newly replicated DNA, located immediately below the replication fork, to become tagged with radiation **(Balakrishnan & Bambara, 2013)**. This experiment demonstrated the temporary presence of DNA fragments, ranging from 1000 to 2000 nucleotides in length, which are now popularly referred to as Okazaki fragments, at the replication fork during DNA replication. Subsequent discovery revealed the presence of analogous replication intermediates in eukaryotes, which are significantly shorter, measuring only 100-200 nucleotides in length. The Okazaki fragments, which were named after the scientist who first identified them, were demonstrated to undergo polymerization only in the 5'-to-3' orientation and then link together following their synthesis to form extended DNA chains.

Consequently, a replication fork exhibits an asymmetrical configuration. The daughter strand of DNA that is generated in a continuous manner is referred to as the leading strand. The synthesis of the lagging strand, which is generated discontinuously, occurs somewhat before the synthesis of the daughter strand (Figure 1.1). The nucleotide polymerization orientation on the lagging strand is opposed to the overall direction of

DNA chain development. The process of synthesizing this strand occurs through a discontinuous "backstitching" method, which necessitates the use of a DNA polymerase that operates in the 5' to 3' direction (**Balakrishnan & Bambara, 2013; Bruce et al., 2015; Zheng & Shen, 2011**).

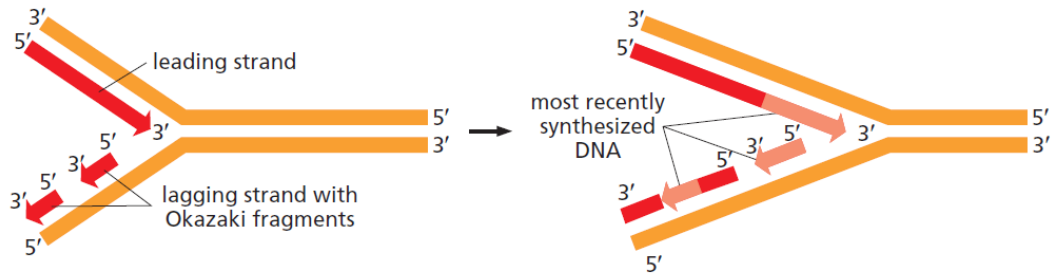


Figure 1.1: Replication fork with newly synthesized DNA highlighted in red (**Bruce et al., 2015**).

Arrows indicate the direction of DNA synthesis, which is from the 5' end to the 3' end. Due to the fact that both daughter DNA strands are synthesized in the 5' to 3' direction, the DNA produced on the lagging strand must first be formed as a sequence of brief DNA molecules known as Okazaki fragments. The Okazaki fragments are generated sequentially on the lagging strand, with the ones closest to the replication fork being the most recently formed (**Bruce et al., 2015, Chapter 5**).

The requirement for precision likely accounts for the fact that DNA replication exclusively takes place in the 5' to 3' direction. If a DNA polymerase were to incorporate deoxyribonucleoside triphosphates in the 3' to 5' direction, the chain would lengthen from its 5' end in a hypothetical scenario. To establish a covalent bond, the entering mononucleotide needs the presence of the activating triphosphate. In this scenario, errors in polymerization cannot be easily eliminated through hydrolysis, as the exposed 5' end of the chain formed would promptly halt DNA synthesis (**Bruce et al., 2015**).

A primer is necessary only at the initiation of replication on the leading strand. After the formation of a replication fork, the DNA polymerase constantly comes across a chain end that is paired with a base, allowing it to add new nucleotides. On the lagging strand of the fork, when the DNA polymerase finishes creating a short DNA Okazaki

fragment, it needs to begin synthesizing a new fragment at a different location on the template strand, as shown in Figure 1.2.1.

A unique method generates the primer strand with complementary base pairing, which is necessary for the DNA polymerase molecules. The process depends on the enzymatic activity of DNA primase, which uses ribonucleoside triphosphates to generate short RNA primers on the lagging strand. In eukaryotes, these primers have a length of around ten nucleotides and are produced at a consistent spacing of 100-200 nucleotides on the lagging strand. When the nucleotide sequences of a strand of RNA and a strand of DNA are compatible, they can generate base pairs and create a DNA-RNA hybrid double helix. Hence, the production of RNA primers is directed by the identical templating principle employed in DNA synthesis. **(Bell & Dutta, 2002)**. An RNA primer can initiate the formation of an Okazaki fragment by providing a nucleotide with a 3'-OH group that can be extended by DNA polymerase. The synthesis of each Okazaki fragment concludes when the DNA polymerase encounters the RNA primer connected to the 5' end of the previous fragment. To generate an uninterrupted DNA chain from the numerous DNA fragments generated on the lagging strand, a specialized DNA repair machinery swiftly eliminates the previous RNA primer and substitutes it with DNA. Afterward, the DNA ligase enzyme joins the 3' end of the newly manufactured fragment of DNA to the 5' end of the previous fragment, therefore completing the procedure **(Bell & Dutta, 2002; Bruce et al., 2015, Chapter 5; Kunkel & Bebenek, 2000)**.

The usage of erasable RNA primer opposite to non-erasable primer is advantageous. The claim that a self-correcting polymerase lacks the ability to start new chains implies that an enzyme capable of launching new chains would not be very efficient at correction itself. Therefore, any enzyme that initiates the production of Okazaki fragments will inevitably create a relatively imprecise replica (with at least one error in every 10⁵ copies). Even if only 5% of the entire genome, such as ten nucleotides out of a 200-nucleic acid DNA fragment, consisted of copies, the resulting increase in the total frequency of mutations would be significant. Therefore, it is probable that the utilization of RNA instead of DNA for initiating confers a substantial advantage to the cell: the presence of ribonucleotides in the primer immediately recognizes those sequences as "potentially flawed copies" so they can be efficiently eliminated and substituted. **(Bruce et al., 2015, Chapter 5)**.

Upon reaching the terminus of a linear chromosome, the replication fork encounters a distinct challenge in the synthesis of the lagging strand. This happens irregularly by a backstitching process that generates tiny fragments of DNA. The RNA primer produced on the template of the lagging strand cannot be substituted with DNA since there is no available 3'-OH end for the repair polymerase. In the absence of a mechanism to address this issue, the DNA would be lost from the terminal regions of all chromosomes during each cell division. This phenomenon is known as the “end replication problem” (Shay, 2016).

The DNA repeated sequences located at the ends of eukaryotic chromosomes are known as telomeres. These telomeres are crucial for maintaining the stability of the genome. The presence of hazard is inherent in the specific positioning of these chromosomal domains inside the terminal region. During each cell cycle, DNA sequences are not fully replicated and are even subject to erosion. This is due to inadequate DNA templated replication and the necessary nuclease processing to create a 3' overhang for the end-capping proteins. Eukaryotes commonly employ telomerase, a specialized enzyme with reverse transcriptase activity, to counteract the degradation of telomeric repeats by synthesizing new DNA (Blackburn & Collins, 2011).

1.3. Telomerase: Reverse Transcriptase Specialist

Telomerase was first discovered in *Tetrahymena* in 1985. Carol Greider and Elizabeth Blackburn from California University found an enzyme that adds tandem repeats (TTGGGG) at the end of the DNA of *Tetrahymena*. They named the enzyme telomere terminal transferase (telomerase) (Greider & Blackburn, 1985). Elizabeth Blackburn, Carol Greider, and Jack Szostak were awarded the Nobel Prize in Physiology or Medicine in 2009 for the discovery of telomerase and telomere's role in protecting the ends of DNA. After the discovery of telomerase, studies on human cells began, and shortly after, the human telomere sequence (TTAGGG) (Moyzis et al., 1988) and human telomerase enzyme (Morin, 1989) were identified. The telomerase activity in *Tetrahymena* can be seen in Figure 1.2.

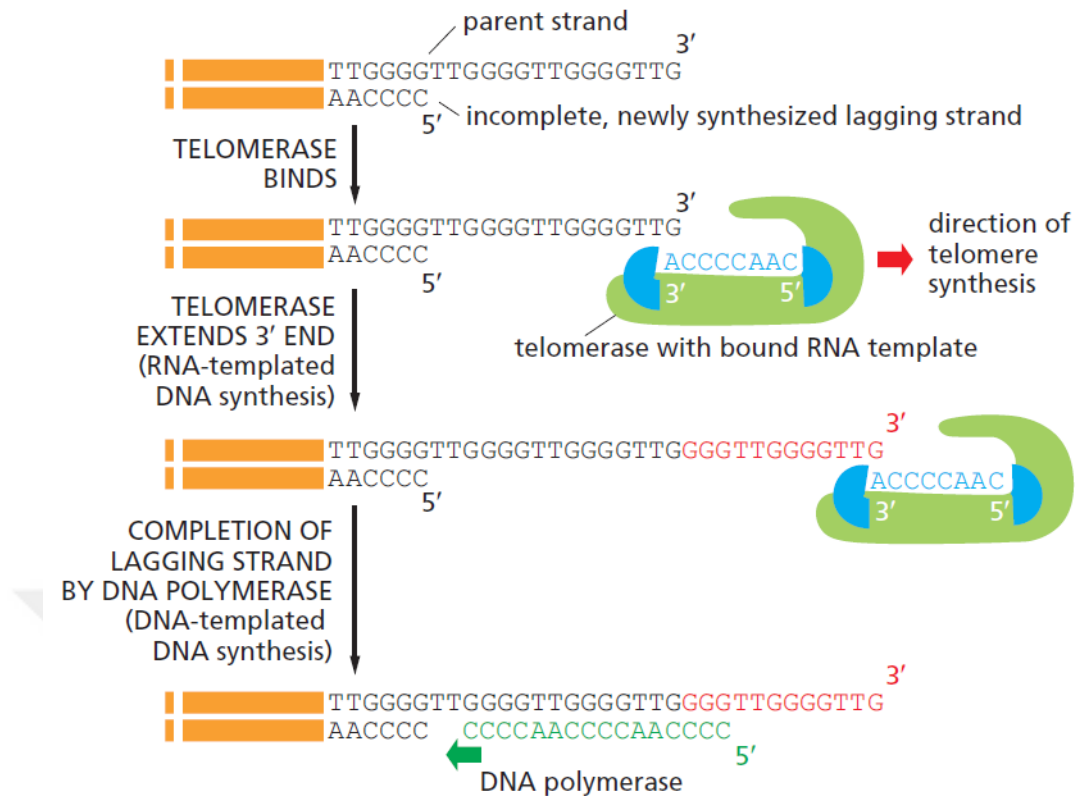


Figure 1.2: The synthesis of the repetitive sequences that constitute the structure of telomeres by telomerase in *Tetrahymena* (Bruce et al., 2015).

1.4. The Structure and Components of Human Telomerase

Telomerase is a ribonucleoprotein enzyme complex that primarily resides in the nucleus of cells. Its activity is tightly regulated and predominantly occurs during the S phase of the cell cycle when DNA replication takes place. The enzyme is especially active in cells with high proliferative capacity, such as stem cells, germ cells, and certain cancer cells. In these cells, telomerase can be found within the nucleoplasm and is particularly associated with Cajal bodies—subnuclear structures that serve as sites for the assembly and modification of the snRNA and snoRNA components of small nuclear ribonucleoproteins (Morris, 2008; Shay & Wright, 2019).

assembly and stability of the telomerase complex. The H/ACA lobe plays a key role in the maturation, trafficking, and overall structural integrity of the telomerase complex, facilitating the correct folding and chemical modification of TERC. Both components of telomerase are essential for its proper function: the catalytic core performs the addition of telomeric repeats, essential for telomere maintenance and cellular immortality, while the H/ACA lobe ensures the structural stability and correct RNA processing that is critical for the assembly of functional telomerase (**Blackburn & Collins, 2011; Ghanim et al., 2021; Greider & Blackburn, 1996; Nguyen et al., 2018**).

Telomerase Reverse Transcriptase (TERT) is a crucial protein in the telomerase complex, responsible for telomere elongation and cell division. Its N-terminal domain regulates the telomerase complex, while its C-terminal domain interacts with the telomerase RNA component (TERC). TERT adds telomeric repeats to chromosome ends by reverse transcribing a segment of TERC RNA. This RNA-dependent DNA polymerase activity counters telomere shortening during DNA replication. TERT's interaction with TERC enhances the enzyme's processivity and fidelity. TERT's activity is tightly regulated in cells, with most somatic cells repressing it, leading to telomere shortening and cellular aging. However, in stem cells, germ cells, and certain cancer cells, TERT expression is upregulated, allowing them to maintain telomere length and divide indefinitely. Understanding TERT's regulation is essential for developing targeted therapies that can modulate telomere length, potentially influencing cancer treatment and the aging process (**Autexier & Lue, 2006; Wyatt et al., 2010; Yuan & Xu, 2019**).

The Telomerase RNA Component (TERC) is a crucial part of the telomerase enzyme complex, serving as the template for synthesizing telomeric repeats. It includes a short template sequence and plays a significant structural and catalytic role within the telomerase complex. TERC's template region ensures accurate telomere repeat synthesis and aligns with the overhanging 3' end of telomere DNA. Its pseudoknot and CR4-CR5 domains are essential for the catalytic activity of the complex and interact with TERT and other telomerase-associated proteins. TERC's expression and stability are tightly regulated, with alterations in its sequence or levels leading to dysfunctional telomere maintenance. Mutations or dysregulations in TERC are associated with various telomeropathies, highlighting its role in cellular aging and disease.

Maintaining TERC integrity and expression levels is essential for telomerase function and cellular immortality in stem cells and certain cancer cells **(Cao et al., 2008; Cayuela et al., 2005; Liu et al., 2019)**.

Dyskerin is a crucial protein in the telomerase complex, playing a crucial role in the stability and function of the TERC. It is a pseudouridine synthase, part of a larger group of proteins that modify RNA molecules, ensuring the proper functioning of RNA structures within the cell. Dyskerin binds to the H/ACA box motif in TERC, facilitating its proper folding and chemical modification. It is part of a ribonucleoprotein particle that supports the telomerase complex's structural integrity and catalytic activity. Dyskerin's interaction with TERC protects the RNA component from degradation, ensuring telomerase maintains telomere length and chromosomal stability. Dyskerin's diverse roles in telomerase function and broader cellular processes highlight its importance in cellular health and longevity. Understanding dyskerin's multifunctional roles can help develop potential therapeutic targets for managing telomere-related diseases **(Corey, 2009; Gu et al., 2009; Montanaro, 2010; Montanaro et al., 2008)**.

NHP2 is a crucial protein in the telomerase complex and other RNPs. It works with proteins like dyskerin, NOP10, and GAR1 to stabilize the TERC. NHP2 binds to specific RNA motifs within H/ACA RNPs, enhancing the stability of the telomerase complex. It also plays a role in ribosomal RNA and small nuclear RNAs, maintaining protein synthesis capacity and RNA splicing fidelity. Mutations in NHP2 are linked to genetic disorders like dyskeratosis congenita, highlighting its importance in genomic stability and cellular longevity **(Pogačić et al., 2000)**.

GAR1 is a crucial protein in the H/ACA ribonucleoprotein complexes, primarily involved in the biogenesis and maintenance of telomeres. It interacts with the TERC, dyskerin, NOP10, and NHP2, ensuring the stability of telomeres, which are essential for cellular longevity and chromosomal integrity. GAR1's structural and functional roles are essential for maintaining cellular function and genomic stability. Dysfunction or improper expression of GAR1 can lead to genetic disorders like dyskeratosis congenita and pulmonary fibrosis. Understanding GAR1's roles and mechanisms could lead to novel therapeutic strategies for age-related diseases and cancer **(Girard et al., 1992)**.

NOP10 is a crucial protein in the human telomerase complex and other RNPs. It forms a core complex with dyskerin, NHP2, and GAR1, binding to specific motifs within the H/ACA RNA pseudouridylation pocket. NOP10's interaction with these proteins enhances the telomerase complex's stability and functionality at telomeres. It is also involved in the pseudouridylation of rRNA and snRNAs, enhancing ribosome function and protein synthesis. NOP10 mutations can lead to genetic disorders like dyskeratosis congenita. Understanding NOP10's role could lead to advances in treating telomere dysfunction and other RNA-processing disorders (**Pogačić et al., 2000; Reichow & Varani, 2008**).

Telomerase Cajal Body Protein 1 (TCAB1) is a crucial protein that directs the telomerase complex to Cajal bodies, specialized subnuclear structures that are key sites for the assembly and maturation of ribonucleoprotein particles, including telomerase. TCAB1's WD40 repeats mediate protein-protein interactions, enabling it to interact effectively with other telomerase complex components, particularly TERC, facilitating the complex's transport to Cajal bodies. This transport is critical as Cajal bodies are the sites where telomerase components are assembled into a functional enzyme capable of elongating telomeres (**Venteicher et al., 2009a; Venteicher & Artandi, 2009b**).

TCAB1 is essential for localizing telomerase and enhancing its efficiency and stability. Dysfunctions or mutations in the TCAB1 gene can lead to mislocalization of telomerase, resulting in inadequate telomere elongation and accelerated cellular aging. These dysfunctions have been linked to various human diseases, including inherited bone marrow failure syndromes and disorders associated with premature aging (**Morris, 2008; Stern et al., 2012; Venteicher & Artandi, 2009**).

TCAB1's role in telomerase trafficking highlights its potential as a therapeutic target. Manipulating TCAB1's expression or function could influence telomerase activity in cells, providing a novel approach to treating diseases related to telomere dysfunction, such as cancer and age-related genetic disorders. Understanding TCAB1's mechanisms and interactions within the telomerase complex is crucial for developing targeted therapies aimed at modulating telomere length and improving cellular longevity (**Chen et al., 2018; Stern et al., 2012; Venteicher et al., 2009**).

1.5. Telomerase Dysfunction and Associated Diseases

Telomerase is a crucial protein that maintains telomere length and chromosomal stability, essential for cellular health and longevity. Dysfunctions in telomerase activity, either through underexpression or overexpression, are linked to serious diseases such as premature aging syndromes, organ-specific degenerative conditions, and cancer. For example, Dyskeratosis Congenita and other telomeropathies are disorders characterized by mutations in telomerase components, leading to short telomeres, premature aging, bone marrow failure, and an increased predisposition to cancers due to genomic instability (**Batista et al., 2011; Walne et al., 2016**). Pulmonary fibrosis and bone marrow failure syndromes, such as idiopathic pulmonary fibrosis and aplastic anemia, are often associated with telomerase mutations, causing cellular senescence and organ failure (**Knight et al., 1999; T. Vulliamy et al., 2002**). In cancer, telomerase reactivates expression, providing telomere maintenance for unlimited cell division and proliferation. This telomere maintenance allows cancer cells to avoid critical shortening, enabling them to grow unchecked. Telomerase also has genomic stability in cancer cells, allowing them to survive and proliferate even under genetic stress. Telomerase's unique expression profile in tumours makes it a valuable target for cancer therapies. Inhibiting telomerase activity could limit growth by inducing telomere shortening and promoting senescence or apoptosis. Several experimental telomerase inhibitors are currently in clinical trials to exploit this vulnerability in various cancers (**Greider & Blackburn, 1996; Kim et al., 1994; Maciejowski & de Lange, 2017; Montanaro, 2010; Montanaro et al., 2008; Shay, 2016; Shay' & Bacchetti2, 1997**).

1.6. Historical Context of Dyskeratosis Congenita Definition

While there is considerable disagreement on the exact year of the initial publication of Dyskeratosis Congenita (DC), either in 1906 or 1910, it is widely agreed that Professor Ferdinand Zinsser authored the first study. Zinsser's paper in 1910 discusses the circumstances of two brothers. The younger sibling who reported having a middle ear infection had pigmentation disorders on his skin, and both the older and younger siblings had white spots in their mouths, under their tongues, and on their inner cheeks. Additionally, structural abnormalities in their nails were found. The same article

mentioned the case that E. Jacobi reported in 1908, highlighting the male patient's unusual skin colouring, thin hair, and presence of white lattice-like areas inside the mouth. Nevertheless, it was reported that there were no anomalies observed in the patient's nails. During the Bern conference in 1906, the term "Poikiloderma vascularis atrophicans" (atrophic vascular poikiloderma) was proposed for this instance. It is probable that the belief that this was the initial documented case of DC stems from this notion. Engman (1926) and Cong et al. (1930) cite comparable instances in their writings, and the disease has been described as "Dyskeratosis Congenita with pigmentation" [Dyskeratosis (Latin): faulty development of the epidermis with abnormal keratinization. Congenital (Latin): inborn, innate, existing from time of birth] or Zinsser–Engman–Cole syndrome. In many studies conducted in the following years, the disease was referred to as Dyskeratosis Congenita. **(Sirinavin & Trowbridge, 1975; Walne & Dokal, 2008).**

1.7. Definition and Clinical Outcomes of DC

Dyskeratosis congenita (DC) is an inherited and extremely pleiotropic syndrome commonly identified by the three key features of mucocutaneous abnormalities, including leukoplakia, nail dystrophy, and irregular skin pigmentation **(Dokal, 2014; Walne et al., 2016)**. DC has been linked to a wide variety of additional characteristics impacting various systems within the body, notably the bone marrow (BM) **(Bessler et al., 2010; Dokal, 2014)**. Figure 1.4 displays some mucocutaneous characteristics of typical DC.

Due to the disease's three types of lineages, (namely X-linked recessive (OMIM #305000), autosomal dominant (OMIM #127550), and autosomal recessive (OMIM #224230)), along with key features (classical form), the clinical symptoms related to each of these genetic variants exhibit significant variations. Table 1.7.1 presents the currently acknowledged clinical characteristics of DC together with their corresponding approximate percentages.



Figure 1.4. Mucocutaneous features commonly found in patients with classical DC. The photos were captured of a 31-year-old male with X-linked recessive DC (dyskerin mutation: Pro40Arg). Images a, b, and c depict abnormal coloration of the skin on the chest, neck, and back, respectively. Furthermore, Image c exhibits indications of premature greying and alopecia. Images d and e exhibit nail degeneration that is impacting the fingers. Image f depicts toenail deterioration. In addition, it highlights the variation in the severity of nail dystrophy throughout the nails; the third nail appears partially preserved, whilst the fourth nail is entirely missing. Image g exhibits leukoplakia on the tongue together with severely distorted teeth. (Walne & Dokal, 2008).

Table 1.1: Clinical abnormality percentages of Dyskeratosis Congenita (Dokal, 2014).

<i>Clinical Abnormalities</i>	<i>Percentage of Patients</i>
<i>Major Features</i>	
Abnormal skin pigmentation	89
Nail dystrophy	88
Bone marrow failure	85.5
Leukoplakia	78
<i>Minor Features</i>	
Epiphora	30.5
Learning difficulties/developmental delay/mental retardation	25.4
Pulmonary disease	20.3
Short stature	19.5
Extensive dental caries/loss	16.9
Esophageal stricture	16.9
Premature hair loss/graying/sparse eyelashes	16.1
Hyperhidrosis	15.3
Malignancy	9.8
Intrauterine growth retardation	7.6
Liver disease/peptic ulceration/enteropathy	7.3
Ataxia/cerebellar hypoplasia	6.8
Hypogonadism/undescended testes	5.9
Microcephaly	5.9
Urethral stricture/phimosis	5.1
Osteoporosis/aseptic necrosis/scoliosis	5.1
Deafness	0.8

The diagnostic inclusion criteria for DC involves the presence of three specific mucocutaneous characteristics, mainly atypical skin pigmentation, nail dystrophy, and oral leukoplakia. Alternatively, a diagnosis can be established if one mucocutaneous characteristic is present together with bone marrow failure and two other somatic symptoms of DC. Additional factors to consider are the occurrence of aplastic anemia, myelodysplastic syndrome, or idiopathic pulmonary fibrosis linked to a harmful telomerase gene mutation. Moreover, the presence of Hoyeraal-Hreidarsson syndrome, which is marked by diminished growth, delayed development, abnormally small head size (microcephaly), underdeveloped cerebellum (cerebellar hypoplasia), failure of the bone marrow, and weakened immune system (immunodeficiency), can serve as an indication of DC. In addition, the diagnostic criteria are met when there are

two or more typically observed characteristics in DC, together with exceptionally short telomeres (less than the 1st percentile) **(Walne et al., 2016)**. Also, the phenomenon that patients with DC have been diagnosed with other diseases over the years (such as aplastic anemia and bone marrow failure) is now generally accepted. Gutman et al. analyzed the literature and found that at least 21 of 60 reported cases had aplastic anemia (AA). Abnormalities typically proceed from ectodermal dystrophy in the first decade to hematopoietic diseases in the second or third decade for about 50% of patients. Early findings indicate haemato-poietic abnormalities as the initial symptoms of DC. An example of this is pancytopenia in a 5-year-old boy. The youngster was diagnosed with aberrant skin pigmentation, nail dystrophy, and leukoplakia at age 10. A 3.5-year-old kid with isolated neutropenia at 13 months is another case. At 2 years, skin pigmentation abnormalities, nail dystrophy, and leukoplakia were observed. These cases demonstrate the potential for misdiagnosis of DC and may explain why many cases have been misdiagnosed as other diseases. The Dyskeratosis Congenita Registry (DCR) has revealed that many individuals initially present with AA. Recently, patients with AA and TERT/TERC mutations have been classified as having DC **(Walne & Dokal, 2008)**.

From the initial description of Dyskeratosis Congenita until 1963, all documented cases of the condition included male individuals. All documented instances included patients under the age of 20, and the majority of symptoms manifested during the first ten years of their lives **(Walne & Dokal, 2008)**. Sorrow and Hitch originally reported the existence of a female patient in 1963 **(Mitchell Sorrow et al., 1963)**. The article reports the identification of pigmentation disease, nail deformity, and severe dental issues in the woman, as well as the presence of whiteness in her oral mucosa. Following the onset of the disease in a woman, speculations arose regarding the potential transmission modes, including autosomal and/or X chromosome-linked. Subsequently, in 1965, three further female patients were recorded, and it was observed that the two women were siblings. This led to the hypothesis that the route of transmission was probably autosomal recessive. Subsequently, research undertaken in a family examined the transmission pattern within the family to assess the plausibility of autosomal dominant transmission. This evaluation was documented in an article published in 1971. The following studies have demonstrated that the mode of transmission is recessive and associated with the X chromosome. Upon compiling

investigations completed on 200 patients till 1995, it was shown that the condition can be transferred through autosomal recessive, autosomal dominant, and X-linked recessive inheritance patterns. Nevertheless, it has been established that the disease is primarily transmitted through an X-linked recessive manner, with a female-to-male ratio varying from 1:10 to 1:13. (Walne & Dokal, 2008).

1.8. Genetics and Inheritance Types of DC

1.8.1. X-Linked Recessive DC and the Høyeraal-Hreidarsson Syndrome

The X-linked recessive mutations occur on the DKC1 gene. The DKC1 gene was initially localized at Xq28 in 1986 and subsequently identified by positional cloning in 1998. The highly conserved gene encodes the protein dyskerin and contributed to the generation of the initial molecular diagnostic test for X-linked DC. The study demonstrated that DC is a diverse condition and that other syndromes with comparable characteristics may possess the same genetic alterations, such as Høyeraal-Hreidarsson (HH) syndrome. Høyeraal-Hreidarsson syndrome (HH) is a complex illness that predominantly affects males and is marked by stunted growth before birth, abnormally small head size, underdeveloped cerebellum, and a condition called aplastic anemia (AA) (Høyeraal et al., 1970; Hreidarsson et al., 1988). Initial reports primarily focused on males with AA. The Dyskeratosis Congenita Registry (DCR) data indicated that certain individuals with classical dyskeratosis congenita (DC) displayed symptoms such as microcephaly, growth and developmental problems, and ataxia. This suggests that the cause of these symptoms may be mutations in the DKC1 gene. Genetic examination of the DKC1 gene in two families affected with HH revealed two previously unidentified missense mutations. This finding supports the idea that HH is a more severe form of DC. This discovery also showed that mutations in DKC1 might lead to a broader range of clinical manifestations than previously believed (Knight et al., 1999). A diagnosis of HH can be established if a patient exhibits at least four of the following primary characteristics: intrauterine growth retardation, developmental delay, microcephaly, cerebellar hypoplasia, immunodeficiency, or AA. Although first believed to exclusively impact males, there have been documented cases of females

with HH, indicating the potential contribution of many genes **(T. J. Vulliamy et al., 2006)**.

1.8.2. Autosomal Dominant DC

Mutations with autosomal dominant effect occur in three genes, and these are TERC, TERT, and TINF2 genes. Currently, heterozygous mutations in three genes (TERC, TERT, and TINF2) have been identified in autosomal dominant DC. The discovery of heterozygous mutations in TERC in 2001 marked a significant advancement in the research of DC, establishing a clear relationship between DC and telomerase. In order to maintain telomere length in rapidly proliferating cells like those in the hematopoietic system, telomerase is necessary due to the semi-conservative nature of DNA replication. Telomerase is mostly found in germ cells, stem cells, activated T cells, and monocytes. Cells lacking telomerase, such as fibroblasts, experience telomere shortening as part of cellular aging **(Diaz de Leon et al., 2010)**. Mutations in autosomal dominant DC patients were first found in TERC. This advancement substantially expanded the DC phenotype to include several hematological and non-hematological illnesses. Heterozygous TERC mutations initially appeared in aplastic anemia patients, followed by myelodysplasia patients. The clinical diagnosis shifted from conventional mucocutaneous symptoms to BM failure as the initial presenting characteristic **(T. Vulliamy et al., 2002; Yamaguchi et al., 2003)**.

Mutations in DKC1 and TERC indicate that defective telomere maintenance is the primary cause of DC. Both dyskerin (encoded by DKC1) and TERC are essential components of telomerase, and patients with these mutations have shorter telomeres than age-matched controls. Further research on the telomerase complex was conducted to determine the genetics of uncharacterized DC patients. Next, alterations were found in TERT, which encodes the telomerase complex's enzymatic component. The clinical appearance among individuals having heterozygous TERT mutations varies greatly, from near DC phenotype to aplastic anemia. Some patients with idiopathic pulmonary fibrosis, hepatic illness, and acute leukemia have heterozygous TERT and TERC mutations **(Calado et al., 2009; Diaz de Leon et al., 2010; Dokal, 2014; Kirwan et al., 2009)**.

1.8.3. Autosomal Recessive DC

It has been determined that sequence changes in seven genes cause autosomal recessive DC disease (**Dokal, 2014**), but only four of them will be examined in this thesis.

TERT has been identified to exhibit biallelic mutations in a specific subgroup of autosomal recessive DC. These mutations result in a significantly altered profile in terms of TRAP activity and telomere length. Both TRAP activity and telomere length are substantially decreased compared to heterozygous TERT mutations. While this particular subtype is not common, individuals with biallelic TERT mutations may exhibit a phenotype that closely resembles HH. Additionally, they may experience substantial immunological insufficiency (**Dokal, 2014; Marrone et al., 2007**).

Two families with autosomal recessive DC have been found to have biallelic mutations in NHP2. Once again, the lengths of telomeres and the levels of TERC are diminished in patients as compared to individuals without any medical conditions. The presence of V126M and Y139H NHP2 mutations has been demonstrated to result in reduced quantities of telomerase RNA in peripheral blood. This provides conclusive proof of the H/ACA RNP component's involvement in the preservation of telomeres in humans (T. Vulliamy et al., 2008). Dyskerin, GAR1, NOP10, and NHP2 are constituents of the H/ACA ribonucleoprotein complex (H/ACA RNP), as previously stated. These four proteins have a high degree of homology across different species and have been demonstrated to play a role in the formation of ribosomes, the process of removing introns from pre-mRNA, and the maintenance of telomeres. Genetic alterations have been detected in all constituents of this H/ACA ribonucleoprotein (RNP) complex in patients with dyskeratosis congenita (DC), except GAR1. The cause of this phenomenon is ambiguous, but it could potentially be attributed to its spatial proximity to the RNA component (**Dokal, 2014**).

NOP10 protein, found in the structure of the human telomerase complex, plays an important role in telomerase stabilization. Only one mutation in this protein has been recorded, causing DC disease to this date. Based on the findings, the p.R34W mutation leads to a decrease in the measurable amounts of TERC and the presence of shorter telomeres (**Walne et al., 2007**).

In 2011, two patients with autosomal recessive DC were identified with biallelic mutations in the TCAB1 gene. Those mutations are F164L, H376Y, R398W and G435R. As discussed earlier, TCAB1 is a protein that acts as a holoenzyme for telomerase. Its main function is to help transport telomerase to Cajal bodies, which are specific locations within the nucleus where nucleoprotein complexes are modified and assembled. TCAB1 mutations interfere with the proper localization of telomerase to Cajal bodies, causing the misplacement of telomerase RNA to nucleoli. As a result, telomerase is unable to lengthen telomeres, leading to their shortening (**Batista et al., 2011; Zhong et al., 2011**).

1.9. Specific TCAB1 Mutations and Their Direct Impact on Dyskeratosis Congenita

Dyskeratosis Congenita (DC) is significantly impacted by mutations in the TCAB1 gene, which is crucial for the proper localization and function of the telomerase complex. Each specific mutation—F164L, H376Y, R398W, and G435R—alters the function of TCAB1, leading to various degrees of telomere maintenance impairment and clinical symptoms associated with DC:

Mutation F164L (Phenylalanine to Leucine at position 164): This mutation affects the structural domain of TCAB1 that is essential for its interaction with the telomerase RNA component (TERC) and other telomerase-associated proteins. The F164L mutation disrupts these interactions, leading to mislocalization of the telomerase complex within the nucleus and reduced telomerase activity at the telomere sites. This results in shorter telomeres and contributes to the accelerated aging symptoms seen in DC (**Zhong et al., 2011**).

Mutation H376Y (Histidine to Tyrosine at position 376): The H376Y mutation impacts the binding efficiency of TCAB1 to Cajal bodies, critical sites for telomerase assembly and processing. By altering the binding affinity, this mutation prevents TCAB1 from effectively anchoring the telomerase complex within these nuclear bodies, thereby hindering the proper maintenance of telomeres and exacerbating the cellular instability and dysfunction characteristic of DC (**Batista et al., 2011; Zhong et al., 2011**).

Mutation R398W (Arginine to Tryptophan at position 398): Located in a crucial regulatory region of TCAB1, the R398W mutation significantly impairs the protein's ability to regulate telomerase access to telomeres during the S phase of the cell cycle.

This leads to inadequate telomere elongation and an increased rate of cell senescence and apoptosis, manifesting in severe cases of DC with widespread tissue dysfunction (**Zhong et al., 2011**).

Mutation G435R (Glycine to Arginine at position 435): This mutation is particularly detrimental as it affects the very terminal region of TCAB1, which is crucial for its stability and interaction with nucleolar transport proteins. The G435R mutation leads to a dysfunctional protein that cannot sustain telomerase within the nucleoli, resulting in drastic reductions in telomere length and highly penetrant DC phenotypes, including severe bone marrow failure and immunodeficiency (**Batista et al., 2011; Zhong et al., 2011**).

Each of these mutations illustrates the delicate and critical role of TCAB1 in telomere maintenance and cellular longevity. The direct consequences of these genetic alterations highlight the importance of TCAB1 in disease pathology and underscore the potential therapeutic targets within the telomerase pathway. Future treatment strategies may include gene therapy to correct these specific mutations or molecular interventions to restore proper telomerase functionality in affected individuals.

1.10. Computational Methods

1.10.1. Molecular Dynamics Simulations

Molecular dynamics (MD) simulations are a sophisticated computational tool used to model the behaviour of biomolecules at an atomic scale, offering critical insights into molecular functions that are otherwise difficult to observe directly. In these simulations, biomolecules—whether proteins, nucleic acids, or lipid bilayers—are depicted as systems composed of interconnected atoms, each defined by parameters such as mass, charge, and initial coordinates (**Hollingsworth & Dror, 2018**).

The fundamental principle guiding MD simulations is the application of classical mechanics to predict the movement of each atom within the system over time. This is achieved by solving Newton's equations of motion for all atoms, considering the forces acting on them. These forces and the potential energies are derived from what is known as a molecular mechanic's force field—a mathematical model that describes the interactions between the atoms in the molecule. Commonly used force fields, such

as AMBER, CHARMM, and GROMOS, incorporate terms for bond stretching, angle bending, torsional angles, van der Waals forces, and electrostatic charges (**Binder et al., 2004; Hansson et al., 2002**).

Each simulation step involves calculating the net force experienced by each atom from its neighbouring atoms and translating these forces into movement, typically using algorithms like the Verlet or Leap-frog integration methods. By repeatedly updating the positions and velocities of all atoms, MD simulations can trace the dynamic trajectory of molecular systems through their configurational space (**Hollingsworth & Dror, 2018; Lindahl, 2008**).

Molecular dynamics (MD) simulations are a powerful computational tool for investigating the structural effects of mutations on proteins at an atomic level. By simulating the physical movements of atoms and molecules over time, MD allows researchers to examine how specific mutations alter the conformational stability, flexibility, and dynamic interactions within the protein structure or between the protein and its binding partners (**Anwar & Choi, 2017**). For instance, simulations can reveal changes in hydrogen bonding patterns, hydrophobic interactions, or salt bridge formations caused by a mutation, thereby providing insights into its potential impact on protein function (**Li et al., 2018**). Additionally, MD enables the exploration of how mutations influence global properties such as the folding energy landscape, R_g , and SASA, offering a comprehensive understanding of their structural and functional consequences. This approach is particularly valuable in studying disease-associated mutations, as it can elucidate molecular mechanisms underlying pathogenicity and inform targeted therapeutic strategies (**Montefiori et al., 2019**).

A key application of MD simulations is in the study of protein dynamics, such as folding, misfolding, and interactions with other molecules (ligands or other proteins). For instance, simulations can provide a time-resolved depiction of the folding pathways of proteins, identifying intermediate states and critical folding events that are elusive in experimental setups. Furthermore, in the field of drug design, MD simulations allow researchers to examine the binding processes between drugs and their target proteins, exploring how molecular adjustments affect binding affinity and specificity (**Binder et al., 2004; Hollingsworth & Dror, 2018; Lindahl, 2008**).

The accuracy of an MD simulation critically depends on the quality of the force field used and the time scale over which the simulation is run. Longer simulations are often necessary to observe biologically relevant phenomena, which occur on timescales of microseconds to milliseconds—a range that has become increasingly accessible due to advances in computational power and algorithms **(Hansson et al., 2002; Lindahl, 2008)**.

Moreover, recent enhancements in hardware, especially the adoption of GPUs and parallel processing architectures, have drastically reduced the computational time required for MD simulations. These technological advancements enable the simulation of larger systems, such as entire viruses or portions of cellular membranes, with greater accuracy and in finer detail. Additionally, specialized techniques like accelerated MD (aMD) and metadynamics introduce modifications to the potential energy landscape, allowing the exploration of rare events that would not occur within the feasible timescales of conventional MD. As computational resources continue to expand, the scope of molecular dynamics simulations is expected to grow, providing deeper and more comprehensive insights into the molecular machinery of life. These simulations bridge the gap between theoretical biochemistry and practical, real-world applications in medicine and biotechnology, highlighting their indispensable role in modern science **(Hollingsworth & Dror, 2018; Páll et al., 2020; Rovigatti et al., 2015)**.

2. AIM OF THE STUDY

The objective of this study is to perform molecular dynamics simulations of the telomerase protein, which have not been conducted previously at such a high capacity and detail, to shed computational light on the functioning of the telomerase enzyme, to examine TCAB1 mutations such as F164L/R398W and H376Y/G435R that have been experimentally proven to inhibit the enzyme's activity through molecular dynamics simulations and to use the insights gained from these studies as a foundation for research aimed at inhibiting the function of the enzyme. In this study, an investigation into the individual effects of the F164L/R398W and H376Y/G435R double mutations is intended, as well as an exploration to ascertain whether the impact of double mutations is cumulative. The hypothesis of the thesis posits that the interface between TCAB1 and the H/ACA RNP complex is expected to be disrupted due to the effects of both single and double mutations.

3. METHODS

Initially, the structure of the human telomerase containing the H/ACA RNP lobe was retrieved from the Protein Data Bank (PDB) (PDB ID: 7bgb). Subsequent steps involved addressing any discontinuities within the structure; this was achieved by aligning the amino acid sequence obtained from UniProt and PDB with the existing structure using the YASARA Structure program to correct any inconsistencies. Following this structural rectification, the TCAB1 protein was isolated from the rest of the complex.

Subsequently, both the TCAB1 protein and the remaining portion of the H/ACA RNP lobe were subjected separately to molecular dynamics simulations under identical conditions—specifically at 300 °K and physiological pH—utilizing the 2021.6 version of the Gromacs software. Each simulation was conducted throughout 100 nanoseconds, reaching a plateau in the root mean square deviation (RMSD) indicative of equilibration. Upon stabilization, cluster structures were derived from the simulations' RMSD equilibrated plateau.

These cluster structures were then reassembled using the YASARA Structure program. This step was crucial as it allowed for the alleviation of any residual stress within the structure that may have been introduced during the initial retrieval and preparation phases. Through the application of molecular dynamics simulations, the integrity and functional accuracy of the reconstituted complex were ensured, providing a more reliable basis for subsequent experimental and computational analyses.

Following the initial phase, the assembled H/ACA RNP complex, incorporating overlaid cluster structures, was subjected to MD simulations at a constant temperature of 300 K and physiological pH for a duration of 100 nanoseconds. After the RMSD of the structure stabilized, a cluster structure from the equilibrated region was selected and designated as the wild-type (WT) H/ACA RNP structure. Subsequent analyses were conducted based on this reference structure.

Further, cluster structures derived from the independently simulated TCAB1 protein were used to sequentially and individually generate the F164L, H376Y, R398W, and G435R single mutant structures, as well as the F164L/R398W and H376Y/G435R double mutant TCAB1 structures. Each of these mutant structures was then simulated

separately under identical conditions of 300 K and physiological pH using the same software and version until equilibration was achieved. From the stabilized RMSD regions, distinct cluster structures were extracted for each mutant. These cluster structures were subsequently integrated with the cluster structure of the H/ACA RNP lobe to form the complete mutant complex structures.

Subsequently, all mutant structures were subjected to MD simulations under the same conditions as the WT complex structure. These simulations were continued until the RMSD values reached a state of equilibrium. This approach ensures that each mutant structure achieves similar stability parameters, facilitating accurate comparative analyses of structural dynamics and functional implications across all variants.

Subsequent to the equilibration of RMSD across all simulations, RMSF analyses were conducted on the stabilized regions. Additionally, throughout the simulation durations, analyses of Rg and SASA were performed on all structures. Following this, cluster structures derived from the equilibrated RMSD plateaus of each simulation were extracted. These structures were then used for protein-protein interaction analyses via the PDB Sum web server. Lastly, snapshots were taken at five ns intervals from the final 50 nanoseconds of each simulation. Each of these snapshots was utilized in YASARA, integrated with the FoldX software, to generate interaction energy graphs.

All simulations mentioned in the thesis were conducted under identical conditions using GROMACS software version 2021.6. For all cluster structures, a cutoff distance of 0.30 nm was employed. Additionally, all graphs presented in the thesis were generated using the MagicPlotStudent 2.9.3 software.

4.RESULTS

4.1. WT and F164L Mutant Protein Results

Both wild-type (WT) and F164L mutant TCAB1 proteins in the complex were subjected to molecular dynamics (MD) simulations for 100 ns and 150 ns, respectively. Root mean square deviation (RMSD) values of each simulation were calculated and plotted (Figure 4.1).

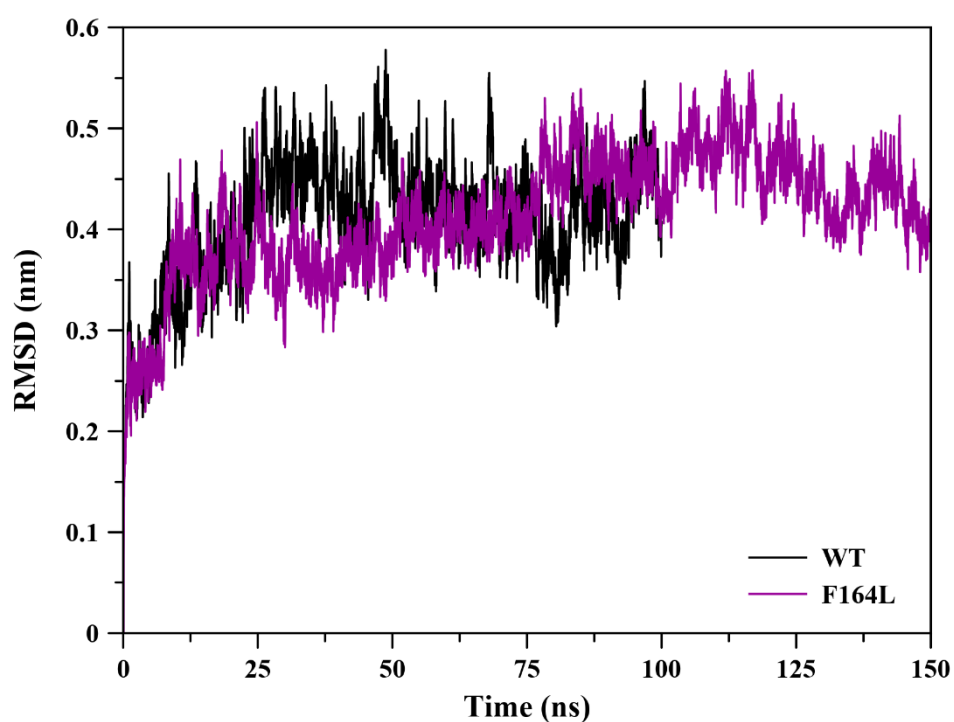


Figure 4.1. RMSD values of WT and F164L mutant complex.

According to the graph in Figure 4.1, the RMSD values of the two simulations are balanced around 0.4 nm. The simulation of the wild-type complex equilibrated in 22 nanoseconds, while the simulation of the G435R mutant took much longer to equilibrate.

Root mean square fluctuation (RMSF) values were calculated for each of the amino acids in the complex, from equilibrated part of the simulations. First, the RMSF values of mutant and wild-type TCAB1 proteins were compared. The RMSF values of the two structures and the relativity of these values to each other can be seen in the Figure 4.2.

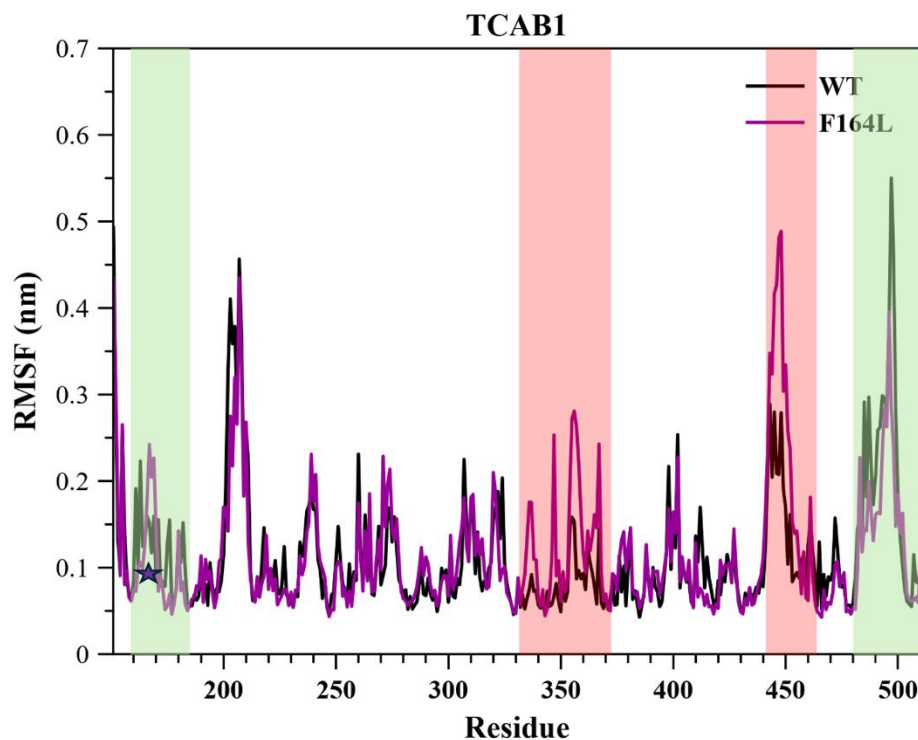


Figure 4.2. Comparison of RMSF values of wild type (WT) and F164L mutant TCAB1 protein in complexes. The star shows the place of mutation.

The RMSF graph in Figure 4.2. compares the structural dynamics between the wild-type (WT) and F164L mutant TCAB1 proteins, specifically highlighting regions of significant fluctuations across the amino acid sequence. In this graph, the black line represents the WT protein, and the purple line indicates the F164L mutant. The graph identifies regions where RMSF values are notably higher for either the mutant or the WT. Specifically, the red background highlights regions from residues 331-373 and 443-461 where the mutant exhibits higher RMSF values, indicating increased flexibility or dynamic behaviour in these segments compared to the WT. Conversely, the green background marks regions from residues 161-183 and 484-510, where the WT protein shows higher RMSF values, suggesting that in these regions, the WT protein is more flexible or dynamic than the mutant.

A critical observation is a noticeable decrease in RMSF values surrounding the mutation site, which could imply that the mutation induces a stabilising effect on the nearby protein structure, potentially affecting the protein's overall dynamics and function. This stabilization might influence how the protein interacts with other molecules or its stability within the cellular environment, suggesting that the F164L

mutation not only affects the localised structure but also has a broader impact on the protein's behaviour.

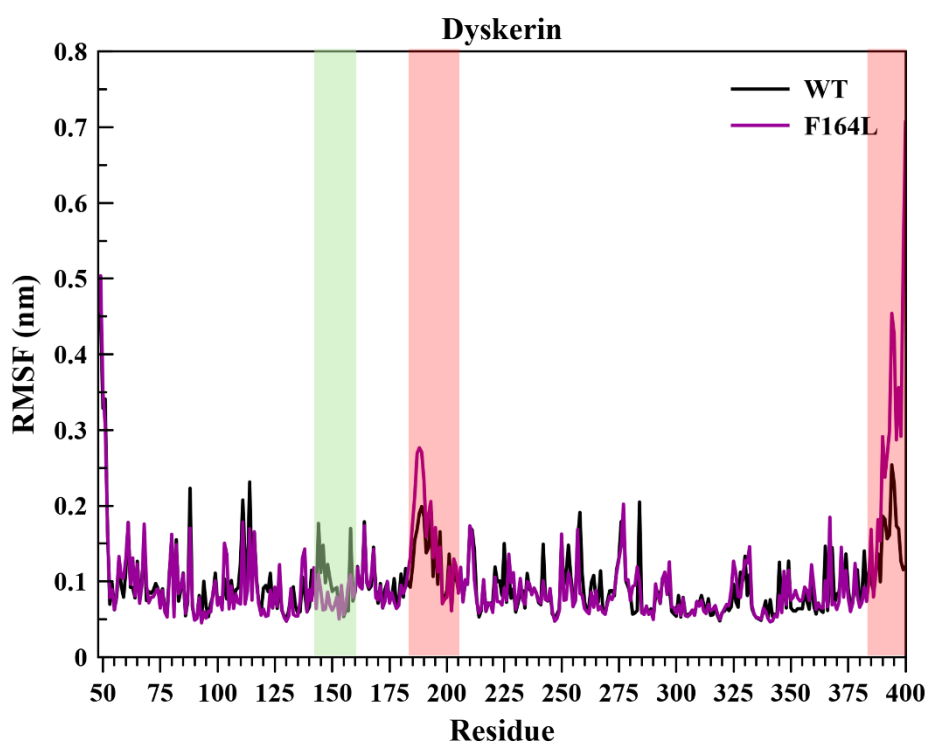


Figure 4.3. Comparison of RMSF values of WT and F164L mutant dyskerin protein in complexes.

In Figure 4.3, the RMSF graph for dyskerin displays the fluctuations in structural dynamics between the WT and the F164L mutant across a range of residues. The graph contrasts the WT, represented by a black line, and the F164L mutant, depicted by a purple line, highlighting regions of higher RMSF values in different colours. The green region, spanning residues 144-152, shows a higher RMSF value for the WT protein, indicating greater flexibility or dynamic motion compared to the mutant in this segment. Conversely, the red regions, which cover residues 184-216 and 385-400, reveal that the F164L mutant exhibits greater fluctuations than the WT. This suggests that these regions in the mutant are less stable or more flexible, which could potentially impact the protein's function or its interactions within the cellular environment.

This pattern of fluctuation suggests that the F164L mutation may introduce changes in the stability and dynamics of specific segments of dyskerin. Higher flexibility in certain regions of the mutant could alter how dyskerin interacts with RNA or other proteins, possibly affecting its role in telomere maintenance and ribosome biogenesis.

Conversely, areas where the WT shows greater flexibility might indicate regions critical for maintaining the structural integrity or functional competence of dyskerin in its natural state. Overall, these variations in RMSF values between the WT and mutant dyskerin provide valuable insights into the potential structural and functional implications of the F164L mutation, highlighting how specific alterations in amino acid sequence can influence the broader dynamics and functionality of a protein.

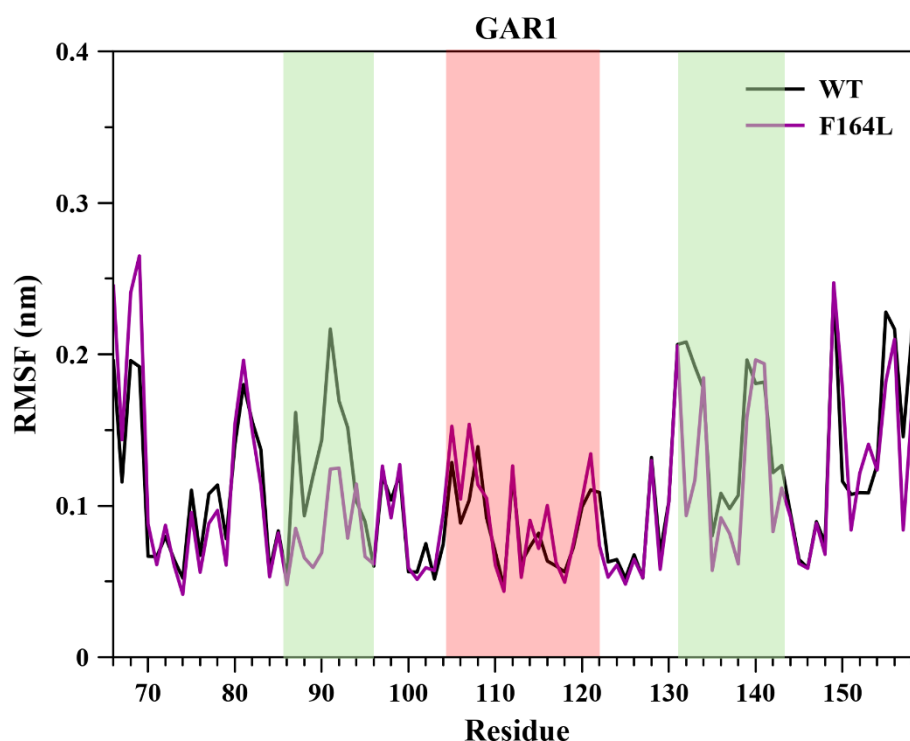


Figure 4.4. Comparison of RMSF values of WT and F164L mutant GAR1 protein in complexes.

The RMSF graph in Figure 4.4 for GAR1 presents a comparison between the wild-type (WT) and F164L mutant across specific amino acid residues, highlighting areas of greater fluctuation in RMSF values. The black line represents the WT protein, and the purple line depicts the F164L mutant. This graph indicates regions where significant differences in RMSF values are observed, albeit the overall variations are relatively minor.

In the green highlighted regions, which span residues 86-94 and 132-145, the WT protein exhibits higher RMSF values compared to the mutant. This suggests that in these regions, the WT protein is more flexible or dynamic, possibly contributing to its functional interactions or stability in its native state. On the other hand, the red

highlighted region from residues 114-121 shows that the mutant has higher RMSF values, indicating increased flexibility or dynamic behaviour in this particular segment of the mutant protein.

Despite these highlighted differences, it is important to note that the overall fluctuations between the WT and the mutant are not markedly distinct across most of the sequence, suggesting that the F164L mutation does not drastically alter the general dynamic behaviour of the GAR1 protein. This subtle variation might imply that while local structural changes are evident, they may not significantly impact the protein's overall flexibility and function. Such insights are crucial for understanding the specific effects of mutations like F164L on protein dynamics and can help infer potential changes in protein function or interaction patterns without causing profound structural disruptions.

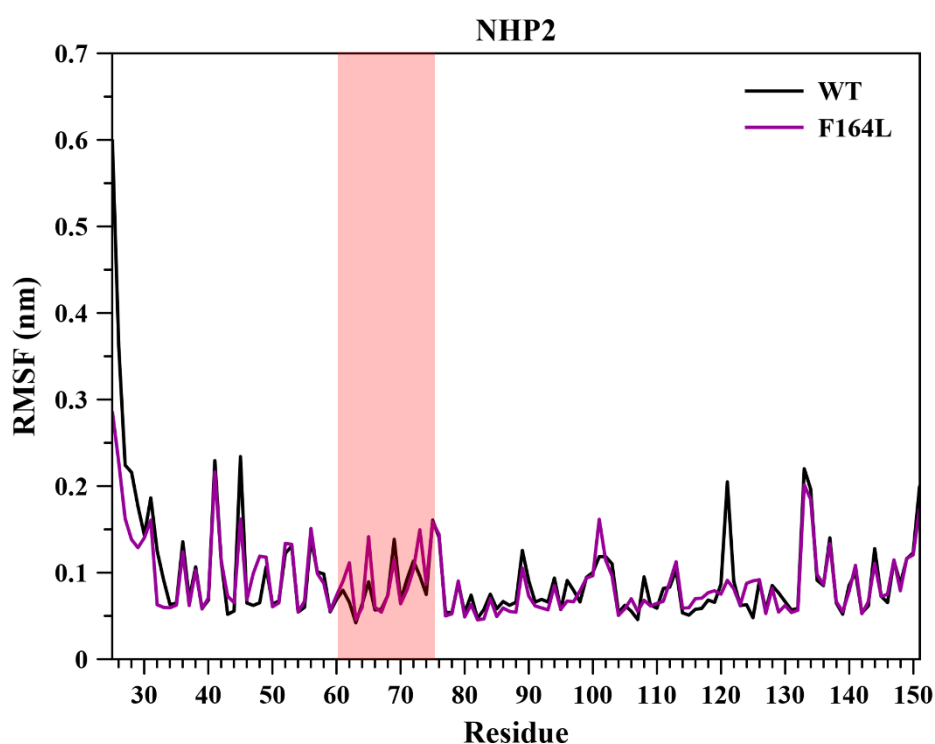


Figure 4.5. Comparison of RMSF values of wild type (WT) and F164L mutant NHP2 protein in complexes.

In Figure 4.5, the RMSF graph for NHP2 shows a comparison between the wild-type (WT) and F164L mutant across residues 30 to 150. The WT is represented by a black line, and the F164L mutant by a purple line. Notably, the red-shaded region from residues 61-73 shows the mutant with slightly higher RMSF values, indicating

increased flexibility. However, the overall differences between the WT and mutant across the sequence are minor, with similar fluctuation patterns suggesting that the F164L mutation does not significantly alter NHP2's general dynamics. This might indicate that the mutation's impact is subtle and localized without causing major functional disruptions.

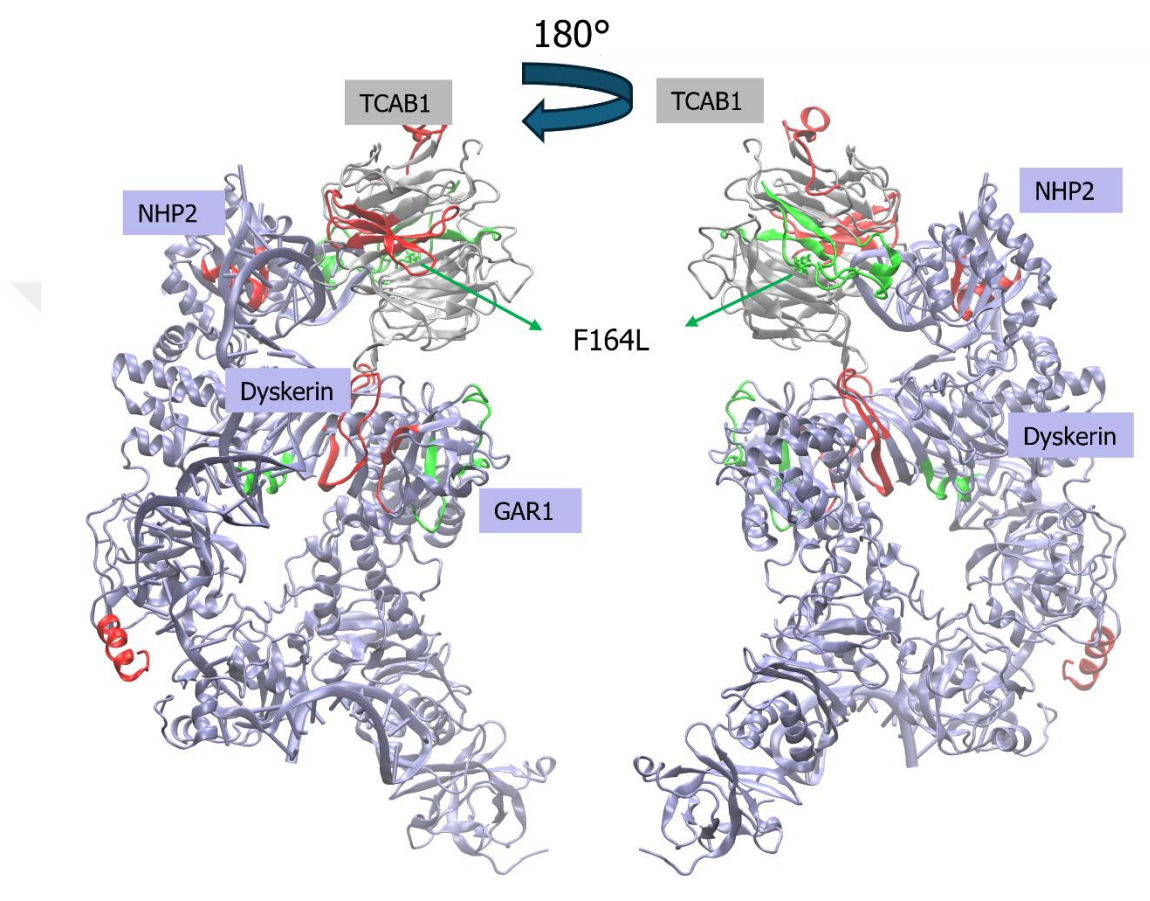


Figure 4.6. F164L complex structure. The protein shown in grey is the TCAB1 protein, the rest of the complex is shown in metallic blue.

In the visual representation depicted in Figure 4.6., the F164L mutant complex comprises various components, including a single TCAB1, one RNA molecule, and two each of dyskerin, GAR1, NHP2, and NOP10 proteins. The F164L mutation, situated on the TCAB1 protein, is indicated by green arrows within the image. The two images presented are rotations of each other, each shown at a 180-degree angle from the other.

This diagram highlights amino acid sequences marked in red, where the mutant protein exhibits higher RMSF values compared to the WT protein. Conversely, sequences

marked in green denote areas where the RMSF values are lower. These highlighted regions correlate precisely with the RMSF value discussions previously detailed for each interacting protein within the complex, as specified in the table descriptions.

Specifically, within the TCAB1 protein's interaction domain with NHP2, a decrease in RMSF is observed, while the region where TCAB1 interacts with RNA shows an increase in RMSF. For the dyskerin protein, significant changes are not observed in the regions where it interacts with TCAB1. However, changes in RMSF are detected in regions where dyskerin interacts with other proteins or is exposed to solvent. In the case of GAR1, a decrease in RMSF values is noted in the regions interacting with TCAB1, whereas an increase is observed in the regions where it interacts with dyskerin. Lastly, changes in the RMSF for the NHP2 protein are confined within its structure and do not impact any interaction domain with adjacent proteins.

This comprehensive analysis provides an enhanced understanding of how the F164L mutation potentially alters structural dynamics and interactions within the protein complex, which could have implications for the biological function and stability of the proteins involved. Such detailed insights are crucial for advancing the understanding of protein structure-function relationships in the context of genetic mutations.

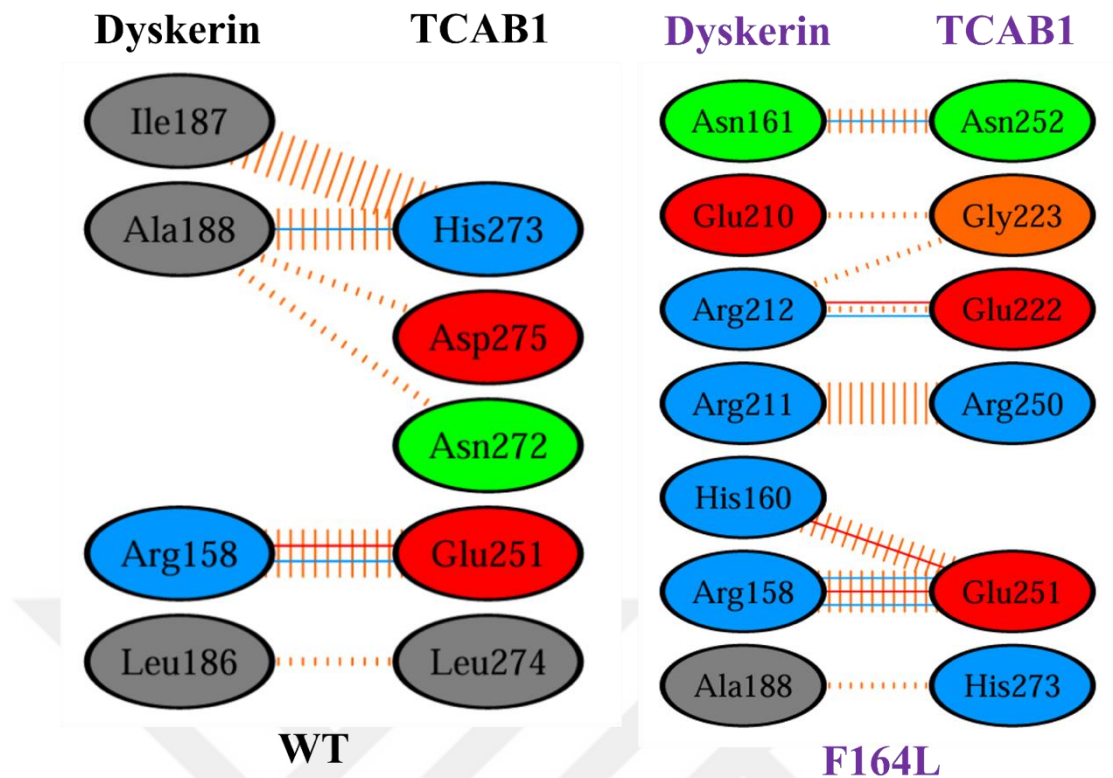


Figure 4.7. Comparative analysis of the interactions between WT TCAB1 and the F164L mutant TCAB1 proteins with the dyskerin protein within the complex structures.

The analysis of the interactions between dyskerin and TCAB1 in both the WT and F164L mutant forms reveals distinct changes in the binding interface. In the WT complex, key stabilizing interactions include hydrogen bonds and non-bonded contacts between Ala188 on dyskerin and His273 on TCAB1, as well as between Arg158 on TCAB1 and Glu251 on dyskerin. These interactions contribute to a stable binding interface, with Asn272 on dyskerin also participating in additional contacts, further strengthening the interaction. The presence of these interactions in the WT complex suggests a well-organized interaction interface that supports a stable and possibly functionally relevant complex between TCAB1 and dyskerin.

In the F164L mutant, the interaction profile undergoes a notable reorganization. While some original contacts are maintained, such as the hydrogen bond between Arg158 on TCAB1 and Glu251 on dyskerin, new interactions emerge that compensate for the mutation-induced structural alterations. For example, additional contacts form between Arg212 and Glu222 and between Arg211 and Arg250 on TCAB1, which are

not observed in the WT complex. These new contacts likely help stabilize the interaction in the F164L mutant form, although the reorganization of the interface suggests that the binding dynamics may be altered.

Moreover, the F164L mutation appears to influence the alignment and positioning of certain residues, as seen with the introduction of interactions involving His160 on dyskerin and Glu222 on TCAB1. This reconfiguration may lead to changes in the binding affinity and stability of the complex, as different residue contacts can affect the overall energy landscape of the protein-protein interaction. Although the mutation disrupts some original WT interactions, such as the specific interaction formations of Ala188 with His273, the new contacts introduced in the F164L mutant suggest an adaptive shift in the binding interface that may help preserve complex stability.

In conclusion, the F164L mutation in TCAB1 significantly modifies the interaction landscape with dyskerin, introducing new stabilizing contacts that replace or compensate for the disrupted WT interactions. This adaptive reconfiguration highlights the role of specific residue interactions in maintaining protein-protein binding stability and suggests that, while the mutation alters the original interaction network, the F164L mutant may still form a stable complex with dyskerin due to the establishment of alternative binding interactions.

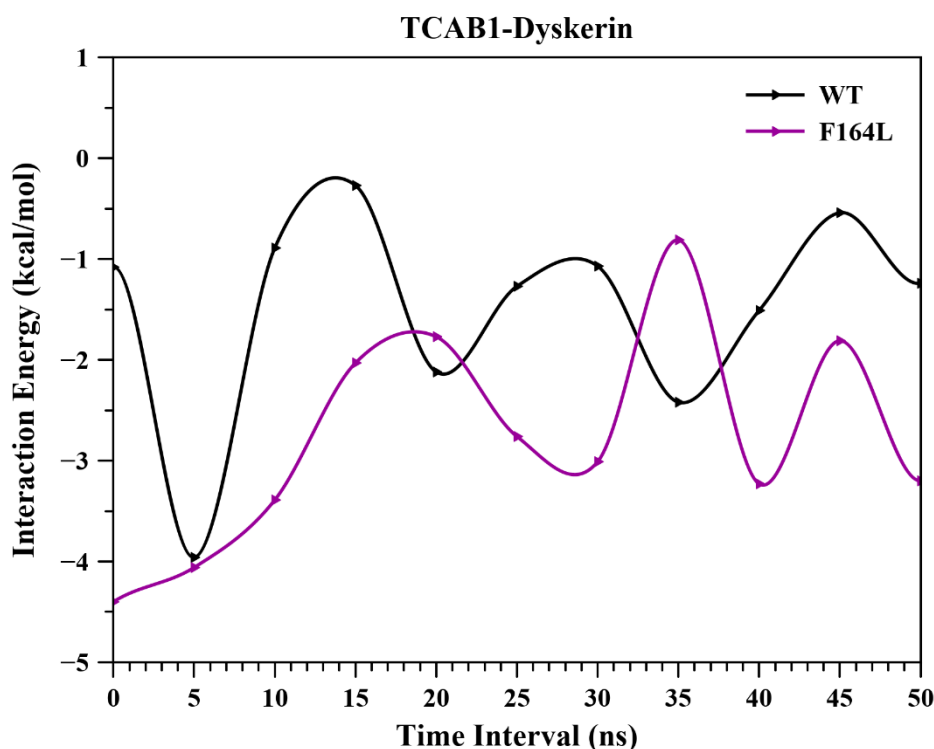


Figure 4.8. Interaction energy profile of WT and F164L mutant TCAB1-Dyskerin complexes over time.

As seen in Figure 4.8., the interaction energy graph for the TCAB1-dyskerin complex, comparing the WT and F164L mutant forms, reveals notable differences in binding stability and affinity. The average interaction energy for the WT complex is -1.5 kcal/mol, which, while negative, indicates relatively weak binding between TCAB1 and dyskerin. The WT energy profile displays fluctuations, with peaks rising close to 0 kcal/mol, indicating moments of reduced binding affinity and potential instability. These energy fluctuations suggest that the WT complex experiences a less stable interaction, with periods where the binding is weaker and possibly transient.

In contrast, the F164L mutant complex has an average interaction energy of -2.7 kcal/mol, which is significantly more negative than the WT. This more negative average energy reflects a stronger and more stable interaction between TCAB1 and dyskerin in the mutant form. The energy profile of the F164L mutant demonstrates reduced fluctuations, stabilizing at consistently lower values. Although the mutant complex also exhibits fluctuations, they remain within a more favourable, negative range, suggesting a more stable binding interface overall. This increased stability aligns with the structural reorganization observed in the F164L mutant, where new

stabilizing interactions are introduced to compensate for the loss of certain WT contacts.

The contrast between the WT and F164L mutant energy profiles highlights the impact of the F164L mutation in enhancing the binding affinity and stability of the TCAB1-dyskerin complex. While the WT form relies on a weak and somewhat unstable interaction, the mutation introduces changes that create a more energetically favorable binding interface. The F164L mutation likely strengthens the interaction through new contacts, which are reflected in the consistently lower energy values and the more stable profile observed. This improvement in binding affinity suggests that the F164L mutation enhances the functional stability of the TCAB1-dyskerin complex, potentially leading to a more effective and resilient protein-protein interaction within a cellular context.

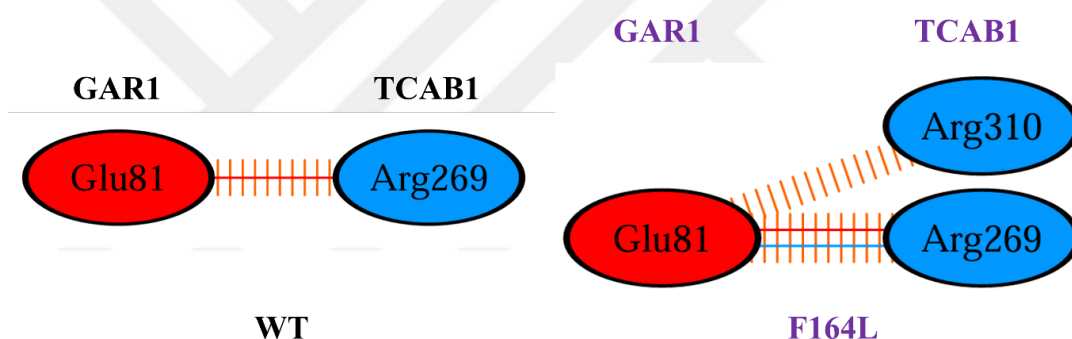


Figure 4.9. Comparative analysis of the interactions between WT TCAB1 and the F164L mutant TCAB1 proteins with the GAR1 protein within the complex structures.

As seen in Figure 4.9., the analysis of the interaction between TCAB1 and GAR1 in both the WT and F164L mutant forms reveals notable changes in the binding interface that impact the stability and strength of the complex. In the WT complex, the interaction is limited to a single non-bonded contact between Glu81 on GAR1 and Arg269 on TCAB1. This minimal interaction suggests a weak and potentially unstable binding affinity, as it lacks additional stabilizing forces, such as multiple hydrogen bonds or additional non-bonded contacts. The limited interaction profile in the WT form may indicate that the TCAB1-GAR1 complex is only weakly bound, with the single contact insufficient for establishing a robust and stable connection.

In contrast, the F164L mutation in TCAB1 introduces a more complex and stabilized interaction profile. In the mutant form, Glu81 on GAR1 not only interacts with Arg269 but also forms additional contacts with Arg310 on TCAB1. This expansion of the binding interface, including a hydrogen bond and multiple non-bonded contacts, suggests that the mutation enhances the overall stability and binding affinity of the complex. The addition of Arg310 as a new interaction partner for Glu81 strengthens the connection, as the increased number of stabilizing contacts likely reduces the likelihood of dissociation and improves the structural integrity of the TCAB1-GAR1 complex. Overall, the F164L mutation shifts the interaction from a weak, single-contact binding in the WT to a more robust and stable multi-contact interface in the mutant, potentially leading to improved functional resilience and efficiency of the TCAB1-GAR1 interaction in cellular processes.

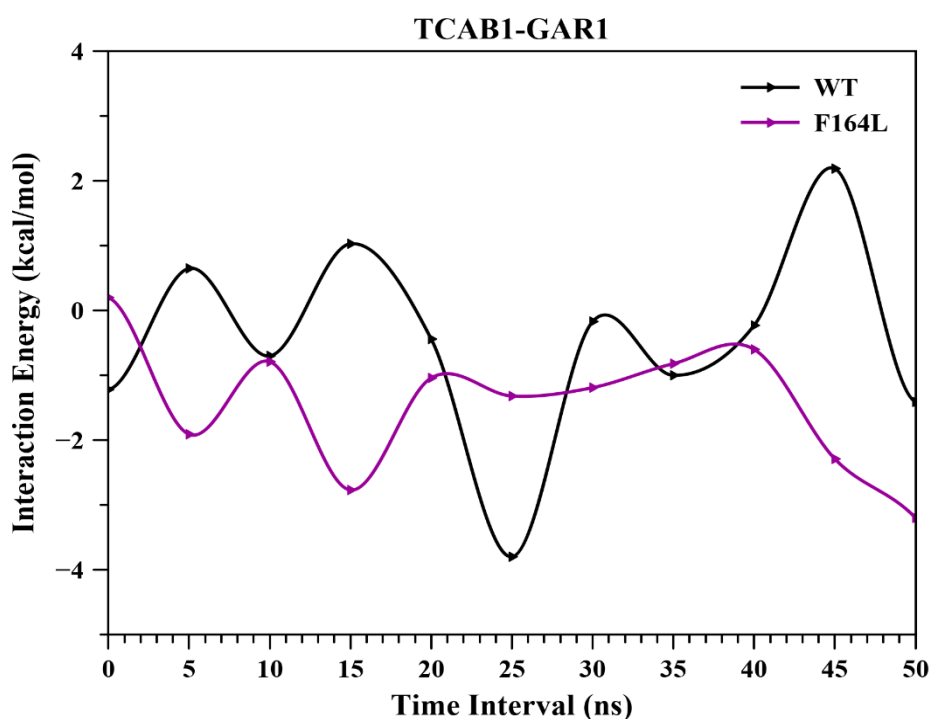


Figure 4.10. Interaction energy profile of WT and F164L mutant TCAB1-GAR1 complexes over time.

The interaction energy graph comparing the WT and F164L mutant forms of the TCAB1-GAR1 complex reveals significant differences in binding stability and affinity. In the WT complex, the average interaction energy is -0.5 kcal/mol, suggesting a relatively weak and fluctuating interaction between TCAB1 and GAR1.

The energy profile for the WT complex shows considerable fluctuations, with peaks frequently rising above 0 kcal/mol, indicating moments of reduced binding affinity and potential instability. This variability suggests that the WT complex struggles to maintain a stable interaction, with transient contacts that contribute to a less stable binding interface.

In contrast, the F164L mutant complex exhibits a more negative average interaction energy of -1.5 kcal/mol, indicating a stronger and more stable interaction. The energy profile of the mutant complex displays reduced fluctuations, stabilizing around more negative values. This consistency suggests that the F164L mutation enhances the stability of the interaction by introducing additional stabilizing contacts, as observed in the interaction profile with added residues such as Arg310 on TCAB1 binding to Glu81 on GAR1. This additional interaction likely strengthens the overall binding affinity, as reflected in the lower average energy and reduced variability, indicating that the mutant complex achieves a more energetically favourable and stable binding interface compared to the WT. Overall, the F164L mutation improves the functional stability of the TCAB1-GAR1 complex, potentially enhancing its resilience and efficiency in cellular processes.

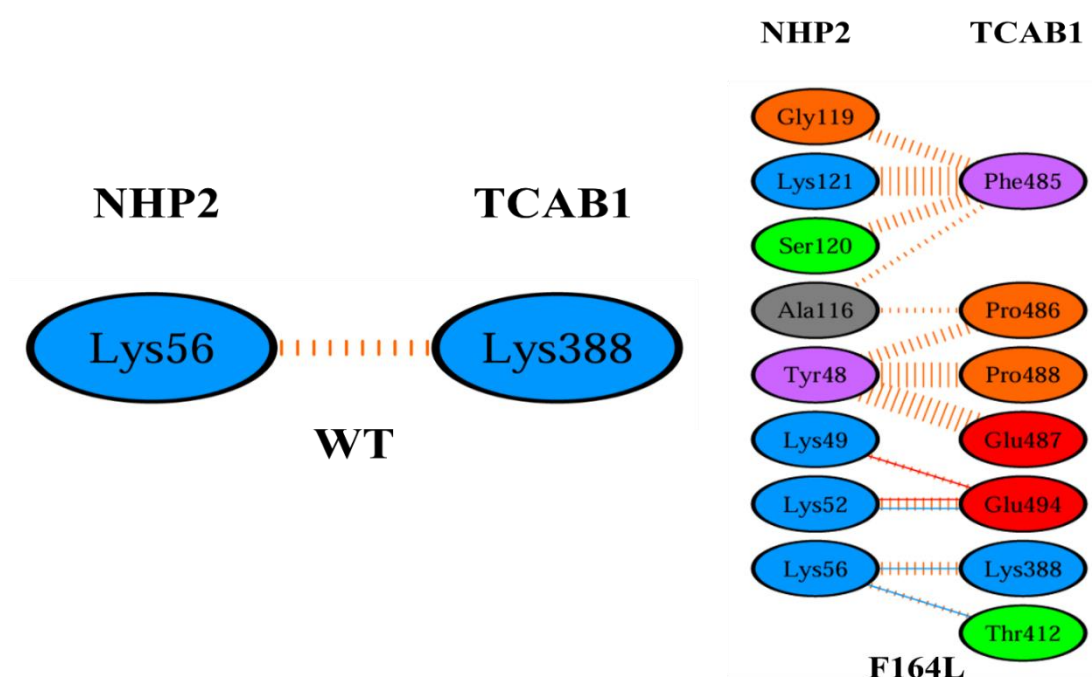


Figure 4.11. Comparative analysis of the interactions between WT TCAB1 and the F164L mutant TCAB1 proteins with the NHP2 protein within the complex structures.

As Figure 4.11 indicates, the interaction between TCAB1 and NHP2 shows a marked difference between the wild-type (WT) and F164L mutant forms. In the WT complex, the interaction is limited to a single non-bonded contact between Lys56 on NHP2 and Lys388 on TCAB1. This minimal interaction suggests a very weak binding affinity between the two proteins, as it lacks additional stabilizing interactions that are typically needed for a more robust protein-protein complex. The single contact indicates that the WT interaction is transient and unlikely to support a stable functional association between TCAB1 and NHP2.

In contrast, the F164L mutation in TCAB1 introduces a complex network of interactions, transforming the interaction landscape between TCAB1 and NHP2. The mutant complex includes multiple non-bonded contacts and hydrogen bonds, with additional interactions involving residues such as Gly119 with Phe485, Lys121 with Phe485, and Ser120 with Phe485. Furthermore, Tyr48 on NHP2 interacts with Pro488 and Pro486 on TCAB1, while Lys49 forms a contact with Glu487, and Lys56 is still involved in non-bonded interactions with Lys388 along with a new contact with Thr412. This extensive network of contacts suggests that the F164L mutation significantly enhances the binding affinity and stability of the TCAB1-NHP2 complex by introducing new stabilizing interactions that were absent in the WT.

Overall, the F164L mutation drastically changes the TCAB1-NHP2 interaction from a weak, single-contact interface to a robust, multi-contact binding interface. This structural reorganization, which includes various non-bonded contacts and hydrogen bonds, likely enhances the overall stability and functional resilience of the complex, potentially increasing its effectiveness in cellular processes where a stable TCAB1-NHP2 interaction is required.

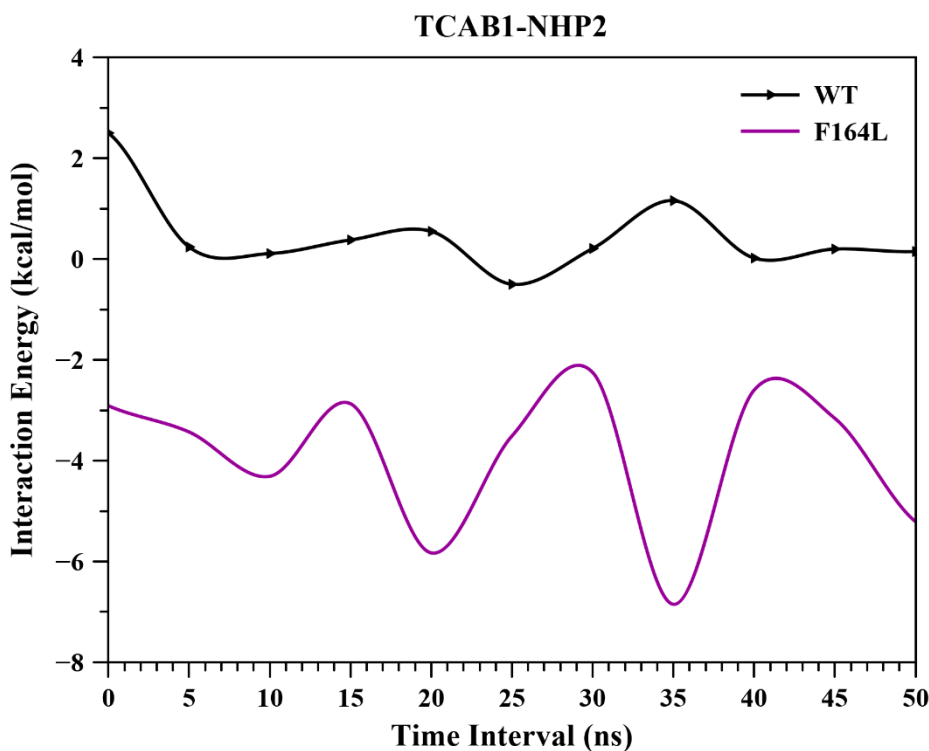


Figure 4.12. Interaction energy profile of WT and F164L mutant TCAB1-NHP2 complexes over time.

The interaction energy graph for the TCAB1-NHP2 complex in both the WT and F164L mutant forms demonstrates a significant impact of the mutation on binding stability and affinity. In the WT complex, the average interaction energy is 0.7 kcal/mol, indicating a weak and unstable interaction between TCAB1 and NHP2. The energy profile for the WT complex shows consistent fluctuations around slightly positive values, suggesting a lack of strong binding affinity. This relatively high average energy implies that the WT interaction is tenuous and prone to dissociation, which aligns with the observation that the WT interaction primarily involves a single non-bonded contact between Lys56 on NHP2 and Lys388 on TCAB1.

In contrast, the F164L mutant complex displays a substantially more negative average interaction energy of -4 kcal/mol, indicating a much stronger and more stable interaction between TCAB1 and NHP2. The energy profile for the mutant complex shows consistently lower values with occasional fluctuations, all within a significantly more negative range. This more stable and energetically favourable profile suggests that the F164L mutation introduces substantial stabilizing interactions, as evidenced by the additional contacts observed in the interaction profile, such as those between

Lys121, Gly119, Tyr48, and other residues on TCAB1 and NHP2. The presence of multiple stabilizing interactions in the F164L mutant is likely responsible for the marked improvement in binding stability.

Overall, the F164L mutation transforms the TCAB1-NHP2 interaction from a weak, single-contact association in the WT form to a robust and stable complex in the mutant form. The significantly more negative interaction energy in the mutant complex reflects the enhanced binding affinity introduced by the mutation, highlighting its impact on the functional stability of the TCAB1-NHP2 interaction. This enhanced stability suggests that the F164L mutation not only strengthens the interaction energetically but also likely contributes to a more resilient and functionally effective complex in cellular contexts.

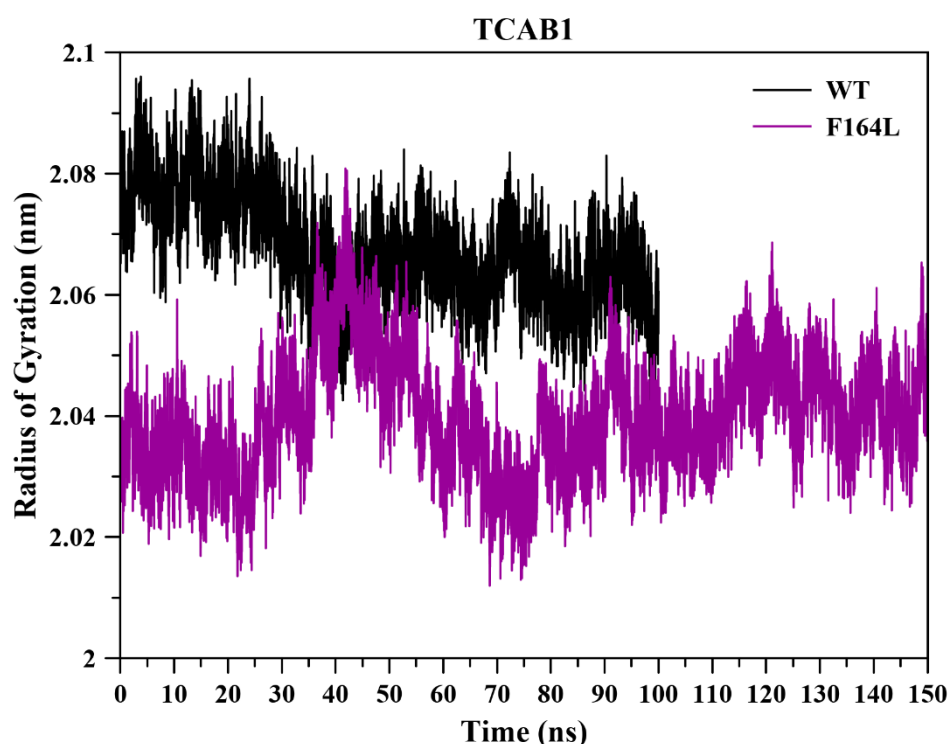


Figure 4.13. Radius of gyration graph of TCAB1 protein in WT complex and F164L mutant complex.

This radius of gyration (Rg) plot compares the Rg values for the wild-type (WT) and F164L mutant versions of the TCAB1 protein. In the graph, the black line represents the WT protein, while the purple line represents the F164L mutant. The y-axis shows the Rg value in nanometres (nm), and the x-axis indicates the simulation time in nanoseconds (ns). The Rg value reflects the structural compactness or expansion of

the protein; lower Rg values indicate a more compact structure, whereas higher Rg values suggest a more extended conformation.

For the WT protein, the Rg value remains relatively stable around 2.06 to 2.08 nm throughout the simulation. This stability implies that the WT protein maintains a structurally compact conformation with minimal structural fluctuations over time. In contrast, the F164L mutant exhibits lower Rg values, approximately within the range of 2.02 to 2.05 nm, indicating a more compact structure compared to the WT. Additionally, the F164L mutant shows more fluctuations in Rg over the course of the simulation, suggesting that this mutant may possess greater structural flexibility or dynamism relative to the WT protein.

In summary, the F164L mutation appears to induce a more compact conformation in the TCAB1 protein and confers increased structural flexibility when compared to the WT. Such changes could potentially impact the protein's functional properties, as structural compactness and dynamic motion are often associated with protein-protein or protein-DNA interactions, as well as with overall functionality. This interpretation is based on the general trends observed in the plot; however, a more comprehensive analysis would require additional structural or functional data for contextual support.

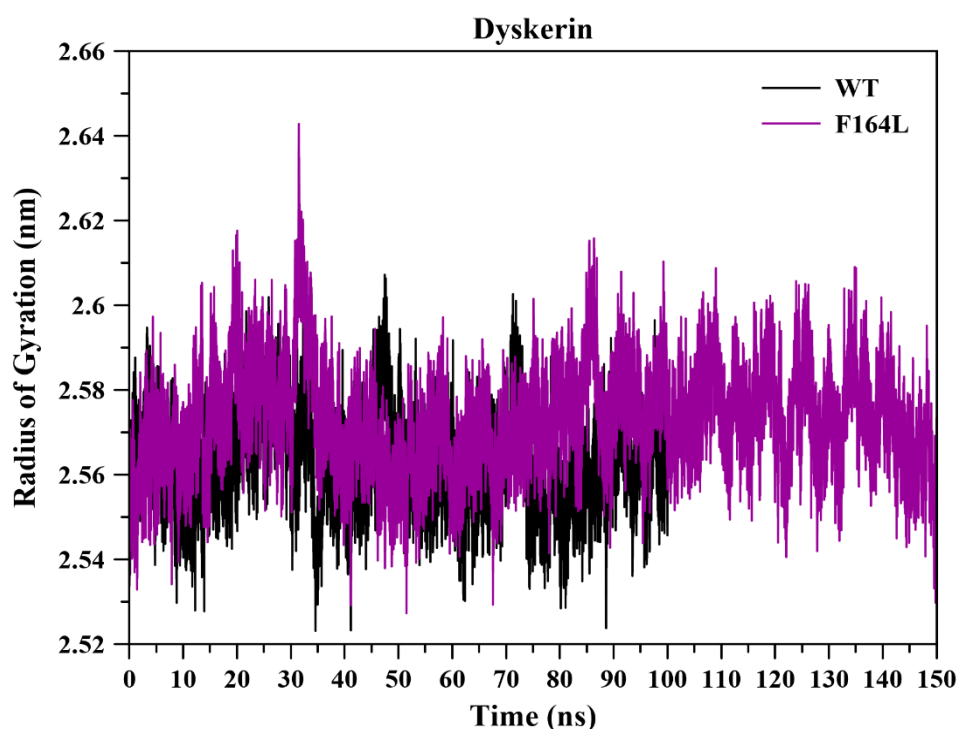


Figure 4.14. Radius of gyration graph of dyskerin protein in WT complex and F164L mutant complex.

As can be understood in Figure 4.14., the Rg values for the WT Dyskerin protein display relatively stable behaviour throughout the simulation, fluctuating between approximately 2.54 and 2.58 nm. This stability suggests that the WT protein maintains a compact structure with minimal structural variations over time. In contrast, the F164L mutant shows slightly higher Rg values, generally ranging from about 2.56 to 2.60 nm, indicating a somewhat less compact conformation. Additionally, the F164L mutant exhibits greater fluctuations in Rg, implying an increased level of flexibility or structural dynamism compared to the WT.

Overall, the F164L mutation appears to result in a modestly expanded and more flexible structure in Dyskerin relative to the WT. These alterations could impact the protein's functional interactions and stability, as shifts in compactness and flexibility can influence the way the protein engages with other molecules and fulfils its biological roles.

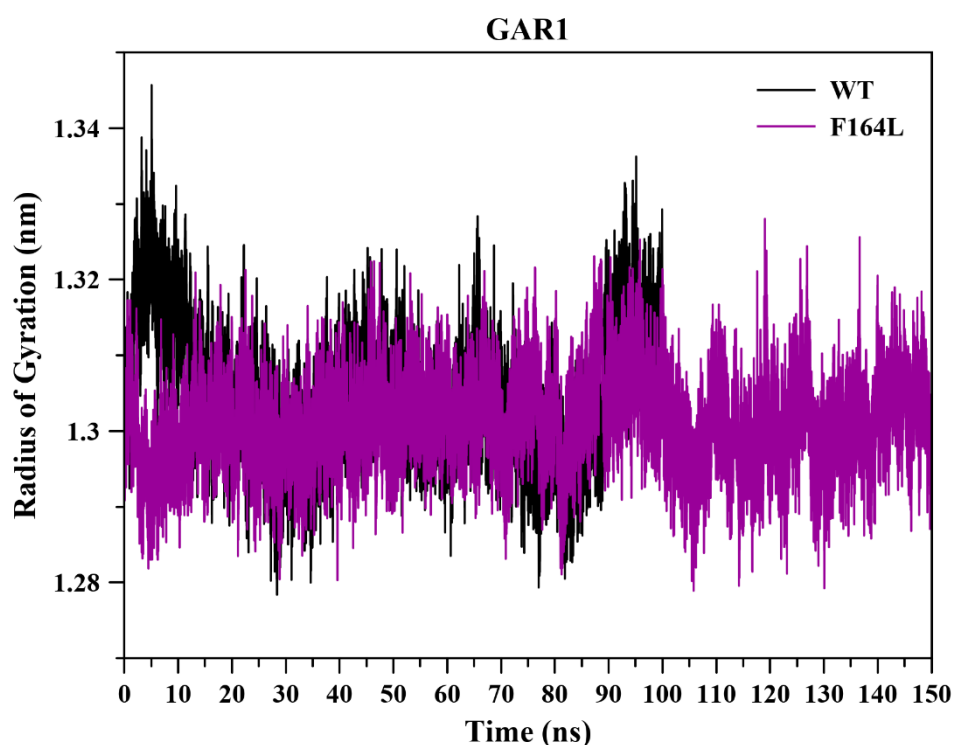


Figure 4.15. Radius of gyration graph of GAR1 protein in WT complex and F164L mutant complex.

As seen in Figure 4.15, the Rg values for the WT GAR1 protein are relatively stable, fluctuating around 1.30 to 1.32 nm, indicating a consistent, compact structure over the

simulation period. In contrast, the F164L mutant generally shows slightly lower Rg values, mostly ranging between 1.28 and 1.30 nm, suggesting a marginally more compact conformation compared to the WT. The Rg fluctuations in the F164L mutant are also slightly more pronounced, which may indicate an increase in flexibility or structural variability.

Overall, the F164L mutation in GAR1 appears to induce a more compact structure with a slight increase in flexibility relative to the WT. These changes could potentially affect the protein's interaction dynamics and functional stability, as variations in compactness and flexibility can influence how proteins engage with other molecular partners.

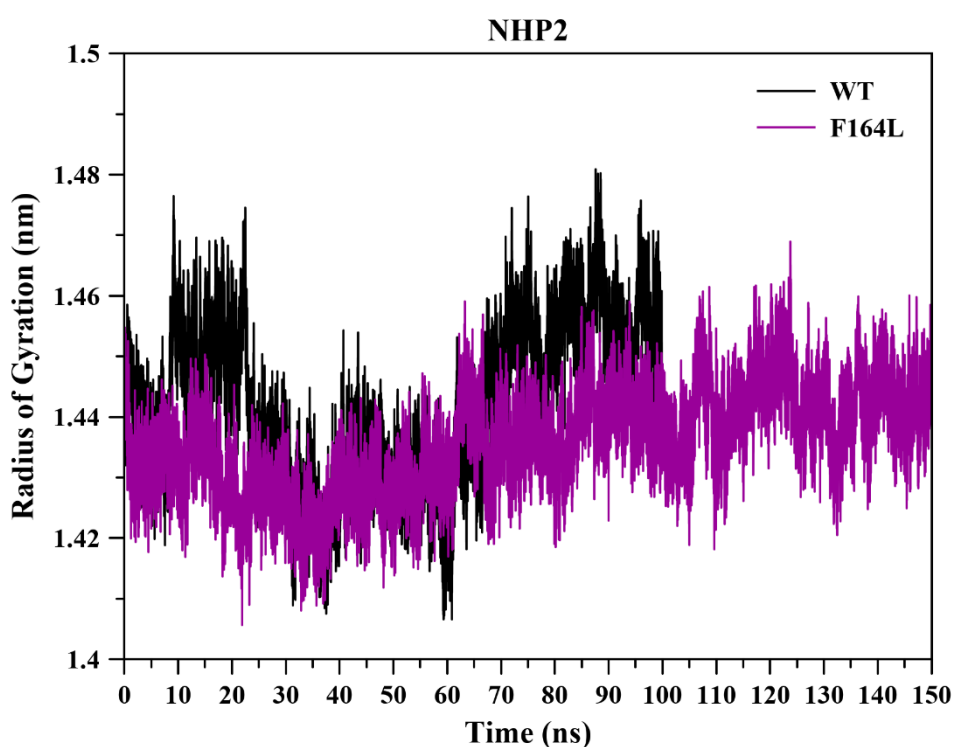


Figure 4.16. Radius of gyration graph of NHP2 protein in WT complex and F164L mutant complex.

In Figure 4.16, the Rg values for the WT NHP2 protein exhibit fluctuations primarily between 1.44 and 1.46 nm, indicating a relatively compact and stable structure throughout the simulation. In comparison, the F164L mutant generally has lower Rg values, fluctuating around 1.42 to 1.44 nm, suggesting a slightly more compact conformation than the WT protein. Additionally, the F164L mutant shows less

variability in Rg values over time, which could imply a more rigid or stable structure relative to the WT.

In summary, the F164L mutation in NHP2 appears to result in a modest increase in compactness and potentially reduces the structural flexibility compared to the WT. These structural changes may influence the protein's functional behaviour, as variations in compactness and rigidity can affect interaction dynamics and stability. However, further experimental or computational analyses would be necessary to fully understand the functional implications of these observed structural modifications.

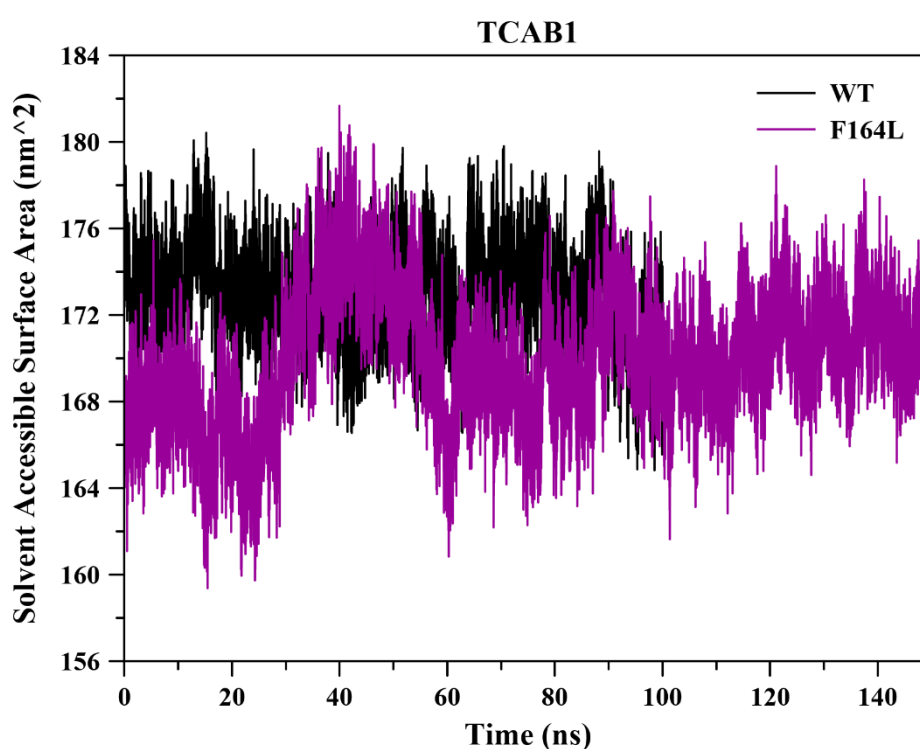


Figure 4.17. Solvent-accessible surface area (SASA) graph of TCAB1 protein in WT complex and F164L mutant complex.

As seen in Figure 4.17., the solvent-accessible surface area (SASA) plot for TCAB1 shows a comparison between the WT and F164L mutant forms over time. SASA values indicate the extent to which a protein's surface is exposed to the solvent, with higher values suggesting a more exposed structure and lower values indicating a more compact or buried conformation.

In this plot, the SASA values for the WT protein fluctuate between approximately 170 and 180 nm², indicating a relatively stable exposure level throughout the simulation.

The F164L mutant, on the other hand, generally displays slightly lower SASA values, mostly ranging from about 166 to 176 nm², suggesting a more compact or less solvent-exposed conformation compared to the WT.

The F164L mutant's reduced SASA values align with the radius of gyration observations, which also indicated a more compact structure. This reduced solvent exposure could affect the protein's interactions with other molecules, as less surface area is available for binding. Additionally, the F164L mutant displays a greater degree of fluctuation in SASA, which may imply increased flexibility in how its surface regions interact with the surrounding environment.

Overall, the F164L mutation in TCAB1 appears to lead to a more compact structure with reduced solvent accessibility, which could impact its functional dynamics and interactions. This interpretation suggests that the mutation might affect the protein's ability to engage in interactions that require an exposed surface, but further studies would be needed to confirm these functional implications.

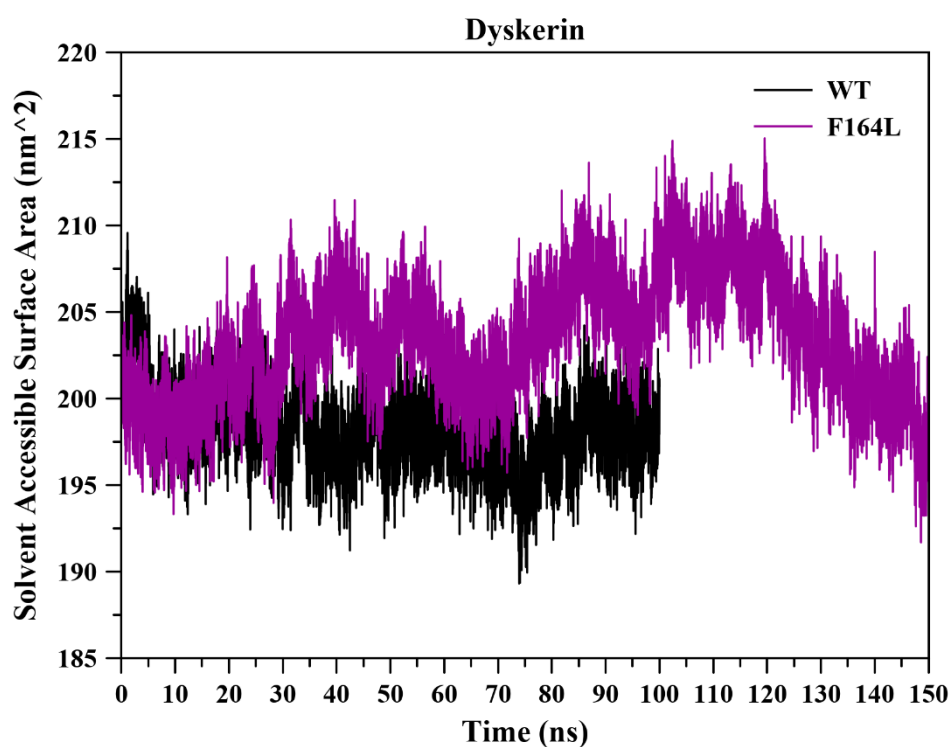


Figure 4.18. Solvent-accessible surface area graph of Dyskerin protein in WT complex and F164L mutant complex.

The SASA plot for Dyskerin shows a clear difference between the WT and F164L mutant forms over time. The WT protein exhibits SASA values that generally fluctuate between 195 and 205 nm², indicating a stable level of solvent exposure. This stability suggests that the WT Dyskerin maintains a consistent degree of surface exposure throughout the simulation.

In contrast, the F164L mutant displays higher SASA values, primarily ranging from 205 to 215 nm², which indicates an overall increase in solvent exposure compared to the WT. This increase suggests that the F164L mutation leads to a more open or extended structure, making more of the protein surface accessible to the solvent. The F164L mutant also shows greater fluctuation in SASA values, particularly in the latter part of the simulation, which could imply increased flexibility or structural variability.

Overall, the F164L mutation in Dyskerin appears to induce a less compact structure with greater solvent accessibility, which may affect its interaction properties and stability. Increased solvent exposure might influence the protein's ability to interact with other molecules or affect its stability in the cellular environment.

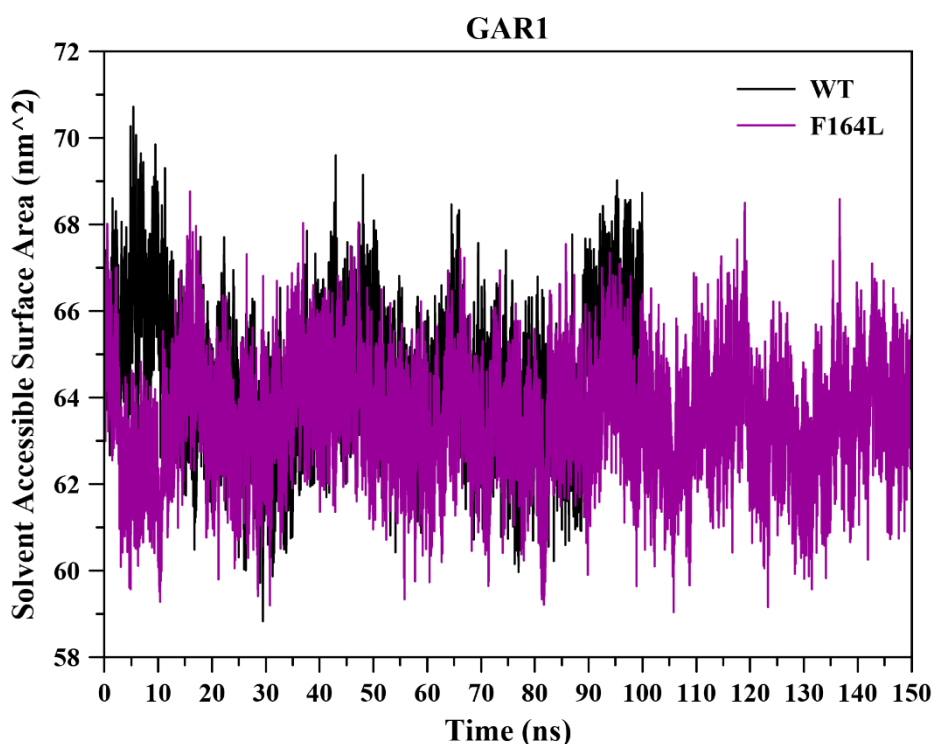


Figure 4.19. Solvent-accessible surface area graph of GAR1 protein in WT complex and F164L mutant complex.

As seen in Figure 4.19., the WT GAR1 protein maintains SASA values generally between 64 and 68 nm², indicating a stable level of solvent accessibility and structural compactness throughout the simulation. This relatively narrow range of fluctuation suggests that the WT form of GAR1 maintains a consistent degree of exposure.

In contrast, the F164L mutant displays slightly lower SASA values, mostly fluctuating between 62 and 66 nm², which indicates a marginally more compact conformation than the WT. This reduced solvent accessibility suggests that the F164L mutation may cause GAR1 to adopt a more closed structure. Additionally, the F164L mutant shows slightly less variability in SASA compared to the WT, potentially indicating a more rigid or stable structure in terms of solvent exposure.

Overall, the F164L mutation in GAR1 appears to lead to a slight reduction in solvent accessibility, which could influence the protein's interaction profile by limiting the available surface area for binding interactions. The reduced SASA might reflect a conformational shift towards a more compact and possibly less flexible structure.

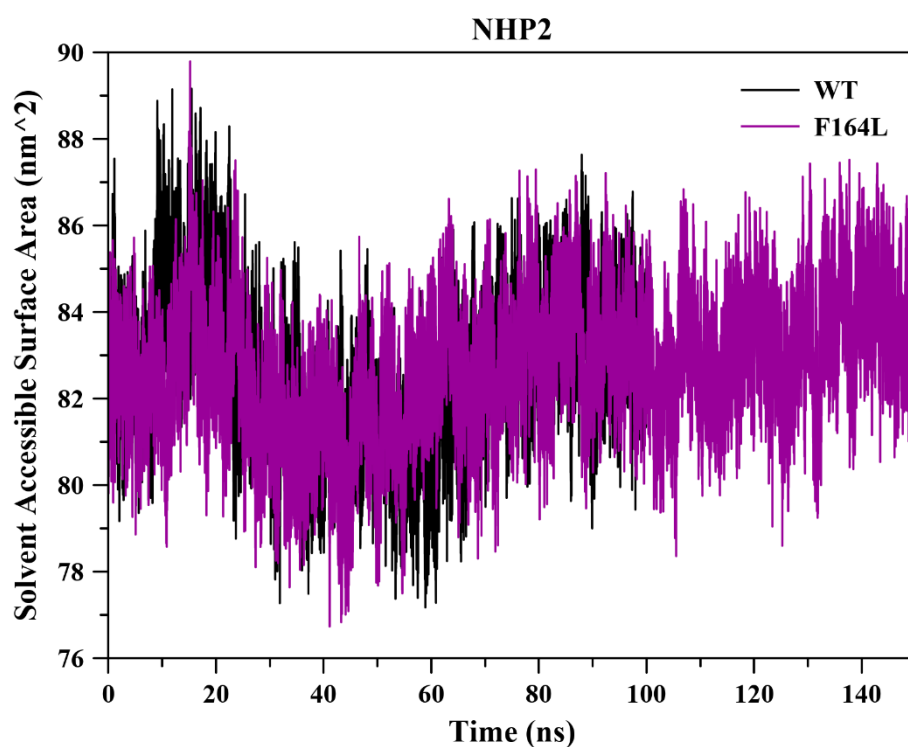


Figure 4.20. Solvent-accessible surface area graph of NHP2 protein in WT complex and F164L mutant complex.

In Figure 4.20., the WT protein generally exhibits SASA values fluctuating between 80 and 86 nm², suggesting a stable level of solvent exposure with slight variations. This indicates that the WT NHP2 maintains a relatively consistent surface area accessible to the solvent throughout the simulation.

In contrast, the F164L mutant displays slightly lower SASA values, mostly ranging between 78 and 84 nm², which suggests a more compact structure with reduced solvent accessibility compared to the WT. The F164L mutant also exhibits greater fluctuations in SASA values, which may reflect an increase in flexibility or structural variability, even though it remains slightly more compact overall.

In summary, the F164L mutation in NHP2 appears to result in a marginally more compact conformation with somewhat reduced solvent accessibility. This structural alteration may impact NHP2's functional properties by potentially reducing available surface area for interactions, although the increased fluctuation could imply dynamic adaptability in specific environmental conditions. Further investigation would be necessary to fully understand the functional implications of these observed changes in SASA.

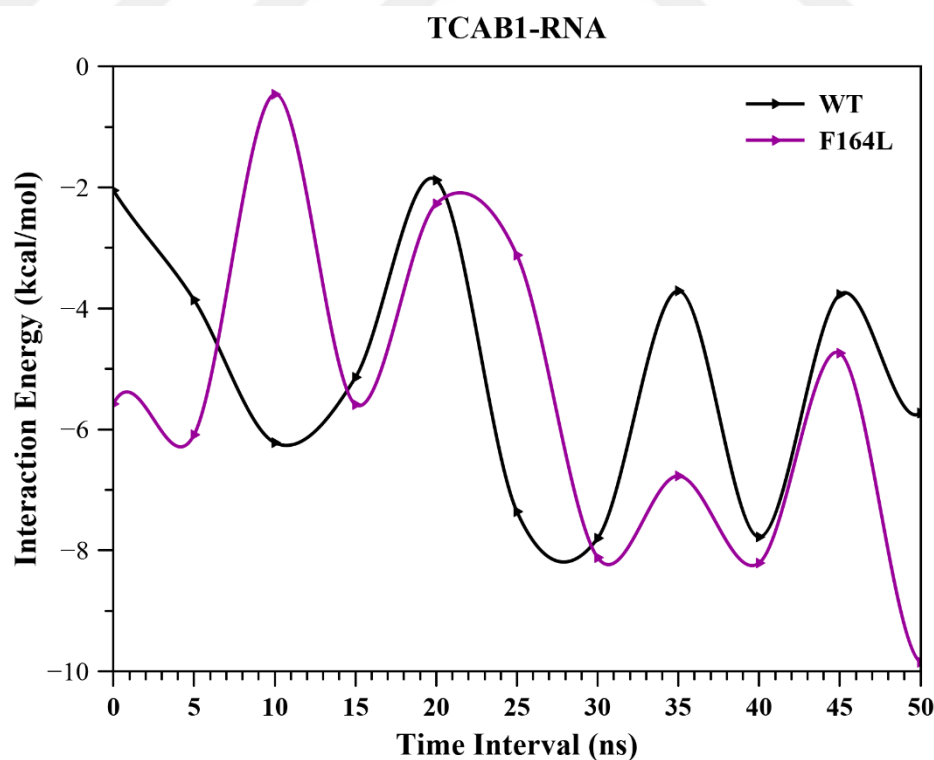


Figure 4.21. Interaction energy profile of WT and F164L mutant TCAB1-RNA complexes over time.

As seen in Figure 4.21., the interaction energy profile for the TCAB1-RNA complex in the WT and F164L mutant displays noticeable differences in stability and binding strength. Both profiles indicate relatively strong interactions, with average energies around -5 kcal/mol for the wild-type and -5.5 kcal/mol for the mutant. The slight increase in the magnitude of the interaction energy in the mutant suggests that the F164L mutation may enhance binding affinity slightly compared to the WT.

Furthermore, the fluctuation patterns reveal that while both the wild-type and mutant interactions exhibit variability, the mutant profile shows somewhat higher peaks and valleys, indicating larger energy fluctuations. These fluctuations suggest that although the F164L mutant may form stronger interactions with RNA, it may also experience dynamic instability, potentially due to conformational adjustments within the binding interface. This combination of stronger binding energy but increased variability may imply a more flexible but less stable interaction in the mutant, allowing for both enhanced binding affinity and increased adaptability in RNA interaction.

4.2. WT and H376Y Mutant TCAB1 Results

Both WT and H376Y mutant TCAB1 proteins in the complex were subjected to 100 ns MD simulations. RMSD values of each simulation were calculated and plotted (Figure 4.22). As seen in Figure 4.22, the H376Y mutant was stabilized at almost the same value as the WT, and not much difference was observed between the RMSD plots. The RMSF graph in Figure 4.23. displayed here represents the comparative analysis of the root mean square fluctuation values for the WT and the H376Y mutant of the TCAB1 protein. The graph distinctly highlights regions with significant fluctuations in the mutant when compared to the WT.

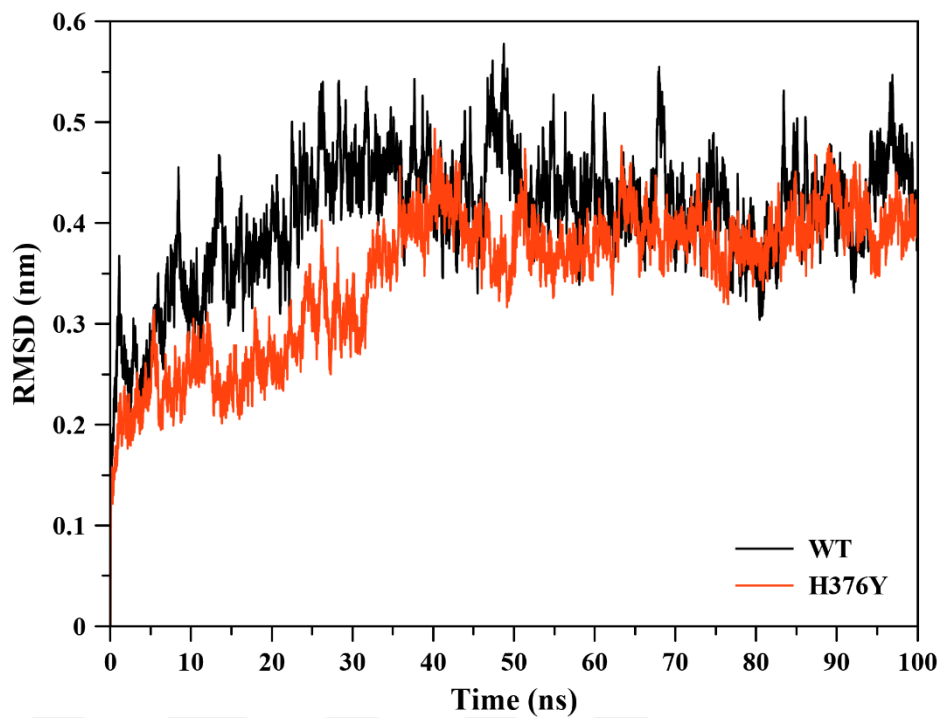


Figure 4.22 RMSD values of WT and H376Y mutant complex.

The regions shaded in red, specifically spanning residues 288 to 426, indicate areas where the H376Y mutant exhibits higher RMSF values than the WT. These elevated RMSF values suggest increased flexibility or dynamic behaviour in this extensive segment of the mutant protein. Such heightened flexibility within this range could potentially influence the mutant protein's structural integrity and functional interactions.

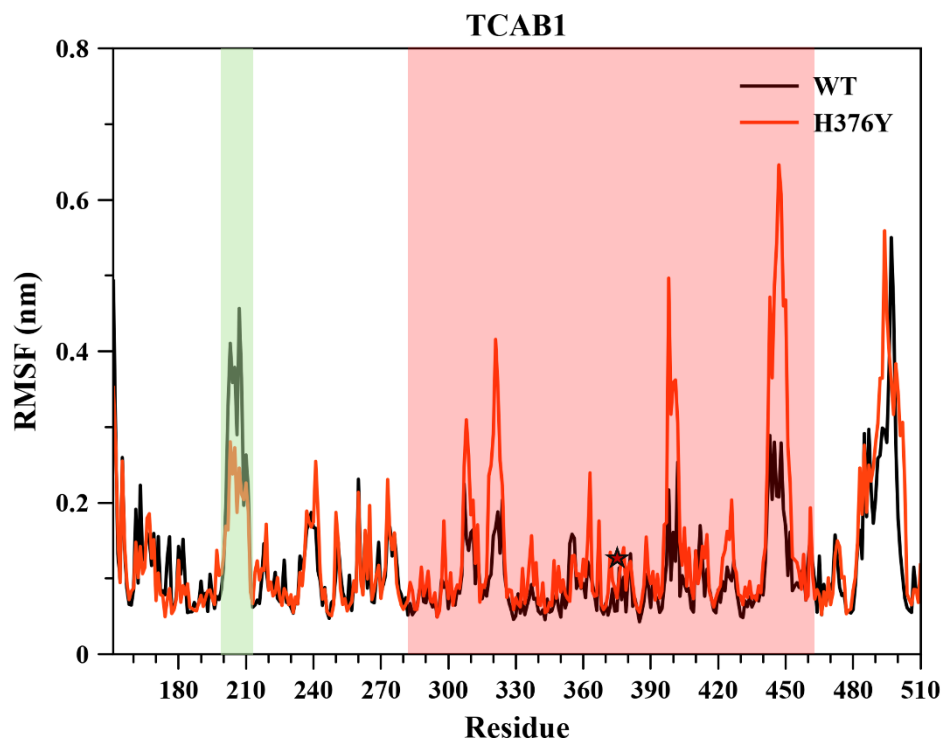


Figure 4.23. Comparison of RMSF values of WT and H376Y mutant TCAB1 protein in complexes. The star shows the place of mutation.

Conversely, the region shaded in green, encompassing residues 202 to 210, shows lower RMSF values in the mutant compared to the wild type. This decreased flexibility in the H376Y mutant within these residues suggests a more rigid and possibly more stable structure in this specific domain.

These variations in RMSF values between the WT and the H376Y mutant underscore the potential structural and functional impacts of the mutation. Increased flexibility in certain segments of the mutant may affect how the protein interacts with other molecules or its overall stability, whereas decreased flexibility in other areas might confer enhanced structural rigidity, possibly altering the protein's interaction capabilities or its role in cellular processes. This detailed RMSF analysis provides crucial insights into how specific mutations like H376Y can affect the dynamic properties of proteins and potentially influence their biological roles.

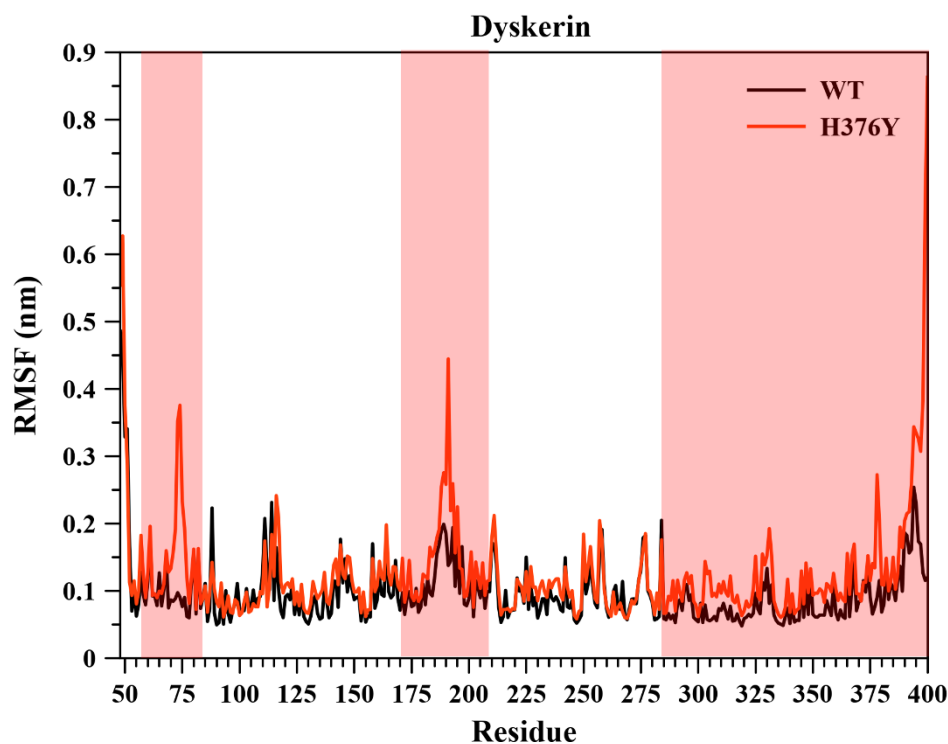


Figure 4.24. Comparison of RMSF values of WT and H376Y mutant dyskerin protein in complexes.

As seen in Figure 4.24, the RMSF graph for the Dyskerin protein highlights notable differences in flexibility between the wild-type (WT) and the H376Y mutant, particularly in regions shaded in red, which correspond to amino acid segments 68-82, 170-207, and 285-400. These regions show significantly higher RMSF values for the H376Y mutant compared to the WT, indicating increased molecular dynamics and flexibility within these domains.

The elevated flexibility observed in these areas suggests that the H376Y mutation may lead to alterations in the structural integrity of Dyskerin. This increased dynamic behaviour could potentially affect the protein's functional roles, including telomere maintenance. Specifically, the heightened flexibility in these segments could impact dyskerin's ability to interact effectively with RNA and other telomere-associated proteins, possibly influencing the stability and functionality of the telomerase complex.

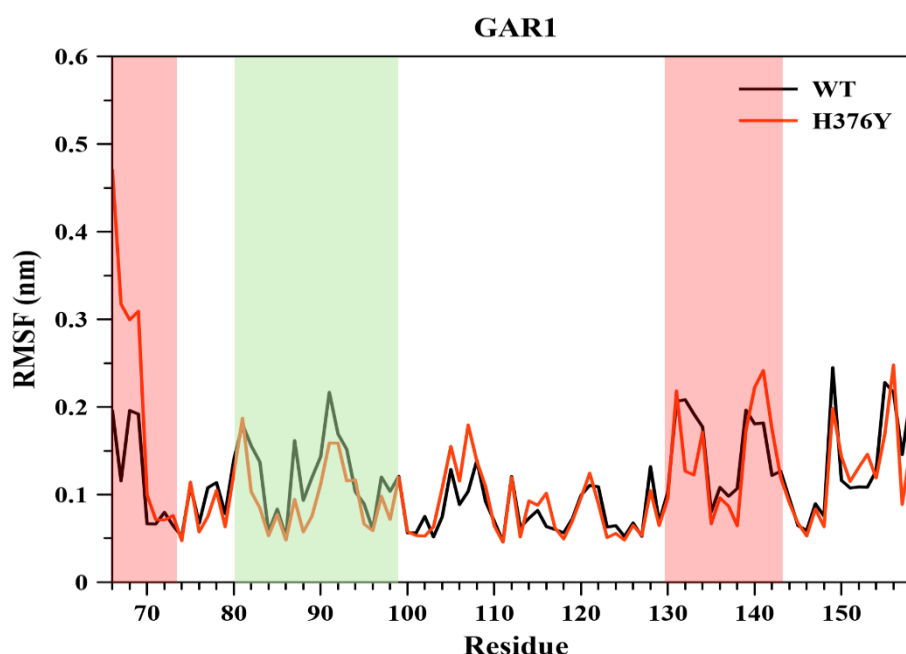


Figure 4.25. Comparison of RMSF values of WT and H376Y mutant GAR1 protein in complexes.

In Figure 4.25., the RMSF graph for the GAR1 protein illustrates the differences in flexibility between the WT and the H376Y mutant across specific amino acid residues. Notably, regions highlighted in red, which include amino acid segments 66-73 and 130-142, exhibit higher RMSF values in the H376Y mutant compared to the WT. This indicates increased flexibility in these segments of the mutant protein, suggesting that the H376Y mutation contributes to enhanced molecular dynamics within these areas.

Conversely, the region shaded in green, spanning residues 80-99, shows lower RMSF values for the mutant compared to the WT. This decreased flexibility in the H376Y mutant within this segment suggests a more rigid and potentially more stable structure in this specific domain.

These variations in RMSF values are indicative of how the H376Y mutation alters the structural dynamics of the GAR1 protein. The increased flexibility in certain regions might affect the mutant's interaction capabilities, potentially influencing its functional roles in cellular processes. Meanwhile, the decreased flexibility in other areas could confer structural stability, which might alter the protein's functional behaviour or interaction with other molecular components. Understanding these dynamics is crucial for assessing the overall impact of the H376Y mutation on the biological functions of GAR1.

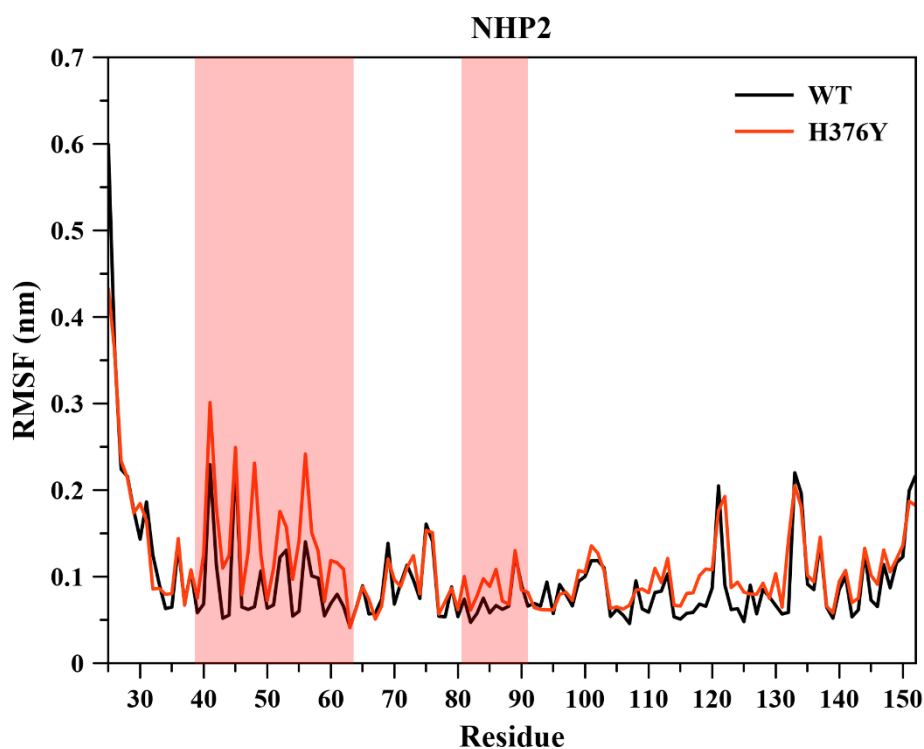


Figure 4.26. Comparison of RMSF values of WT and H376Y mutant NHP2 protein in complexes.

The RMSF graph for the NHP2 protein highlights significant differences in flexibility between the WT and the H376Y mutant, particularly within specific regions that are marked in red on the graph. These regions, spanning residues 38-62 and 81-89, exhibit higher RMSF values in the H376Y mutant compared to the WT, indicating an increase in flexibility. This enhanced molecular dynamics within these segments suggests that the H376Y mutation contributes to a more flexible and potentially less stable structure in these parts of the NHP2 protein.

The increase in flexibility associated with the H376Y mutation in these areas may influence the structural integrity of the NHP2 protein and potentially affect its interaction with other molecular components within the cell. Such changes in the dynamic properties of the protein could alter its functional roles, particularly in processes where NHP2 is a critical participant.

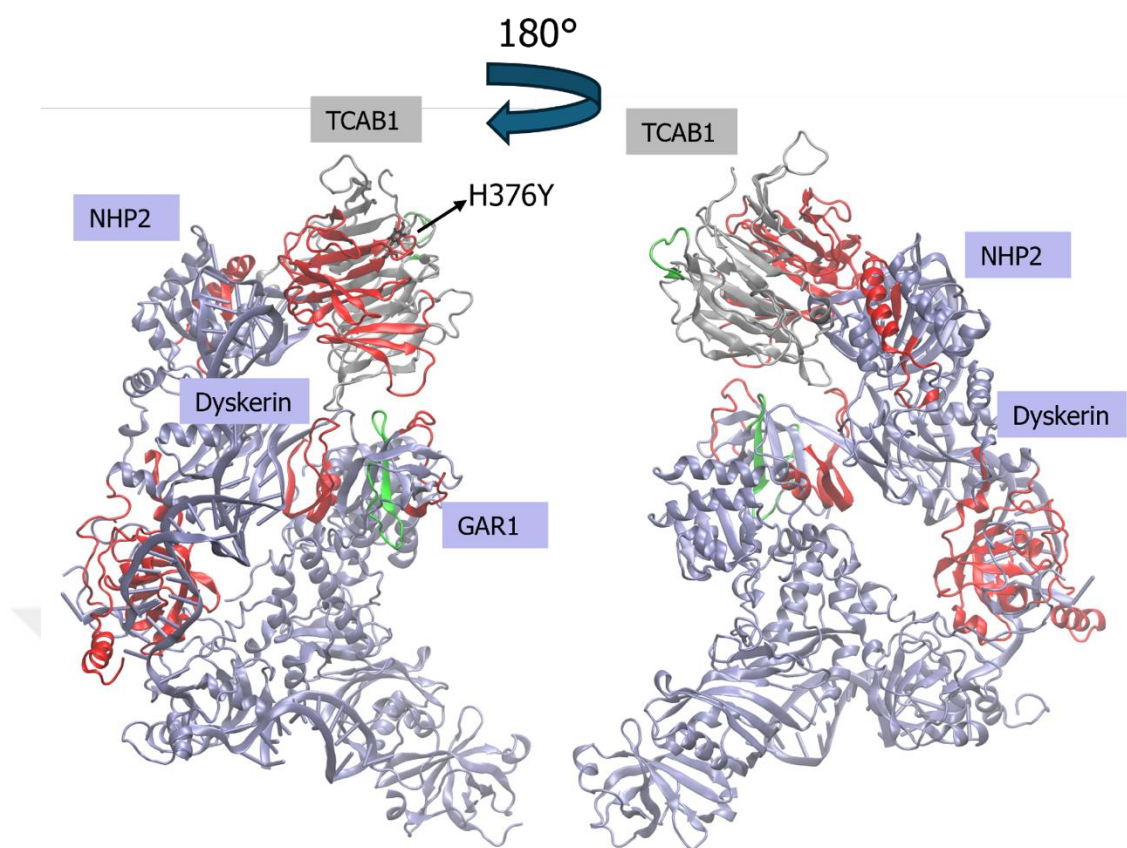


Figure 4.27. H376Y complex structure. The protein shown in grey is the TCAB1 protein, the rest of the complex is shown in metallic blue.

Figure 4.27 displays the H376Y mutant complex, which comprises a single TCAB1 molecule, one RNA molecule, and two copies each of dyskerin, GAR1, NHP2, and NOP10 proteins. The H376Y mutation is located on the TCAB1 protein and is indicated in the image by a black arrow. The two images presented are rotations of each other, each shown at a 180-degree angle.

In this visual representation, amino acid sequences marked in red denote areas where the RMSF (root mean square fluctuation) values of the mutant protein are higher than those of the wild-type (WT) protein. Conversely, sequences highlighted in green indicate regions where the RMSF values are lower in the mutant compared to the WT. These regions align with the areas described in the RMSF value table discussions for each protein interacting with TCAB1.

According to the detailed analysis, an increase in RMSF values is observed in nearly all the regions across the complex. Specifically, all regions showing changes in the NHP2 and dyskerin proteins exhibit increased RMSF. In contrast, only a single region

in both the TCAB1 and GAR1 proteins shows a decrease in RMSF. Notably, the area of reduced RMSF in the GAR1 protein corresponds to its interaction domain with TCAB1, whereas the region of decreased RMSF in TCAB1 does not align with any interaction domains with other proteins. An increase in RMSF has been detected across all domains of TCAB1 that interact with other proteins and RNA.

This comprehensive visualization and analysis underscore the significant impact of the H376Y mutation on the structural dynamics of the TCAB1 protein and its interactions within the complex. The observed alterations in RMSF values suggest modifications in the protein's flexibility and stability, potentially affecting its functional interactions and overall biological roles within the cellular environment.

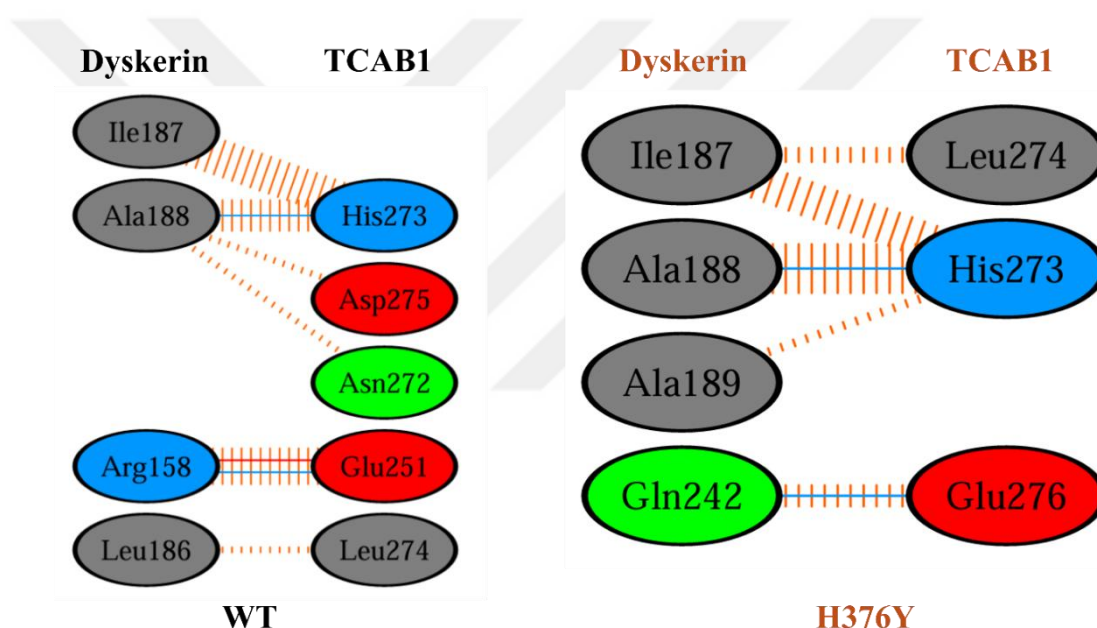


Figure 4.28. Comparative analysis of the interactions between WT TCAB1 and the H376Y mutant TCAB1 proteins with the dyskerin protein within the complex structures.

As seen in Figure 4.28, the comparison of interactions between TCAB1 and dyskerin in the WT and H376Y mutant forms reveals how the mutation influences the binding interface. In the WT complex, there are multiple stabilizing interactions, including non-bonded contacts and hydrogen bonds. Specifically, Ala188 on dyskerin forms hydrogen bonds with His273 on TCAB1, while Arg158 on TCAB1 interacts with Glu251 on dyskerin through both hydrogen bonds and non-bonded contacts. Additionally, Asn272 on dyskerin forms a stabilizing contact, further strengthening

the WT interaction. These interactions suggest a moderately stable binding interface in the WT complex, supported by various contacts.

In the H376Y mutant, the interaction landscape changes, with some interactions being preserved while others are modified. Ala188 continues to interact with His273, but additional contacts are now observed, such as the new interactions between Gln242 on dyskerin and Glu276 on TCAB1. This new interaction may serve to stabilize the mutant complex further, potentially compensating for any structural disruptions introduced by the H376Y mutation. The presence of Gln242-Glu276 interaction, which was absent in the WT, suggests that the H376Y mutation reconfigures the interface to introduce new stabilizing interactions, possibly enhancing the binding affinity between TCAB1 and dyskerin.

Overall, the H376Y mutation appears to restructure the binding interface, maintaining certain WT interactions while introducing new contacts that could contribute to a more robust interaction. The additional interactions likely enhance stability by providing alternative pathways for molecular interactions, thereby compensating for the structural effects of the mutation. This suggests that the H376Y mutation, despite altering the original interface, may support a functionally resilient complex due to these adaptive interaction changes.

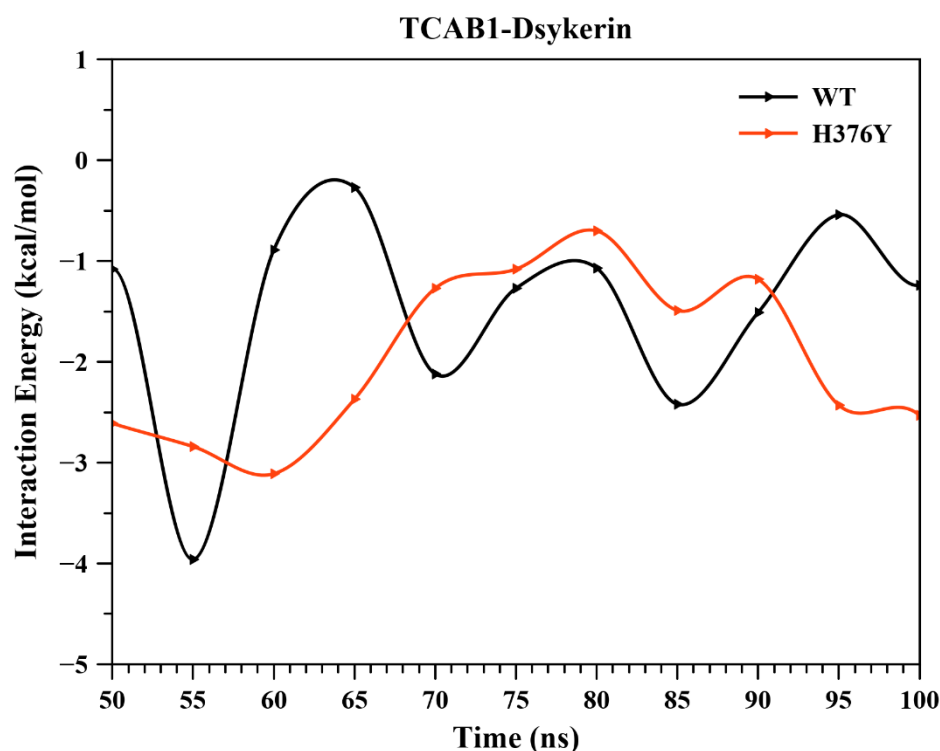


Figure 4.29. Interaction energy profile of WT and H376Y mutant TCAB1-Dyskerin complexes over time.

In Figure 4.29, the interaction energy graph for the TCAB1-dyskerin complex in both the wild-WT and H376Y mutant forms indicates that the H376Y mutation has a stabilizing effect on the complex. The average interaction energy in the WT complex is -1.5 kcal/mol, suggesting a moderately stable but fluctuating interaction. The WT energy profile shows considerable fluctuations, with energy levels periodically rising closer to 0 kcal/mol, which reflects moments of reduced binding affinity and transient stability in the interaction between TCAB1 and dyskerin.

In contrast, the H376Y mutant complex has a more negative average interaction energy of -2 kcal/mol, indicating an increase in binding affinity and stability compared to the WT. The energy fluctuations in the H376Y mutant, although still present, tend to stabilize at a lower, more favourable energy level than in the WT. This suggests that the mutation enhances the interaction, likely due to the additional stabilizing contacts observed in the mutant, such as the new interaction between Gln242 on dyskerin and Glu276 on TCAB1. This additional interaction may contribute to the more stable binding interface seen in the H376Y mutant, helping to maintain a more energetically favourable association.

Overall, the H376Y mutation appears to strengthen the TCAB1-dyskerin interaction by introducing new stabilizing contacts, leading to a more consistently negative interaction energy profile. This indicates a more robust binding interaction in the mutant complex, suggesting that the H376Y mutation enhances the stability and potentially the functional efficiency of the TCAB1-dyskerin complex. The decrease in energy fluctuations and more negative average interaction energy highlight the impact of the mutation in improving binding affinity and stability within the complex.

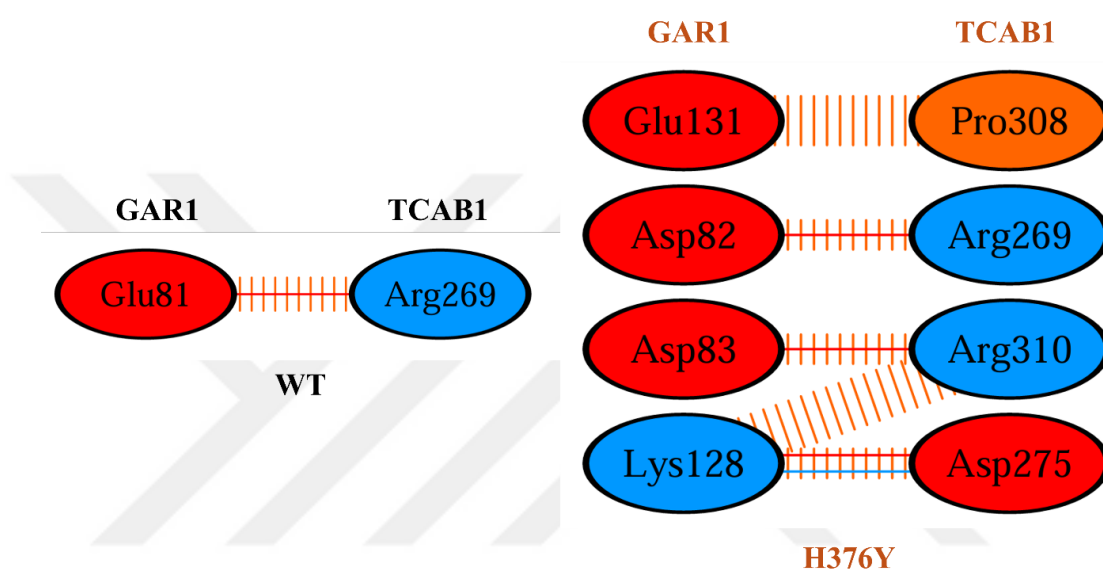


Figure 4.30. Comparative analysis of the interactions between WT TCAB1 and the H376Y mutant TCAB1 proteins with the GAR1 protein within the complex structures.

The interaction between TCAB1 and GAR1 shows significant enhancement in the H376Y mutant compared to the WT. In the WT form, the interaction is limited to a single non-bonded contact between Glu81 on GAR1 and Arg269 on TCAB1. This singular contact suggests a weak binding affinity in the WT complex, as it lacks additional stabilizing interactions that are typically essential for robust protein-protein binding. Such a limited interaction profile implies a transient and potentially unstable association between TCAB1 and GAR1 in the WT form.

In contrast, the H376Y mutation in TCAB1 introduces a range of additional contacts, transforming the interaction landscape with GAR1. In the mutant, Glu81 on GAR1 continues to interact with Arg269 on TCAB1, but further interactions are introduced.

Notably, Asp82 and Asp83 on GAR1 establish contacts with Arg310 on TCAB1, and Lys128 interacts with Asp275. Moreover, Glu131 on GAR1 forms multiple non-bonded contacts with Pro308 on TCAB1. This expansion of the binding interface significantly strengthens the connection between TCAB1 and GAR1 in the H376Y mutant, as the additional stabilizing interactions likely contribute to an increase in binding affinity and stability.

Overall, the H376Y mutation changes the TCAB1-GAR1 interaction from a weak, single-contact association in the WT to a robust, multi-contact interface in the mutant. This structural reorganization, featuring additional non-bonded contacts, suggests that the H376Y mutation enhances the binding affinity by introducing more stabilizing interactions, thereby potentially improving the functional stability and resilience of the TCAB1-GAR1 complex in a cellular environment.

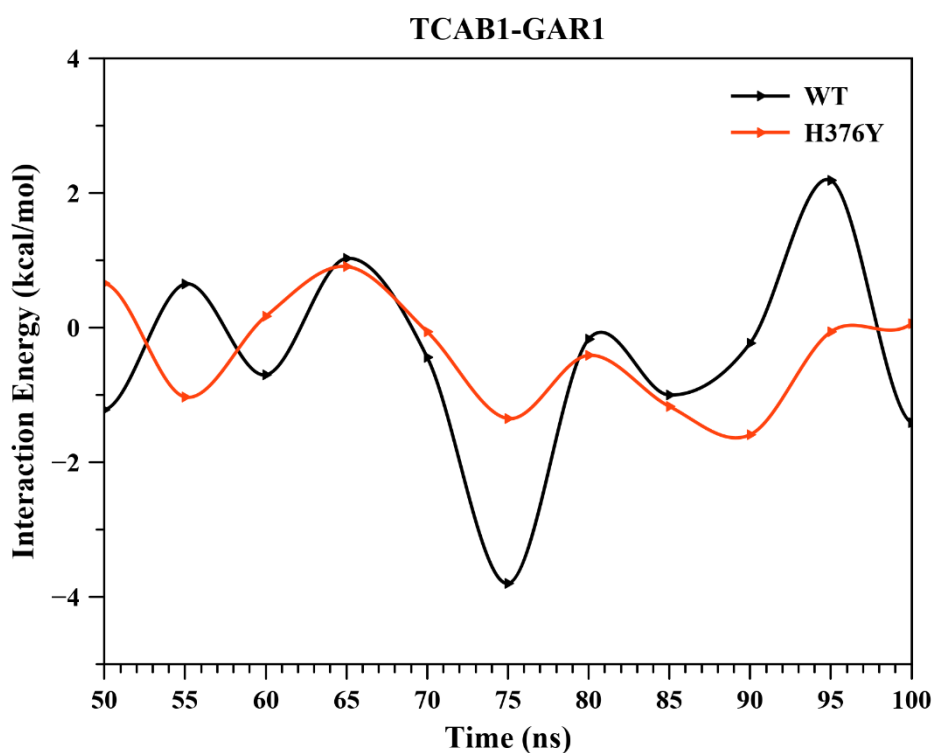


Figure 4.31. Interaction energy profile of WT and H376Y mutant TCAB1-GAR1 complexes over time.

As seen in Figure 4.31, the interaction energy graph for the TCAB1-GAR1 complex in both the WT and H376Y mutant forms indicates minimal difference in binding affinity between the two forms. The average interaction energy for the WT complex is

0.5 kcal/mol, while the H376Y mutant shows a slightly lower average of 0.4 kcal/mol. This similarity suggests that, overall, the H376Y mutation does not substantially alter the binding stability between TCAB1 and GAR1. Both profiles show energy values that oscillate around slightly positive values, which typically indicates a weaker and more transient interaction.

Despite the additional non-bonded contacts and stabilizing interactions introduced in the H376Y mutant, as observed in the residue interaction profile, these modifications do not result in a significantly stronger binding affinity. The energy fluctuations in both the WT and mutant complexes remain within a similar range, suggesting that while the H376Y mutation does introduce new contacts, these do not drastically enhance the binding stability of the complex. Thus, the H376Y mutation, although structurally altering the interface between TCAB1 and GAR1, has a limited impact on the energetic stability, maintaining a comparable interaction strength to that of the WT complex.

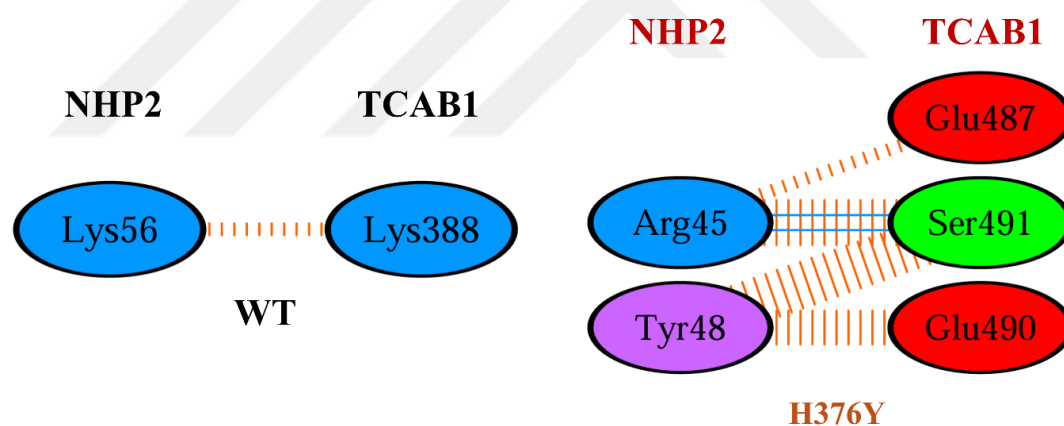


Figure 4.32. Comparative analysis of the interactions between WT TCAB1 and the H376Y mutant TCAB1 proteins with the NHP2 protein within the complex structures.

As seen in Figure 4.32, the interaction comparison between TCAB1 and NHP2 in the WT and H376Y mutant forms reveals a substantial increase in binding interactions within the H376Y mutant. In the WT complex, the interaction is limited to a single non-bonded contact between Lys56 on NHP2 and Lys388 on TCAB1, indicating a relatively weak association. This single contact suggests minimal stability and a

transient interaction in the WT complex, as it lacks multiple interactions that could strengthen the binding interface.

In the H376Y mutant, however, several additional interactions are observed. The mutant complex shows Arg45 on NHP2 forming hydrogen bonds with Ser491 on TCAB1 and Tyr48 on NHP2 engaging with Glu490 on TCAB1 through multiple non-bonded contacts. Additionally, Glu487 on TCAB1 establishes contacts with both Arg45 and Tyr48 on NHP2, creating a more intricate network of interactions. These added contacts likely contribute to enhanced binding stability and affinity in the H376Y complex.

Overall, the transition from a single-contact interaction in the WT to a multi-contact binding interface in the H376Y mutant may have suggests a significant strengthening of the TCAB1-NHP2 complex. This structural reorganization most likely increases the interaction's stability, as the new interactions in the mutant may provide additional points of contact that were absent in the WT.

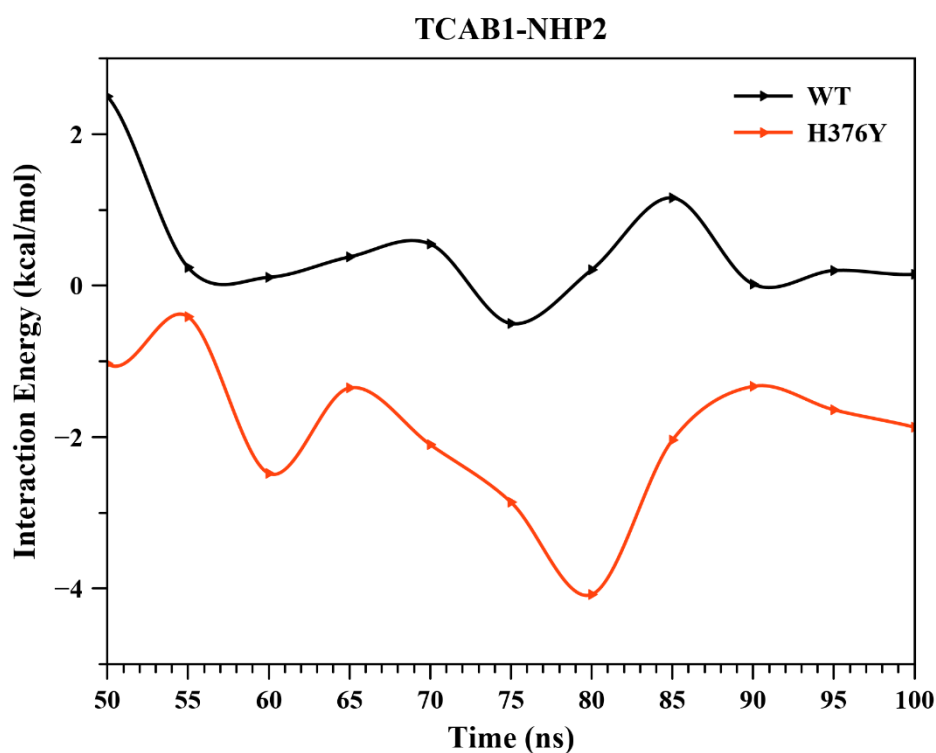


Figure 4.33. Interaction energy profile of WT and H376Y mutant TCAB1-NHP2 complexes over time.

As seen in Figure 4.33, the interaction energy profile between TCAB1 and NHP2 reveals a significant increase in binding affinity in the H376Y mutant compared to the wild-type (WT) form. The average interaction energy for the WT complex is 0.7 kcal/mol, indicating a relatively weak and unstable association. The WT energy fluctuates around positive values, suggesting a lack of strong binding interactions that would stabilize the TCAB1-NHP2 complex in its native state.

In contrast, the H376Y mutant exhibits an average interaction energy of -2 kcal/mol, with values consistently in the negative range. This lower interaction energy implies a much stronger and more stable binding in the mutant complex. The addition of new contacts, as observed in the residue interaction analysis (where multiple non-bonded interactions were introduced in the H376Y form), likely contributes to this enhanced binding affinity. The mutant's negative interaction energy reflects these additional stabilizing interactions, resulting in a more energetically favourable complex.

Overall, the H376Y mutation transforms the TCAB1-NHP2 interaction from a weak and transient association in the WT form to a significantly more stable and resilient complex. This shift is evidenced by the lower and more consistent negative energy values in the H376Y mutant, highlighting the mutation's impact on increasing the binding affinity between TCAB1 and NHP2.

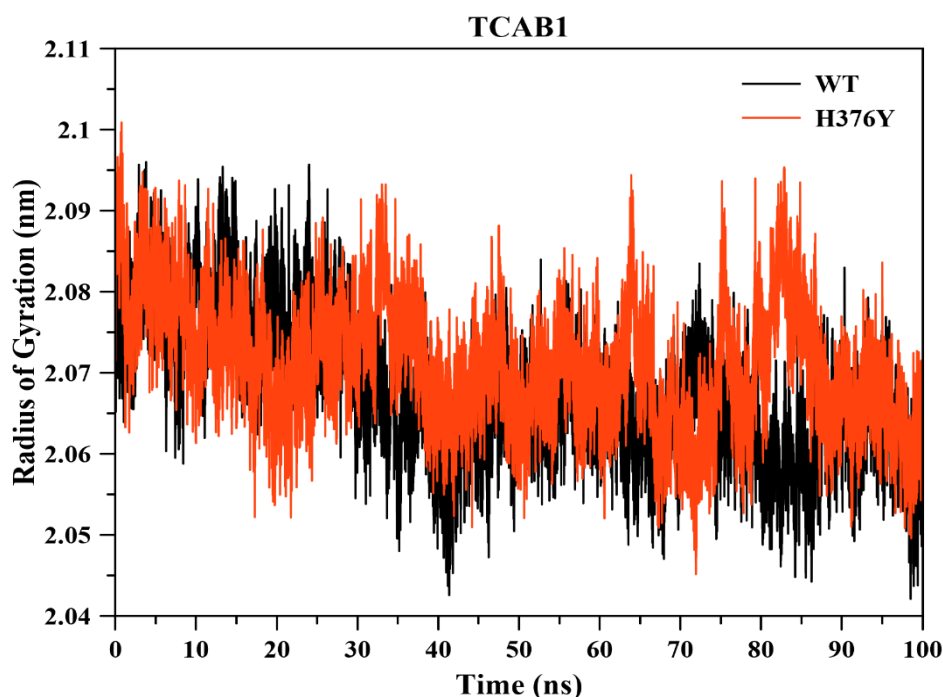


Figure 4.34. Radius of gyration graph of TCAB1 protein in WT complex and H376Y mutant complex.

As seen in the Figure 4.34, the Rg values for the WT protein fluctuate around 2.05 to 2.09 nm, indicating a relatively stable and compact structure. This stability suggests that the WT maintains a consistent conformation with minimal variations in compactness throughout the simulation.

The H376Y mutant, on the other hand, shows Rg values that are slightly higher, generally fluctuating between 2.06 and 2.10 nm. This slight increase suggests that the H376Y mutation results in a marginally expanded conformation compared to the WT, indicating a less compact structure. Additionally, the H376Y mutant displays more pronounced fluctuations in Rg, implying an increased level of structural flexibility or dynamism relative to the WT.

In summary, the H376Y mutation in TCAB1 appears to induce a modest expansion in structure and greater flexibility. These structural alterations may influence the protein's functional interactions by providing a slightly more open conformation, which could affect its stability and interaction with other molecules.

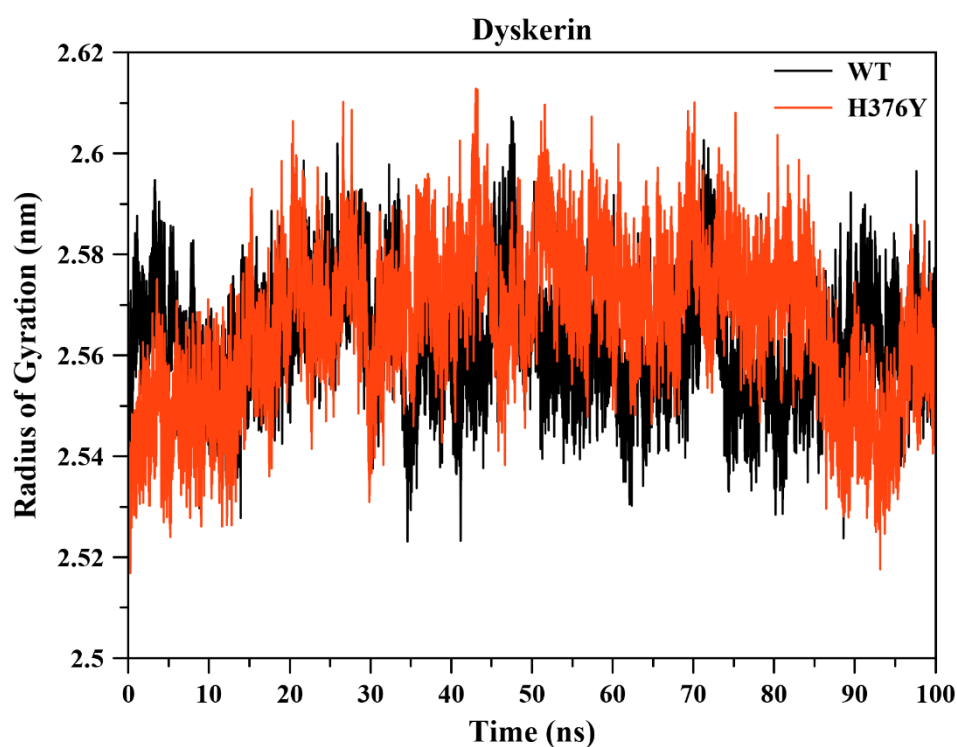


Figure 4.35. Radius of gyration graph of dyskerin protein in WT complex and H376Y mutant complex.

The WT Dyskerin protein exhibits relatively stable Rg values, generally fluctuating around 2.54 to 2.58 nm. This suggests that the WT form maintains a stable, compact structure throughout the simulation with minimal variations.

In comparison, the H376Y mutant shows slightly higher Rg values, fluctuating around 2.55 to 2.60 nm. This increase indicates that the H376Y mutation may induce a more expanded or less compact structure relative to the WT. Additionally, the H376Y mutant displays more pronounced fluctuations in Rg, suggesting an increase in structural flexibility or dynamic behaviour compared to the WT.

In summary, the H376Y mutation in Dyskerin appears to lead to a modestly expanded conformation and greater flexibility. These structural changes may impact the protein's functional dynamics, as increased flexibility and reduced compactness could alter its interaction capabilities and stability.

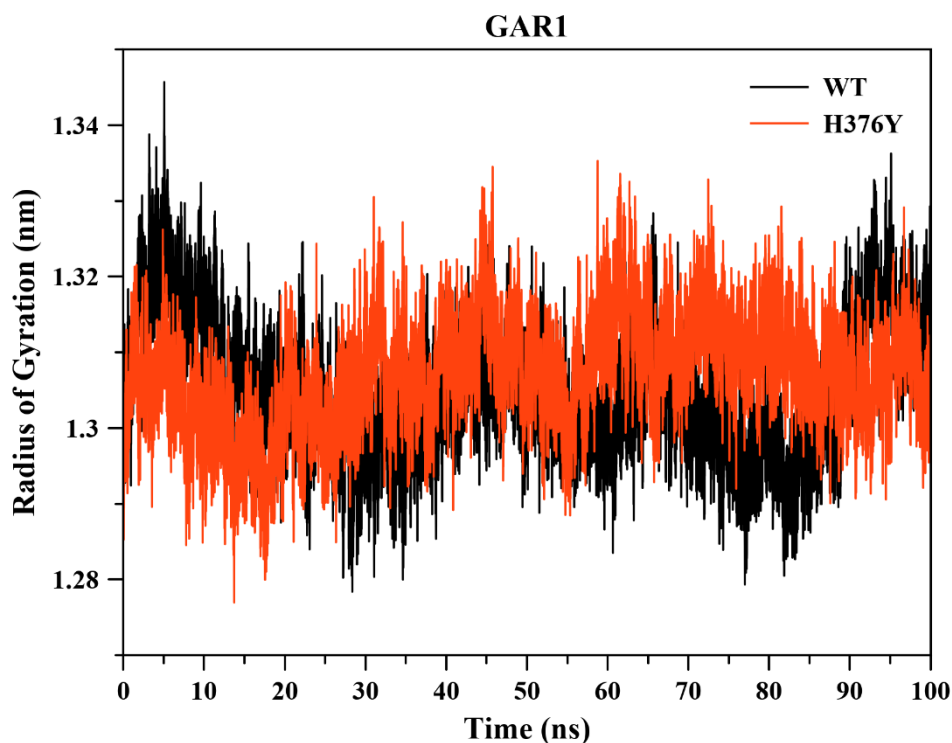


Figure 4.36. Radius of gyration graph of GAR1 protein in WT complex and H376Y mutant complex.

The WT GAR1 protein maintains relatively stable Rg values around 1.29 to 1.32 nm, suggesting a consistent, compact structure throughout the simulation.

In contrast, the H376Y mutant exhibits Rg values that are generally slightly higher, fluctuating between approximately 1.30 and 1.33 nm. This increase indicates that the H376Y mutation may induce a somewhat expanded conformation compared to the WT, suggesting a less compact structure. Additionally, the H376Y mutant shows more variability in Rg, indicating increased structural flexibility or dynamic behaviour.

In summary, the H376Y mutation in GAR1 appears to result in a modestly expanded structure and greater flexibility. These changes may influence the protein's interaction potential and stability, as alterations in compactness and flexibility can affect binding dynamics.

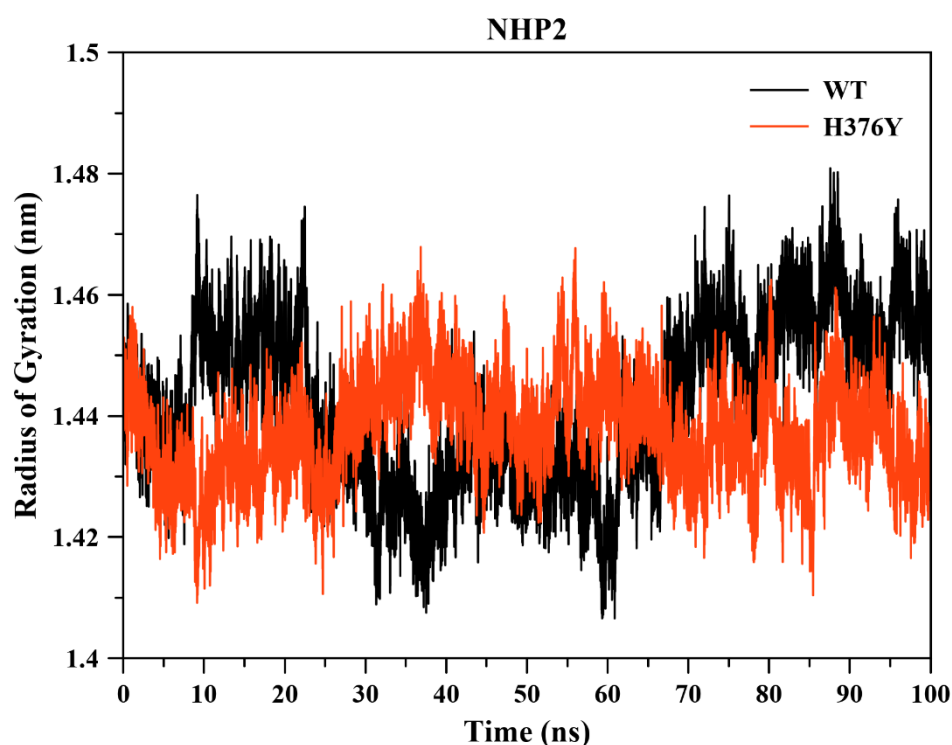


Figure 4.37. Radius of gyration graph of NHP2 protein in WT complex and H376Y mutant complex.

In Figure 4.37, the WT NHP2 protein displays Rg values fluctuating around 1.44 to 1.46 nm, indicating a relatively stable, compact structure with minimal structural variations.

In contrast, the H376Y mutant shows a slightly more compact structure, with Rg values primarily ranging from 1.42 to 1.44 nm. This slight reduction in Rg suggests that the H376Y mutation induces a more compact conformation in NHP2 relative to

the WT. Additionally, the H376Y mutant exhibits less fluctuation in Rg values over time, which may imply increased structural stability or rigidity.

In summary, the H376Y mutation in NHP2 appears to lead to a more compact and potentially more rigid structure compared to the WT. These changes may affect the protein's functional properties, as alterations in compactness and rigidity can influence binding interactions and stability.

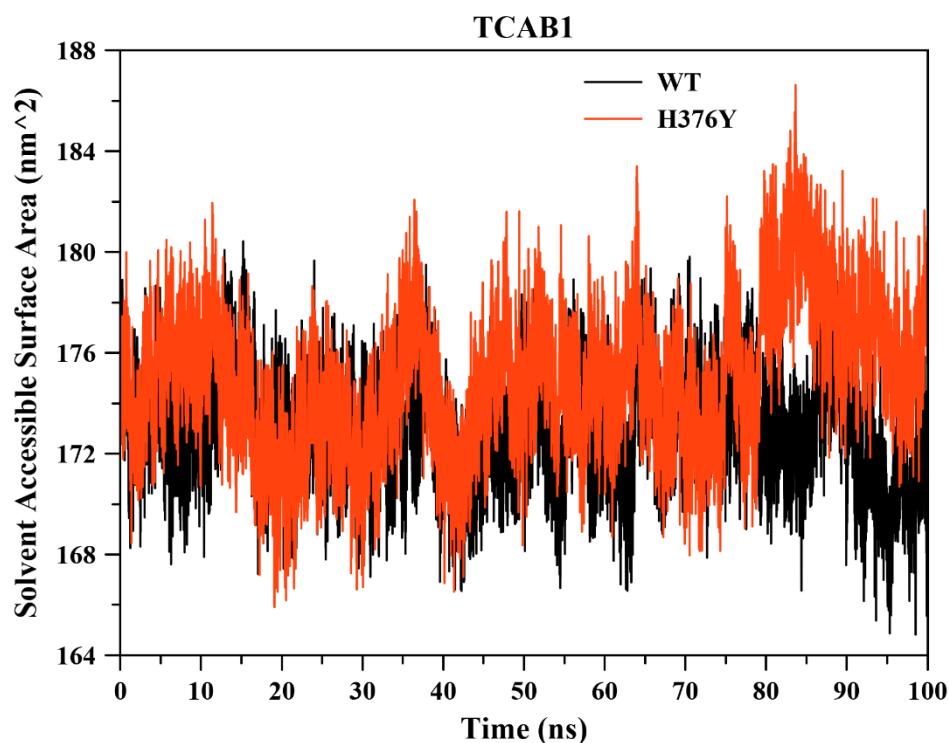


Figure 4.38. Solvent-accessible surface area graph of TCAB1 protein in WT complex and H376Y mutant complex.

The WT protein exhibits SASA values fluctuating between approximately 170 and 178 nm², indicating a stable level of solvent accessibility, which suggests a consistent degree of structural compactness.

In contrast, the H376Y mutant generally displays higher SASA values, fluctuating mostly between 174 and 184 nm². This increase in SASA suggests that the H376Y mutation results in a more solvent-exposed conformation, indicating a less compact structure than the WT. Additionally, the H376Y mutant shows greater fluctuations in SASA, implying increased flexibility or structural variability.

In summary, the H376Y mutation in TCAB1 appears to induce a more open structure with greater solvent accessibility. This change could impact the protein's interactions and stability, as increased solvent exposure may influence how the protein interacts with other molecules or its environment.

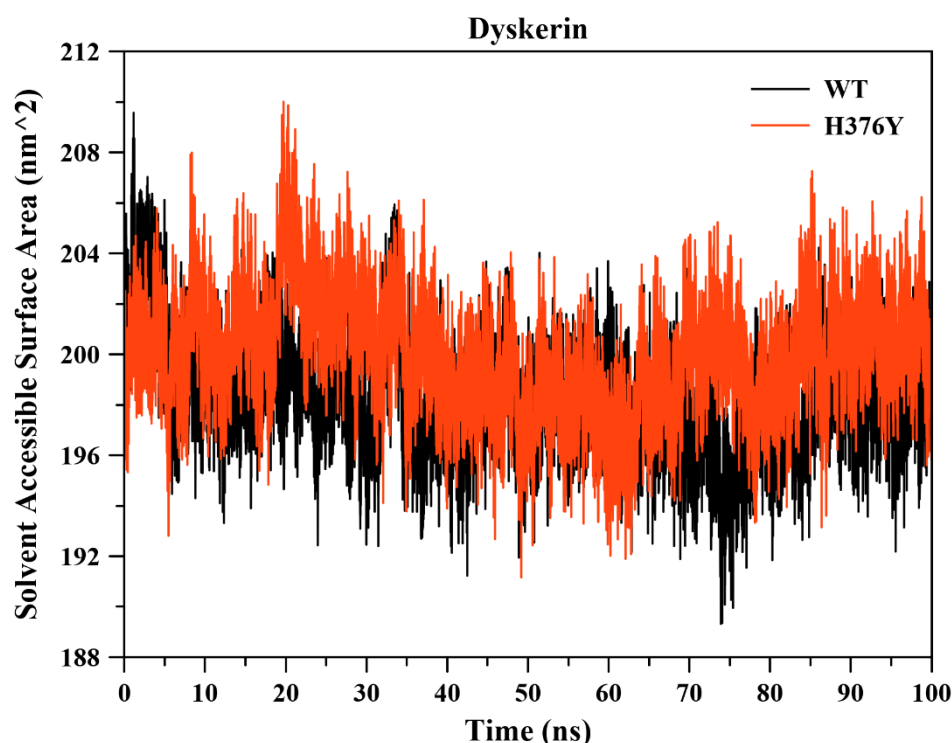


Figure 4.39. Solvent-accessible surface area graph of Dyskerin protein in WT complex and H376Y mutant complex.

As seen in Figure 4.39 the WT protein maintains SASA values generally ranging from 196 to 204 nm², indicating a stable level of solvent accessibility and suggesting a compact and consistent structure.

In contrast, the H376Y mutant consistently exhibits higher SASA values, fluctuating primarily between 200 and 208 nm². This increase in SASA implies that the H376Y mutation leads to a more solvent-exposed structure, indicating a less compact conformation compared to the WT. Additionally, the H376Y mutant shows more significant fluctuations in SASA, suggesting increased structural flexibility or dynamic variability.

In summary, the H376Y mutation in Dyskerin appears to result in a more open and flexible structure, with greater solvent accessibility than the WT. This alteration could

impact the protein's functional interactions by increasing the accessible surface area, which might influence binding capabilities or stability.

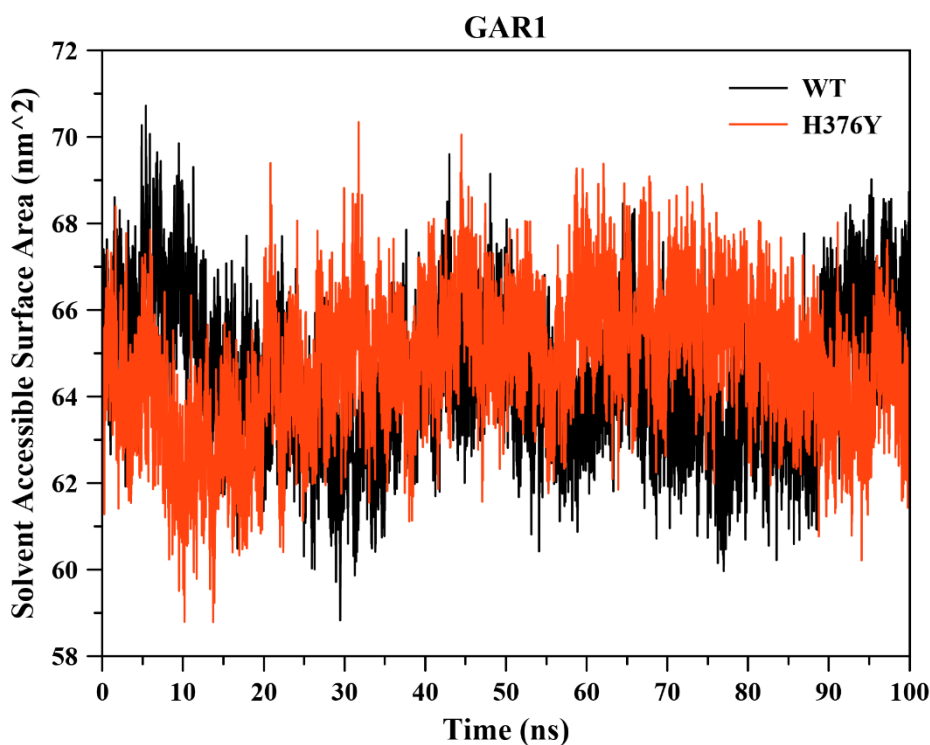


Figure 4.40. Solvent-accessible surface area graph of GAR1 protein in WT complex and H376Y mutant complex.

In Figure 4.40, the WT protein shows SASA values primarily fluctuating between 62 and 68 nm², indicating a stable level of solvent accessibility, which suggests a compact structure with minimal exposure.

The H376Y mutant, however, consistently exhibits slightly higher SASA values, ranging mostly from 63 to 70 nm². This increase suggests that the H376Y mutation induces a more solvent-exposed structure, indicating a less compact conformation compared to the WT. Additionally, the H376Y mutant displays more pronounced fluctuations in SASA values, implying increased structural flexibility or variability.

In summary, the H376Y mutation in GAR1 appears to result in a more open and flexible conformation, with greater solvent accessibility than the WT. These structural changes could impact GAR1's interaction dynamics and stability, as increased solvent exposure may affect binding interactions and overall functionality.

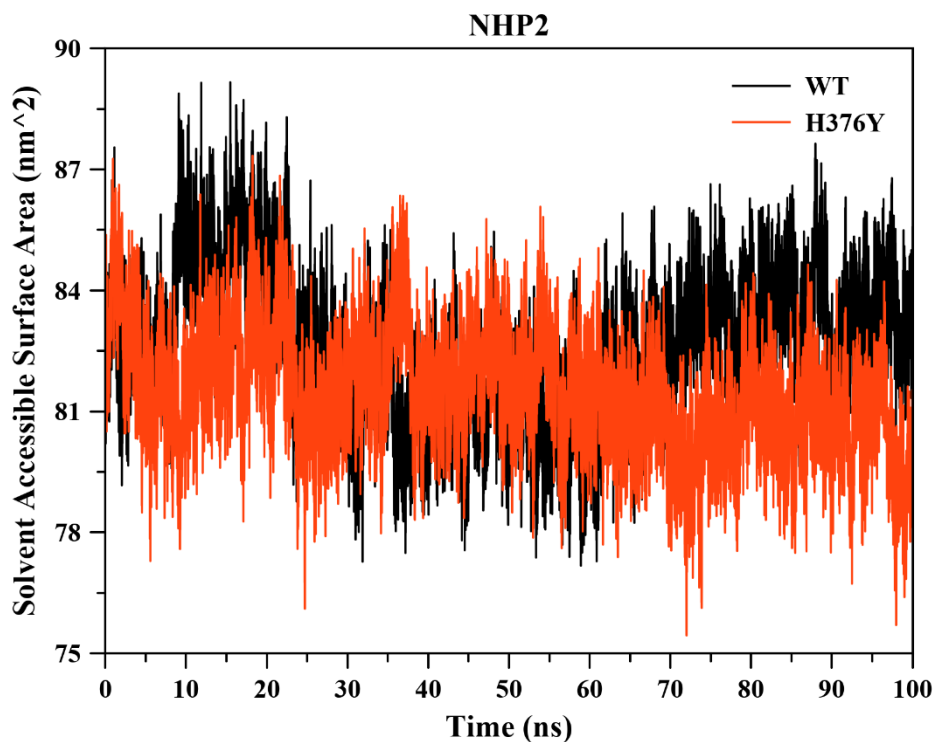


Figure 4.41. Solvent-accessible surface area graph of NHP2 protein in WT complex and H376Y mutant complex.

As seen in Figure 4.41, the WT protein generally displays SASA values fluctuating between 82 and 87 nm², suggesting a stable and consistent level of solvent exposure. In contrast, the H376Y mutant exhibits slightly lower SASA values, mostly ranging between 78 and 84 nm². This reduction in SASA suggests that the H376Y mutation results in a more compact structure with reduced solvent accessibility compared to the WT. The lower SASA values also imply that the mutant may have a more closed or compact conformation, which could influence its interaction with the solvent environment.

Overall, the H376Y mutation in NHP2 appears to induce a slightly more compact structure with reduced solvent exposure. This structural adjustment could potentially impact the protein's interaction capabilities, as a decrease in solvent-accessible surface area might limit binding interactions with other molecules.

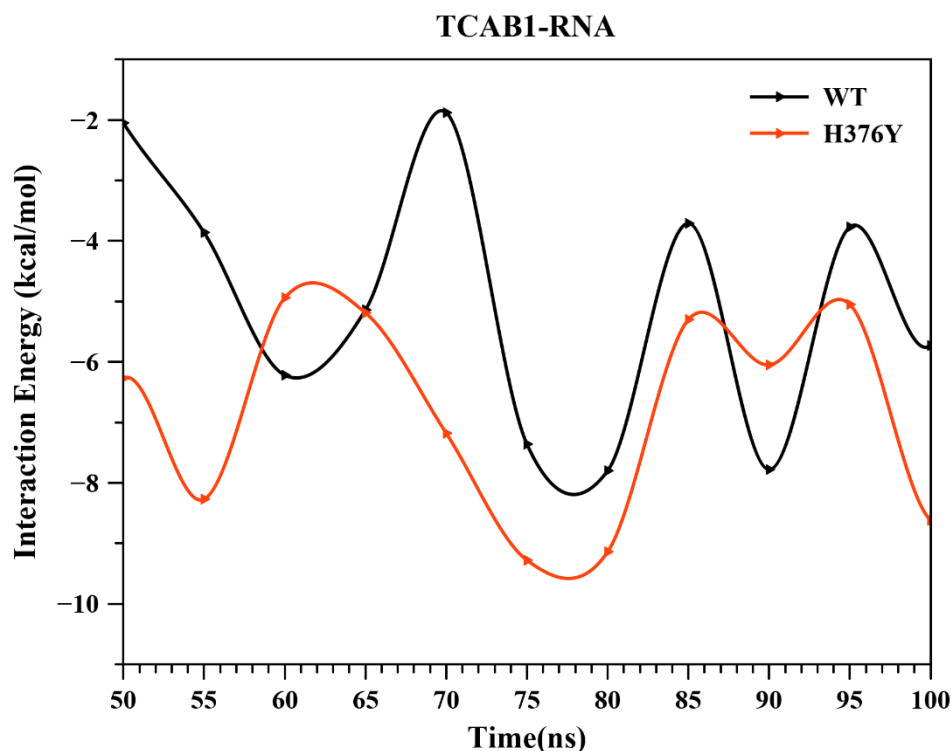


Figure 4.42. Interaction energy profile of WT and H376Y mutant TCAB1-RNA complexes over time.

According to Figure 4.42., in the interaction energy profile between TCAB1 and RNA, the WT shows an average binding energy of approximately -5 kcal/mol, while the H376Y mutant exhibits a more negative average energy at -7 kcal/mol. This increased negative energy in the mutant indicates a stronger binding affinity between TCAB1 and RNA in the H376Y variant compared to the WT.

The fluctuation patterns reveal that the WT profile varies between -2 and -8 kcal/mol, suggesting some degree of instability in the interaction, with peaks indicating moments of weaker binding. Conversely, the H376Y mutant profile consistently maintains a lower energy level, reflecting a more stable binding pattern with less pronounced peaks and troughs. This stability, combined with a stronger binding affinity, suggests that the H376Y mutation enhances TCAB1's interaction with RNA, potentially due to conformational changes in the binding interface that favour stronger contacts.

4.3. WT and R398W Mutant Protein Results

Both WT and R398W mutant TCAB1 proteins in the complex were subjected to MD simulations for 100 ns and 200 ns, respectively. The root mean square deviation (RMSD) values of each simulation were calculated and plotted (Figure 4.43).

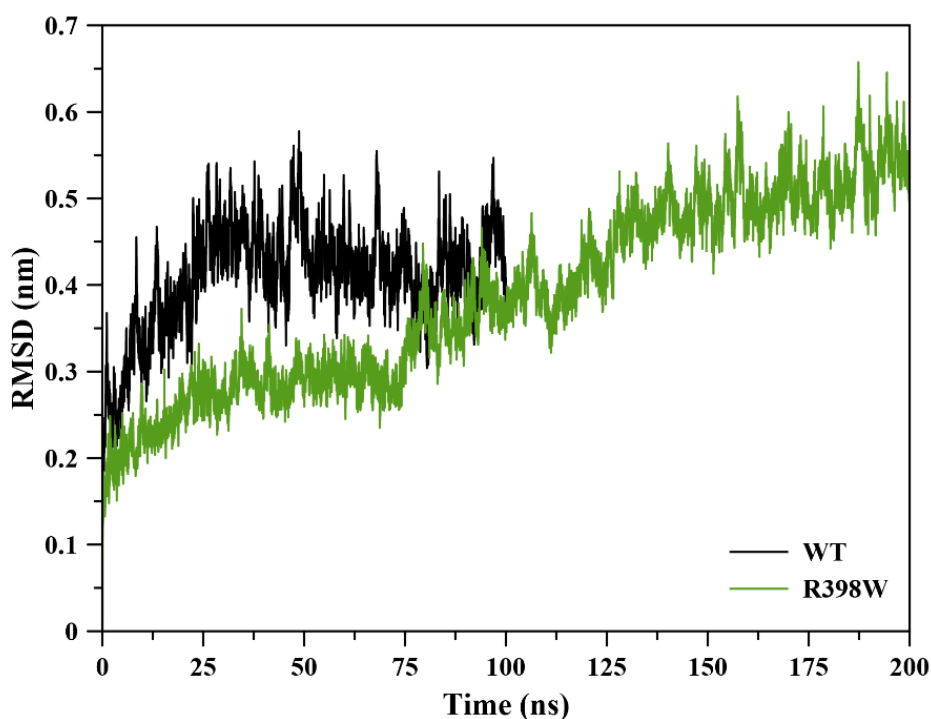


Figure 4.43. RMSD values of WT and R398W mutant complex.

As seen in Figure 4.43, the RMSD values of the WT complex simulation stabilized after the 25th nanosecond, while in the simulation of the R398W mutant TCAB1-bound telomerase complex, the RMSD values appeared to stabilize between the 25th and 75th nanoseconds, but gradually increased after the 75th nanosecond. However, the RMSD value calculated as 0.49 nm in the 128th nanosecond was measured as 0.52 in the 200th nanosecond and considering that many values in the 128-200 ns range oscillated between this 0.49-0.52 range, it was concluded that the simulation had reached sufficient balance.

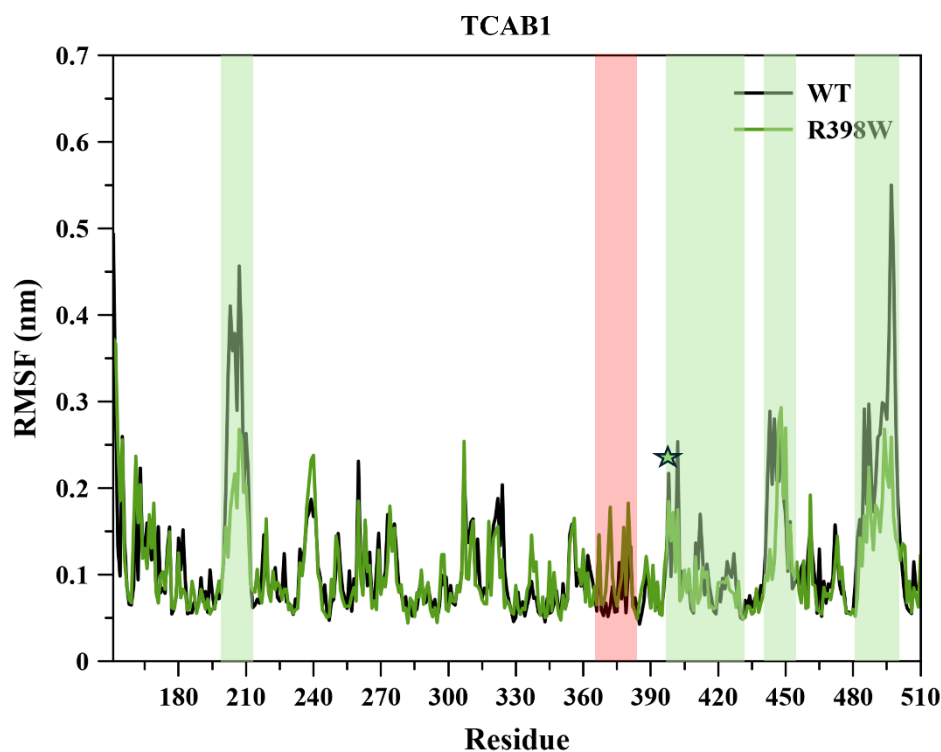


Figure 4.44. Comparison of RMSF values of WT and R398W mutant TCAB1 protein in complexes. The star shows the place of mutation.

In Figure 4.44, the regions shaded in green, which include amino acid segments 200-210, 398-429, 440-452, and 483-502, demonstrate lower RMSF values for the R398W mutant compared to the WT. This suggests that these segments in the mutant protein exhibit decreased flexibility, potentially indicating a more rigid and possibly more stable structure in these areas. Conversely, the region highlighted in red, spanning residues 367-380, shows higher RMSF values for the R398W mutant. This increased flexibility in the mutant could suggest alterations in the protein's structural integrity and could influence its interaction with other molecular components.

These variations in RMSF values are crucial for understanding the structural and functional implications of the R398W mutation within TCAB1. The increased flexibility in certain regions may affect the protein's ability to interact with other components of the cellular machinery, potentially altering its functional roles. Meanwhile, the decreased flexibility in other areas might confer structural stability, influencing the protein's behaviour and interaction dynamics in the cellular context. This detailed analysis provides insights into how specific mutations can impact protein dynamics and their potential consequences for the protein's biological functions.

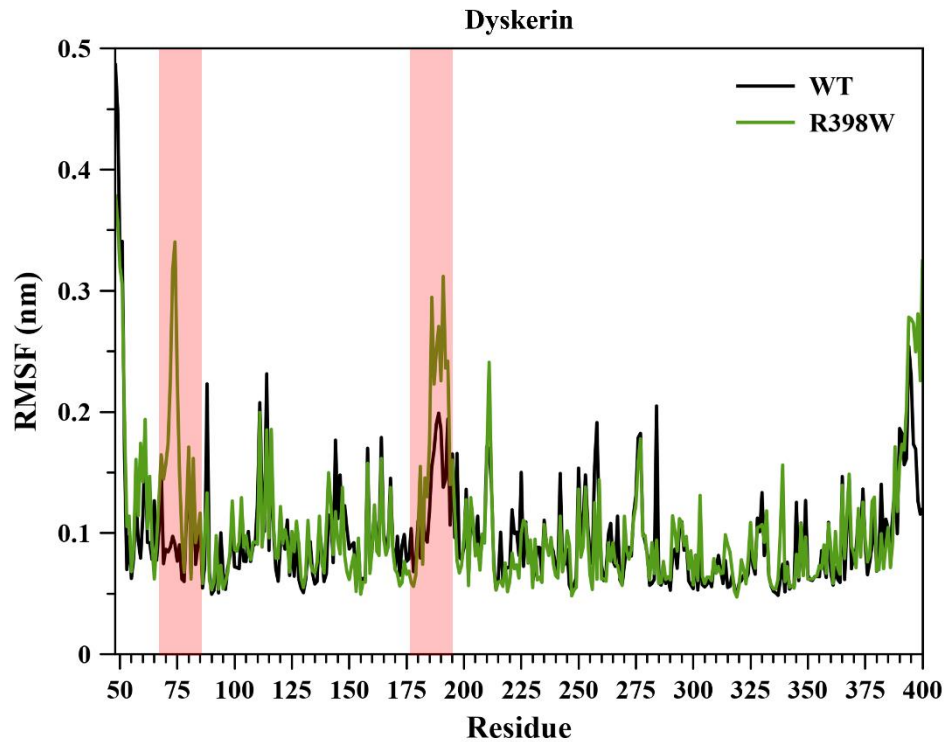


Figure 4.45. Comparison of RMSF values of WT and R398W mutant dyskerin protein in complexes.

In Figure 4.45, the RMSF graph for the Dyskerin protein provides a comparative analysis of root mean square fluctuation values between the wild-type (WT) and the R398W mutant, highlighting distinct regions where differences in flexibility are observed. Specifically, the graph delineates areas shaded in red—residue segments 67-85 and 181-195—where the R398W mutant exhibits higher RMSF values compared to the WT. This increased flexibility within these segments suggests that the R398W mutation introduces greater molecular dynamics, which may impact the structural integrity and functional capabilities of the Dyskerin protein.

The enhanced flexibility in these areas of the mutant could potentially influence Dyskerin's roles in critical cellular functions, such as RNA processing and telomere maintenance. The increased RMSF values indicate a potential alteration in the protein's ability to maintain stable interactions with RNA or other telomere-associated proteins. Such variations are crucial for understanding the mutation's impact on the protein's biological activities and its broader implications for cellular stability and disease progression.

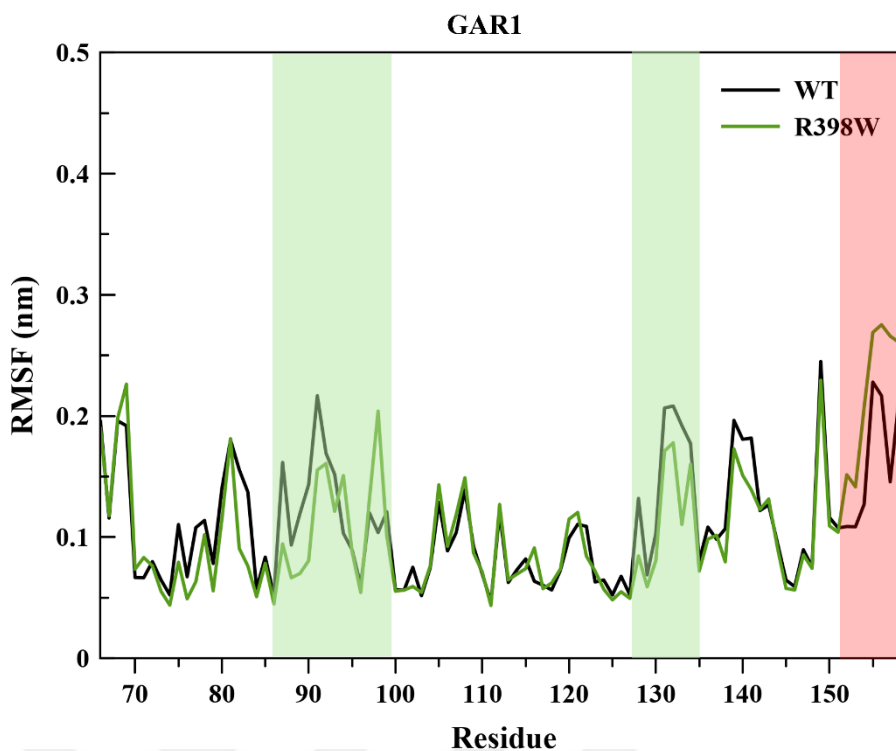


Figure 4.46. Comparison of RMSF values of WT and R398W mutant GAR1 protein in complexes.

As seen in Figure 4.46., in the regions shaded in green, specifically spanning residues 87-99 and 128-135, the WT protein demonstrates higher RMSF values than the R398W mutant. This increased flexibility in the WT suggests that these segments are more dynamic, potentially facilitating interactions with other molecular components or influencing the protein's functional stability and conformational adaptability.

Conversely, the region shaded in red, covering residues 151-159, shows higher RMSF values for the R398W mutant compared to the WT. This indicates that the mutation introduces enhanced flexibility in this segment, which might impact the mutant protein's structural integrity and possibly its functional interactions within the cellular environment.

These observations are critical for understanding the specific effects of the R398W mutation on GAR1's structural dynamics. The variations in RMSF values highlight potential differences in how the WT and mutant proteins may interact with other molecular entities, possibly affecting their roles in cellular processes.

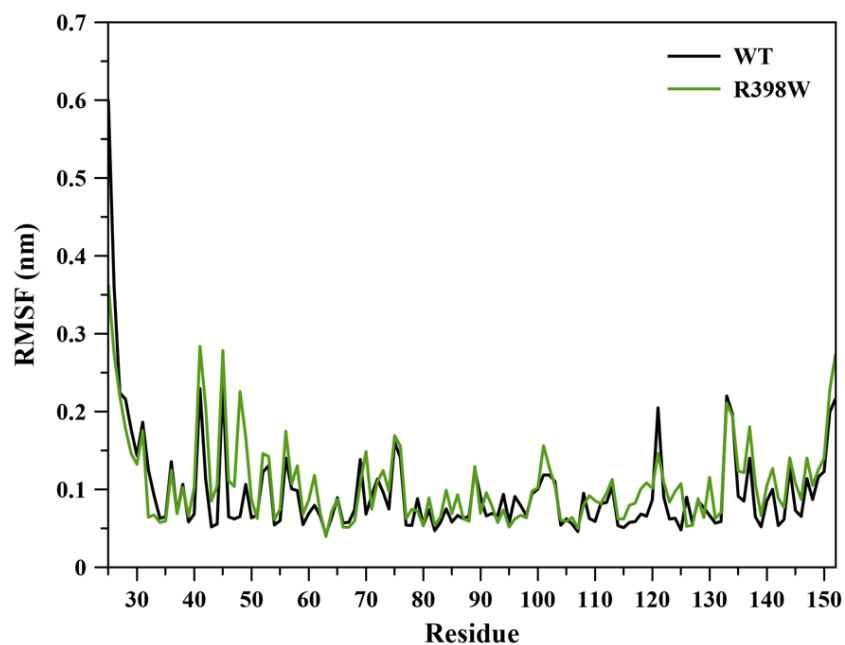


Figure 4.47. Comparison of RMSF values of WT and R398W mutant NHP2 protein in complexes.

According to Figure 4.47, the RMSF values of the NHP2 protein interacting with WT TCAB1 and the NHP2 protein interacting with the R398W mutant followed a similar rhythm and did not differ in a wide enough range to be called a region.

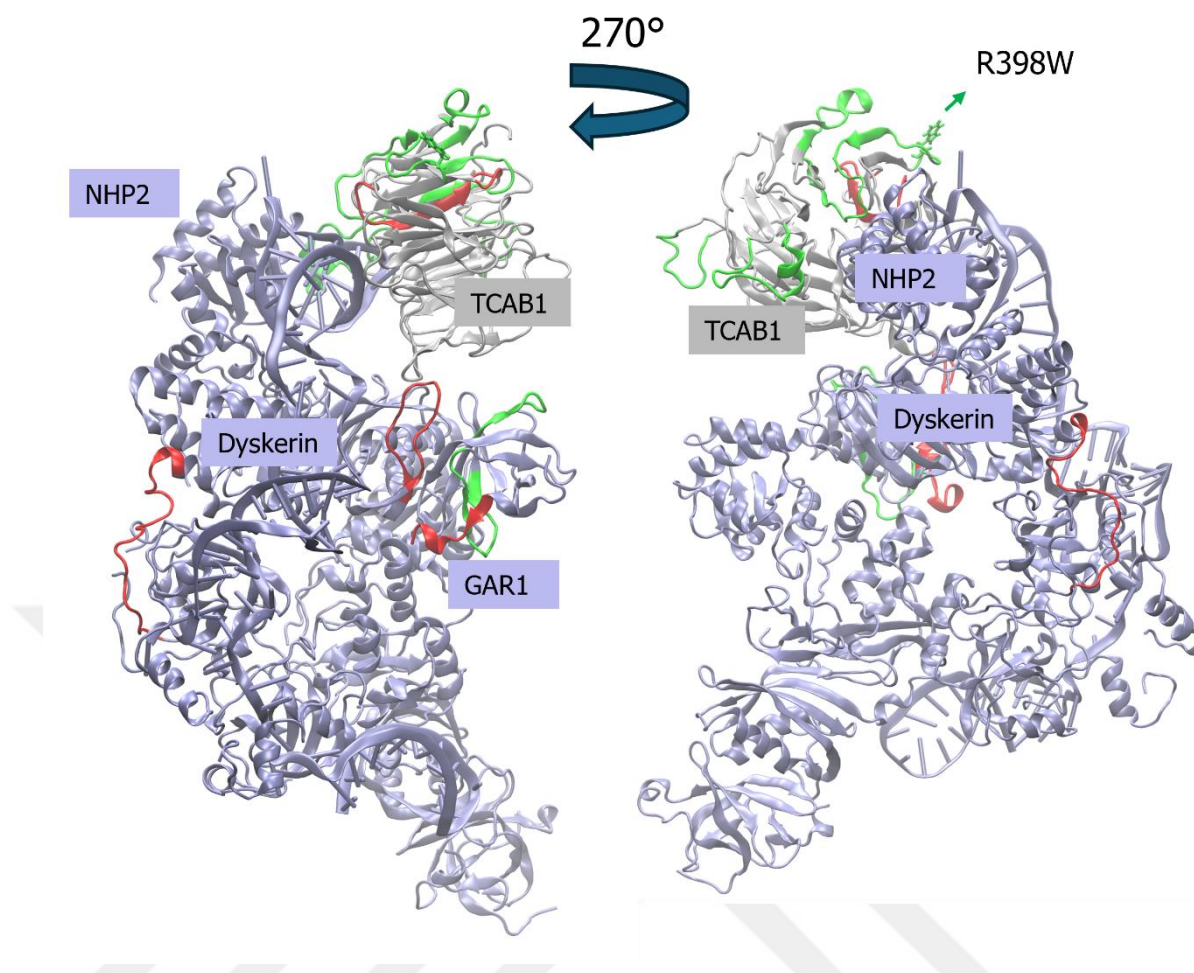


Figure 4.48. R398W complex structure. The protein shown in grey is the TCAB1 protein, the rest of the complex is shown in metallic blue.

According to Figure 4.48., the visualization displays the R398W mutant complex, comprising a single TCAB1 molecule, one RNA molecule, and two copies each of dyskerin, GAR1, NHP2, and NOP10 proteins. The R398W mutation, situated on the TCAB1 protein, is highlighted with a green arrow within the image. The two depicted views are rotated 270 degrees relative to each other, providing a comprehensive perspective of the complex's spatial arrangement.

In this graphical representation, amino acid sequences marked in red indicate regions where the RMSF (root mean square fluctuation) values of the mutant protein are elevated compared to the wild-type (WT) protein, suggesting increased flexibility in these segments. Conversely, sequences highlighted in green denote areas where the RMSF values are comparatively lower in the mutant, indicating a decrease in flexibility. These regions correlate directly with those specified in the RMSF value table descriptions for each protein interacting with TCAB1.

Notably, in areas corresponding to TCAB1's interaction with RNA, there is an observed increase in RMSF values, suggesting enhanced dynamic interaction at this interface. In contrast, the interfaces where TCAB1 interacts with other proteins exhibit a decrease in RMSF values, potentially indicating a stabilization of these interaction domains. Consequently, this alteration has led to an increased interaction between TCAB1 and the dyskerin and GAR1 proteins. Specifically, modifications in the RMSF values within the dyskerin domain interacting with TCAB1 have been observed, indicating changes in flexibility that could affect their mutual interaction dynamics.

Furthermore, while the RMSF values in the GAR1 interaction domain with TCAB1 have decreased, suggesting enhanced stability, no significant changes have been detected in the NHP2 protein. This observation leads to the hypothesis that the interaction between NHP2 and TCAB1 may have been disrupted, potentially affecting the functional synergy within the complex.

This detailed analysis not only underscores the impact of the R398W mutation on the structural dynamics of TCAB1 and its interactions but also highlights the potential functional implications these changes may have on the overall stability and activity of the protein complex within cellular processes.

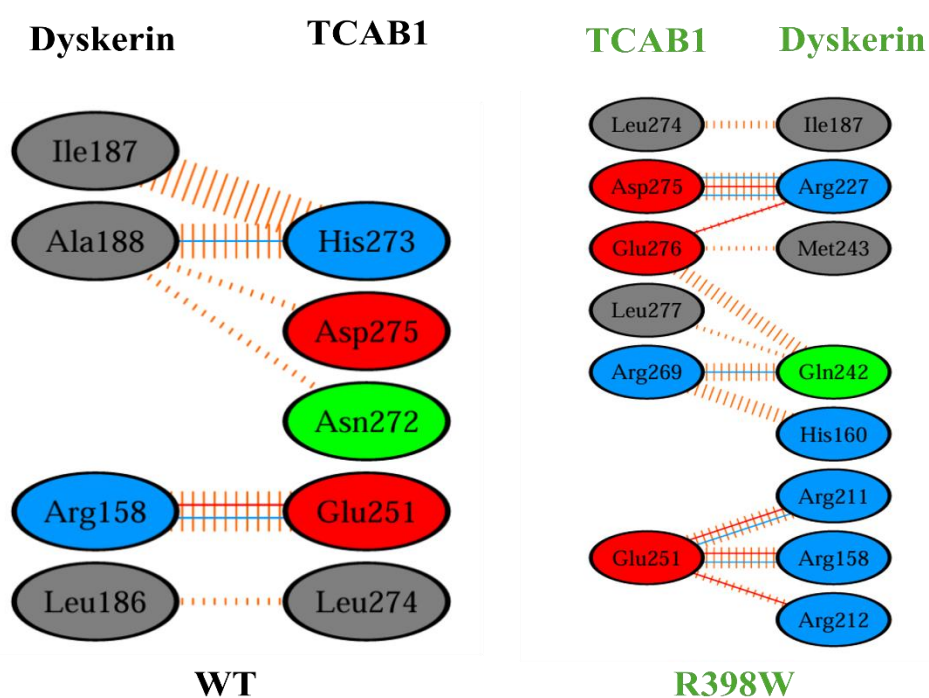


Figure 4.49. Comparison of dyskerin and TCAB1 protein interactions in complexes with WT and R398W mutant TCAB1 proteins.

According to Figure 4.49, in the WT complex, Ala188 and Ile187 on dyskerin form critical interactions with His273 on TCAB1 through both hydrogen bonds and non-bonded contacts. Additionally, Asp275 on dyskerin interacts with His273 on TCAB1, further stabilizing the complex. The presence of hydrogen bonds between Asn272 on dyskerin and Asp275 on TCAB1, as well as between Glu251 on dyskerin and Arg158 on TCAB1, indicates a well-established binding interface that is crucial for the stability of the WT complex.

In the R398W mutant complex, significant changes in the interaction landscape are observed. New hydrogen bonds form between Asp275 on TCAB1 and Arg227 on dyskerin and between Arg269 on TCAB1 and Gln242 on dyskerin. These new interactions suggest that the mutation has reorganized the binding interface, potentially compensating for any lost interactions from the WT configuration. The mutant complex also exhibits additional non-bonded contacts involving Leu274, Glu276, and Leu277 on TCAB1, which interact with Ile187, Met243, and Gln242 on dyskerin. These additional contacts likely enhance the stability of the mutant complex by introducing new interaction points that strengthen the overall binding.

The R398W mutation leads to a restructuring of the interaction network between TCAB1 and dyskerin. While the WT complex relies on specific hydrogen bonds and non-bonded contacts to maintain stability, the mutant complex introduces new interactions that appear to compensate for any disrupted contacts. The formation of new hydrogen bonds and non-bonded interactions in the mutant complex likely enhances the overall binding affinity and stability, making the mutant complex potentially more robust than the WT.

The loss of some original WT interactions, such as those involving His273 and Asn272, is offset by the introduction of new stabilizing forces, particularly through the involvement of residues like Arg269 and Glu276 on TCAB1. This reorganization suggests that the mutation enables the complex to achieve a more stable configuration.

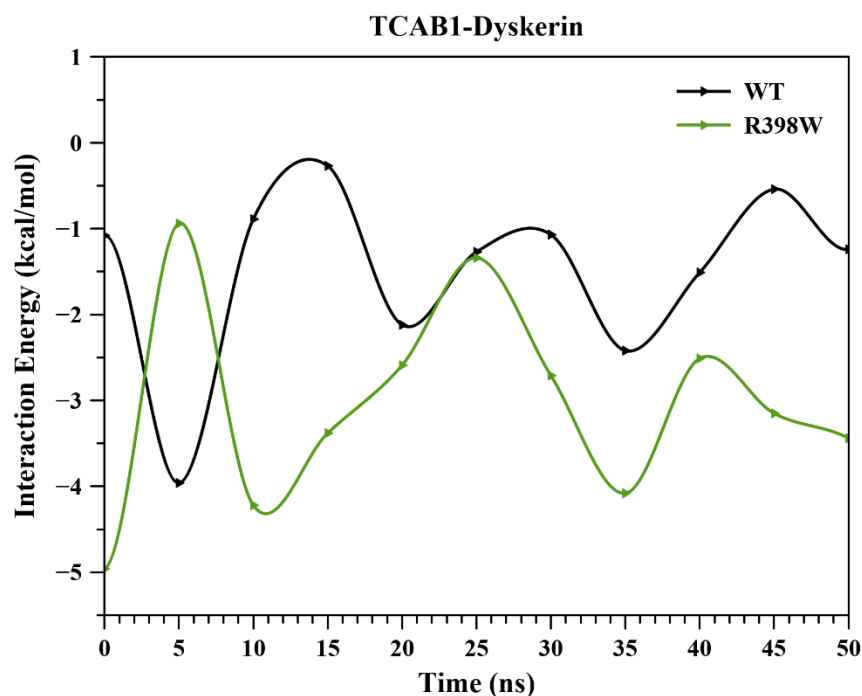


Figure 4.50. Interaction energy profile of WT and R398W mutant TCAB1-Dyskerin complexes over time.

According to Figure 4.50., the interaction energy analysis between WT TCAB1 and dyskerin, compared to the R398W mutant, reveals significant differences in binding stability and affinity. The WT complex exhibits an average binding free energy of -1.48 kcal/mol, which aligns with the observed fluctuations in the graph. This relatively higher average energy suggests that the WT complex experiences periods of weaker binding, with energy levels occasionally approaching fewer negative values, indicating potential instability or partial dissociation within the complex. The frequent fluctuations around this average further underscore the dynamic and less stable nature of the WT interaction, with the binding affinity being moderate at best.

In contrast, the R398W mutant complex shows a much more negative average binding free energy of -3.02 kcal/mol, reflecting a significantly stronger and more stable interaction between TCAB1 and dyskerin. The energy profile of the mutant complex demonstrates consistently lower values, indicating that the mutation enhances the overall binding affinity and reduces the likelihood of dissociation or unstable interactions. The less pronounced fluctuations and more consistently negative energy values in the R398W complex suggest that this mutation induces structural changes that create a more favourable and stable binding interface. This increase in binding

stability is likely due to the introduction of additional stabilizing interactions or a reorganization of the binding interface that compensates for any destabilizing effects observed in the WT complex.

Overall, the average binding free energy values, combined with the interaction energy profiles, clearly indicate that the R398W mutation significantly enhances the binding stability and affinity of the TCAB1-dyskerin complex, leading to a more robust and functionally stable interaction compared to the WT form. As seen in Figure 3.3.9, in the comparison between the WT and R398W mutant interactions of TCAB1 with GAR1, the figure highlights significant differences in how these proteins interact within the complex. In the WT complex, the interaction is primarily characterized by non-bonded contacts between Glu81 on GAR1 and Arg269 on TCAB1. These interactions, which are weaker and non-covalent, suggest a relatively simple binding interface that may contribute to a less stable and weaker overall interaction between WT TCAB1 and GAR1. The lack of additional stabilizing interactions, such as hydrogen bonds, indicates that the WT complex might not achieve the same level of stability as the mutant complex.

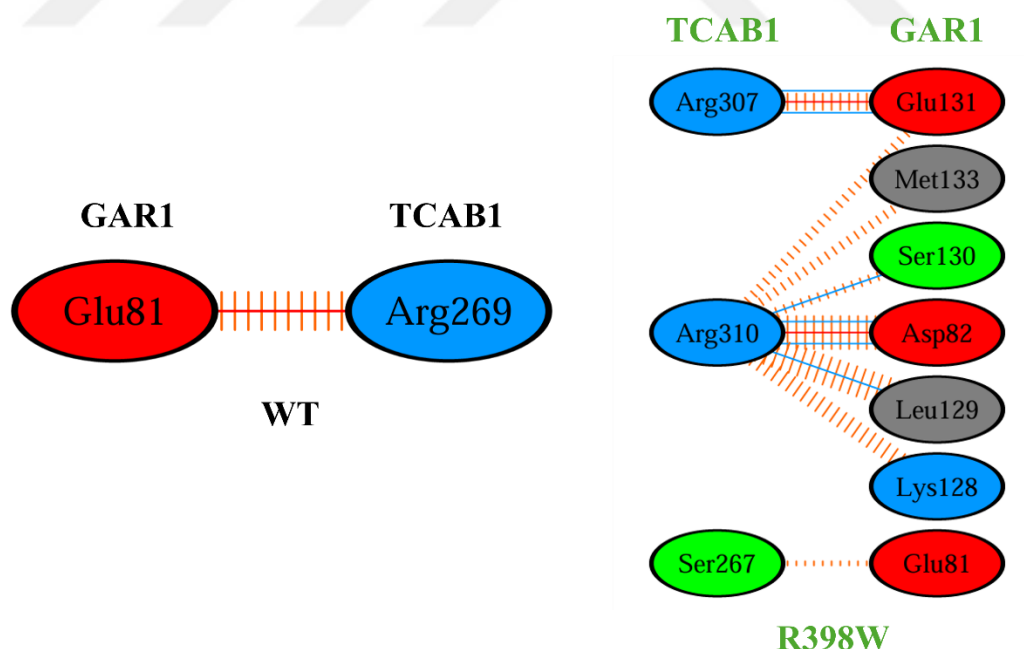


Figure 4.51. Comparison of TCAB1 and GAR1 protein interactions in complexes with WT and R398W mutant TCAB1 proteins.

The R398W mutation introduces a more complex and robust interaction landscape. In the mutant complex, multiple residues on TCAB1, including Arg307, Arg310, and Ser267, establish interactions with corresponding residues on GAR1, such as Glu131, Asp82, Ser130, and Glu81. Notably, Arg310 on TCAB1 forms hydrogen bonds with Asp82 on GAR1 and non-bonded contacts with several other residues, including Ser130, Leu129, and Lys128. This extensive network of interactions suggests a significantly more stable and stronger binding interface compared to the WT complex. Additionally, Arg307 on TCAB1 forms a non-bonded contact with Glu131 on GAR1, further enhancing the stability of the complex. The interaction between Ser267 on TCAB1 and Glu81 on GAR1, which was present in the WT but appears more complex and involved in the mutant, also contributes to the overall stability.

The R398W mutation enhances the interaction between TCAB1 and GAR1 by increasing the number and types of interactions between the two proteins. The introduction of new hydrogen bonds and an increased number of non-bonded contacts not only strengthens existing interactions but also creates new interaction sites, leading to a more robust and stable complex. The mutation-induced reorganization of the interaction interface suggests a compensatory effect, where new stabilizing interactions are formed, potentially enhancing the functional integrity of the TCAB1-GAR1 complex by reinforcing the protein-protein interface.

In conclusion, the R398W mutation significantly alters and enhances the binding between TCAB1 and GAR1. While the WT complex relies on a relatively simple and potentially weaker non-bonded interaction between Glu81 and Arg269, the mutant complex introduces a more complex and stable interaction network involving multiple residues and hydrogen bonds.

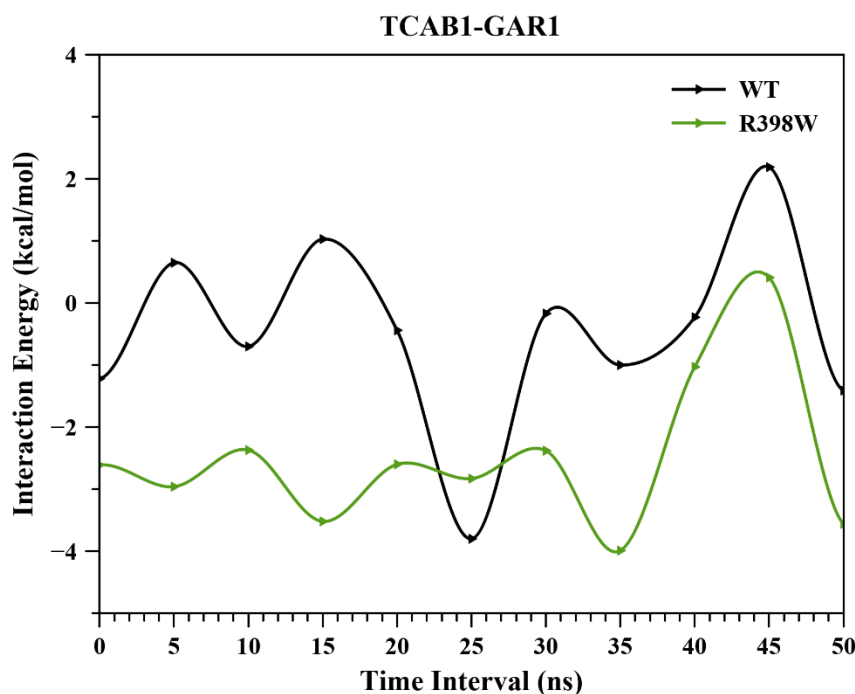


Figure 4.52. Interaction energy profile of WT and R398W mutant TCAB1-GAR1 complexes over time.

As seen in Figure 4.52, for the WT complex, the average interaction energy is -0.46 kcal/mol, indicating relatively weak and unstable binding between TCAB1 and GAR1. The energy profile shows frequent fluctuations, with peaks often reaching positive values, which suggests moments where the binding affinity decreases or the interaction becomes less stable. The fact that the energy occasionally approaches or exceeds 0 kcal/mol indicates that the WT complex is prone to instability, with the potential for partial dissociation or weak binding interactions. These fluctuations reflect a dynamic interaction that may be less effective in maintaining a stable protein-protein interface.

In contrast, the R398W mutant complex exhibits a significantly more negative average interaction energy of -2.50 kcal/mol, suggesting a much stronger and more stable binding interaction between TCAB1 and GAR1. The energy profile for the mutant complex shows less pronounced fluctuations compared to the WT, with consistently lower energy values. These findings indicate that the R398W mutation enhances the overall stability of the complex, reducing the likelihood of dissociation and promoting a more stable binding interface. The consistently negative energy values suggest that the mutant complex achieves and maintains a more energetically favourable interaction state throughout the time course of the simulation.

The comparison between the WT and R398W mutant interaction energies highlights the substantial impact of the R398W mutation on the binding affinity and stability of the TCAB1-GAR1 complex. While the WT complex is characterized by a relatively weak and unstable interaction, the R398W mutant demonstrates a more robust and energetically favourable binding, as indicated by the more negative average interaction energy and reduced fluctuations. The mutation appears to induce structural changes that enhance the interaction between TCAB1 and GAR1, leading to a more stable and stronger protein-protein interface. Overall, the R398W mutation significantly improves the binding stability and affinity of the TCAB1-GAR1 complex, as reflected in the more negative interaction energy profile.

In the R398W TCAB1 mutant, no interaction was found between TCAB1 and NHP2 proteins. Figure 3.2.11 shows the interaction energy graph comparing the WT TCAB1-NHP2 complex to the R398W mutant form, revealing critical insights into the effect of the R398W mutation on the binding affinity and stability of the protein-protein interaction. The WT complex, with an average interaction energy of 0.45 kcal/mol, shows significant fluctuations throughout the simulation. These fluctuations, ranging from slightly positive values to near zero or even negative values, suggest a weak and unstable interaction. The variability in energy levels indicates that the WT complex experiences moments of weak binding, where the interaction is unstable and may be prone to dissociation.

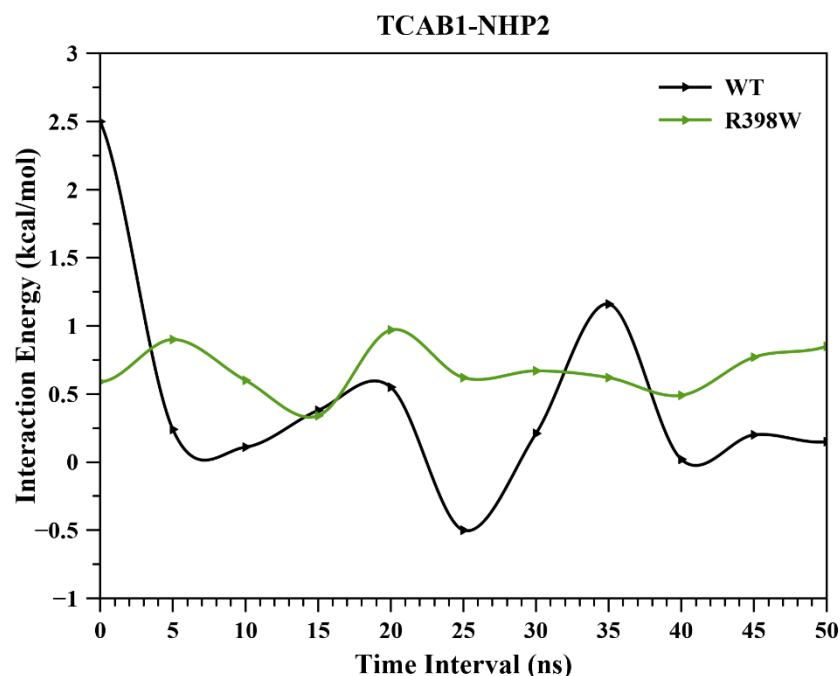


Figure 4.53. Interaction energy profile of WT and R398W mutant TCAB1-NHP2 complexes over time.

In the R398W mutant complex, the average interaction energy is slightly higher at 0.67 kcal/mol, but this increase in energy does not indicate an improvement in binding affinity. Instead, the interaction energy stabilizes at a more positive value, suggesting that the mutation leads to a consistently weak interaction rather than a stable binding interface. The energy profile of the R398W complex, while more stable in its lack of fluctuations, actually reflects a less favourable interaction because it remains at a higher, more positive energy level throughout the simulation.

This consistent positive energy indicates that the R398W mutation does not support the formation of a strong or stable interaction between TCAB1 and NHP2. Instead of enhancing binding affinity, the mutation results in an interaction that is weak and unlikely to contribute meaningfully to complex formation. The absence of significant energy fluctuations in the R398W complex does not denote stability in a positive sense but rather reflects a lack of dynamic binding interactions that would typically lower the energy to more negative, favourable levels. Therefore, the R398W mutation does not improve the interaction between TCAB1 and NHP2 and instead seems to contribute to an energetically unfavourable, weak association between the two proteins.

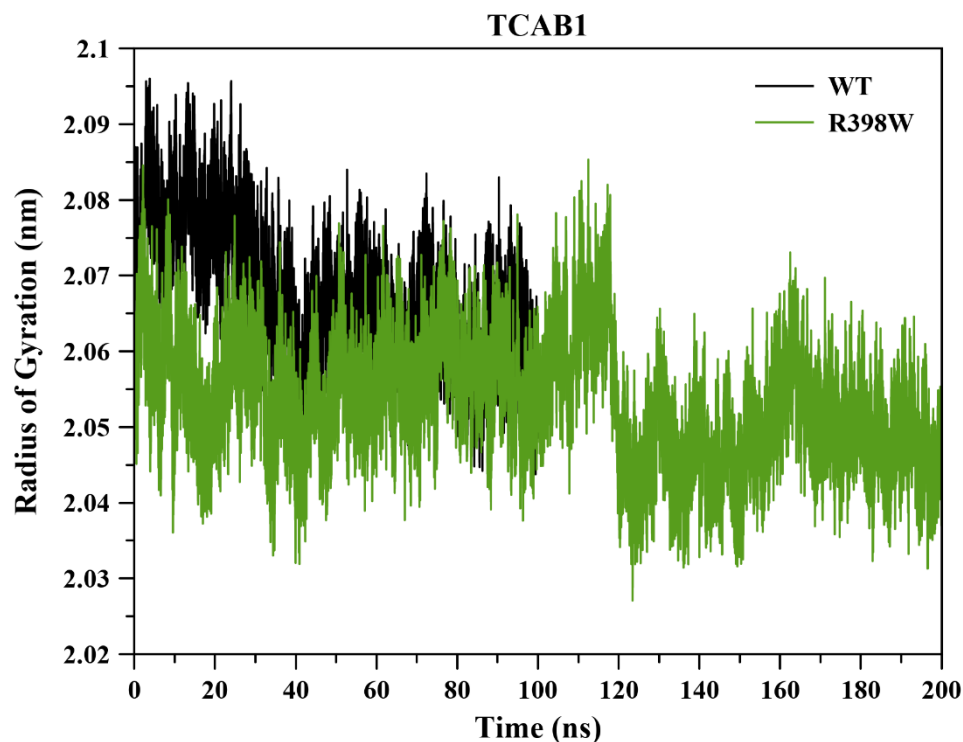


Figure 4.54. Radius of gyration graph of TCAB1 protein in WT complex and R398W mutant complex.

According to Figure 4.54, the WT protein generally maintains Rg values around 2.07 to 2.08 nm, indicating a stable, compact structure with minimal fluctuations over time. In contrast, the R398W mutant exhibits lower Rg values, primarily fluctuating between 2.03 and 2.06 nm. This reduction in Rg suggests that the R398W mutation induces a more compact conformation in TCAB1 relative to the WT. Additionally, the R398W mutant shows greater variability in Rg values as the simulation progresses, indicating possible structural adjustments or increased flexibility over time.

In summary, the R398W mutation appears to result in a more compact and potentially more flexible structure in TCAB1. This structural alteration could impact the protein's functional properties, as increased compactness and flexibility may influence its stability and interactions with other molecules.

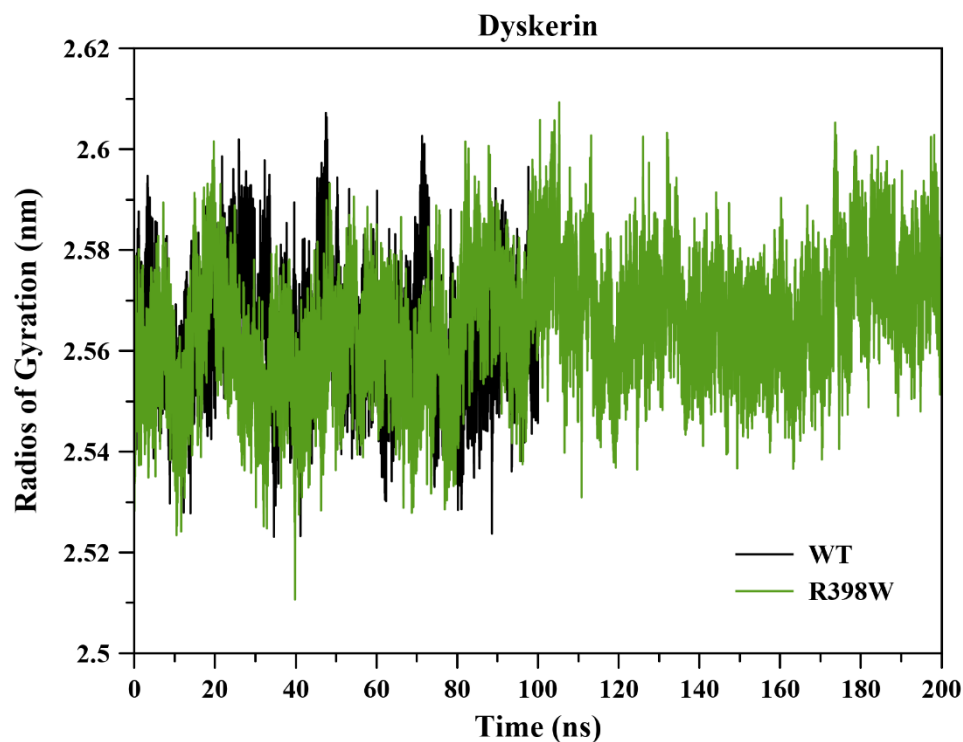


Figure 4.55. Radius of gyration graph of dyskerin protein in WT complex and R398W mutant complex.

According to Figure 4.55, the WT protein maintains Rg values around 2.54 to 2.58 nm, indicating a relatively stable and compact structure throughout the simulation.

In contrast, the R398W mutant exhibits slightly lower Rg values, generally fluctuating around 2.53 to 2.57 nm. This slight reduction in Rg suggests that the R398W mutation induces a marginally more compact structure in Dyskerin compared to the WT. Additionally, the R398W mutant shows more pronounced fluctuations as the simulation progresses, indicating potential structural adjustments or increased flexibility over time.

In summary, the R398W mutation in Dyskerin appears to lead to a more compact structure with increased flexibility. This structural change could impact the protein's stability and interaction capabilities, as a more compact and flexible structure may alter binding interactions or functional dynamics.

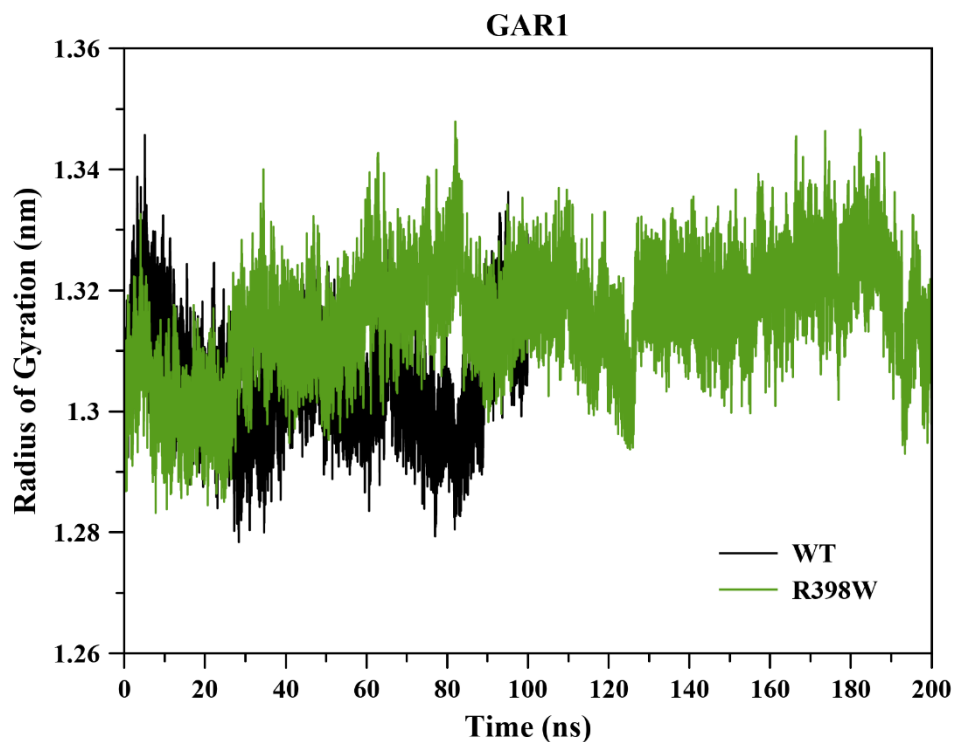


Figure 4.56. Radius of gyration graph of GAR1 protein in WT complex and R398W mutant complex.

According to Figure 4.56., the WT protein generally maintains Rg values around 1.29 to 1.32 nm, indicating a stable and compact structure with limited fluctuations.

In contrast, the R398W mutant exhibits higher Rg values, fluctuating between 1.30 and 1.34 nm. This increase in Rg suggests that the R398W mutation induces a more expanded or less compact conformation in GAR1 relative to the WT. Additionally, the R398W mutant shows greater fluctuations as the simulation progresses, indicating increased flexibility or dynamic structural changes over time.

In summary, the R398W mutation in GAR1 appears to result in a slightly expanded and more flexible structure compared to the WT. These changes could potentially affect GAR1's interaction dynamics, as a more open and flexible structure may alter its stability and binding interactions.

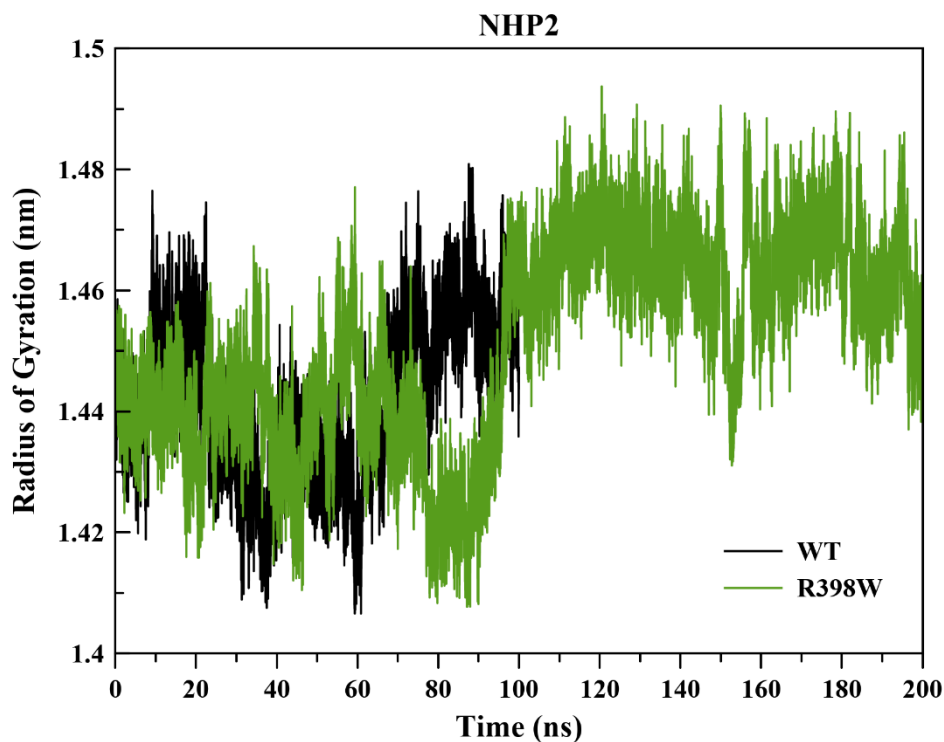


Figure 4.57. Radius of gyration graph of NHP2 protein in WT complex and R398W mutant complex.

According to Figure 4.57., the WT protein generally maintains Rg values around 1.43 to 1.46 nm, indicating a relatively stable and compact structure with minimal fluctuations over time.

In contrast, the R398W mutant exhibits higher Rg values, fluctuating between approximately 1.44 and 1.48 nm. This increase in Rg suggests that the R398W mutation results in a more expanded or less compact conformation in NHP2 compared to the WT. Additionally, the R398W mutant shows greater fluctuations, especially in the latter part of the simulation, indicating potential structural flexibility or dynamic adjustments.

In summary, the R398W mutation in NHP2 appears to induce a slightly expanded and more flexible structure. These structural changes may impact NHP2's functional interactions, as an increase in flexibility and a reduction in compactness could alter its stability and binding dynamics.

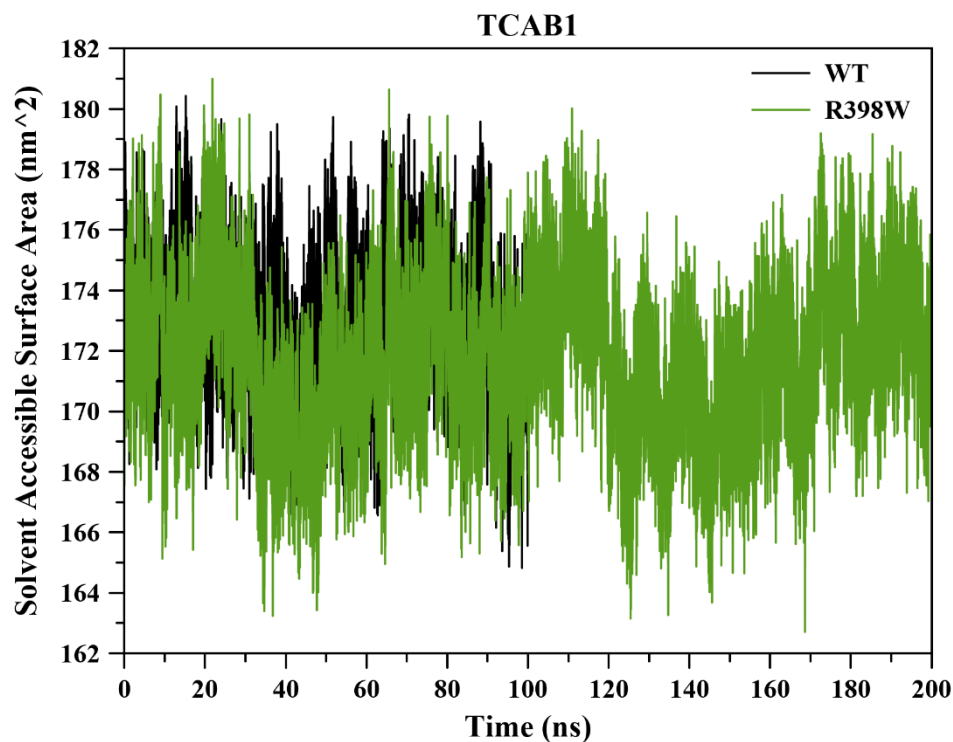


Figure 4.58. Solvent-accessible surface area graph of TCAB1 protein in WT complex and R398W mutant complex.

According to Figure 4.58., the WT protein generally maintains SASA values between 170 and 178 nm², indicating a stable level of solvent accessibility, which suggests a compact and consistent structure over time.

In contrast, the R398W mutant exhibits slightly lower SASA values, primarily fluctuating between 168 and 176 nm². This reduction in SASA suggests that the R398W mutation results in a more compact structure with decreased solvent exposure relative to the WT. Additionally, the R398W mutant shows greater variability in SASA values as the simulation progresses, indicating possible structural adjustments or increased flexibility.

In summary, the R398W mutation in TCAB1 appears to induce a more compact and potentially more flexible structure, with reduced solvent accessibility. This structural alteration could influence the protein's functional interactions and stability, as a decrease in solvent-exposed surface area may impact binding capabilities.

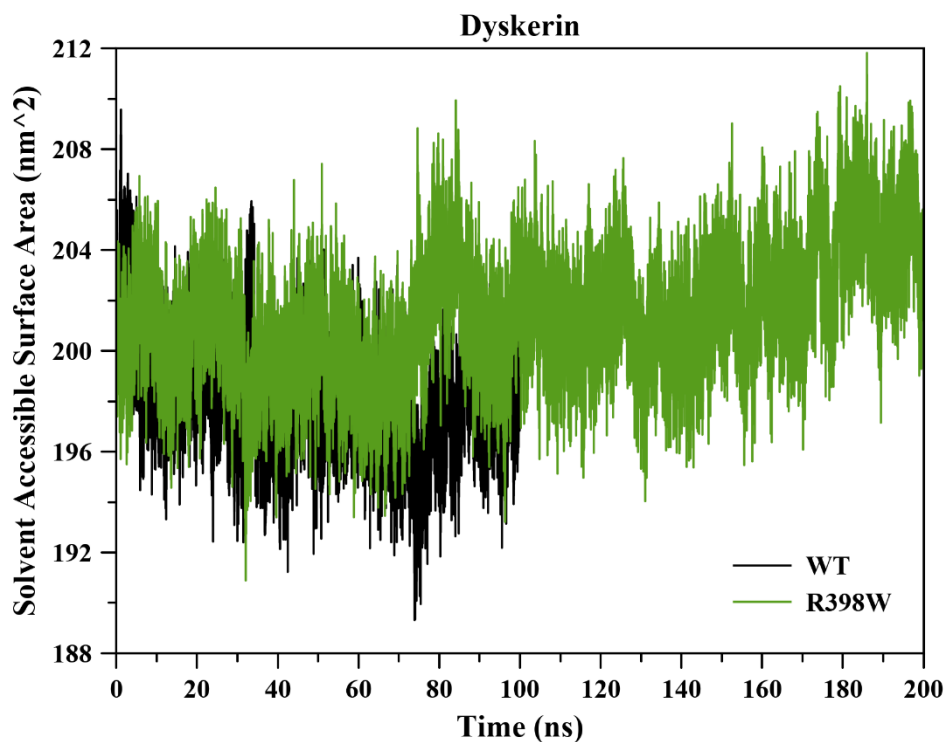


Figure 4.59. Solvent-accessible surface area graph of dyskerin protein in WT complex and R398W mutant complex.

According to Figure 4.59, the WT protein generally maintains SASA values between 194 and 202 nm², suggesting a stable level of solvent accessibility that reflects a compact structure.

The R398W mutant, however, exhibits higher SASA values, primarily fluctuating between 200 and 208 nm². This increase in SASA indicates that the R398W mutation may result in a more solvent-exposed and less compact structure compared to the WT. Additionally, the R398W mutant displays greater fluctuations in SASA, indicating increased flexibility or dynamic variability in its structure.

In summary, the R398W mutation in Dyskerin appears to induce a more open and flexible conformation with higher solvent accessibility. These structural changes may impact the protein's interactions and stability, as increased solvent exposure could influence binding interactions or its overall functional behaviour.

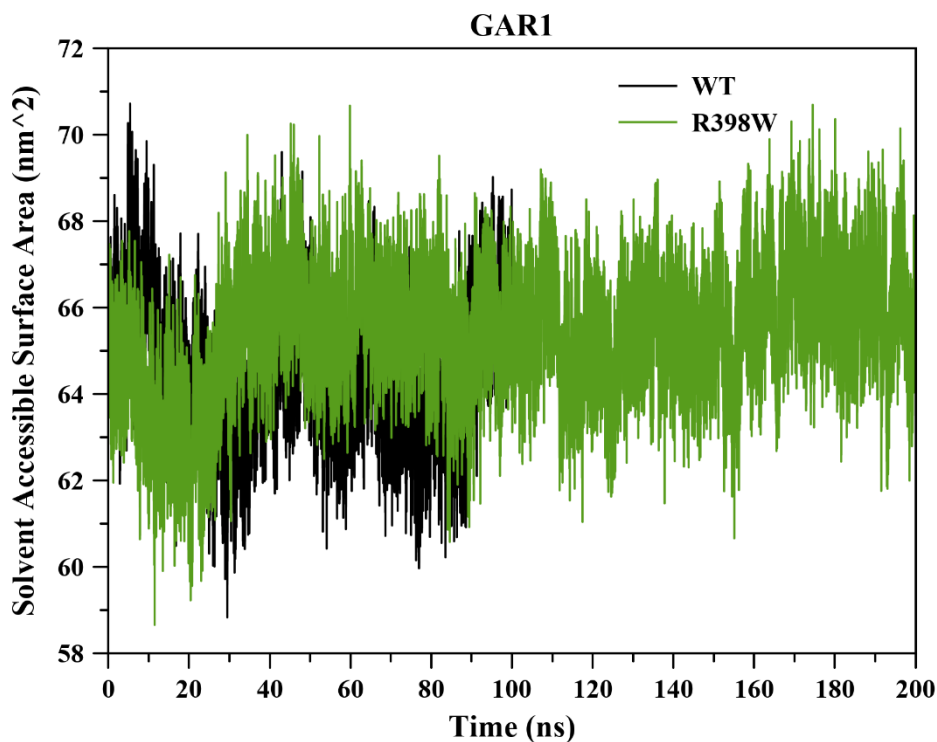


Figure 4.60. Solvent-accessible surface area graph of GAR1 protein in WT complex and R398W mutant complex.

According to Figure 4.60, the WT protein generally displays SASA values between 62 and 66 nm², indicating a stable and compact structure with minimal solvent accessibility.

In contrast, the R398W mutant shows consistently higher SASA values, fluctuating mostly between 64 and 70 nm². This increase suggests that the R398W mutation results in a more solvent-exposed and less compact structure compared to the WT. The R398W mutant also exhibits greater variability in SASA values, particularly as the simulation progresses, which may reflect increased structural flexibility or dynamic adjustments.

In summary, the R398W mutation in GAR1 appears to lead to a more open and flexible conformation with enhanced solvent accessibility. These structural changes may influence the protein's interaction dynamics and stability, as increased solvent exposure can affect its binding capabilities and functional behaviour.

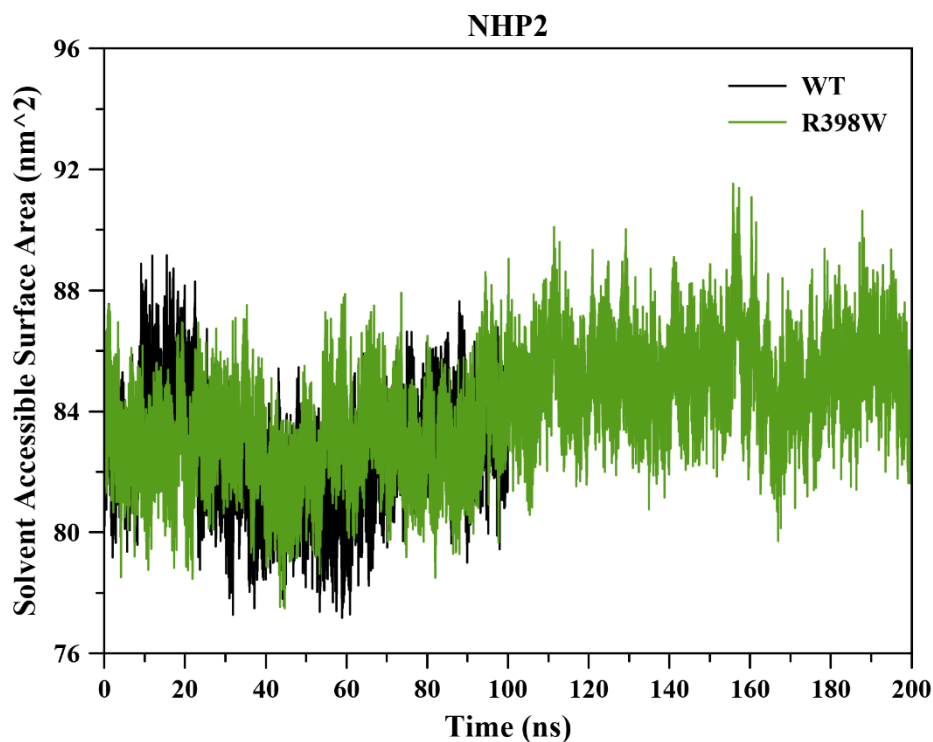


Figure 4.61. Solvent-accessible surface area graph of NHP2 protein in WT complex and R398W mutant complex.

According to Figure 4.61, the WT protein generally displays SASA values fluctuating between 80 and 86 nm², indicating a compact structure with moderate solvent accessibility.

In contrast, the R398W mutant consistently shows higher SASA values, mostly fluctuating between 84 and 90 nm². This increase suggests that the R398W mutation leads to a more solvent-exposed and less compact structure in NHP2 compared to the WT. The R398W mutant also exhibits greater variability in SASA, particularly in the latter part of the simulation, suggesting increased flexibility or dynamic structural changes over time.

In summary, the R398W mutation in NHP2 appears to result in a more open and flexible conformation with enhanced solvent accessibility. These structural alterations could impact the protein's interaction capabilities and stability, as a more exposed surface area may affect binding dynamics and functional behaviour.

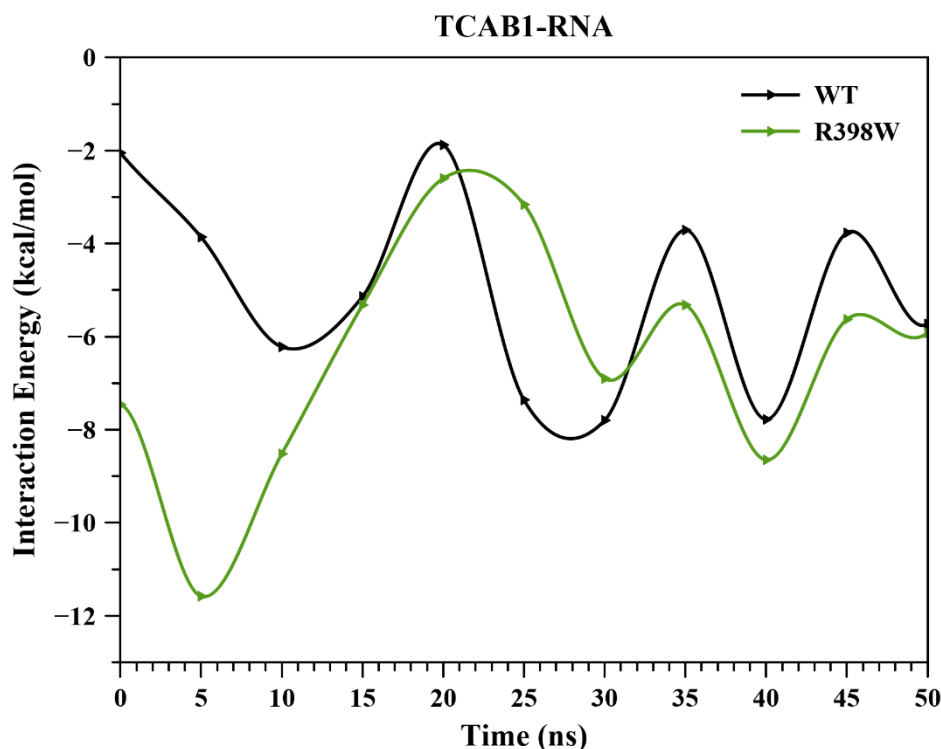


Figure 4.62. Interaction energy profile of WT and R398W mutant TCAB1-RNA complexes over time.

According to Figure 4.62, in the interaction energy profile between TCAB1 and RNA, both the wild-type (WT) and R398W mutant show similar patterns, with the average binding energy for WT at -5 kcal/mol and for R398W at -6.5 kcal/mol. While the R398W mutant exhibits slightly stronger interactions on average, fluctuations across the profile indicate variable binding stability.

Interestingly, both profiles converge toward a similar energy level by the end of the simulation period, suggesting that, despite the R398W mutant's initially stronger interaction, the binding affinity levels out over time. The convergence implies that the mutation might not lead to a significantly enhanced binding strength in practical terms, as the final binding energy values align closely with those of the WT. This observation suggests that the mutation affects the early dynamics of the interaction but does not sustain a notably stronger or more stable binding throughout the interaction timeframe.

4.4. WT and G435R Mutant Protein Results

Both WT and G435R mutant TCAB1 proteins in the complex were subjected to 100 ns molecular dynamics simulations. RMSD values of each simulation were calculated and plotted.

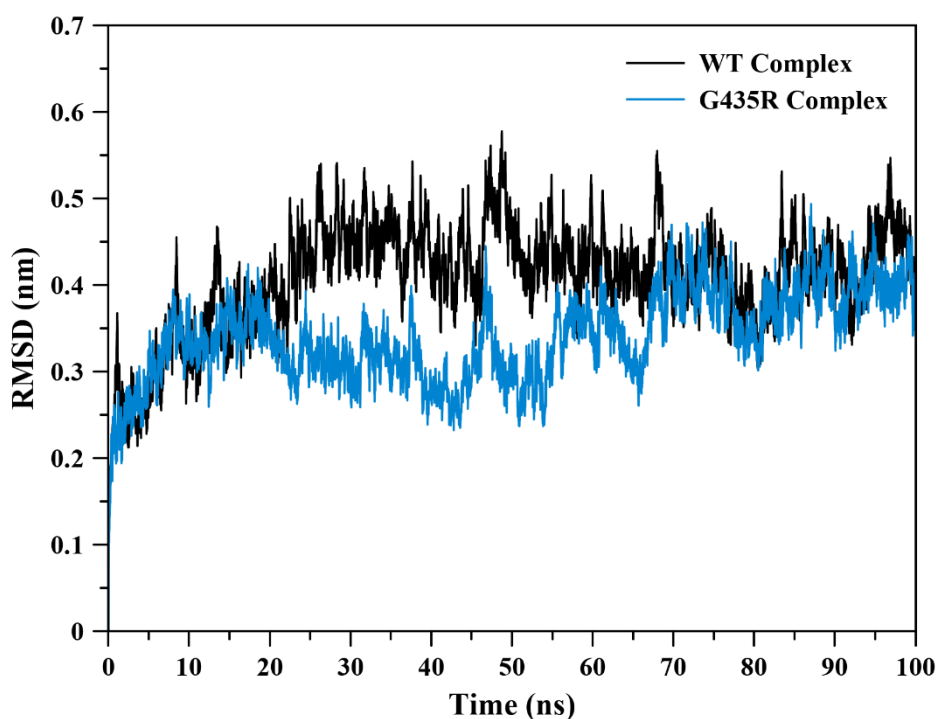


Figure 4.63. The graph shows the RMSD values of each simulation.

According to the graph in Figure 4.63, the RMSD values of the two simulations are balanced around 0.4 nm. The simulation of the wild-type complex equilibrated in 22 nanoseconds, while the simulation of the G435R mutant took 67 nanoseconds to equilibrate.

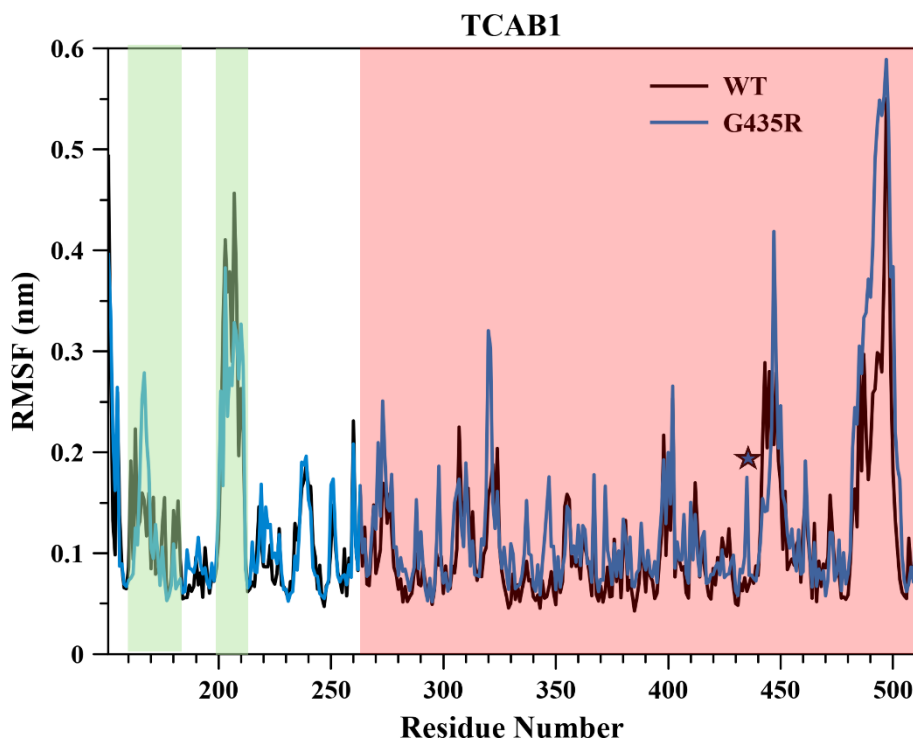


Figure 4.64. Comparison of RMSF values of WT and G435R mutant TCAB1 protein in complexes. The star shows the place of mutation.

In Figure 4.64., the RMSF graph for TCAB1 offers a detailed comparison of the root mean square fluctuation values between the wild-type (WT) and the G435R mutant across the protein's amino acid sequence. The graph displays a notable divergence in the flexibility profiles of the WT and mutant proteins, particularly highlighted by regions color-coded in green and red.

The areas shaded in green, encompassing residues 161-182 and 203-208, show lower RMSF values for the G435R mutant compared to the WT. This indicates reduced flexibility in these segments of the mutant protein, suggesting enhanced structural rigidity that may influence the protein's functional interaction domains or its stability.

Conversely, the extensive region shaded in red, spanning residues 263-510, exhibits significantly higher RMSF values for the G435R mutant. This increased flexibility across a substantial portion of the mutant protein suggests that the G435R mutation introduces substantial alterations in molecular dynamics, potentially affecting the structural integrity and functional capabilities of TCAB1. The elevated RMSF values indicate that the mutant protein may have altered interactions with other cellular components, potentially impacting its role in cellular processes.

The star marker within this red region further emphasizes a specific site where the RMSF peak is notably pronounced, suggesting a critical area where the mutation may have a pronounced effect on the protein's dynamics and interaction properties.

Overall, this analysis underscores the profound impact of the G435R mutation on TCAB1's structural dynamics, highlighting areas of increased and decreased flexibility that could have significant implications for the protein's biological functions. Such insights are crucial for understanding the mutation's role in altering protein behaviour and its potential consequences within the cellular environment.

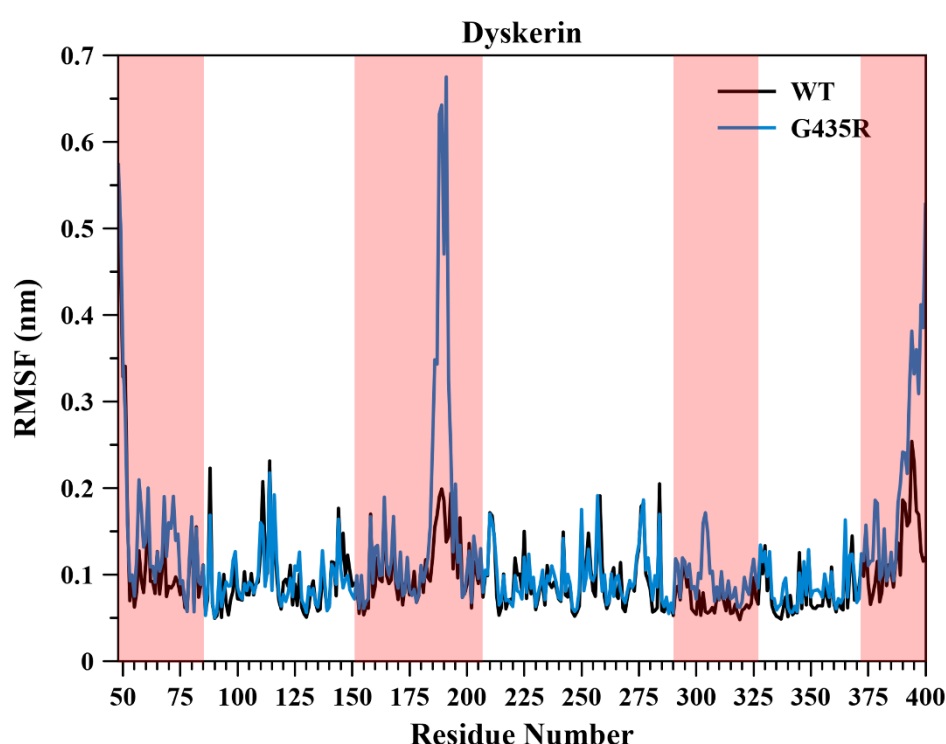


Figure 4.65. Comparison of RMSF values of WT and G435R mutant dyskerin protein in complexes.

The graph in Figure 4.65. reveals that in the regions shaded in red—spanning residues 48-85, 152-206, 291-328, and 374-400—the G435R mutant exhibits higher RMSF values compared to the WT.

This increased flexibility in the mutant suggests that the G435R mutation substantially affects the molecular dynamics of Dyskerin, potentially altering its structural integrity and interaction capabilities. The regions with elevated RMSF values are crucial as they may indicate areas where the mutant protein's altered flexibility impacts its function.

The absence of any regions shaded in green indicates that there are no segments where the mutant displays decreased flexibility compared to the WT, which is notable as it underscores the mutation's pervasive impact on increasing protein dynamics.

These observed increases in flexibility could influence dyskerin's role in critical cellular functions such as RNA processing and telomere maintenance. The heightened RMSF values in these specific domains suggest potential changes in how the G435R mutant interacts with RNA or other telomere-associated proteins. This alteration in dynamics may affect the protein's ability to maintain stable interactions, potentially impacting the overall functionality and stability of the telomerase complex.

Understanding these changes is vital for assessing the G435R mutation's broader implications on Dyskerin's biological activities and its contributions to cellular health and disease processes.

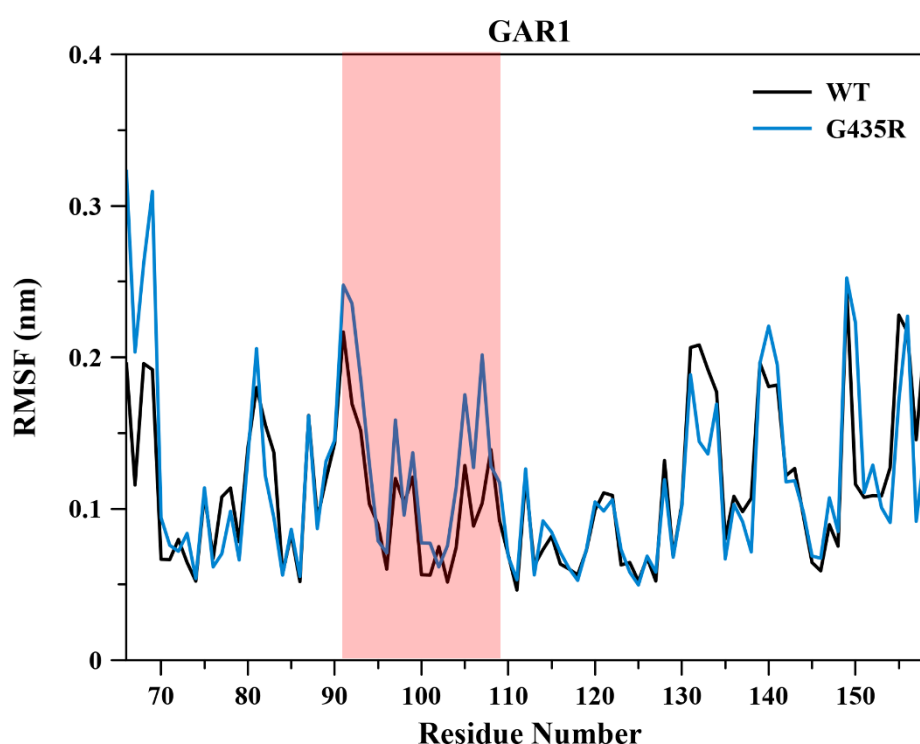


Figure 4.66. Comparison of RMSF values of WT and G435R mutant GAR1 protein in complexes.

The RMSF graph in Figure 4.66., for the GAR1 protein provides a detailed comparison of root mean square fluctuation values between the WT and the G435R mutant across specific regions of the protein sequence. Particularly notable is the region shaded in

red, spanning residues 91-109, where the G435R mutant exhibits higher RMSF values compared to the WT. This increase in flexibility within this segment suggests that the G435R mutation contributes to enhanced molecular dynamics, potentially affecting the structural integrity and functional interactions of the GAR1 protein.

The absence of any regions shaded in green indicates that there are no segments where the mutant displays decreased flexibility compared to the WT. This observation underscores the mutation's overall impact on increasing the dynamics across the examined sequence. The heightened RMSF values in the red-shaded region may imply alterations in how the mutant GAR1 interacts with other cellular components, possibly impacting its role in cellular processes such as RNA processing or the assembly of ribonucleoprotein complexes.

This analysis highlights the significance of the G435R mutation in altering the structural dynamics of GAR1, suggesting that the mutation may lead to changes in the protein's interaction capabilities and stability. Such insights are crucial for understanding the functional implications of mutations on protein behaviour.

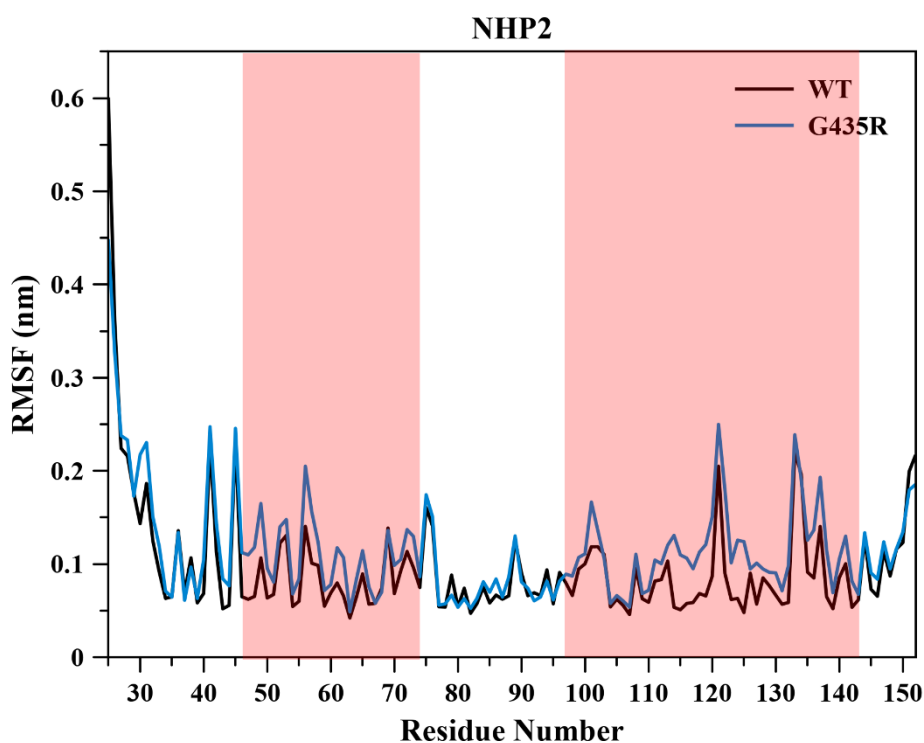


Figure 4.67. Comparison of RMSF values of WT and G435R mutant NHP2 protein in complexes.

The graph in Figure 4.67., highlights two primary regions, shaded in red—spanning residues 46-74 and 97-143—where the G435R mutant exhibits significantly higher RMSF values compared to the WT. This increased flexibility within these segments suggests that the G435R mutation introduces substantial alterations in the molecular dynamics of NHP2, potentially affecting its structural and functional properties.

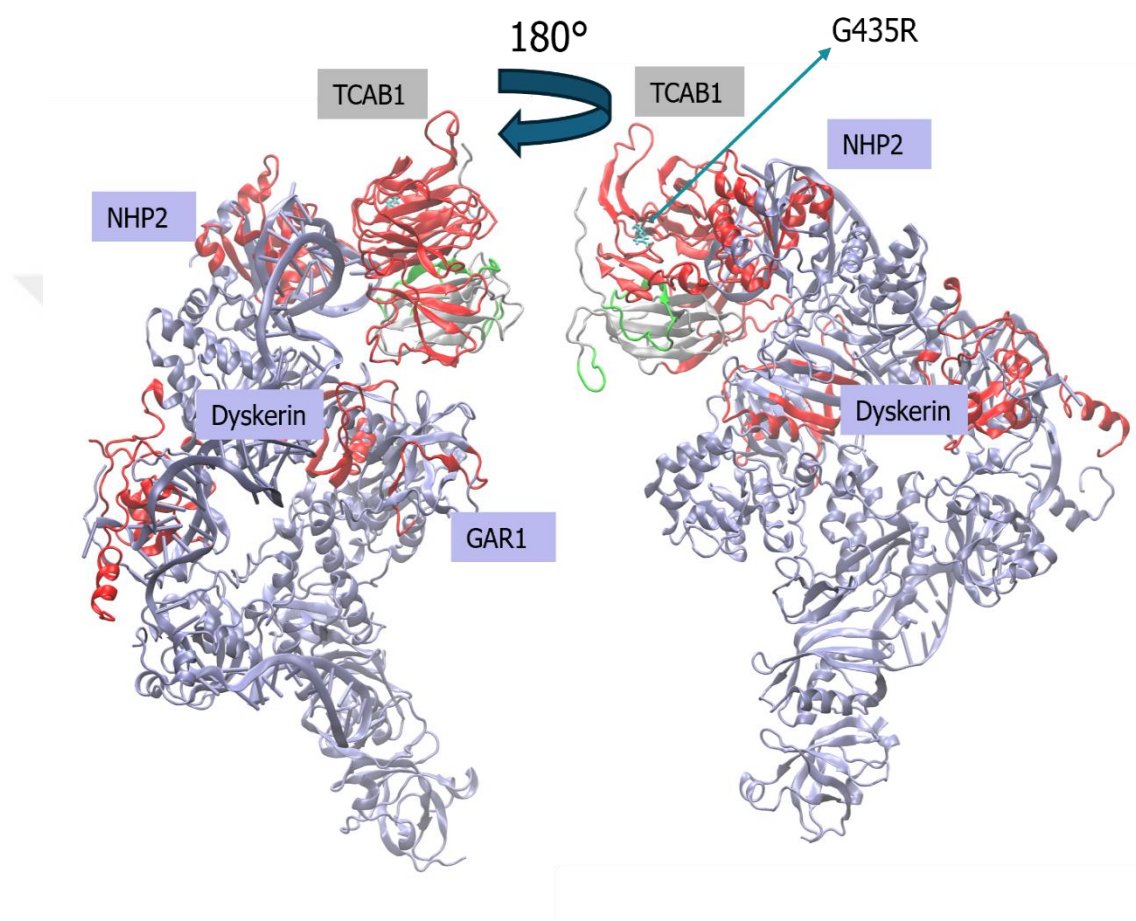


Figure 4.68. G435R complex structure. The protein shown in grey is the TCAB1 protein, the rest of the complex is shown in metallic blue.

The absence of green-shaded areas indicates that there are no segments where the G435R mutant shows decreased flexibility relative to the WT across the examined sequence. This finding underscores the mutation's pervasive impact on increasing the dynamic behavior of the protein, particularly in the red-highlighted regions. The elevated RMSF values in these areas may indicate a potential alteration in the protein's ability to maintain stable interactions with other molecular components or its role in RNA processing and telomere maintenance.

The increased flexibility in these specific domains of NHP2 could lead to changes in how the mutant protein interacts with other components of the telomerase complex or affect the overall stability and functionality of the protein within cellular processes. This detailed analysis is crucial for understanding the implications of the G435R mutation on NHP2's biological activities.

The visualization in Figure 4.68. portrays the G435R mutant complex, consisting of one TCAB1 molecule, one RNA molecule, and two copies each of dyskerin, GAR1, NHP2, and NOP10 proteins. The G435R mutation is located on the TCAB1 protein and is indicated in the image by a blue arrow. The two views presented are rotations of each other, each displayed at a 180-degree angle, providing a comprehensive perspective on the spatial arrangement of the complex.

In this graphical representation, amino acid sequences highlighted in red denote regions where the RMSF values of the mutant protein are higher than those of the WT protein, suggesting increased flexibility in these segments. Conversely, sequences marked in green indicate areas where the RMSF values are lower in the mutant compared to the WT, signifying decreased flexibility. These highlighted regions correspond precisely to the RMSF values described in the table discussions for each protein interacting with TCAB1.

Specifically, in the regions where TCAB1 interacts with NHP2, a decrease in RMSF values has been observed, indicating a potential stabilization of this interaction interface in the mutant. However, the interaction domains of TCAB1 with dyskerin and GAR1 show increased RMSF values, suggesting enhanced flexibility and possibly altered interaction dynamics in these areas. Notably, the dyskerin protein exhibits increased RMSF across all its binding regions with RNA, TCAB1, and GAR1, with the TCAB1 interaction site showing the highest increase among all mutations analyzed. Similarly, the GAR1 protein's interface with dyskerin has also experienced an increase in RMSF, indicating a potential destabilization or increased dynamic behaviour at this interaction site. Minor increases in RMSF have been detected in NHP2 at its binding sites with TCAB1 and RNA, which may suggest slight alterations in the interaction dynamics within these regions.

This detailed analysis underscores the significant impact of the G435R mutation on the structural dynamics of the proteins within the complex, highlighting variations in

flexibility that could influence the functional interactions and overall stability of the complex. Such insights are crucial for understanding the biological implications of mutations within the complex and their potential effects on cellular functionality and disease progression.

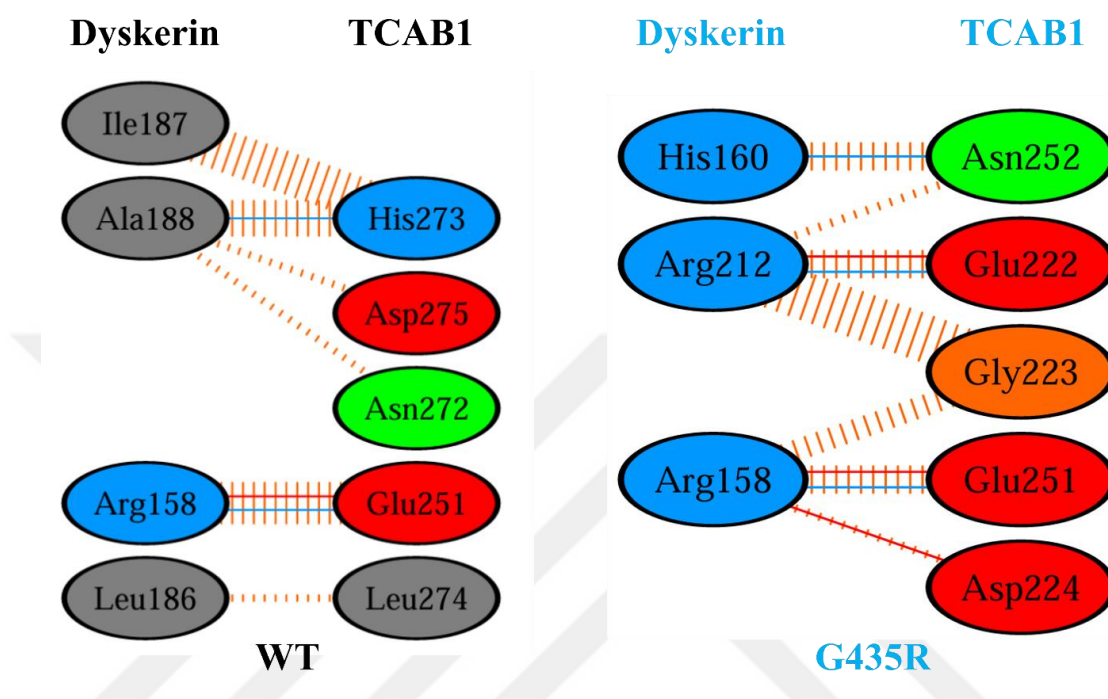


Figure 4.69. Comparative analysis of the interactions between the G435R mutant TCAB1 and WT TCAB1 proteins with the dyskerin protein within the complex structures.

In Figure 4.69, the interactions between the WT TCAB1 protein and its G435R mutant with the dyskerin protein are compared. The orange lines represent non-bonded contacts (non-covalent interactions), the red lines represent salt bridges, and the blue lines represent hydrogen bonds.

In the WT, His273 forms non-bonded contacts with Ala188 and Asp275 while also forming a hydrogen bond with Asn272. These interactions are absent in the mutant. Glu251 forms a hydrogen bond and non-bonded contact with Arg158 in both cases. In the WT, Glu251 also forms a non-bonded contact with Leu274, whereas, in the G435R mutant, it forms a salt bridge with Asp224.

In the G435R mutant, His160 and Arg212 form non-bonded contacts with Asn252. Additionally, Arg212 forms a hydrogen bond with Glu222 and a non-bonded contact with Gly223. These interactions are not present in the wild type.

Overall, there are significant differences in binding between the WT and G435R mutants. The mutant develops some new interactions (His160, Glu222, Gly223) while losing some of the original interactions (His273, Asn272). In conclusion, most of the interactions in both the wild-type and the G435R mutant occur between hydrophilic amino acids. However, the hydrophobic interactions present in the wild type (e.g., Leu274) are lost in the G435R mutant. This suggests that the mutant protein has acquired a more hydrophilic character, which could alter its interaction dynamics with dyskerin.

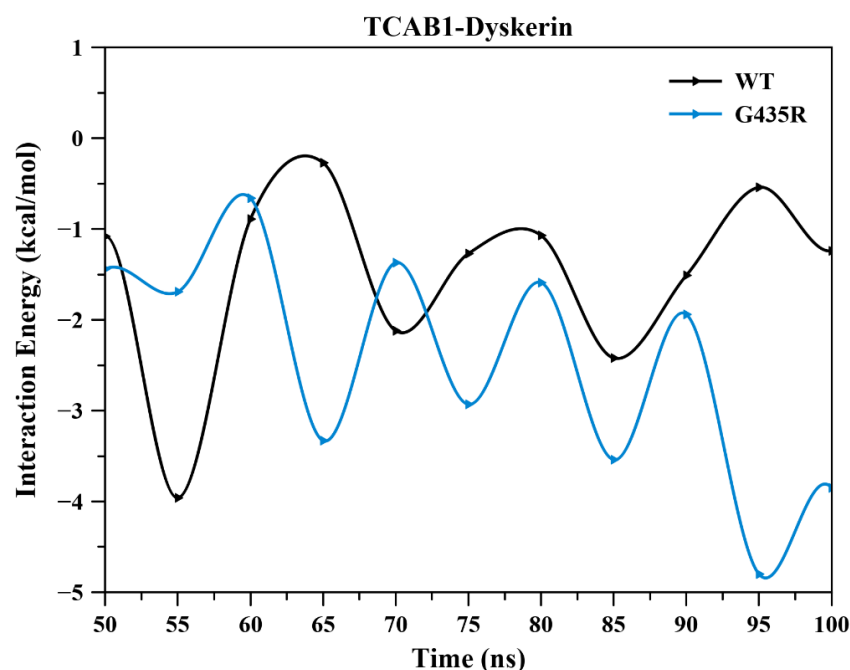


Figure 4.70. Interaction energy profile of WT and G435R mutant TCAB1-Dyskerin complexes over time.

As seen in Figure 4.70, the binding energy for the WT TCAB1-dyskerin complex fluctuated around an average of -1.4 kcal/mol. These energy values exhibited significant variability, occasionally approaching 0 kcal/mol, suggesting periods of weakened binding affinity or even partial dissociation. In contrast, the G435R mutant-dyskerin complex demonstrated a more stable binding profile, with an average energy of -2.4 kcal/mol. Fluctuations in the G435R complex were less pronounced, remaining

consistently within a negative range, indicative of a stronger and more stable interaction.

These results indicate that the G435R mutation enhances the binding affinity between TCAB1 and dyskerin. The more negative average binding energy observed in the G435R mutant suggests that the mutation facilitates a more favourable interaction, leading to a stronger and more stable complex formation. The reduced fluctuations in binding energy further support the conclusion that the mutant complex remains consistently stable throughout the simulation, with minimal risk of dissociation.

In contrast, the WT TCAB1-dyskerin complex exhibits a less stable interaction. The higher average binding energy and notable fluctuations, including values approaching 0 kcal/mol, suggest that the WT complex experiences periods of instability where the binding affinity is significantly weakened. These fluctuations may reflect transient conformational changes or partial dissociation events that reduce the overall stability of the complex.

The findings suggest that the G435R mutation enhances the interaction between TCAB1 and dyskerin by increasing binding affinity and stabilizing the complex. In contrast, the less stable binding energy profile observed in the WT complex suggests a more dynamic and potentially weaker interaction with dyskerin.

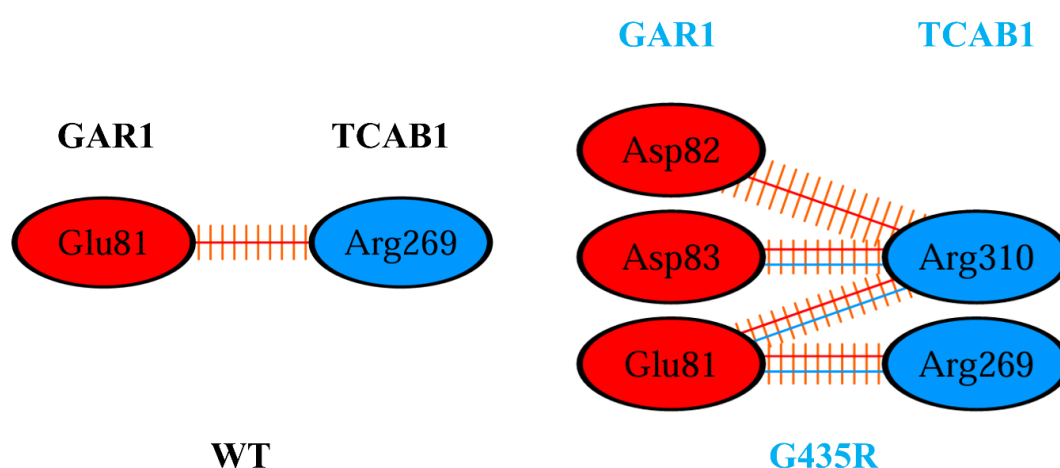


Figure 4.71. Comparative analysis of the interactions between the G435R mutant TCAB1 and WT TCAB1 proteins with the GAR1 protein within the complex structures.

In Figure 4.71, the interactions between the WT TCAB1 protein and its G435R mutant with the GAR1 protein are compared. In the wild-type TCAB1, the Glu81 residue forms a non-bonded contact with Arg269. Glu81 is a hydrophilic amino acid with a negatively charged side chain, which allows it to form a strong electrostatic interaction with the positively charged Arg269. In the G435R mutant, more interactions are observed. Arg310 and Arg269 (both hydrophilic and positively charged) form multiple non-bonded contacts and hydrogen bonds with Glu81, Asp82, and Asp83 (all negatively charged, hydrophilic amino acids).

In the G435R mutant, whereas the wild-type shows only the Glu81-Arg269 interaction, the mutant protein reveals new binding sites. Asp82 and Asp83 become involved in these interactions, forming interactions with new partners such as Arg310. This suggests that the G435R mutant has an increased binding capacity with GAR1, including more salt bridges and hydrogen bonds.

From a hydrophobic/hydrophilic interaction perspective, all the interacting residues are hydrophilic. Thus, the interactions occur between hydrophilic amino acids, and in the G435R mutant, hydrophilic interactions increase significantly. This indicates that the mutation could significantly affect the protein's interaction with water or its functional conformation.

In conclusion, the G435R mutant appears to have increased its binding strength with GAR1 compared to the wild type by forming more interactions.

Figure 4.72 illustrates the interaction energies between WT and G435R mutant TCAB1 proteins with GAR1 over the final 50 nanoseconds of molecular dynamics simulation.

The WT TCAB1-GAR1 complex exhibits interaction energies that frequently hover near or above 0 kcal/mol, with an average value of -0.46 kcal/mol. These relatively high energy levels suggest that the WT complex generally displays weak binding, with a tendency towards instability or transient interactions. In contrast, the G435R mutant complex consistently demonstrates more negative interaction energies, averaging -1.16 kcal/mol. This indicates that the mutant complex forms a stronger and more stable interaction with GAR1.

The fluctuations observed in the WT complex's energy levels, including periodic rises to positive values, suggest instability in the interaction, with moments where the

binding may weaken or partially dissociate. Conversely, the G435R mutant complex shows lower energy levels with less pronounced fluctuations, indicative of a more stable and consistently strong interaction with GAR1.

In terms of binding affinity, the more negative average interaction energy of the G435R mutant (-1.16 kcal/mol) compared to the WT (-0.46 kcal/mol) suggests that the mutation enhances the binding affinity and stability of the TCAB1-GAR1 interaction. The WT complex's tendency to reach positive energy values indicates weaker binding and potential instability in the interaction.

In conclusion, the WT TCAB1 protein exhibits a weaker and less stable interaction with GAR1, as evidenced by its higher average interaction energy and significant fluctuations that sometimes reach positive values. These fluctuations indicate that the complex experiences periods of instability, where the binding is weak or potentially dissociative. In contrast, the G435R mutant shows a stronger and more stable interaction with GAR1, as indicated by its more negative average interaction energy and reduced fluctuations. These findings suggest that the G435R mutation strengthens the TCAB1-GAR1 interaction, leading to a more stable complex formation.

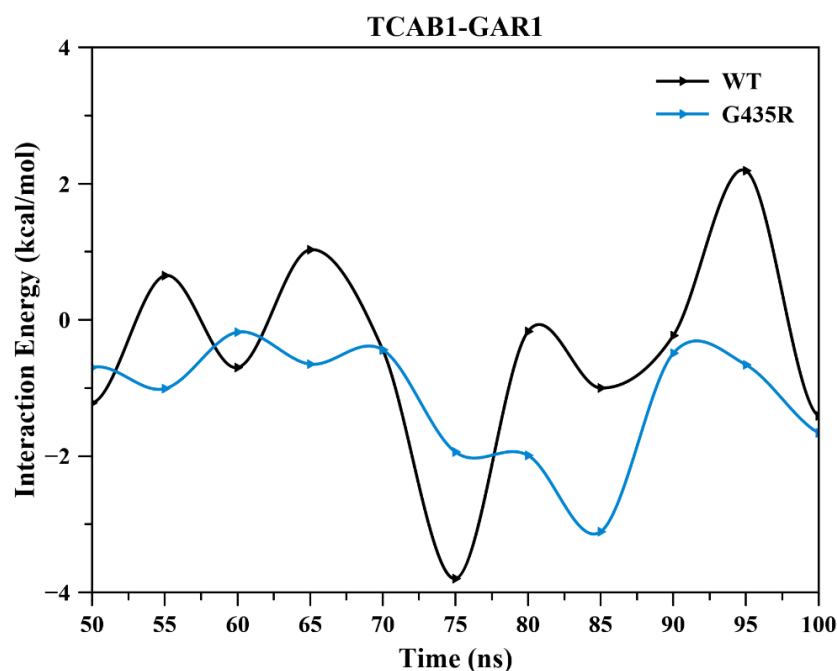


Figure 4.72. Interaction energy profile of WT and G435R mutant TCAB1-GAR1 complexes over time. This illustrates the interaction energies between WT and G435R mutant TCAB1 proteins with GAR1 over the final 50 nanoseconds of molecular dynamics simulation.

The WT TCAB1-GAR1 complex exhibits interaction energies that frequently hover near or above 0 kcal/mol, with an average value of -0.46 kcal/mol. These relatively high energy levels suggest that the WT complex generally displays weak binding, with a tendency towards instability or transient interactions. In contrast, the G435R mutant complex consistently demonstrates more negative interaction energies, averaging -1.16 kcal/mol. This indicates that the mutant complex forms a stronger and more stable interaction with GAR1.

The fluctuations observed in the WT complex's energy levels, including periodic rises to positive values, suggest instability in the interaction, with moments where the binding may weaken or partially dissociate. Conversely, the G435R mutant complex shows lower energy levels with less pronounced fluctuations, indicative of a more stable and consistently strong interaction with GAR1.

In terms of binding affinity, the more negative average interaction energy of the G435R mutant (-1.16 kcal/mol) compared to the WT (-0.46 kcal/mol) suggests that the mutation enhances the binding affinity and stability of the TCAB1-GAR1 interaction. The WT complex's tendency to reach positive energy values indicates weaker binding and potential instability in the interaction.

In conclusion, the WT TCAB1 protein exhibits a weaker and less stable interaction with GAR1, as evidenced by its higher average interaction energy and significant fluctuations that sometimes reach positive values. These fluctuations indicate that the complex experiences periods of instability, where the binding is weak or potentially dissociative. In contrast, the G435R mutant shows a stronger and more stable interaction with GAR1, as indicated by its more negative average interaction energy and reduced fluctuations. These findings suggest that the G435R mutation strengthens the TCAB1-GAR1 interaction, leading to a more stable complex formation.

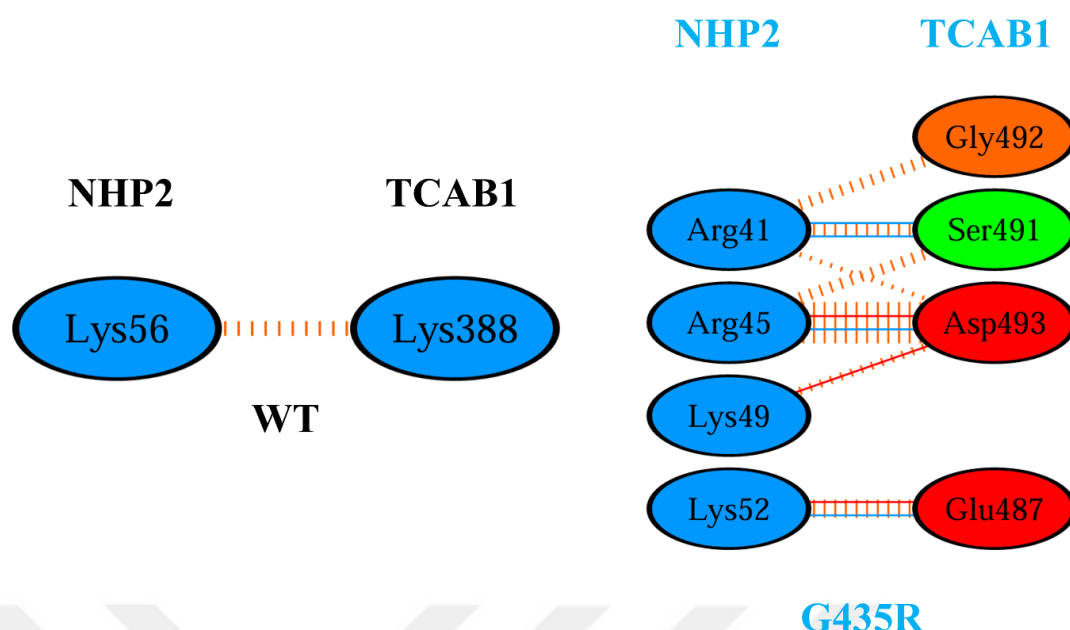


Figure 4.73. Comparative analysis of the interactions between the G435R mutant TCAB1 and WT TCAB1 proteins with the NHP2 protein within the complex structures.

As seen in Figure 4.73, the interactions between the WT TCAB1 protein and its G435R mutant with the NHP2 protein are compared. In the WT TCAB1, a non-bonded contact is formed between Lys56 and Lys388. Lysine is a hydrophilic and positively charged amino acid. Despite both being positively charged, this interaction occurs between the side chain of Lys388 and carboxyl oxygen of Lys56 amino acid and may be important for maintaining the overall conformation of the proteins.

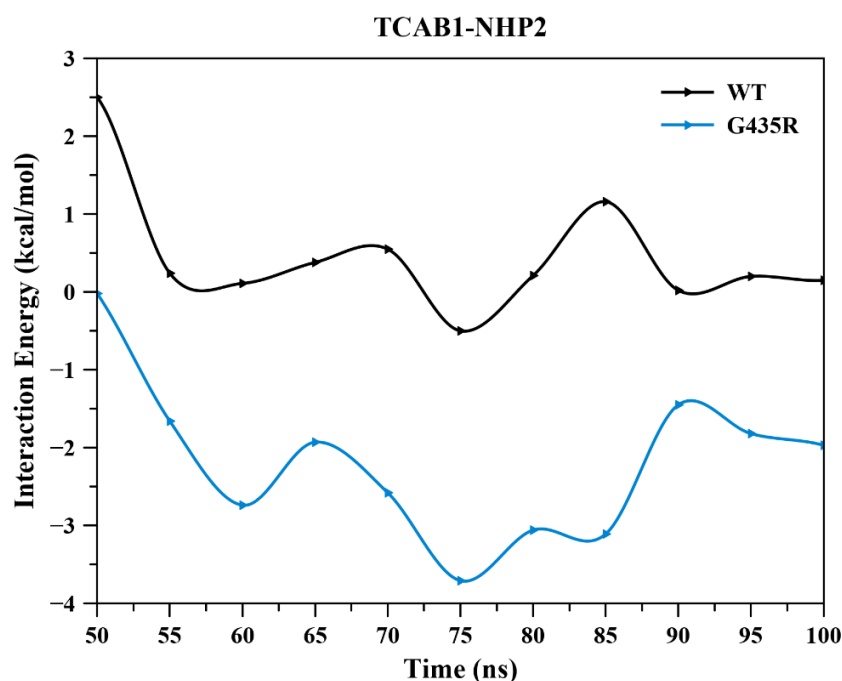


Figure 4.74. Interaction energy profile of WT and G435R mutant TCAB1-NHP2 complexes over time.

In the G435R mutant, more complex and numerous interactions with the NHP2 protein are observed. A hydrogen bond is present between Arg41 and Ser491, while Arg45 forms a salt bridge and non-bonded contacts with Asp493. Lys49 and Lys52 form salt bridges and non-bonded contacts with Asp493 and Glu487, respectively. The interactions primarily occur between hydrophilic amino acids. Most of the interacting residues in both the WT and G435R mutant are hydrophilic. Notably, the G435R mutant shows strong electrostatic interactions, such as salt bridges and hydrogen bonds. In conclusion, the G435R mutant establishes more and stronger interactions with the NHP2 protein compared to the wild type.

Figure 4.74 displays the interaction energies between WT and G435R mutant TCAB1 proteins with NHP2 over the final 50 nanoseconds of molecular dynamics simulation. In the WT TCAB1-NHP2 complex, the average interaction energy is 0.45 kcal/mol, indicating a relatively weak and potentially unstable interaction. The positive energy values suggest that the interaction is not energetically favourable, and the binding affinity is low. The energy values hover around or slightly above zero throughout the simulation, indicating that the proteins may not be forming a strong or stable complex. In contrast, the G435R mutant TCAB1-NHP2 complex shows an average interaction energy of -2.18 kcal/mol, which is significantly more negative. This suggests a much

stronger and more stable interaction between TCAB1 and NHP2 in the presence of the G435R mutation. The more negative energy values indicate a higher binding affinity and the interaction is more energetically favourable compared to the WT complex.

The WT complex exhibits relatively small fluctuations, with energy values consistently around the 0 kcal/mol mark. These minimal fluctuations suggest that while the interaction is weak, it remains relatively consistent over time, though not strong enough to indicate a stable or robust binding interaction.

On the other hand, the G435R mutant complex displays larger fluctuations, with energy values dipping as low as -1.5 kcal/mol and as high as 1.2 kcal/mol. Despite these fluctuations, the overall average energy is much more negative, indicating that the complex is capable of achieving stronger binding states, even if it occasionally fluctuates towards less favourable interactions.

The comparison between WT and G435R mutant TCAB1 interactions with NHP2 clearly shows that the G435R mutation significantly enhances the binding affinity and stability of the complex. While the WT complex exhibits weak and energetically unfavourable interactions, the G435R mutant complex demonstrates a much stronger and more stable interaction profile, as indicated by its more negative average interaction energy and its capacity to reach stronger binding states despite some fluctuations. This suggests that the G435R mutation introduces structural or electrostatic changes that favour stronger and more energetically favourable binding between TCAB1 and NHP2.

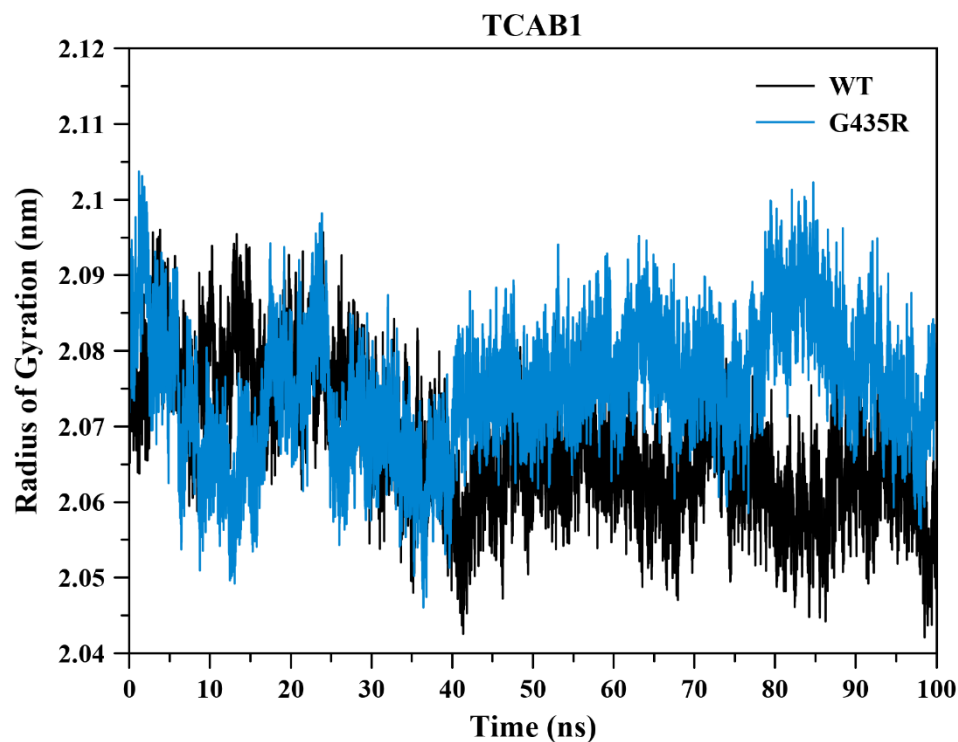


Figure 4.75. Radius of gyration graph of TCAB1 protein in WT complex and G435R mutant complex.

According to Figure 4.75., the WT protein maintains Rg values around 2.07 to 2.09 nm, indicating a stable and compact structure with limited fluctuations. In contrast, the G435R mutant exhibits slightly lower Rg values, primarily fluctuating between 2.05 and 2.08 nm. This reduction in Rg suggests that the G435R mutation may induce a more compact conformation in TCAB1 compared to the WT. Additionally, the G435R mutant shows greater fluctuations in Rg values throughout the simulation, which may indicate increased flexibility or dynamic structural adjustments.

In summary, the G435R mutation in TCAB1 appears to lead to a marginally more compact and flexible structure. These structural alterations could affect the protein's interaction dynamics and stability, as increased flexibility and compactness might influence binding capabilities and functional properties.

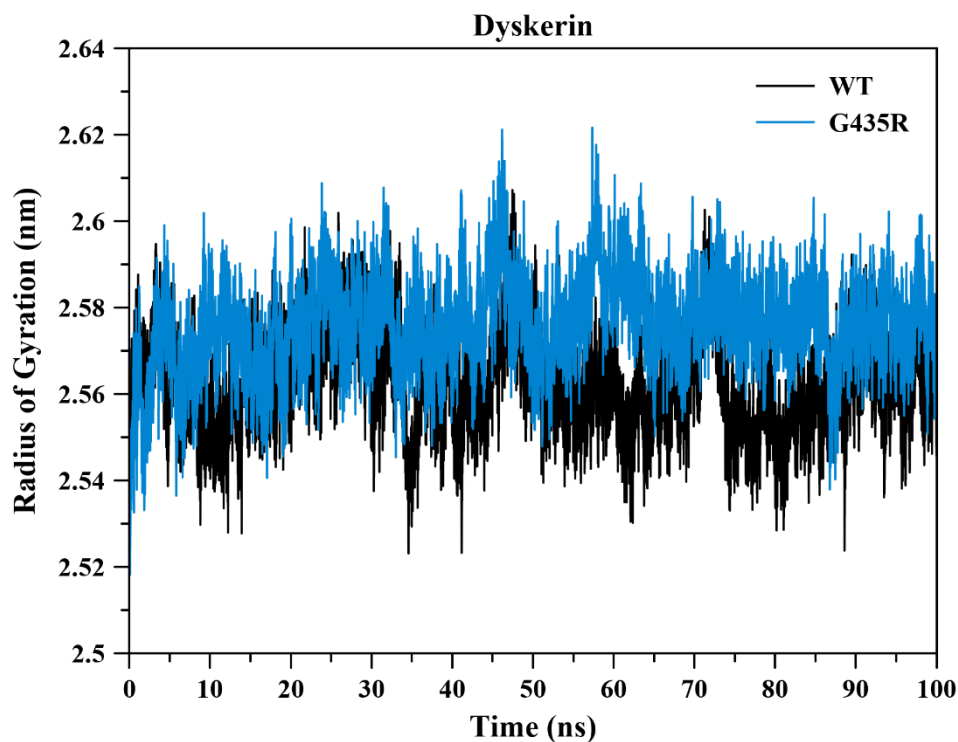


Figure 4.76. Radius of gyration graph of dyskerin protein in WT complex and G435R mutant complex.

In Figure 4.76, the WT protein maintains Rg values primarily around 2.54 to 2.58 nm, indicating a stable, compact structure. The G435R mutant, however, exhibits slightly higher Rg values, fluctuating between 2.56 and 2.61 nm. This increase suggests that the G435R mutation may result in a more expanded or less compact conformation in Dyskerin relative to the WT. Additionally, the G435R mutant shows greater fluctuations in Rg values, indicating increased structural flexibility or dynamic adjustments.

In summary, the G435R mutation in Dyskerin appears to lead to a slightly expanded and more flexible structure. These structural changes may impact Dyskerin's functional dynamics, as an increase in flexibility and expansion could alter its stability and interaction potential.

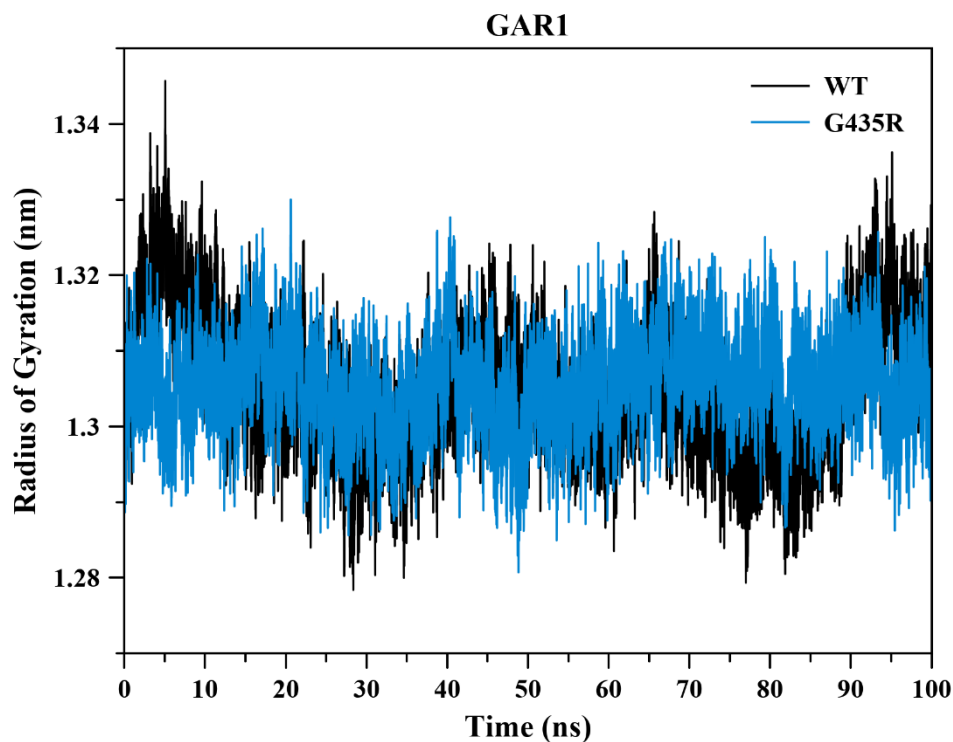


Figure 4.77. Radius of gyration graph of GAR1 protein in WT complex and G435R mutant complex.

The Rg plot in Figure 4.77 for GAR1 compares the WT and G435R mutant forms, showing differences in structural compactness over the simulation. The WT protein generally maintains Rg values around 1.29 to 1.32 nm, indicating a stable, compact structure. In comparison, the G435R mutant exhibits similar Rg values, fluctuating between approximately 1.30 and 1.33 nm, suggesting that the G435R mutation does not cause a significant change in compactness. However, the G435R mutant displays slightly more fluctuation in Rg, indicating a potential increase in flexibility compared to the WT.

In summary, while the G435R mutation in GAR1 does not drastically alter compactness, it appears to introduce a slight increase in flexibility. This change may have implications for GAR1's interaction dynamics and stability.

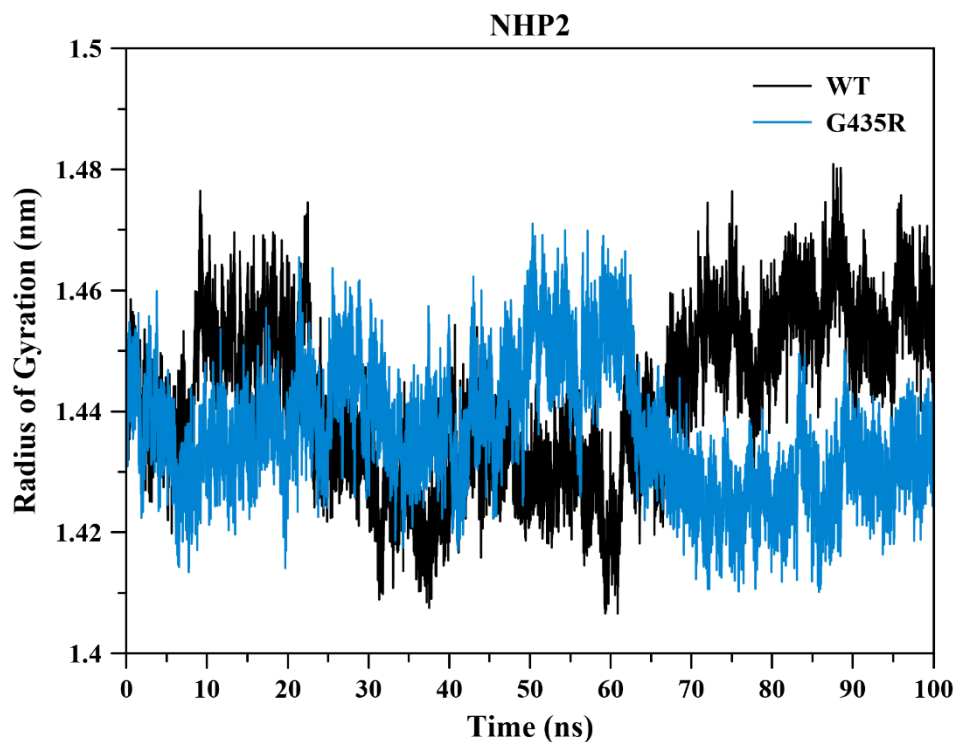


Figure 4.78. Radius of gyration graph of NHP2 protein in WT complex and G435R mutant complex.

As seen in Figure 4.78, the WT protein shows Rg values primarily around 1.43 to 1.46 nm, indicating a compact and stable structure. The G435R mutant exhibits similar Rg values, fluctuating slightly lower, mostly between 1.42 and 1.45 nm. This slight reduction suggests that the G435R mutation may induce a marginally more compact structure in NHP2. Additionally, the G435R mutant shows some fluctuation, particularly in the early stages of the simulation, indicating potential structural adjustments. In summary, the G435R mutation in NHP2 appears to result in a slightly more compact structure with minimal changes in flexibility.

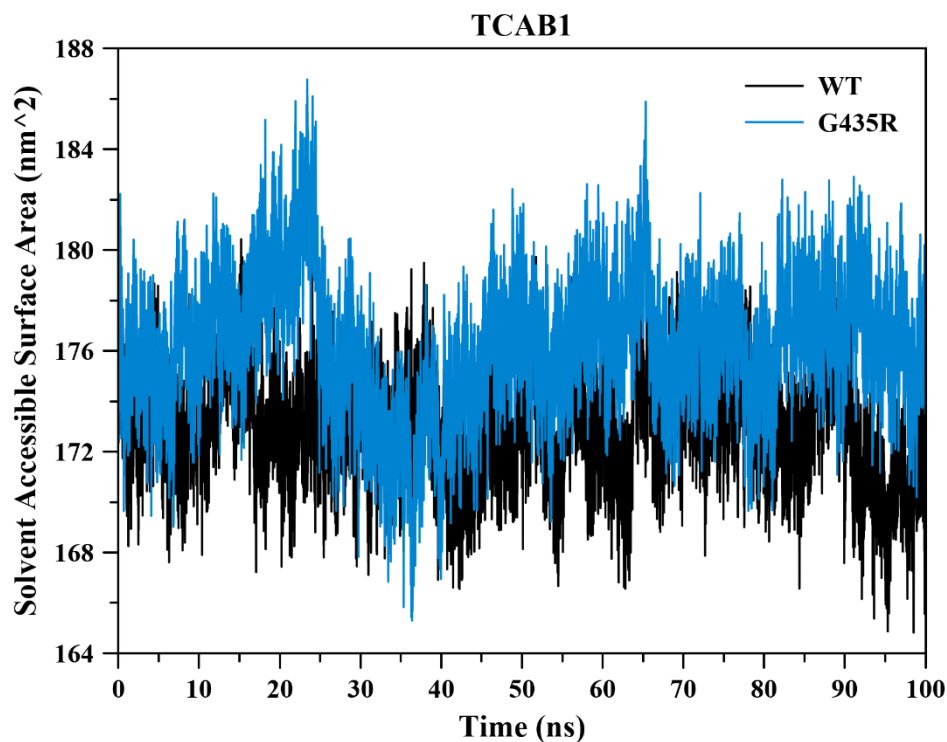


Figure 4.79. Solvent-accessible surface area graph of TCAB1 protein in WT complex and G435R mutant complex.

As seen in Figure 4.79, the WT protein generally displays SASA values fluctuating between 170 and 178 nm², indicating a stable level of solvent accessibility. In contrast, the G435R mutant exhibits higher SASA values, mostly fluctuating between 175 and 184 nm². This increase suggests that the G435R mutation results in a more solvent-exposed structure, indicating a slightly less compact conformation compared to the WT. The G435R mutant also shows greater fluctuations in SASA, which may reflect increased structural flexibility or dynamic adjustments over time.

In summary, the G435R mutation in TCAB1 appears to lead to a more open structure with enhanced solvent accessibility. These structural changes could impact the protein's interaction potential and stability, as increased solvent exposure may influence binding interactions or functional dynamics.

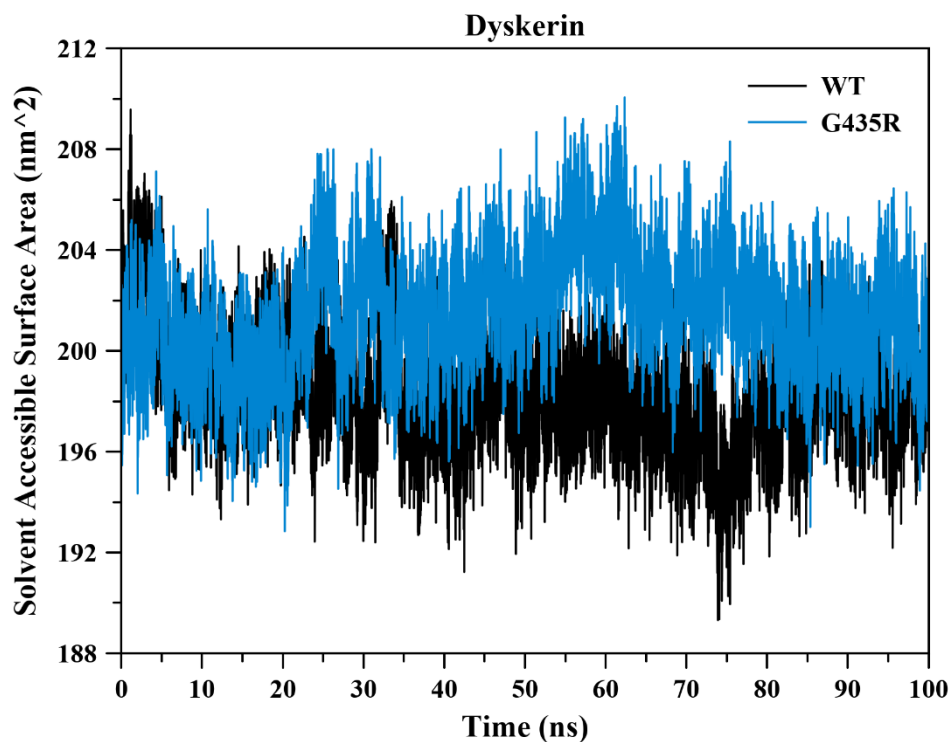


Figure 4.80. Solvent-accessible surface area graph of dyskerin protein in WT complex and G435R mutant complex.

According to Figure 4.80, the WT protein generally displays SASA values between 196 and 202 nm², suggesting a stable and compact structure. In contrast, the G435R mutant exhibits higher SASA values, primarily fluctuating between 200 and 208 nm². This increase in SASA suggests that the G435R mutation results in a more solvent-exposed structure, indicating a slightly expanded conformation compared to the WT. The G435R mutant also shows greater fluctuations, which may reflect increased structural flexibility or dynamic adjustments.

In summary, the G435R mutation in Dyskerin appears to lead to a more open and flexible structure with enhanced SASA. These structural changes could affect the protein's interaction dynamics and stability, as increased solvent exposure may influence binding capabilities and functional properties.

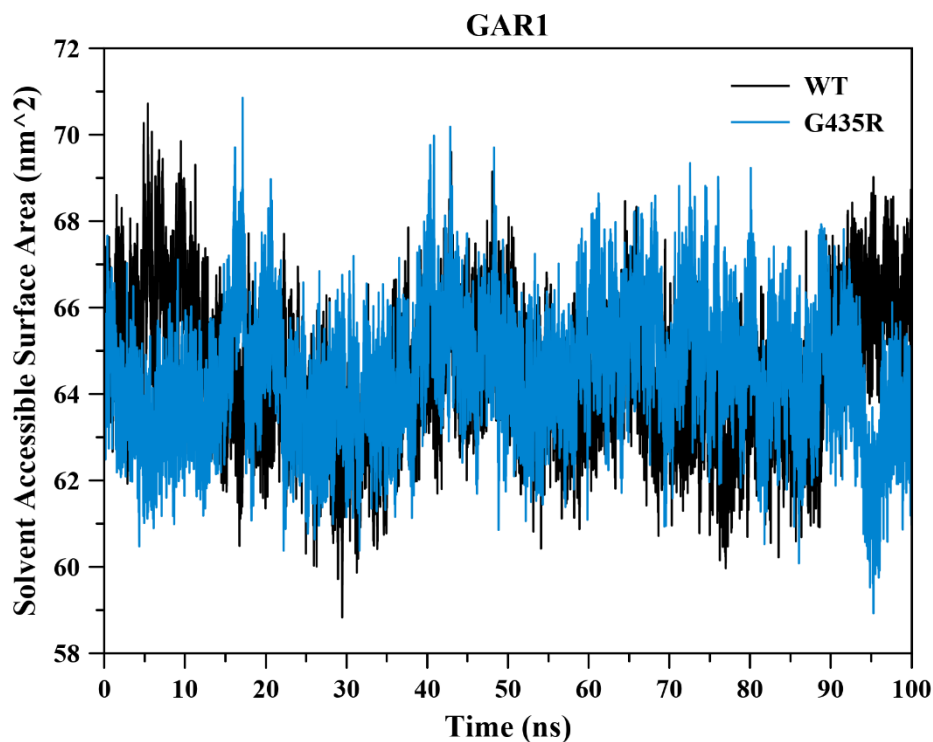


Figure 4.81. Solvent-accessible surface area graph of GAR1 protein in WT complex and G435R mutant complex.

According to Figure 4.81., the WT protein generally displays SASA values between 62 and 68 nm², indicating a compact structure with stable solvent accessibility. In contrast, the G435R mutant shows slightly higher SASA values, mostly fluctuating between 64 and 70 nm². This increase suggests that the G435R mutation results in a more solvent-exposed structure, indicating a less compact conformation compared to the WT. Additionally, the G435R mutant exhibits greater variability in SASA, which may reflect increased flexibility or dynamic adjustments.

In summary, the G435R mutation in GAR1 appears to lead to a slightly expanded and more flexible structure with enhanced SASA. These structural changes could influence GAR1's interaction dynamics and stability, as increased solvent exposure may impact binding interactions and overall functionality.

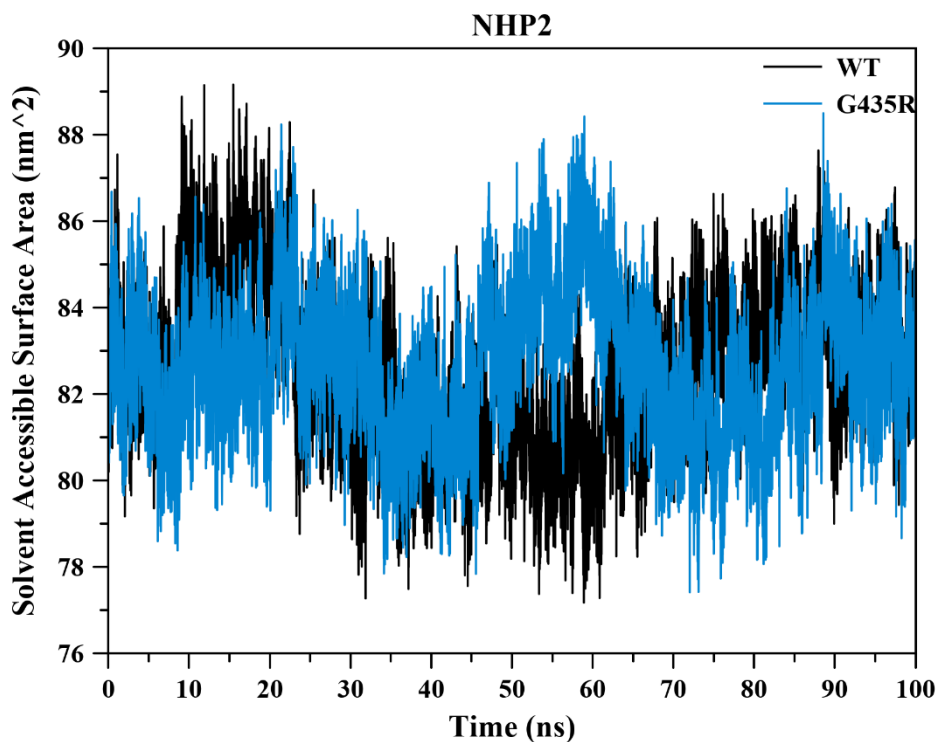


Figure 4.82. Solvent-accessible surface area graph of NHP2 protein in WT complex and G435R mutant complex.

According to Figure 4.82, the WT protein maintains SASA values generally between 80 and 86 nm², indicating a relatively compact and stable structure. The G435R mutant, however, displays higher SASA values, fluctuating mostly between 82 and 88 nm². This increase suggests that the G435R mutation results in a more solvent-exposed structure, indicating a slightly expanded conformation compared to the WT. The G435R mutant also exhibits more variability in SASA, implying increased structural flexibility or dynamic adjustments throughout the simulation.

In summary, the G435R mutation in NHP2 appears to lead to a more open and flexible structure with higher SASA. These structural changes could affect NHP2's interaction dynamics and stability, as an increase in solvent exposure may influence binding potential and overall functionality.

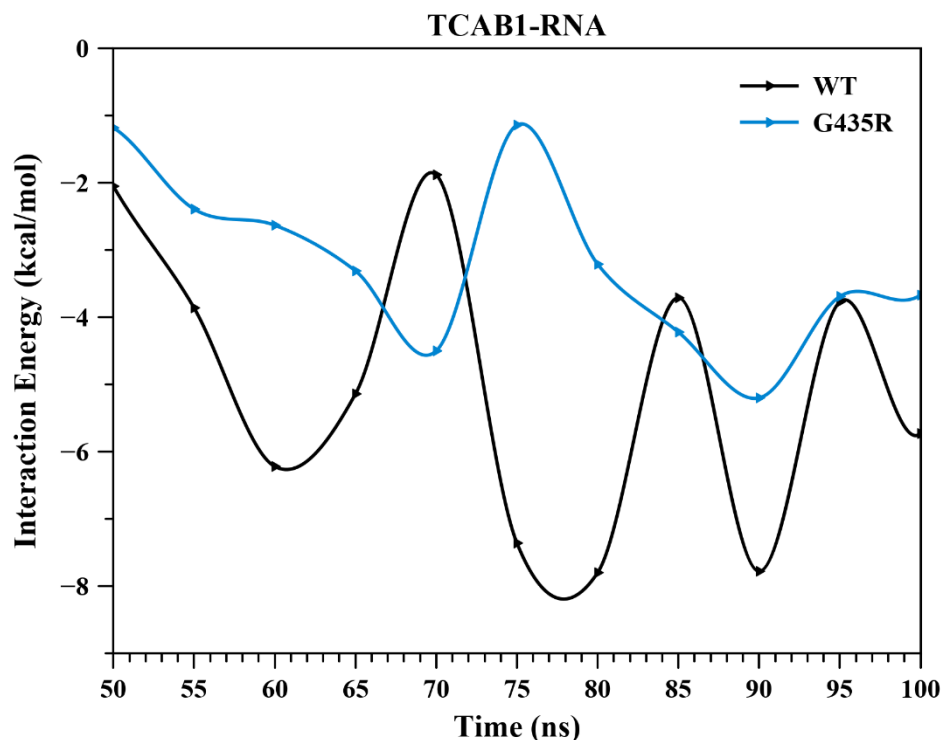


Figure 4.83. Interaction energy profile of WT and G435R mutant TCAB1-RNA complexes over time.

In this interaction energy profile in Figure 4.83., between TCAB1 and RNA, the WT has an average binding energy of -5 kcal/mol, while the G435R mutant shows a less negative average binding energy of -3 kcal/mol. This difference indicates that the G435R mutation leads to a weaker interaction with RNA compared to the WT.

Throughout the simulation, the WT consistently displays more negative energy values, suggesting a stronger binding affinity and a more stable interaction profile with RNA. In contrast, the G435R mutant demonstrates a relatively higher fluctuation range and less negative binding energies, reinforcing the notion of a reduced affinity. The upward shift in the average energy levels for the G435R mutant implies that this mutation may disrupt the optimal binding conformation or reduce the strength of interactions with RNA, possibly impacting TCAB1's functionality in RNA binding and related cellular processes.

4.5. WT and F164L/R398W Mutant Protein Results

Both WT and F164L/R398W mutant TCAB1 proteins in the complex were subjected to molecular dynamics simulations for 100 ns and 200 ns, respectively. RMSD values of each simulation were calculated and plotted.

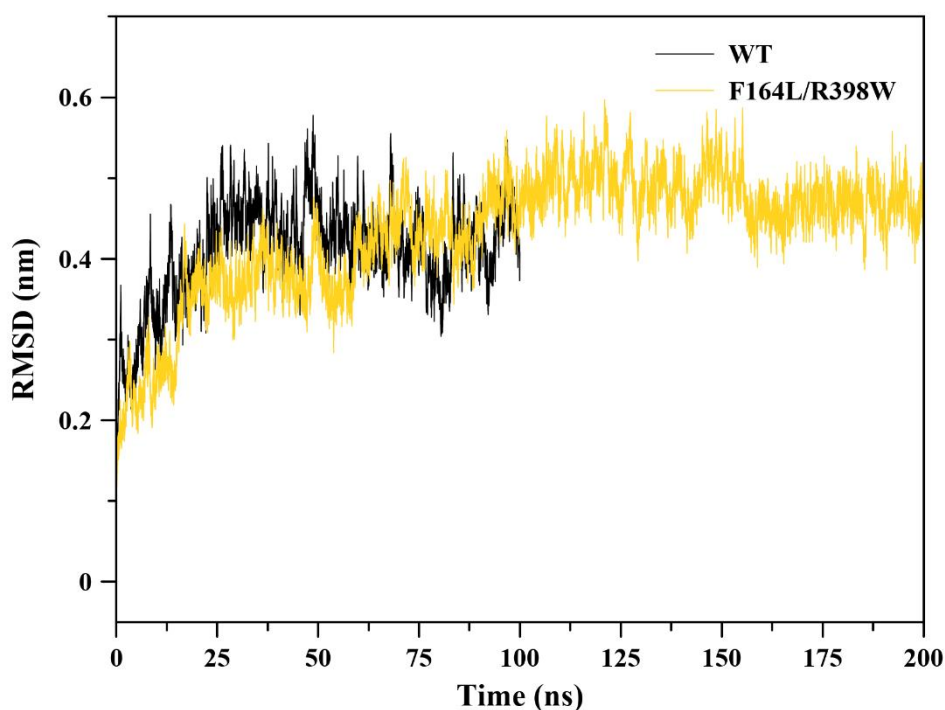


Figure 4.84. RMSD values of WT and F164L/R398W biallelic mutant complex.

As seen in Figure 4.84, the equilibration of the F164L/R398W biallelic mutant took twice as much time than the equilibration of the WT, and the equilibrated RMSD value of the mutant complex is higher than the WT complex.

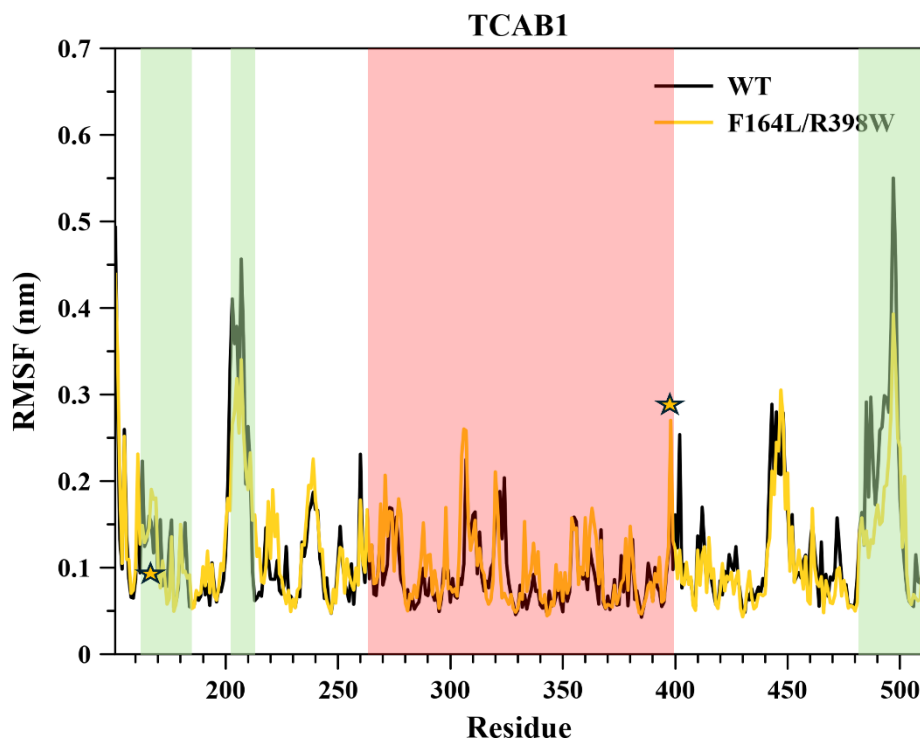


Figure 4.85. Comparison of RMSF values of WT and F164L/R398W mutant TCAB1 protein in complexes. The stars show the place of mutation.

In Figure 4.85, the RMSF graph for TCAB1 presents a comparative analysis between the WT and the double mutant F164L/R398W, focusing on distinct variations in flexibility across the protein's sequence. The graph identifies specific regions with significant differences in RMSF values, which are highlighted in green and red.

In the regions shaded in green—spanning residues 163-182, 203-211, and 483-510—the F164L/R398W mutant exhibits lower RMSF values compared to the WT. This indicates a reduction in flexibility in these segments of the mutant protein, suggesting enhanced structural rigidity that might influence the protein's functional interaction domains or its stability within the cellular environment.

Conversely, the extensive region shaded in red, covering residues 263-398, shows significantly higher RMSF values for the F164L/R398W mutant. This increased flexibility across a large portion of the mutant protein suggests that the combined effects of the F164L and R398W mutations introduce substantial alterations in molecular dynamics, potentially affecting the structural integrity and functional capabilities of TCAB1. The elevated RMSF values indicate that the mutant protein

may have altered interactions with other cellular components, potentially impacting its role in cellular processes.

This analysis highlights the profound impact of the F164L and R398W mutations on the structural dynamics of TCAB1, underscoring areas of increased and decreased flexibility that could have significant implications for the protein's biological functions. Such insights are crucial for understanding the mutations' roles in altering protein behaviour and their potential consequences for cellular functionality and disease progression.

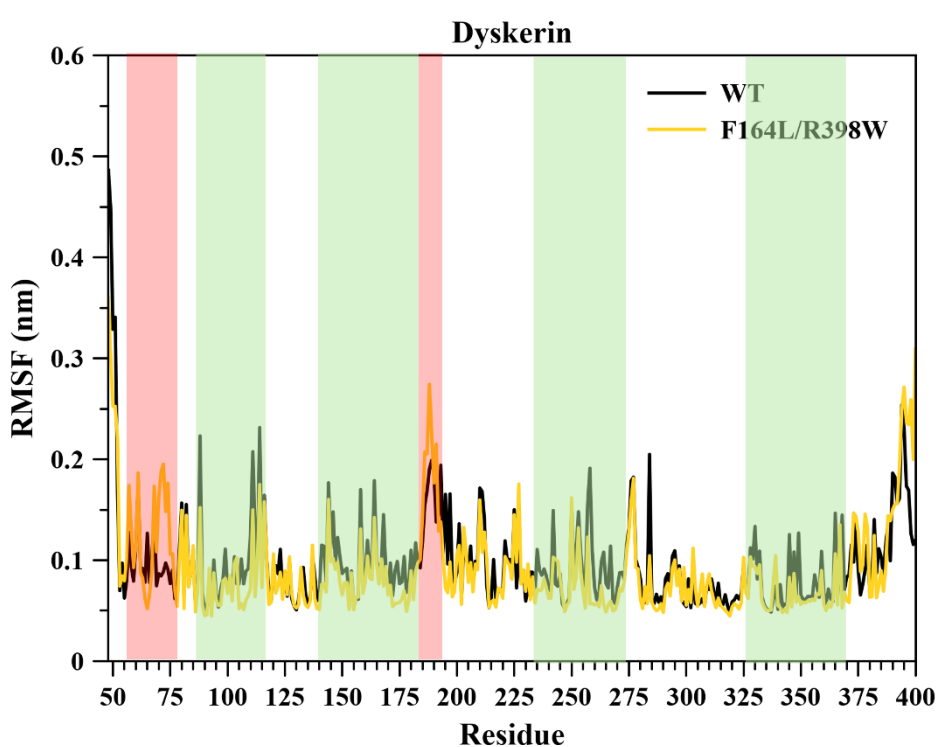


Figure 4.86. Comparison of RMSF values of WT and F164L/R398W mutant dyskerin protein in complexes. The star shows the place of mutation.

In Figure 4.86, the detailed analysis reveals distinct regions where the mutant exhibits variations in RMSF compared to the WT, specifically highlighted by areas coloured in green and red. In the regions shaded in green—residues 88-114, 144-182, 233-271, and 330-368—the F164L/R398W mutant displays lower RMSF values compared to the WT. These decreased RMSF values suggest that the mutant protein exhibits enhanced rigidity in these segments, potentially reflecting a more stable and less dynamic structure at these sites. This reduction in flexibility may influence the

mutant's functional interactions or its stability within the cellular machinery, potentially impacting its role in critical processes such as RNA processing or telomere maintenance.

Conversely, the regions shaded in red—covering residues 57-76 and 183-191—show higher RMSF values in the mutant. The increased flexibility in these areas indicates that the F164L/R398W mutations introduce significant alterations in the structural dynamics of Dyskerin. This enhanced molecular mobility might affect the protein's interaction capabilities and could have implications for its structural integrity and functional effectiveness.

Overall, the graph underscores the significant impact of the F164L/R398W mutations on Dyskerin's structural dynamics, highlighting both increases and decreases in flexibility that could have profound implications for the protein's biological functions.

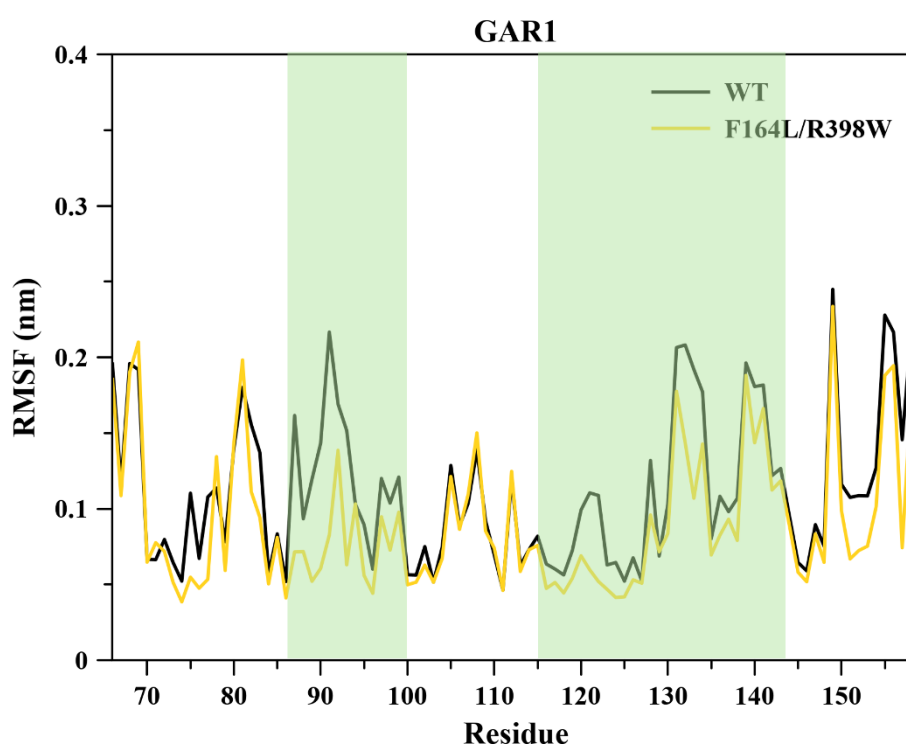


Figure 4.87. Comparison of RMSF values of WT and F164L/R398W mutant GAR1 protein in complexes. The star shows the place of mutation.

The RMSF graph in Figure 4.87, for the GAR1 protein provides a comparative analysis between the WT and the F164L/R398W mutant, highlighting regions where the mutant exhibits notably reduced flexibility. Specifically, the segments spanning residues 87 -

99 and 115-143, marked in green, show decreased RMSF values in the mutant compared to the WT. This reduction in flexibility suggests an increased structural rigidity within these domains of the mutant GAR1.

The lower RMSF values in these regions indicate that the F164L/R398W mutations may confer a more stable, less dynamic structure to the GAR1 protein. This stabilization could potentially influence the mutant's functional interactions, possibly affecting its ability to engage in dynamic interactions necessary for its role in RNA processing or integration into ribonucleoprotein complexes.

Given the absence of any regions with increased flexibility (commonly highlighted in red in other analyses), the focus here shifts entirely to understanding the implications of decreased flexibility. Such changes could have a significant impact on the protein's functional landscape, particularly in how it interacts with other molecular components.

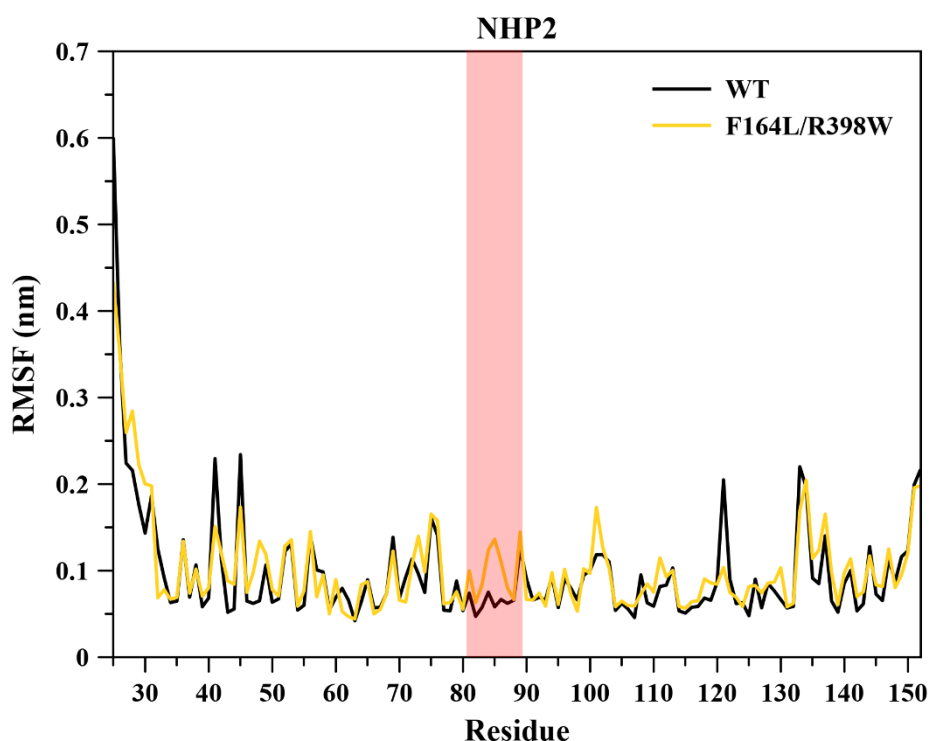


Figure 4.88. Comparison of RMSF values of WT and F164L/R398W mutant NHP2 protein in complexes. The star shows the place of mutation.

The RMSF graph in Figure 4.88, for the NHP2 protein provides a detailed comparison between the WT and the F164L/R398W mutant, focusing on the differences in flexibility across the protein sequence. A particular region of interest, shaded in red

and spanning residues 81-89, shows higher RMSF values for the F164L/R398W mutant compared to the WT. This indicates an increased flexibility in this segment of the mutant protein, suggesting that the F164L/R398W mutations contribute to significant alterations in the molecular dynamics of this region.

The increased flexibility within this specific segment could potentially affect the mutant protein's interaction capabilities and structural integrity. Such changes in dynamics may influence the protein's role in cellular processes, particularly those involving RNA processing and the assembly of ribonucleoprotein complexes. The heightened flexibility in this region might lead to altered binding affinities or changes in the structural framework of the protein complex in which NHP2 participates.

Given the absence of regions showing decreased flexibility (commonly highlighted in green), the focus shifts entirely to the implications of increased flexibility due to the mutations.

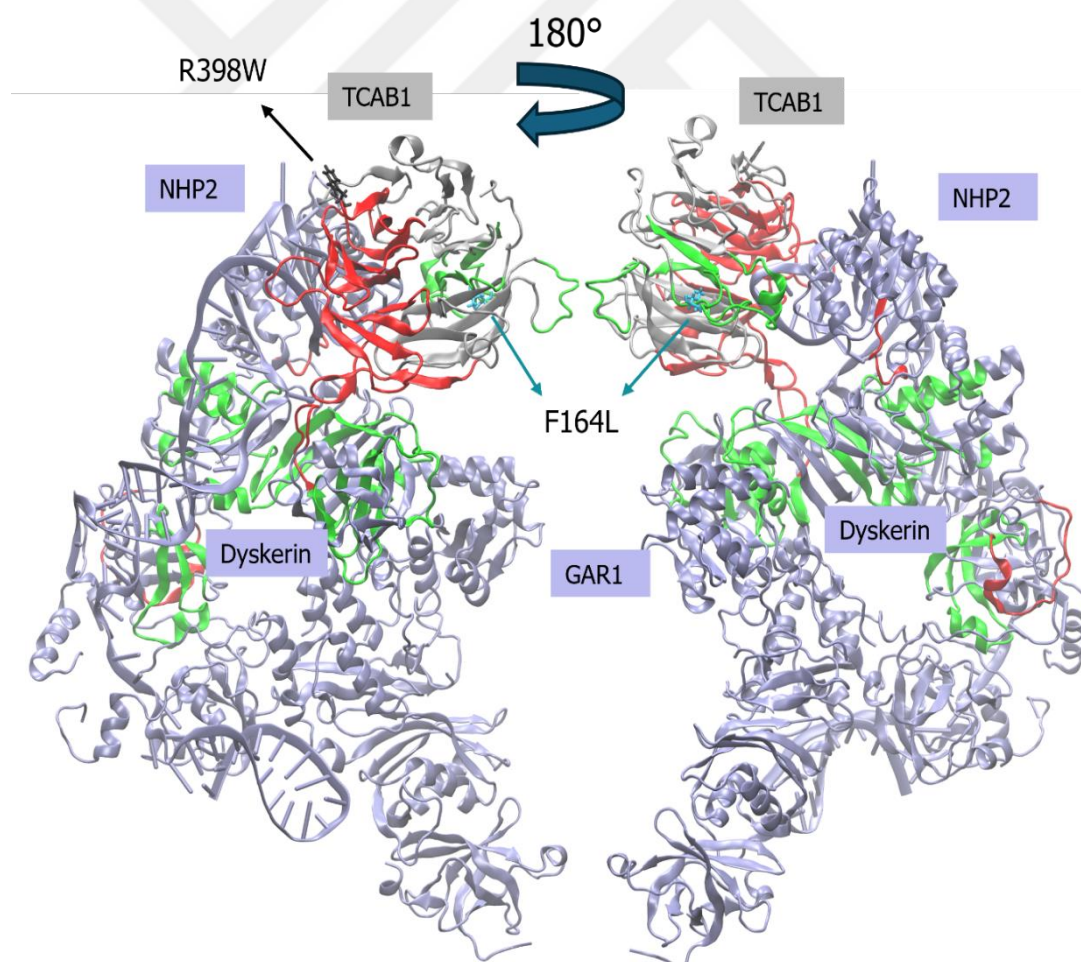


Figure 4.89. F164L/R398W complex structure. The protein shown in grey is the TCAB1 protein, the rest of the complex is shown in metallic blue.

The visualization in Figure 4.89., depicts the F164L/R398W double mutant complex, which comprises a single TCAB1 molecule, one RNA molecule, and two copies each of dyskerin, GAR1, NHP2, and NOP10 proteins. The R398W mutation is situated on the TCAB1 protein and is highlighted by blue and black arrows in the image. The two images provided are rotations of one another, each shown at a 180-degree angle to offer a comprehensive view of the complex's spatial arrangement.

In this graphical representation, amino acid sequences marked in red indicate regions where the RMSF values of the mutant protein are higher than those of the WT. Conversely, sequences highlighted in green denote areas where the RMSF values are lower in the mutant compared to the WT. These regions correspond to the areas described in the RMSF value discussions for each protein interacting with TCAB1, as specified in the accompanying RMSF value table.

Specifically, an increase in RMSF values has been observed in the regions where TCAB1 interacts with dyskerin and GAR1, suggesting enhanced flexibility and potentially altered interaction dynamics within these segments of the mutant complex. However, the interaction domain between TCAB1 and NHP2 exhibits a decrease in RMSF, indicating a stabilization or reduced flexibility in this particular interface.

In contrast, the dyskerin protein shows a decrease in RMSF in regions interacting with RNA, which may signify a tighter, more stable interaction profile, while the areas of interaction with TCAB1 show an increase in RMSF, suggesting increased dynamics and potentially altered binding characteristics. Furthermore, while the GAR1 protein's interaction domain with TCAB1 shows a decrease in RMSF, indicative of enhanced stability or rigidity, the interaction domain of NHP2 with dyskerin displays an increase in RMSF, suggesting heightened flexibility and dynamic changes in this segment.

This detailed analysis underlines the significant impact of the F164L/R398W mutations on the structural dynamics of the complex, emphasizing the variable effects these mutations have on the flexibility and interaction capabilities of the involved proteins. Such insights are critical for advancing our understanding of the functional implications of mutations within protein complexes, highlighting the need for further investigations into their potential effects on cellular functionality and disease mechanisms.

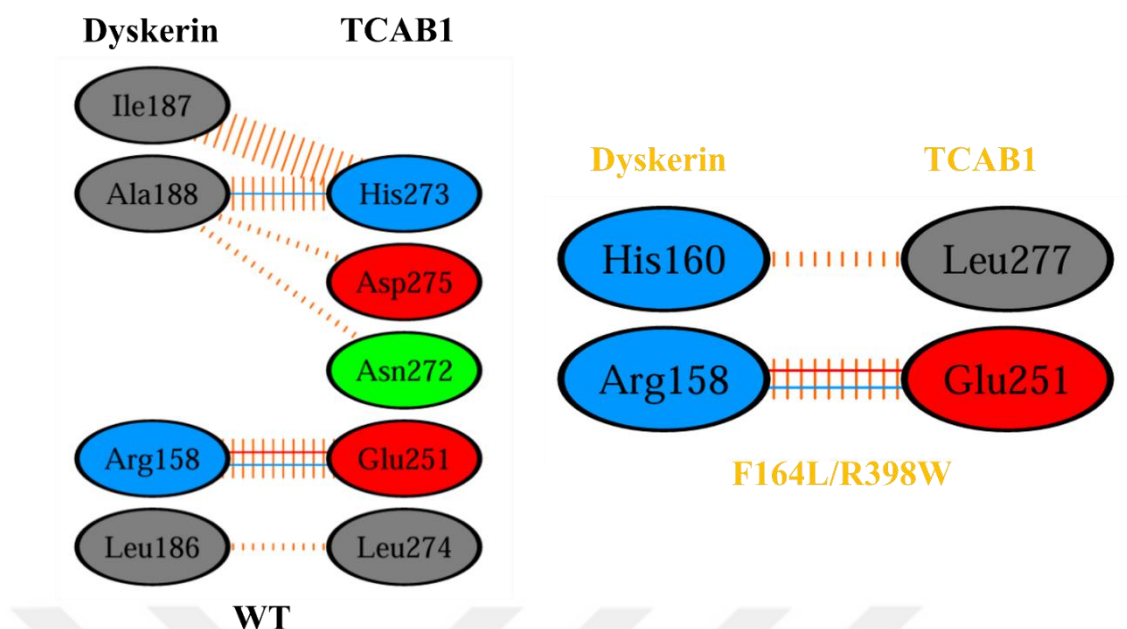


Figure 4.90. Comparative analysis of the interactions between WT TCAB1 and the F164L/R398W mutant TCAB1 proteins with the dyskerin protein within the complex structures.

According to Figure 4.90, the analysis of interactions between dyskerin and TCAB1 in the wild-type and F164L/R398W mutant reveals significant differences in binding characteristics. In the wild-type complex, there are multiple non-bonded contacts, hydrogen bonds, and salt bridges facilitating the interaction. Notably, interactions are observed between Ile187 and Ala188 from dyskerin with His273 in TCAB1, Asp275, and Asn272 from dyskerin with TCAB1 residues, and strong salt bridges between Arg158 (dyskerin) and Glu251 (TCAB1). These connections involve both hydrophobic and hydrophilic residues, allowing a stable interface that maintains a balanced interaction profile.

In the F164L/R398W mutant, the interaction landscape changes markedly. Non-bonded contacts and hydrogen bonds are reduced, with some key interactions either significantly weakened or absent. The salt bridge between Arg158 and Glu251 remains intact, suggesting that this particular interaction contributes to a foundational aspect of binding. However, other previous interactions involving Ile187 and Ala188 with His273 and Asp275 are notably reduced or absent. Additionally, His160 and Leu277 interactions replace some of the former interactions, shifting the binding interface dynamics.

The alteration in amino acid interactions suggests that the F164L/R398W mutations lead to a decrease in binding affinity due to the loss of stabilizing contacts, particularly hydrophilic interactions that were prominent in the wild-type complex. Overall, the shift in interaction types, combined with fewer non-bonded contacts and hydrogen bonds, indicates a weakened binding in the mutant complex relative to the wild-type form.

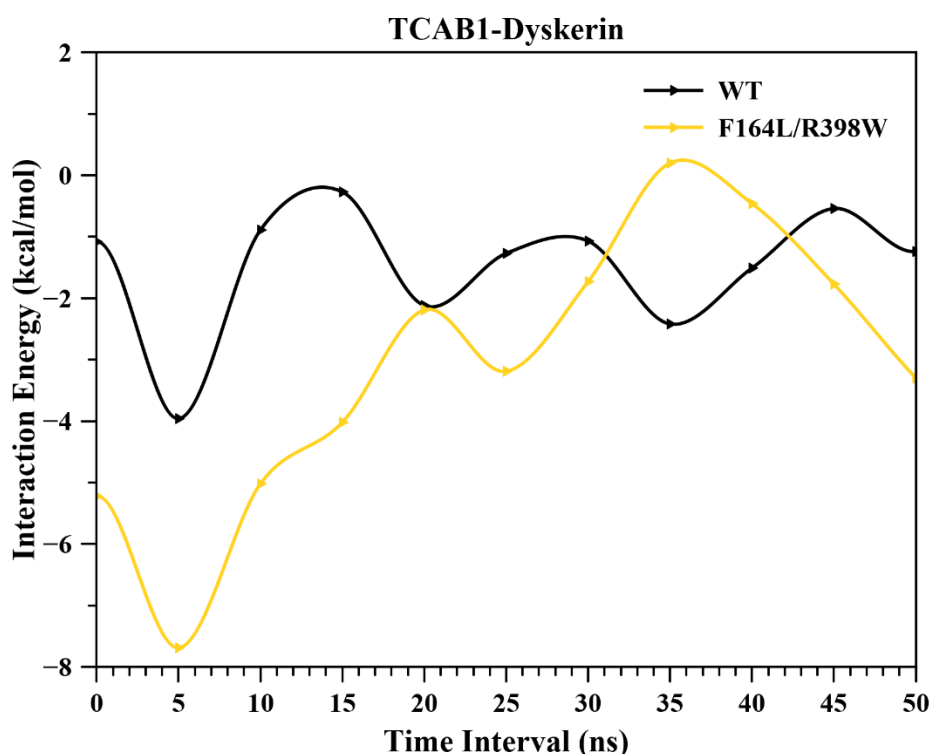


Figure 4.91. Interaction energy profile of WT and F164L/R398W mutant TCAB1-Dyskerin complexes over time.

As seen in Figure 4.91., the interaction energy profile between TCAB1 and dyskerin reveals a more dynamic and less stable binding pattern for the F164L/R398W mutant complex compared to the WT. In the WT complex, the interaction energy stabilizes around an average of -1.5 kcal/mol, with moderate fluctuations that suggest a relatively stable interaction. The energy remains within a narrow range, indicating consistent binding strength with minimal disruption over time.

In contrast, the F164L/R398W mutant complex exhibits a more negative average interaction energy of -3 kcal/mol, which initially suggests a potentially stronger binding affinity. However, the energy profile also shows more pronounced fluctuations, with larger swings between peaks and troughs throughout the simulation.

This increased variability in energy indicates a less stable interaction despite the lower average energy value. The frequent energy oscillations suggest that the binding is more susceptible to transient destabilizations, which could imply a less resilient interaction under dynamic conditions.

Thus, while the F164L/R398W mutations may introduce interactions that deepen the average binding energy, the increased energy fluctuations imply a trade-off with stability. The mutant complex's profile suggests that, although it might exhibit stronger transient binding at certain points, it lacks the stability seen in the WT complex, making it potentially weaker and more prone to dissociation under variable conditions. This dynamic instability could be due to altered residue interactions that fail to maintain a steady, stabilizing influence on the complex.

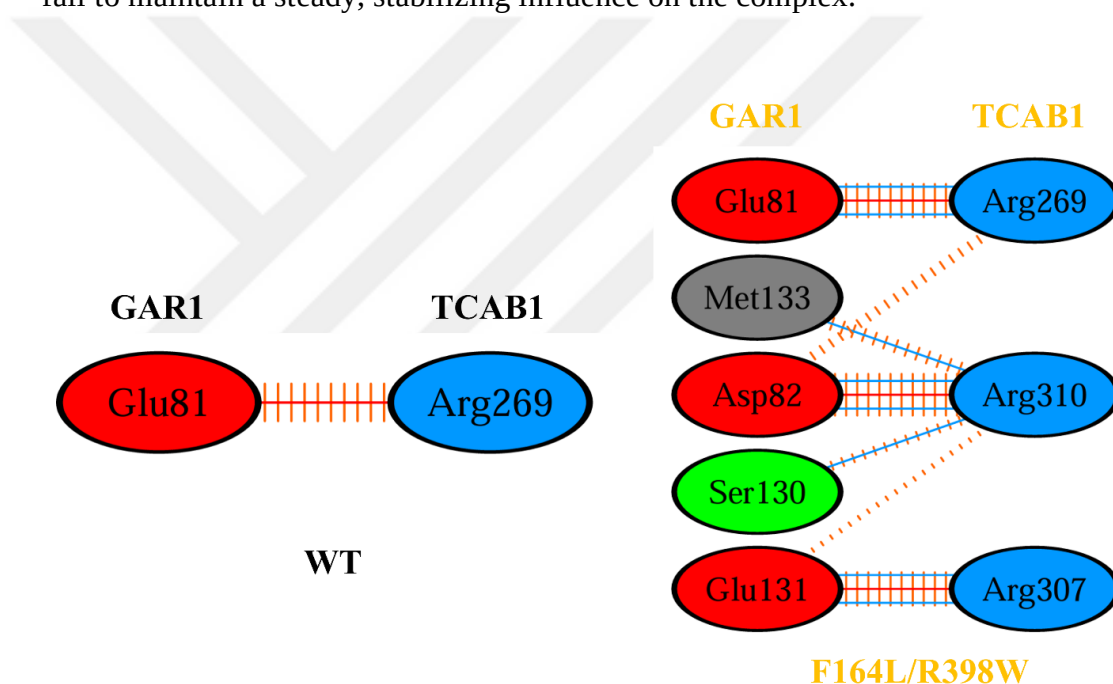


Figure 4.92. Comparative analysis of the interactions between WT TCAB1 and the F164L/R398W mutant TCAB1 proteins with the GAR1 protein within the complex structures.

In the interaction map in Figure 4.92., for the F164L/R398W mutant TCAB1-GAR1 complex, significant changes in bonding patterns and interaction types can be observed when compared to the wild-type (WT) structure. The total number of non-bonded contacts, salt bridges, and hydrogen bonds has increased, suggesting a denser network of interactions in the mutant complex.

For non-bonded contacts, the F164L/R398W mutation introduces additional interactions between residues such as Arg310 and Asp82 and Arg307 with Glu131. This increase in non-bonded contacts contributes to a tighter interaction profile between TCAB1 and GAR1, implying a potentially stronger binding affinity.

The salt bridge count is also notably higher in the mutant, with new salt bridges forming, particularly between Glu131 and Arg307 and Asp82 and Arg310. These new electrostatic interactions are likely enhancing the stability of the mutant complex through strengthened ionic interactions, which may contribute to the increased binding strength observed.

Regarding hydrogen bonds, additional hydrogen bonds have been introduced, notably between Ser130 and Arg310. This hydrogen bonding pattern, along with the salt bridges, contributes to the hydrophilic character of the interaction interface, further stabilizing the complex in a cellular environment.

The increase in both hydrophilic contacts and hydrogen bonding interactions suggests a shift towards a more polar interface, potentially enhancing interaction stability. However, it should be noted that the increased binding strength, while beneficial for the interaction affinity, may also lead to a more rigid and less flexible interface, which could affect dynamic adaptability under varying physiological conditions. Overall, the F164L/R398W mutation appears to increase the binding affinity and stability of the TCAB1-GAR1 interaction, primarily through enhanced electrostatic and hydrogen-bond interactions.

The interaction energy graph in Figure 4.94., for the TCAB1-GAR1 complex reveals a marked difference between the WT and the F164L/R398W mutant, with an average interaction energy of -0.5 kcal/mol for WT and -3 kcal/mol for the mutant. The more negative average interaction energy in the mutant indicates a stronger binding affinity between TCAB1 and GAR1 when the F164L/R398W mutations are present.

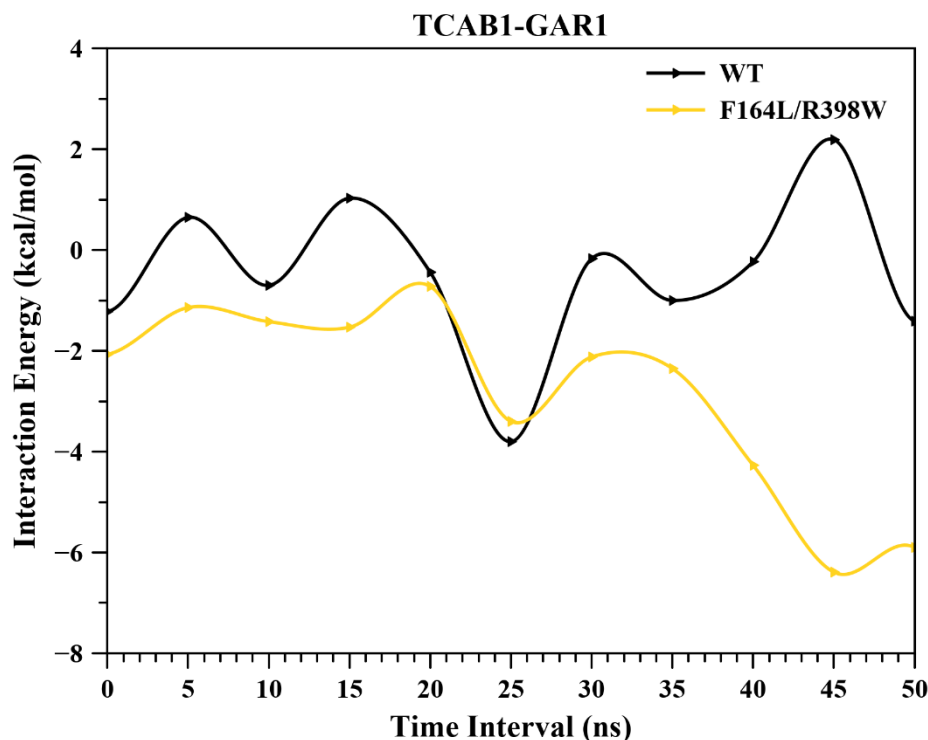


Figure 4.93. Interaction energy profile of WT and F164L/R398W mutant TCAB1-GAR1 complexes over time.

Additionally, the mutant shows reduced fluctuations in interaction energy compared to WT, particularly the last 25 ns, where the WT energy profile displays larger oscillations, even reaching positive values, indicating weaker or more transient interactions. In contrast, the F164L/R398W mutant maintains a relatively stable and consistently negative interaction energy. This stability and increased binding affinity in the mutant suggest that the mutations enhance the electrostatic and possibly hydrophobic interactions between TCAB1 and GAR1, leading to a stronger and more stable complex. However, this increase in stability may also result in reduced flexibility at the interface, potentially impacting the functional dynamics of the interaction. As seen in Figure 4.95., the interaction profile between NHP2 and TCAB1 in the F164L/R398W mutant reveals notable differences in non-bonded contacts, salt bridges, and hydrogen bonds compared to the WT. In the mutant complex, there are increased non-bonded interactions, primarily involving residues such as Lys52, Arg45, and Arg41 on NHP2, which interact with Glu494, Ser491, and Glu487 on TCAB1. Additionally, several hydrogen bonds are formed between these residues, indicating enhanced electrostatic interactions. Notably, Glu487 forms multiple interactions with both Arg45 and Arg41, and Asp493 forms a hydrogen bond with Lys49 on NHP2.

The mutant complex also shows an increase in salt bridges, as Glu494 and Glu487 form stable interactions with positively charged residues like Arg45 and Lys52. This increase in salt bridge interactions suggests a stronger electrostatic component, contributing to the overall binding affinity. Furthermore, new hydrogen bonds are observed in the mutant structure, particularly involving Ser491, Thr413, and Asp493, which are absent in the wild-type complex.

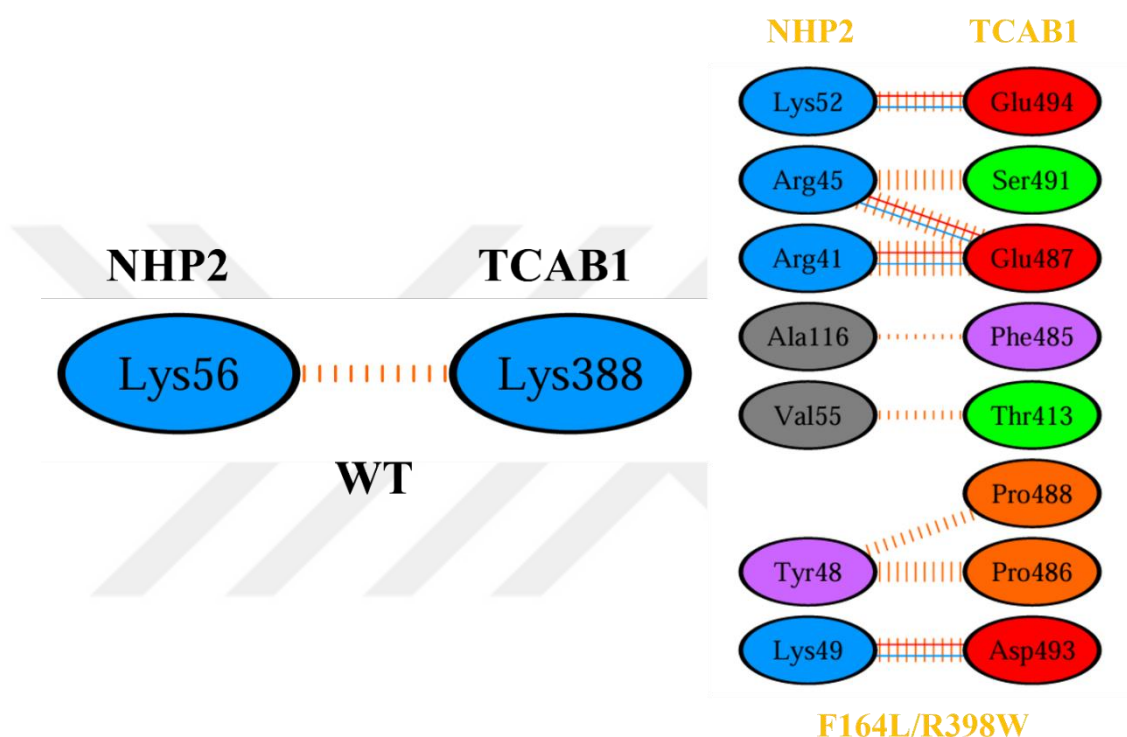


Figure 4.94. Comparative analysis of the interactions between WT TCAB1 and the F164L/R398W mutant TCAB1 proteins with the NHP2 protein within the complex structures.

From a hydrophobicity perspective, the mutant complex introduces interactions between hydrophilic residues such as Ser491 and Thr413 on TCAB1, which may alter the hydration dynamics at the interface. Overall, these changes in bonding patterns and electrostatic interactions likely enhance the binding affinity of the mutant TCAB1-NHP2 complex, as evidenced by the increased number of stabilizing interactions. However, this enhanced binding might come at the cost of reduced flexibility due to the increased number of stable contacts, potentially impacting the dynamic behaviour of the complex.

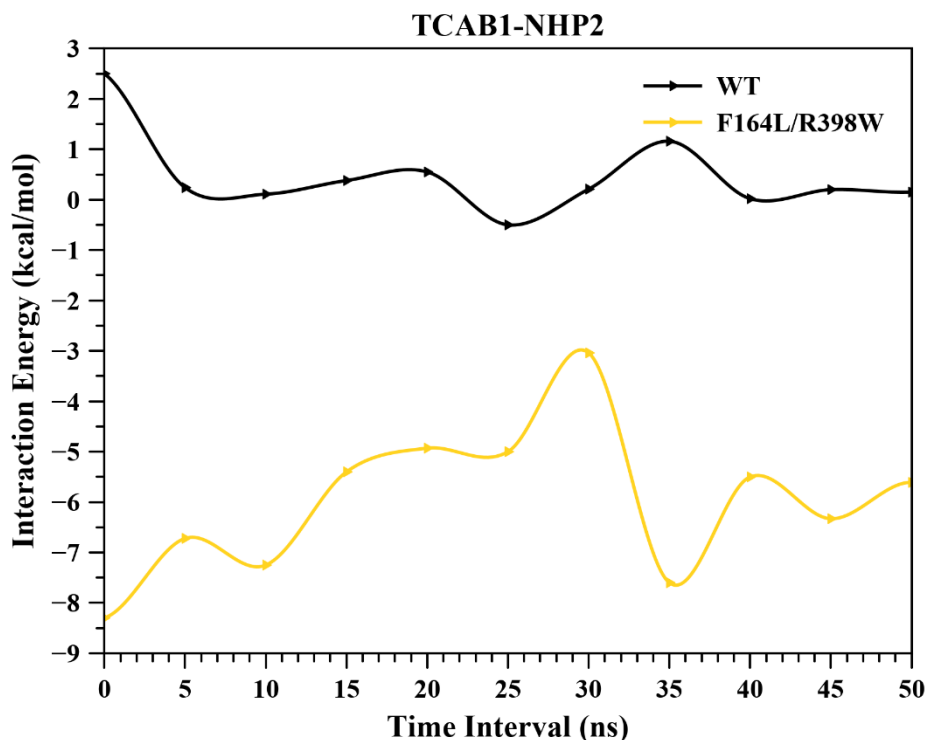


Figure 4.95 Interaction energy profile of WT and F164L/R398W mutant TCAB1-NHP2 complexes over time.

The interaction energy graph in Figure 4.95., for the TCAB1-NHP2 complex demonstrates a marked difference in binding affinity between the wild-type (WT) and the F164L/R398W mutant. In the WT, the average interaction energy is around 0.5 kcal/mol, with minimal fluctuations that mostly stay near the baseline. This profile suggests a relatively weak interaction, as the energy remains close to zero, indicative of limited binding strength and a stable, albeit loose, association between TCAB1 and NHP2.

In contrast, the F164L/R398W mutant shows a significantly stronger binding affinity, with an average interaction energy of approximately -6 kcal/mol. Notably, all fluctuations in the mutant complex remain in the negative energy range, signifying that the interaction is consistently stable and energetically favourable throughout the simulation. Although the fluctuations are somewhat more pronounced compared to the WT, they do not diminish the binding strength; rather, they reflect a dynamic but robust interaction. This stability in the negative energy range suggests that the mutant establishes stronger, likely additional, non-covalent interactions, such as hydrogen bonds or salt bridges, enhancing the overall binding affinity.

Thus, the F164L/R398W mutation strengthens the TCAB1-NHP2 interaction, creating a more energetically favourable and stable binding interface, even though the interaction retains some flexibility due to the observed fluctuations. This enhanced binding affinity may lead to altered functional dynamics in the mutant complex, potentially impacting the biological function of the TCAB1-NHP2 interaction.

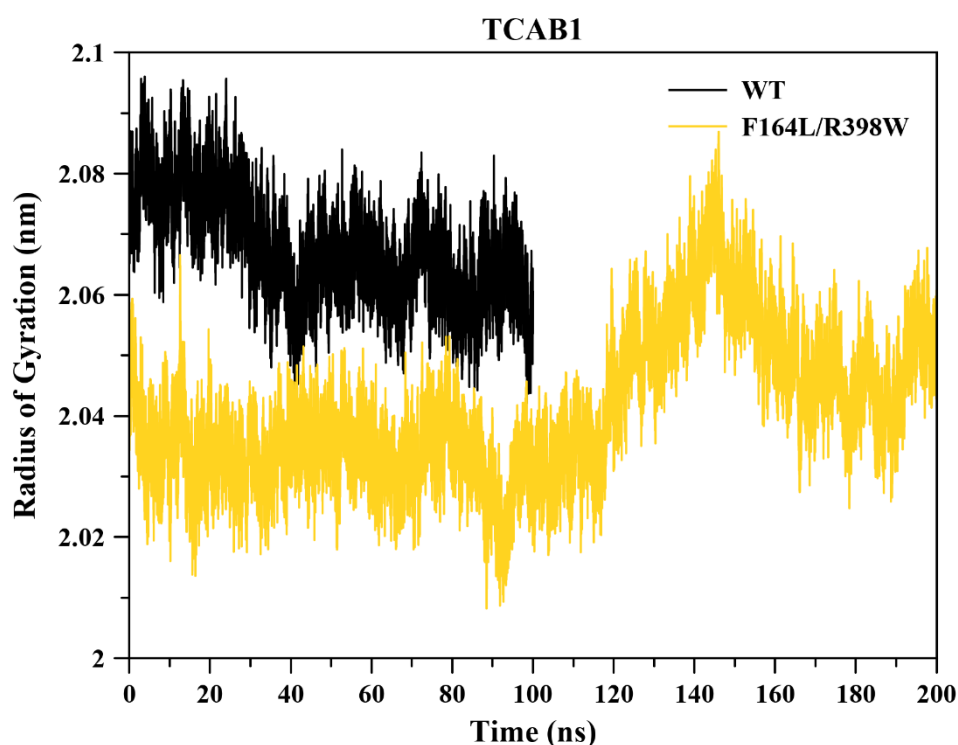


Figure 4.96. Radius of gyration graph of TCAB1 protein in WT complex and F164L/R398W mutant complex.

According to Figure 4.96., the WT protein generally maintains Rg values between 2.06 and 2.08 nm, indicating a stable and compact structure. The F164L/R398W mutant, on the other hand, displays lower Rg values, fluctuating around 2.03 to 2.05 nm for the majority of the simulation. This suggests a more compact conformation relative to the WT. However, a noticeable increase in Rg occurs around 140 ns, with values rising to 2.07 nm, indicating a possible structural expansion or flexibility in the double mutant during the later stages of the simulation.

In summary, the F164L/R398W mutation in TCAB1 appears to induce a more compact structure initially, with a potential increase in flexibility or expansion as the simulation

progresses. These structural changes may impact the protein's stability and interaction properties.

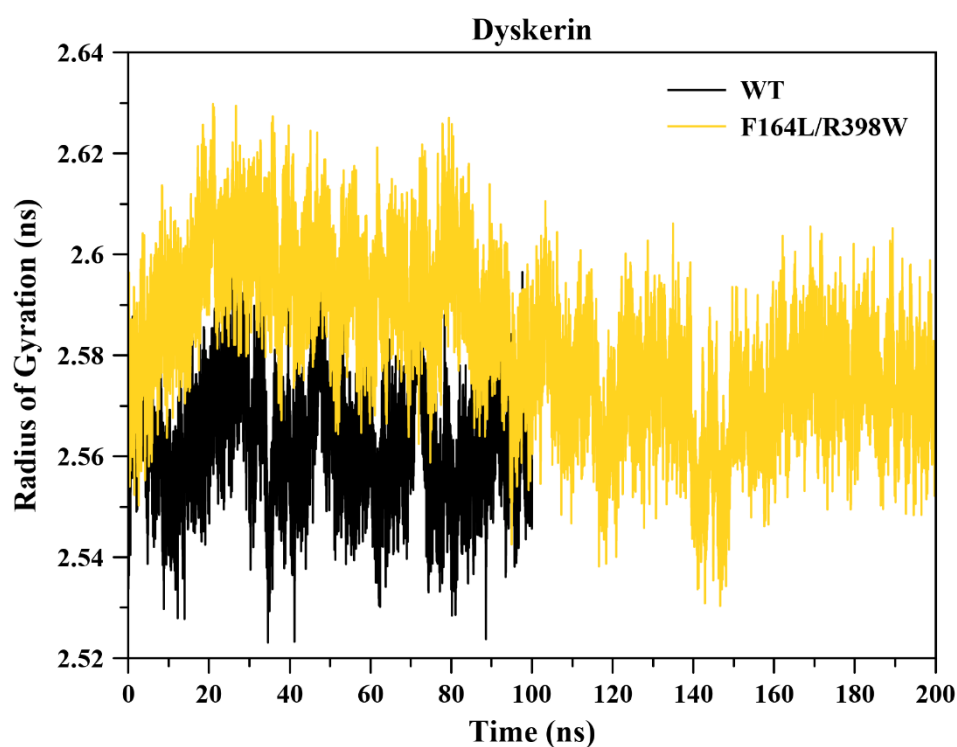


Figure 4.97. Radius of gyration graph of dyskerin protein in WT complex and F164L/R398W mutant complex.

According to Figure 4.97., the WT protein displays Rg values fluctuating between 2.54 and 2.58 nm, indicating a stable and compact structure. In contrast, the F164L/R398W mutant has higher Rg values, primarily fluctuating between 2.58 and 2.62 nm, suggesting a more expanded or less compact structure compared to the WT. This difference is evident throughout the simulation and implies that the double mutation results in a structural configuration that is more open.

In summary, the F164L/R398W mutation in Dyskerin appears to induce a more expanded conformation with increased flexibility. This structural alteration could affect the protein's stability and interaction potential, as a more open configuration may influence binding dynamics and functionality.

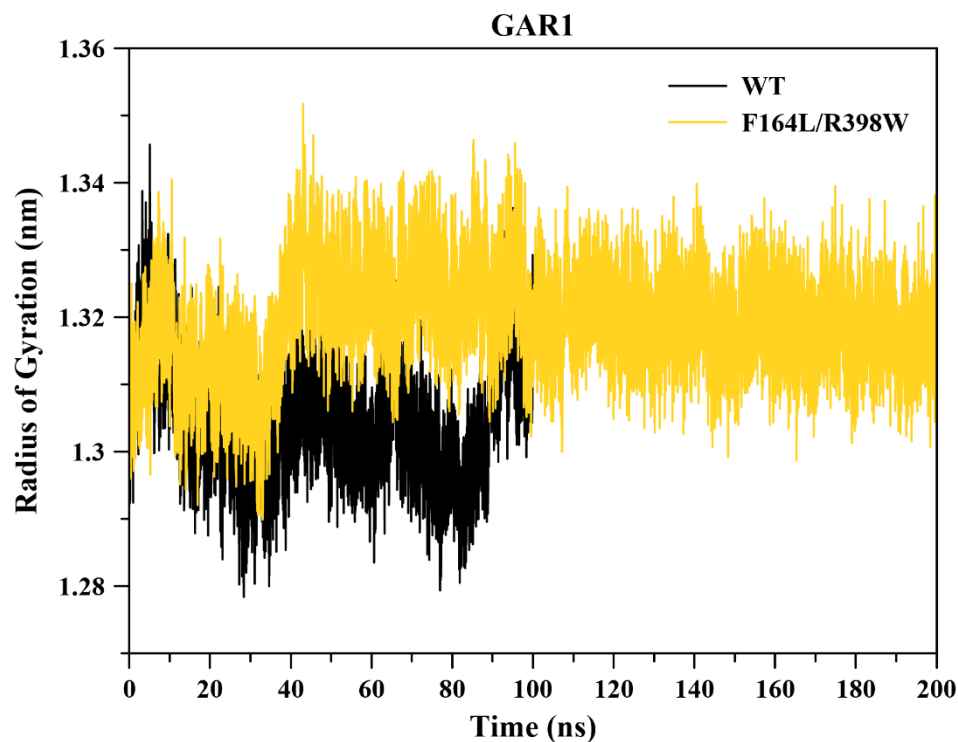


Figure 4.98. Radius of gyration graph of GAR1 protein in WT complex and F164L/R398W mutant complex.

According to Figure 4.98., the WT protein maintains Rg values primarily around 1.29 to 1.32 nm, indicating a compact and stable structure. In contrast, the F164L/R398W mutant displays higher Rg values, fluctuating between 1.32 and 1.34 nm, suggesting a more expanded or less compact structure than the WT. This increased Rg is consistent throughout the simulation, implying that the double mutation results in a structurally more open conformation.

In summary, the F164L/R398W mutation in GAR1 appears to lead to an expanded and more flexible structure. This structural change could affect GAR1's interaction dynamics and stability, as a more open configuration may influence binding potential and functional properties.

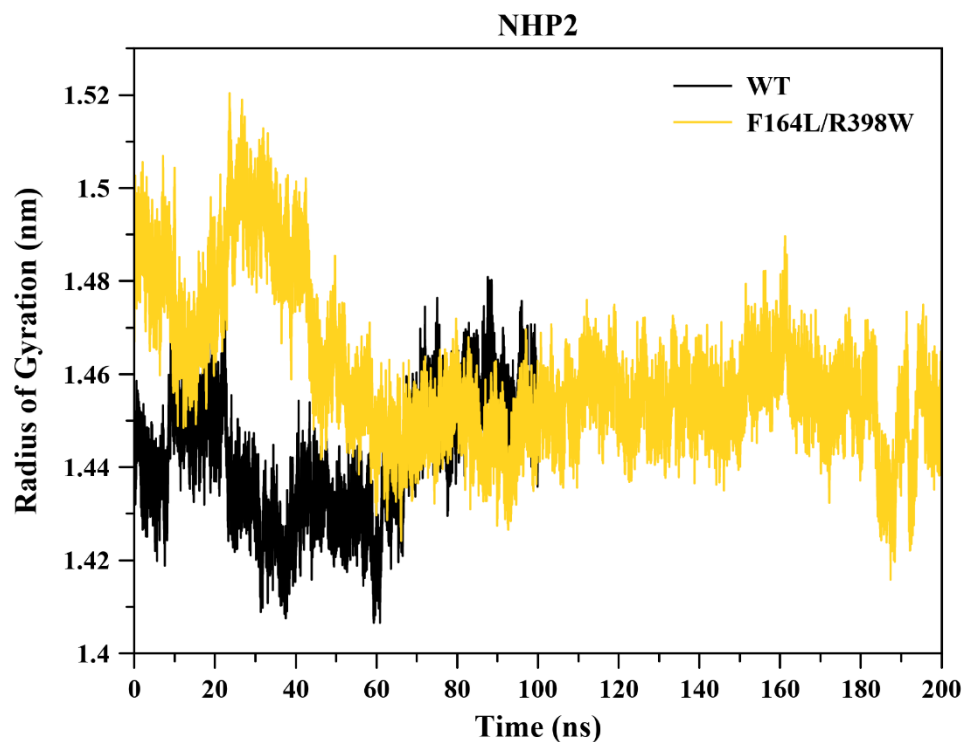


Figure 4.99. Radius of gyration graph of NHP2 protein in WT complex and F164L/R398W mutant complex.

According to Figure 4.99., the WT protein maintains Rg values mostly around 1.43 to 1.47 nm, indicating a compact and stable structure. The F164L/R398W mutant, however, displays higher Rg values, fluctuating primarily between 1.46 and 1.50 nm, suggesting a more expanded structure than the WT. This difference persists throughout the simulation, implying that the double mutation results in a configuration that is consistently more open.

In summary, the F164L/R398W mutation in NHP2 appears to lead to an expanded and possibly more flexible structure. This structural shift could impact NHP2's stability and interaction dynamics, as increased openness may influence its binding properties and functional roles.

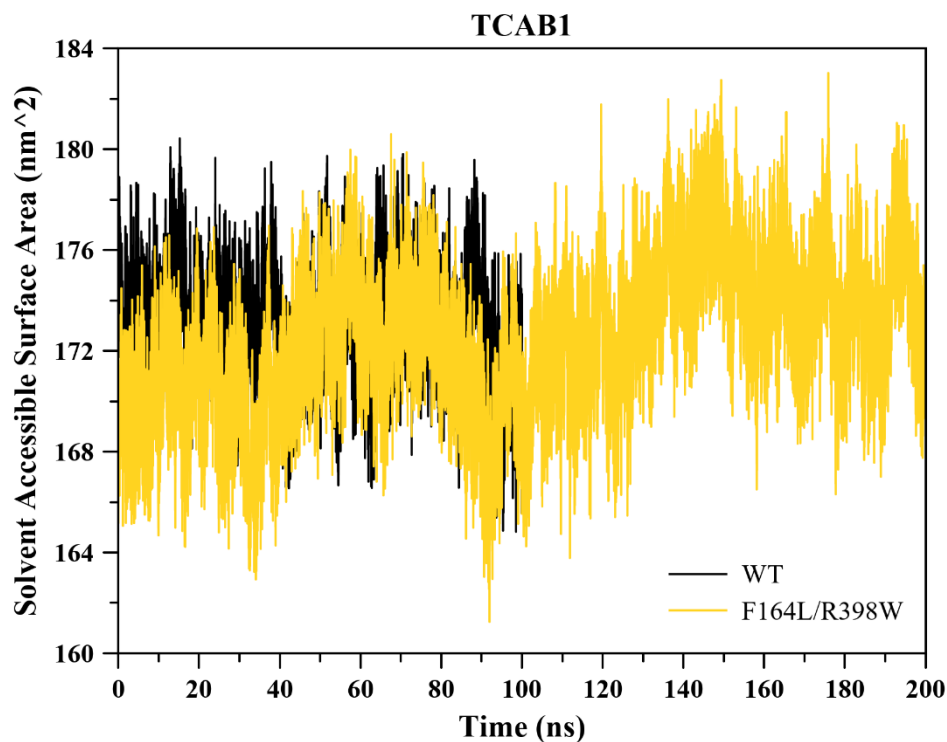


Figure 4.100. SASA graph of TCAB1 protein in WT complex and F164L/R398W mutant complex.

According to Figure 4.100., the WT protein generally maintains SASA values between 168 and 176 nm², indicating a stable level of solvent accessibility and compactness.

In contrast, the F164L/R398W mutant displays an increasing trend in SASA, starting at similar levels to the WT but rising to values between 174 and 182 nm² after approximately 100 ns. This rise in SASA suggests that the double mutation results in a more solvent-exposed and possibly expanded conformation over time, indicating structural changes that lead to greater exposure to the solvent.

In summary, the F164L/R398W mutation in TCAB1 appears to induce a progressively more open structure with higher SASA. These structural changes could affect TCAB1's stability and interactions, as increased solvent exposure may influence its binding dynamics and functional properties.

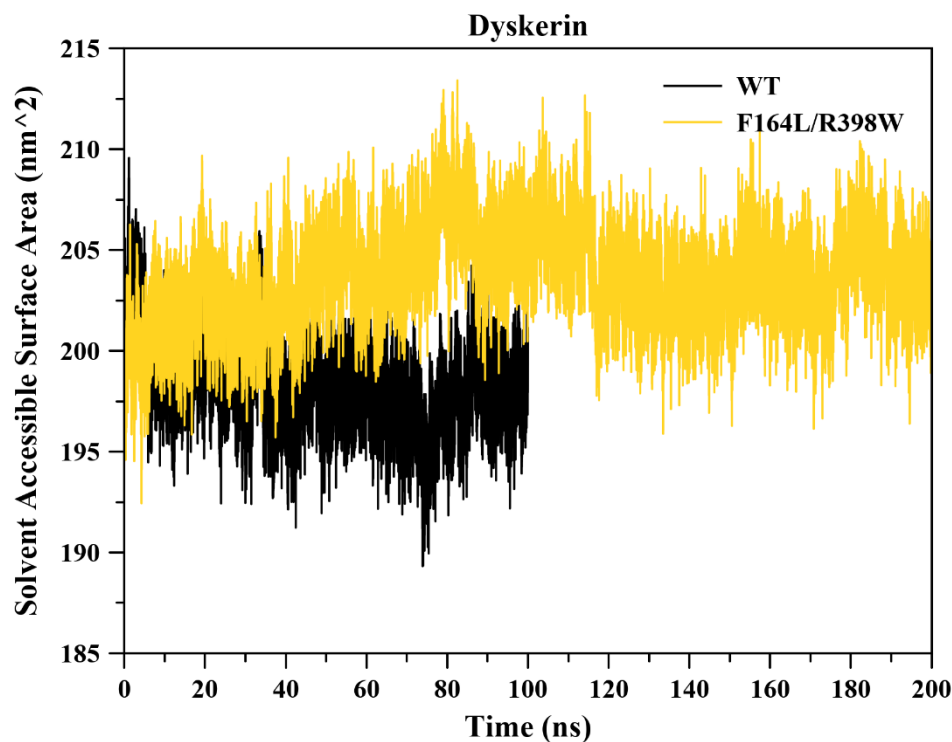


Figure 4.101. SASA graph of dyskerin protein in WT complex and F164L/R398W mutant complex.

According to Figure 4.101., the WT protein primarily exhibits SASA values between 195 and 200 nm², indicating a relatively compact structure with stable solvent accessibility. In contrast, the F164L/R398W mutant shows higher SASA values, fluctuating mostly between 200 and 210 nm². This suggests a more solvent-exposed structure, indicating an expanded or less compact conformation compared to the WT. The elevated SASA values for the mutant are consistent over time, suggesting that the double mutation leads to a sustained increase in structural openness.

In summary, the F164L/R398W mutation in Dyskerin appears to induce a more open conformation, with greater solvent accessibility throughout the simulation. This structural change may impact Dyskerin's stability and binding interactions, as an increase in SASA can affect its functional properties.

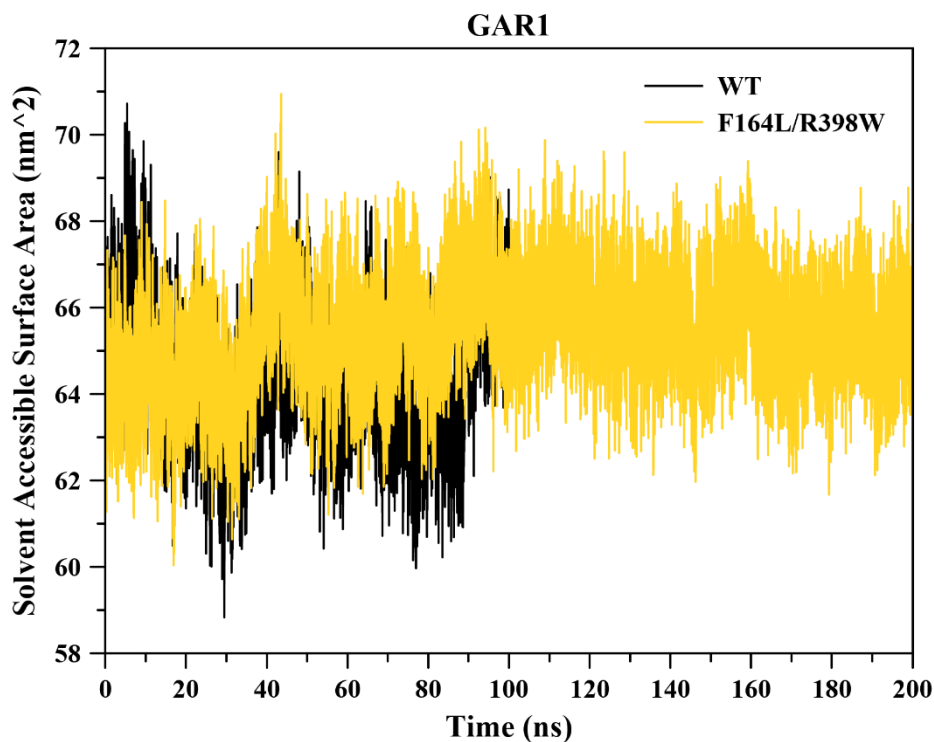


Figure 4.102. SASA graph of GAR1 protein in WT complex and F164L/R398W mutant complex.

According to Figure 4.102., the WT protein generally maintains SASA values between 62 and 66 nm², suggesting a compact structure with limited solvent accessibility.

In contrast, the F164L/R398W mutant shows higher SASA values, ranging primarily between 66 and 70 nm². This elevated SASA implies a more expanded structure with increased solvent exposure compared to the WT. This difference remains stable over the simulation period, indicating that the double mutation in GAR1 consistently leads to a more open conformation.

In summary, the F164L/R398W mutation in GAR1 seems to result in a structure with increased SASA, suggesting greater solvent accessibility and structural flexibility. This alteration could influence GAR1's stability and interaction potential, potentially affecting its functional behaviour.

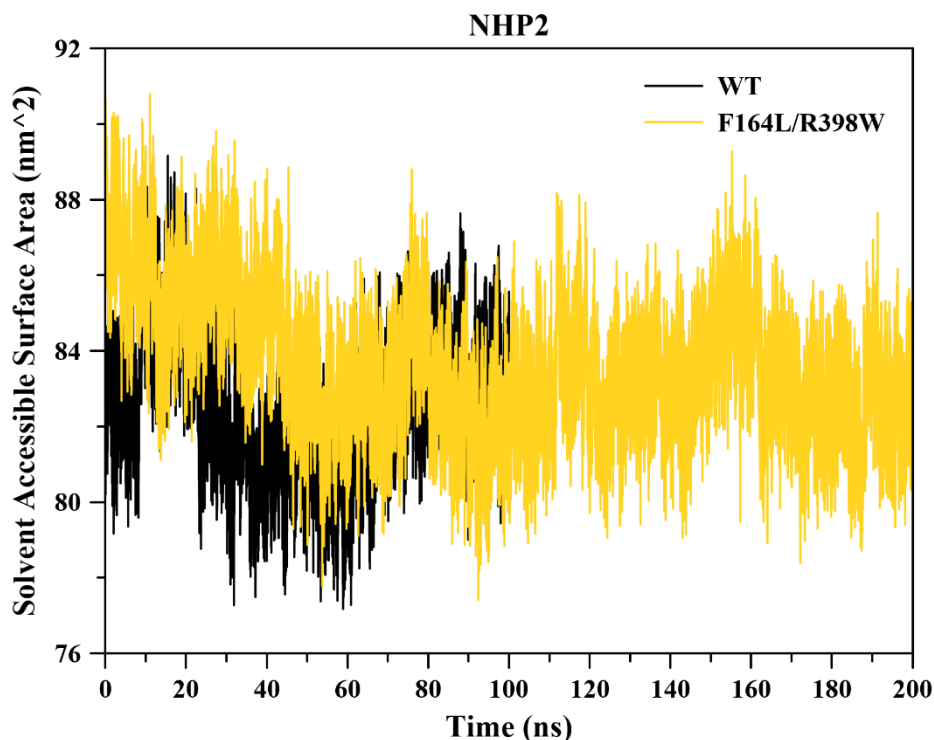


Figure 4.103. SASA graph of NHP2 protein in WT complex and F164L/R398W mutant complex.

According to Figure 4.103., the WT NHP2 generally fluctuates between 80 and 84 nm², indicating a relatively compact structure with moderate solvent accessibility throughout the simulation. In contrast, the F164L/R398W mutant demonstrates consistently higher SASA values, ranging from 84 to 88 nm². This increased SASA suggests a more expanded or flexible structure in the mutant form, allowing greater solvent exposure than the WT. Over time, the F164L/R398W mutant does not return to the lower SASA values observed in the WT, maintaining a more open conformation throughout the simulation.

These observations imply that the F164L/R398W mutations may induce a structural change in NHP2, increasing its surface accessibility and potentially impacting its stability or interaction properties.

According to Figure 4.105, in this interaction energy profile between TCAB1 and RNA, the WT exhibits an average binding energy of -5 kcal/mol, whereas the F164L/R398W mutant has a higher average of -3.5 kcal/mol. This suggests a weaker binding affinity in the mutant compared to the WT.

The WT consistently shows more negative values throughout the simulation, indicating stronger and more stable binding to RNA. The F164L/R398W mutant, however, displays a less negative energy profile, reflecting reduced interaction strength. Additionally, the mutant profile appears to have fewer fluctuations compared to the WT, suggesting a different interaction stability. While the WT may achieve deeper binding stability, the mutant's relatively higher energy values imply that the combined F164L and R398W mutations disrupt optimal RNA binding, potentially altering TCAB1's functional efficiency in RNA interaction.

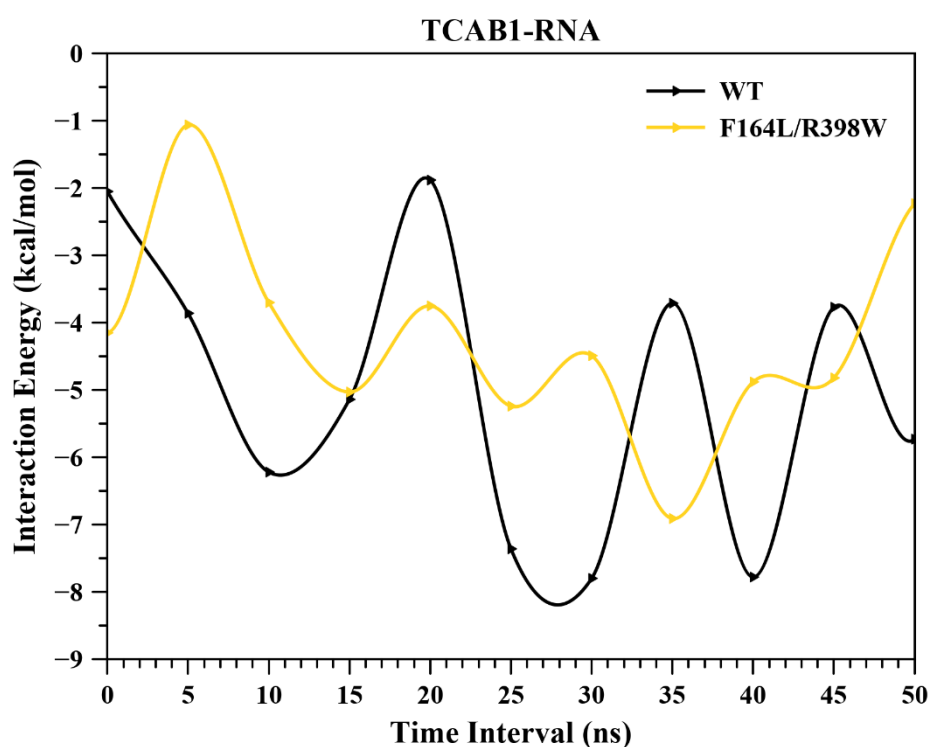


Figure 4.104. Interaction energy profile of WT and F164L/R398W mutant TCAB1-RNA complexes over time.

4.6. WT and H376Y/G435R Mutant Protein Results

Both WT and H376Y/G435R mutant TCAB1 proteins in the complex were subjected to 100 ns MD simulations. RMSD values of each simulation were calculated and plotted (Figure 4.105).

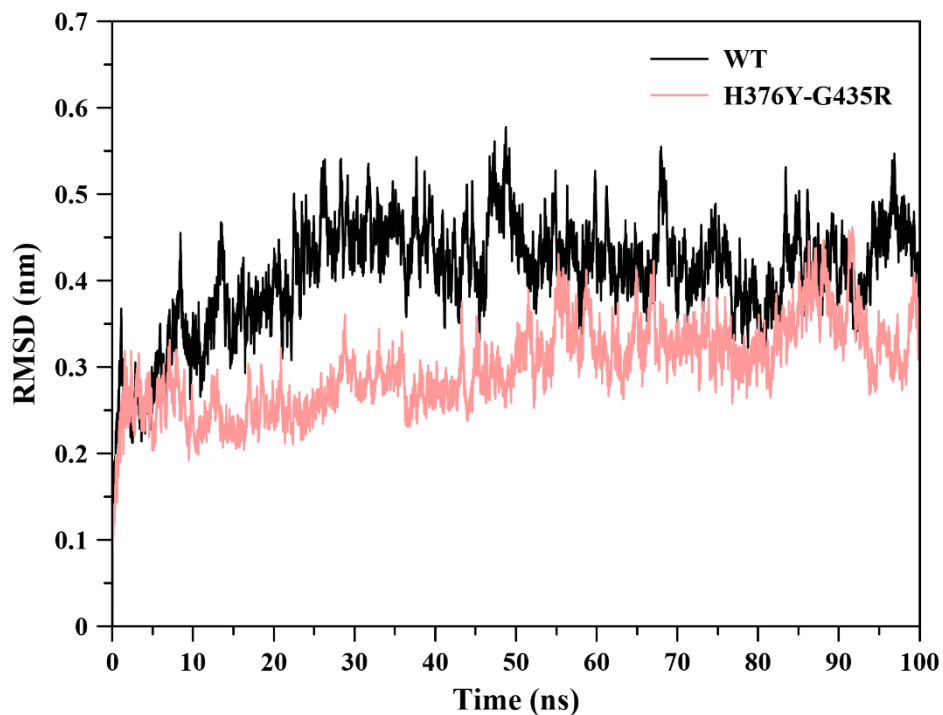


Figure 4.105. RMSD values of WT and H376Y/G435R biallelic mutant complex.

As seen in Figure 4.105 although the RMSD stabilization of the H376Y/G435R mutant took almost the same time as the WT, the equilibration value was lower than the WT. The simulation of the wild-type complex equilibrated in 22 nanoseconds, while the simulation of the H376Y/G435R mutant took 55 nanoseconds to equilibrate.

According to Figure 4.106., in regions shaded in green—spanning residues 160-184, 202-210, 307-313, and 485-510—the H376Y/G435R mutant exhibits lower RMSF values compared to the WT. These decreased RMSF values suggest a reduction in flexibility, implying enhanced structural rigidity in these segments of the mutant protein. This increased rigidity could potentially stabilize the protein structure in these domains, possibly affecting the mutant's functional interactions or its stability within the cellular machinery.

Conversely, the extensive region shaded in red, covering residues 321-461, shows significantly higher RMSF values in the mutant. This increase in flexibility suggests that the H376Y/G435R mutations contribute to a more dynamic and less stable structure in this segment of TCAB1. The elevated RMSF values indicate that the mutant protein may have altered interactions with other cellular components, potentially impacting its role in cellular processes.

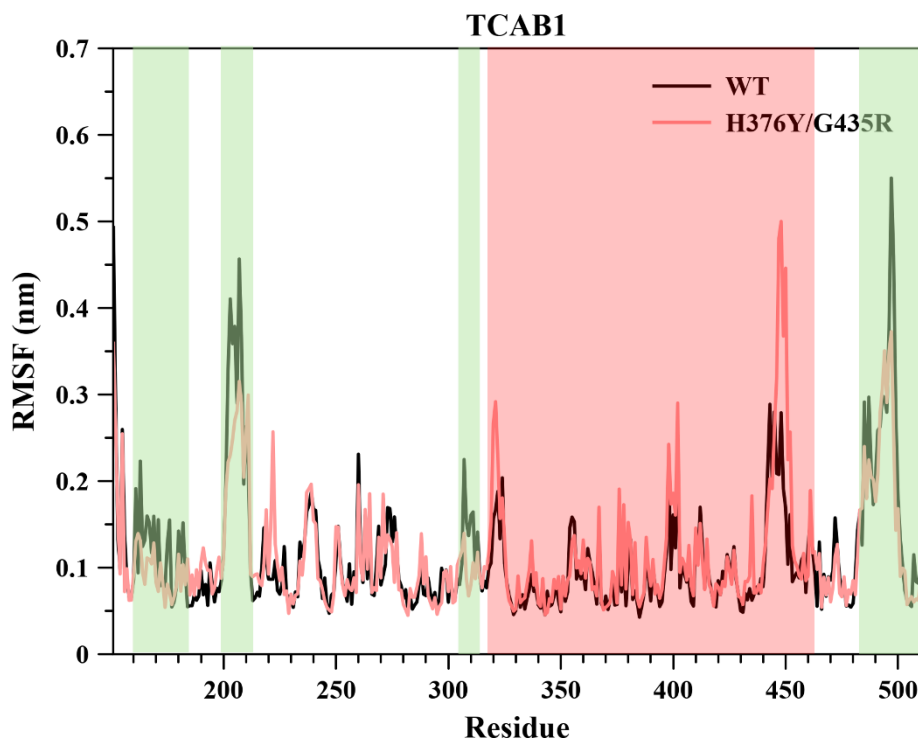


Figure 4.106. Comparison of RMSF values of WT and H376Y mutant TCAB1 protein in complexes.

This analysis underscores the significant impact of the H376Y/G435R mutations on the structural dynamics of TCAB1, highlighting areas of increased and decreased flexibility that could have substantial implications for the protein's biological functions. The variable effects of these mutations on the flexibility and interaction capabilities of TCAB1 are crucial for understanding the functional implications of these genetic alterations, emphasizing the need for further investigations into their potential effects on protein behaviour and cellular functionality. According to Figure 4.108., in the regions shaded in red—spanning residues 57-82, 161-171, and 351-400—the H376Y/G435R mutant exhibits higher RMSF values compared to the WT. This increase in flexibility within these segments suggests that the H376Y/G435R mutations contribute to significant alterations in the structural dynamics of Dyskerin. The elevated RMSF values indicate that the mutant protein may have altered interactions with other cellular components, potentially impacting its role in cellular processes such as RNA processing and the assembly of the telomerase complex.

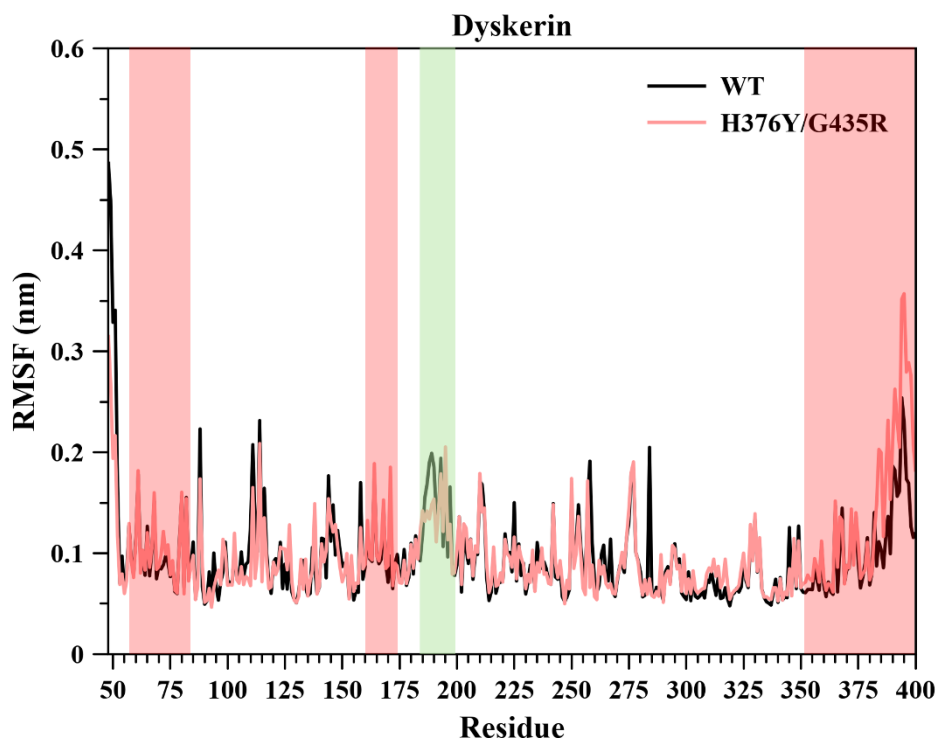


Figure 4.107. Comparison of RMSF values of WT and H376Y/G435R mutant dyskerin protein in complexes.

Conversely, the region shaded in green—covering residues 186-197—shows lower RMSF values in the mutant compared to the WT. This decreased flexibility suggests an enhanced structural rigidity within this segment of the mutant protein. The increased rigidity could potentially stabilize the protein structure in this domain, possibly affecting the mutant's functional interactions or its stability within the cellular machinery.

Overall, this analysis underscores the significant impact of the H376Y/G435R mutations on the structural dynamics of Dyskerin, highlighting areas of both increased and decreased flexibility that could have substantial implications for the protein's biological functions.

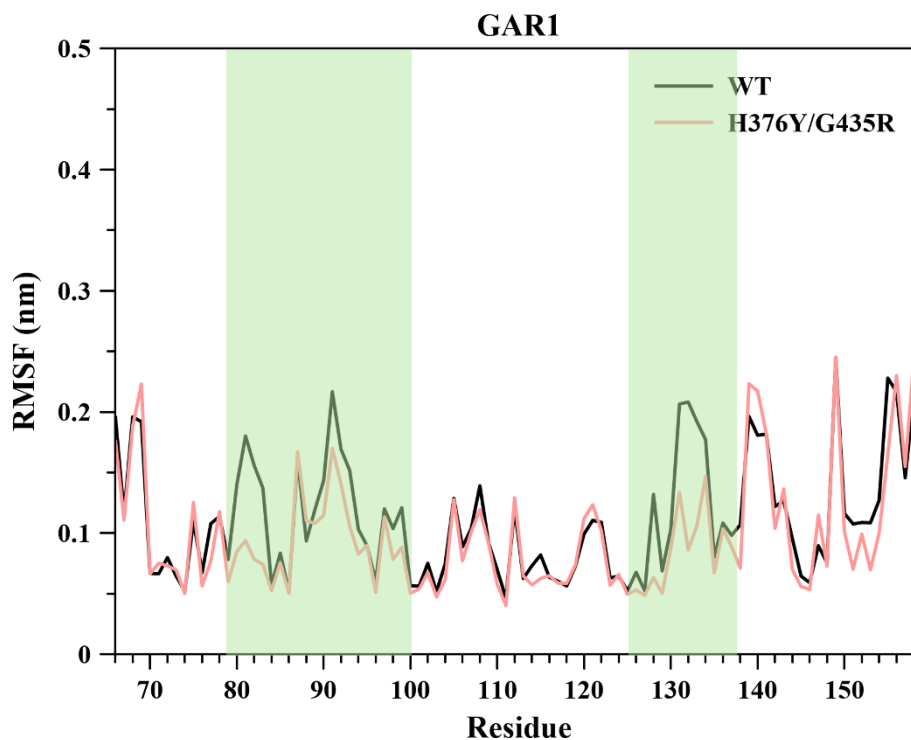


Figure 4.108. Comparison of RMSF values of WT and H376Y/G435R mutant GAR1 protein in complexes.

According to Figure 4.108., the areas shaded in green—spanning residues 79-100 and 126-137—demonstrate lower RMSF values for the H376Y/G435R mutant compared to the WT. These decreased RMSF values in the mutant suggest a reduction in flexibility, indicating increased structural rigidity within these segments. This enhanced rigidity may stabilize the protein structure in these domains, potentially affecting the mutant's functional interactions or its stability within the cellular environment.

Notably, the absence of any regions with increased RMSF (commonly highlighted in red) emphasizes the overall stabilizing effect of the H376Y/G435R mutations on these specific segments of the GAR1 protein. The decreased flexibility could contribute to altered functional dynamics, possibly impacting GAR1's role in cellular processes such as RNA processing or its interaction within ribonucleoprotein complexes.

This analysis underscores the significant impact of the H376Y/G435R mutations on the structural dynamics of GAR1, highlighting areas of decreased flexibility that could have substantial implications for the protein's biological functions.

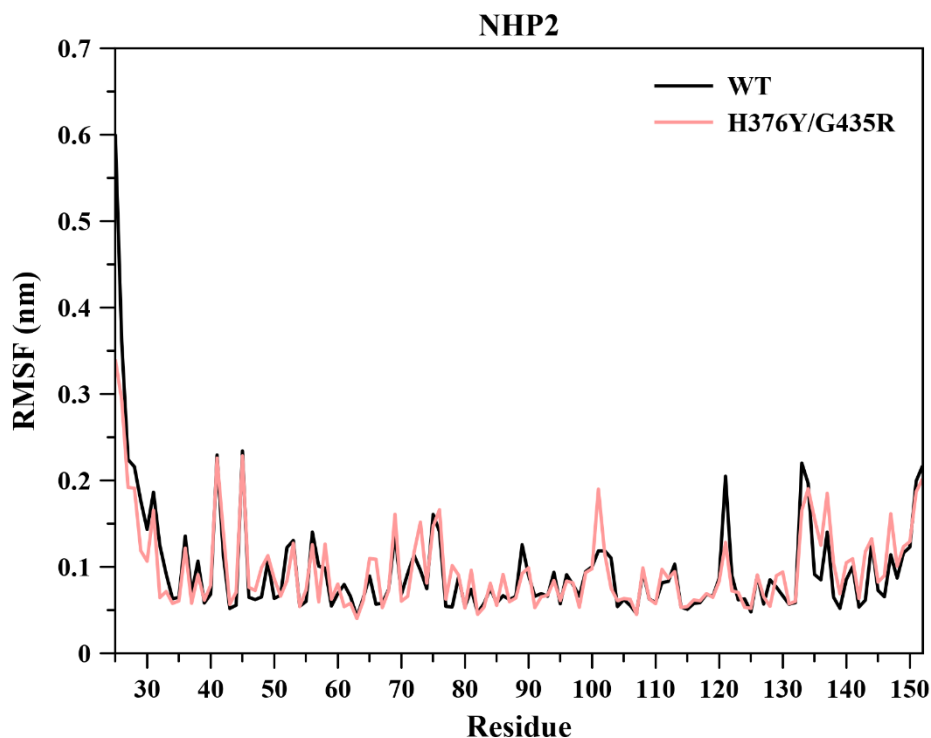


Figure 4.109. Comparison of RMSF values of WT and H376Y/G435R mutant NHP2 protein in complexes.

Both the WT and the mutant demonstrate parallel trends in RMSF values, indicating that while mutations have been introduced, they do not significantly impact the molecular dynamics in terms of increasing or decreasing the flexibility across the observed residue range. This could imply that the H376Y/G435R mutations maintain the structural integrity and functional interactions of NHP2 similar to the wild type, suggesting minimal impact on the protein's role within cellular processes. This stability in flexibility profiles could be crucial for maintaining the functional consistency of NHP2 in its biological roles, potentially preserving its interactions with RNA and other components of the ribonucleoprotein complexes.

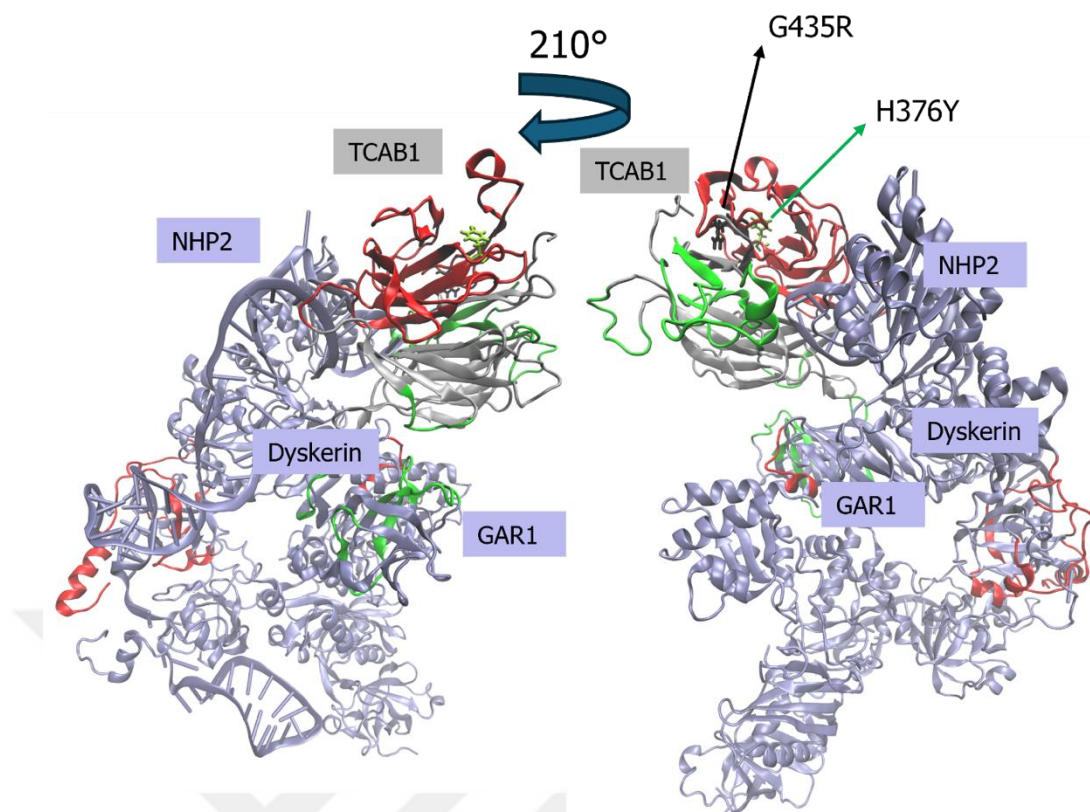


Figure 4.110. H376Y/G435R complex structure. The protein shown in grey is the TCAB1 protein, the rest of the complex is shown in metallic blue.

The visualization in Figure 4.110. depicts the H376Y/G435R mutant complex, which is composed of one TCAB1 molecule, one RNA molecule, and two copies each of dyskerin, GAR1, NHP2, and NOP10 proteins. Notably, the R398W mutation is located on the TCAB1 protein, as indicated by green and black arrows within the image. The two images provided are rotations of one another, each displayed at a 210-degree angle, offering a comprehensive perspective on the spatial arrangement of the complex.

In this graphic representation, amino acid sequences highlighted in red signify regions where the RMSF values of the mutant protein are higher than those of the WT, suggesting increased flexibility in these segments. Conversely, sequences marked in green denote areas where the RMSF values are lower in the mutant compared to the WT, indicating decreased flexibility. These highlighted regions correspond precisely to the RMSF values described in the table discussions for each protein interacting with TCAB1.

Specifically, an increase in RMSF values has been observed across the entire RNA binding region of TCAB1, indicating enhanced molecular dynamics and potentially altered interaction dynamics within this segment of the mutant complex. In contrast, the interaction domains of TCAB1 with GAR1 and NHP2 exhibit a decrease in RMSF, suggesting a stabilization or reduced flexibility in these particular interfaces.

Furthermore, the interaction domain of GAR1 with TCAB1 shows a decrease in RMSF, implying enhanced stability or rigidity, while the RMSF changes in the dyskerin protein do not appear to have a significant impact on its interaction dynamics or structural stability.

This detailed analysis underlines the significant impact of the H376Y/G435R mutations on the structural dynamics of the complex, emphasizing the variable effects these mutations have on the flexibility and interaction capabilities of the involved proteins. Such insights are critical for advancing our understanding of the functional implications of mutations within protein complexes, highlighting the need for further investigations into their potential effects on cellular functionality and disease mechanisms.

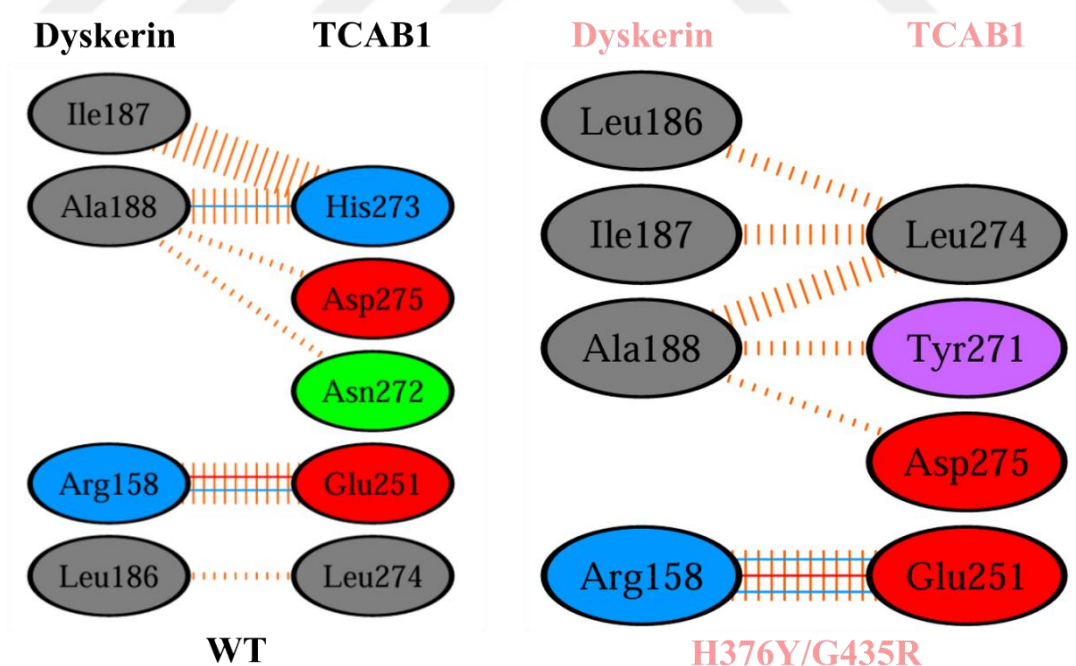


Figure 4.111. Comparative analysis of the interactions between WT TCAB1 and the H376Y/G435R mutant TCAB1 proteins with the dyskerin protein within the complex structures.

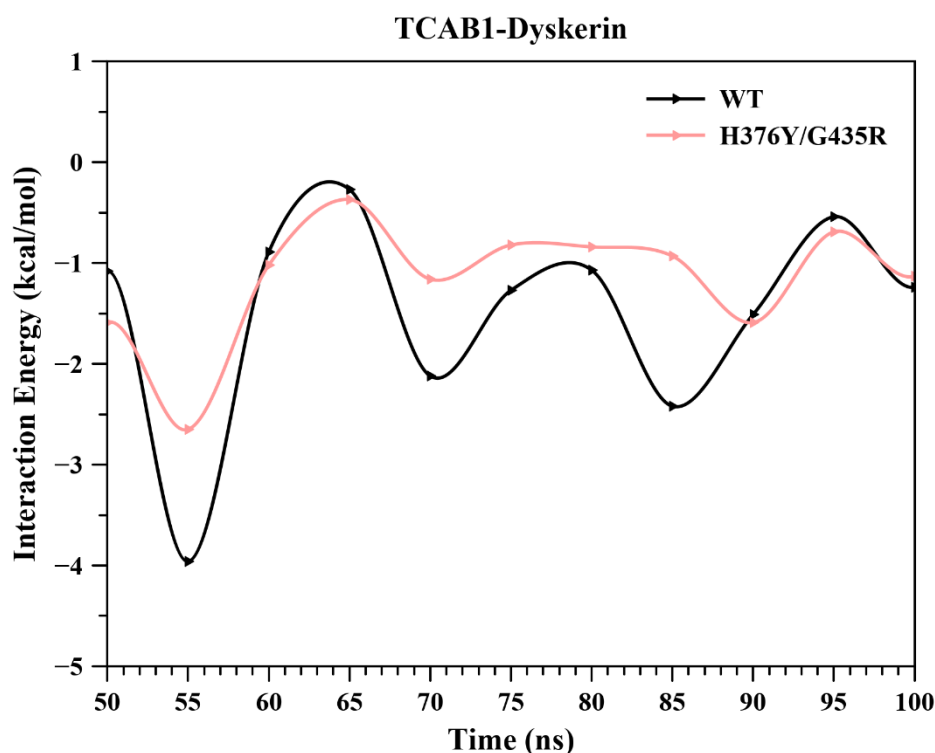


Figure 4.112. Interaction energy profile of WT and H376Y/G435R mutant TCAB1-Dyskerin complexes over time.

The interaction analysis between the TCAB1 and Dyskerin proteins in the H376Y/G435R double mutant, compared to the WT, reveals notable differences in non-bonded contacts, salt bridges, and hydrogen bonds. In the WT complex, the key interactions include multiple non-bonded contacts and a consistent salt bridge between Arg158 and Glu251, stabilizing the interface. Additionally, hydrogen bonds between residues like Ala188 and His273 contribute to the interaction stability. This configuration yields a balanced hydrophilic and hydrophobic interaction profile, supporting a stable but moderate binding affinity.

In the H376Y/G435R mutant, the interaction profile is altered significantly. The number of non-bonded contacts increases, particularly involving Leu186 and Tyr271, suggesting an enhanced hydrophobic interface in this mutant. Furthermore, the salt bridge between Arg158 and Glu251 is retained, but additional hydrogen bonds appear with Asp275 and Tyr271, intensifying the interaction network. This increase in hydrogen bonds and hydrophobic contacts strengthens the overall binding affinity, making the mutant complex more robust. However, the shift towards hydrophobic interactions, combined with the increase in binding strength, could impact the

complex's dynamic stability, potentially leading to decreased flexibility compared to the WT. This enhanced binding affinity may contribute to altered functional properties in the H376Y/G435R mutant. As seen in Figure 3.6.8., in comparing the interaction energies between the WT and H376Y/G435R mutant TCAB1-Dyskerin complexes, a shift towards a slightly less stable binding affinity in the mutant is observed. The average interaction energy for the WT complex is -1.5 kcal/mol, while the mutant displays a higher, less negative average at -1.2 kcal/mol. This indicates a reduction in binding strength in the H376Y/G435R mutant relative to the WT. The energy fluctuations throughout the simulation show that the mutant maintains a more consistent energy profile, with fewer extreme variations compared to the WT. However, this steadier profile does not compensate for the decrease in binding affinity. The slight reduction in the interaction energy suggests that while the mutant complex remains bound, it does so with weaker interactions, possibly due to altered inter-residue contacts or a change in the network of hydrogen bonds and non-bonded contacts. This reduced binding strength may affect the functional stability of the TCAB1-Dyskerin complex in the presence of the H376Y/G435R mutation.

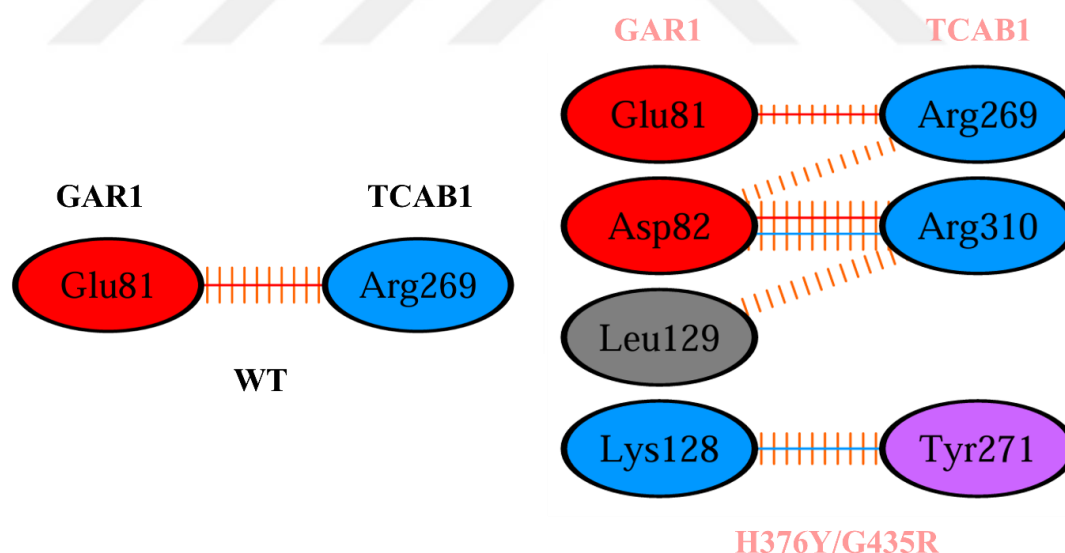


Figure 4.113. Comparative analysis of the interactions between WT TCAB1 and the H376Y/G435R mutant TCAB1 proteins with the GAR1 protein within the complex structures.

In Figure 4.113., the interaction analysis between GAR1 and TCAB1 in the WT and H376Y/G435R mutant reveals distinct differences in non-bonded contacts, salt

bridges, and hydrogen bonds, impacting the binding stability and affinity. In the WT structure, a single salt bridge is observed between Glu81 and Arg269, forming a stable ionic interaction that contributes to the binding affinity. This salt bridge is retained in the H376Y/G435R mutant, with additional contacts formed in the mutated complex. In the H376Y/G435R mutant, multiple interactions are present, including new non-bonded contacts and hydrogen bonds between Asp82-Arg310, Lys128-Tyr271, and Glu81-Arg269. These additional interactions likely increase the overall binding strength, with the presence of Asp82-Arg310 introducing a new salt bridge that enhances the electrostatic component of the interaction. Furthermore, the introduction of Tyr271, a hydrophobic residue, in proximity to Arg269 may add stability through hydrophobic effects, though it also introduces potential changes in interaction dynamics due to differing polarities compared to other residues. Overall, the mutant displays increased binding strength, evidenced by the presence of new interactions, although these interactions may alter the structural flexibility and local dynamics at the interface.

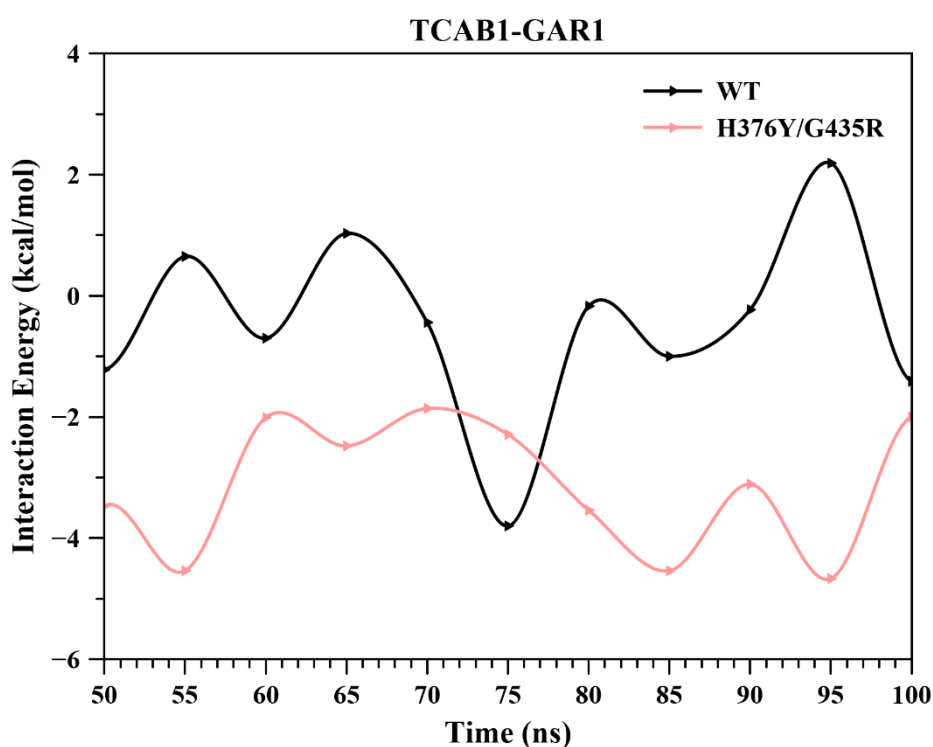


Figure 4.114. Interaction energy profile of WT and H376Y/G435R mutant TCAB1-GAR1 complexes over time.

As seen in Figure 4.114., the interaction energy profile between WT and H376Y/G435R mutant TCAB1-GAR1 complexes indicates notable differences in binding strength and stability. The WT complex shows an average interaction energy of -0.5 kcal/mol, with fluctuations that reach positive energy regions, suggesting a less stable interaction with periodic weakening in the binding affinity. In contrast, the H376Y/G435R mutant maintains a lower, more consistently negative interaction energy, averaging -3 kcal/mol. This negative energy profile, combined with smaller fluctuations, implies that the mutant complex exhibits stronger and more stable binding compared to the WT.

The decreased fluctuation in the mutant energy profile suggests that the additional non-bonded contacts and salt bridges, particularly between residues such as Asp82-Arg310 and Glu81-Arg269, stabilize the interaction. The introduction of these interactions not only increases binding affinity but also reduces the dynamic instability observed in the WT complex. Thus, the H376Y/G435R mutations appear to enhance both the strength and resilience of the TCAB1-GAR1 interaction, as indicated by the consistently more negative and stable energy values throughout the simulation.

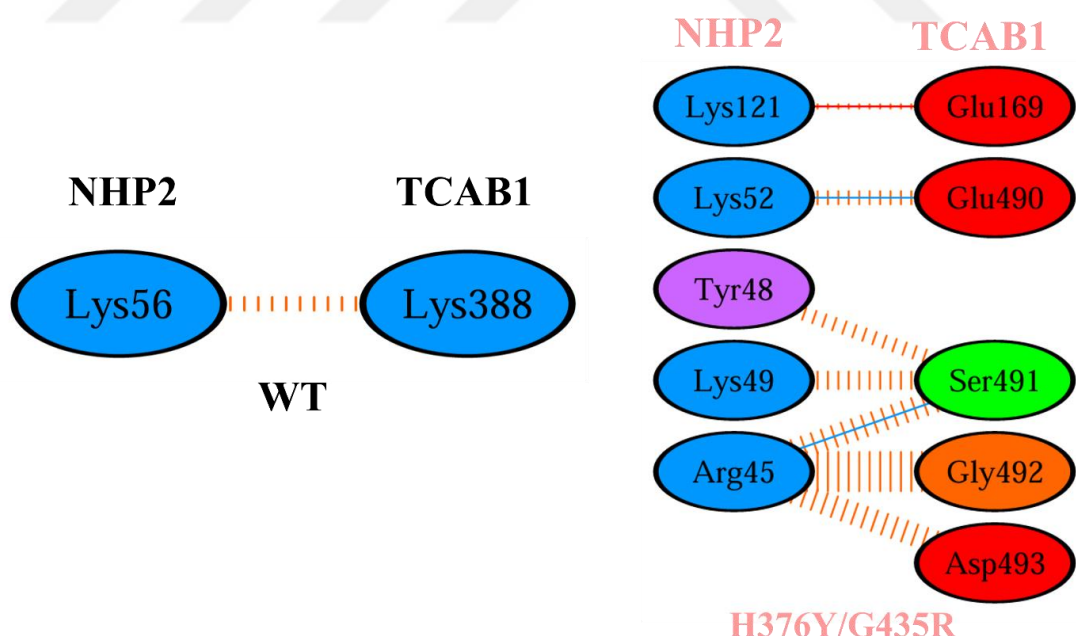


Figure 4.115. Comparative analysis of the interactions between WT TCAB1 and the H376Y/G435R mutant TCAB1 proteins with the NHP2 protein within the complex structures.

According to Figure 4.115., in the interaction between the H376Y/G435R TCAB1 and NHP2, there is a notable increase in the number and type of interactions compared to the wild type. The mutant complex forms a total of six non-bonded contacts, including both salt bridges and hydrogen bonds, which is a significant enhancement from the minimal interactions seen in the wild type. Specifically, the presence of new salt bridges between Lys121-Glu169 and Lys52-Glu490 suggests increased electrostatic stability in the complex, reinforcing the binding strength.

Hydrogen bonds are also more abundant in the mutant, with interactions observed between Lys49-Ser491, Arg45-Gly492, and Arg45-Asp493. These hydrogen bonds introduce additional points of contact, enhancing the overall binding stability and affinity. Hydrophilic residues, such as Ser491 and Asp493 on TCAB1, play a significant role in interacting with charged residues on NHP2, indicating a shift toward more hydrophilic interactions in the mutant complex. This shift, alongside the increase in electrostatic contacts and hydrogen bonds, suggests that the H376Y/G435R mutation strengthens the TCAB1-NHP2 binding, resulting in a more stable and potentially functionally relevant interaction.

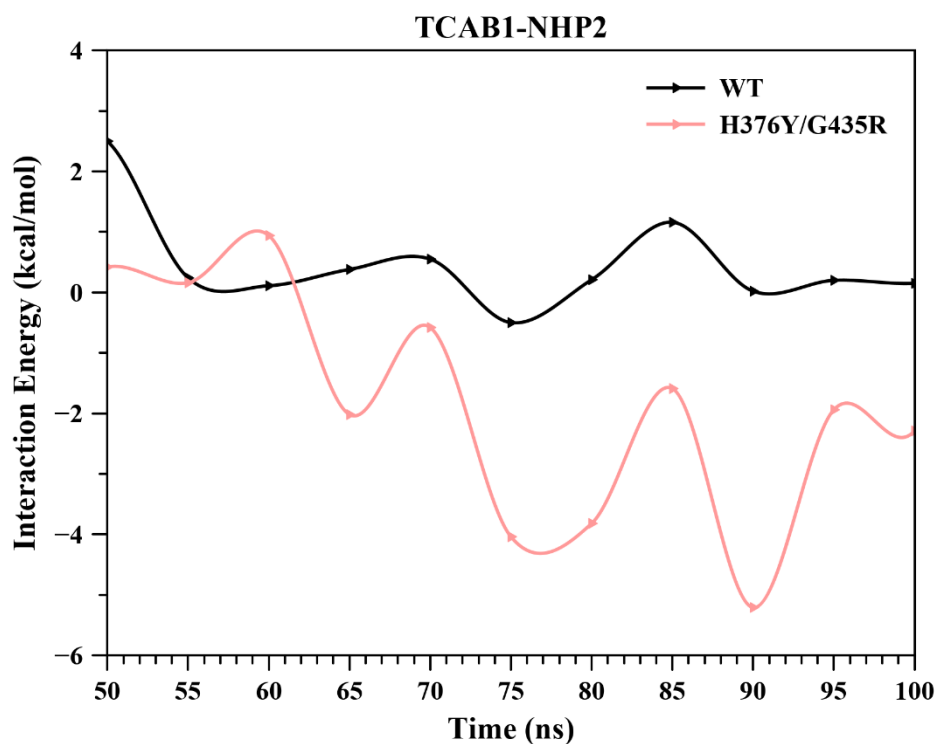


Figure 4.116. Interaction energy profile of WT and H376Y/G435R mutant TCAB1-NHP2 complexes over time.

In Figure 4.116., the interaction energy profile of the H376Y/G435R TCAB1-NHP2 complex shows a significant shift compared to the wild-type interaction. While the wild-type complex has an average interaction energy of approximately 0.5 kcal/mol, indicating a relatively weak and less stable interaction, the mutant complex displays an average interaction energy of -2 kcal/mol, which reflects a stronger binding affinity. This negative shift in energy suggests an increase in binding strength due to the mutations.

Additionally, the mutant complex shows greater fluctuation in energy values, particularly within the negative range, indicating an unstable yet strong interaction. The fluctuations, though higher in amplitude, consistently remain below the energy level of the WT, which further supports the enhanced binding strength of the mutant complex. However, the increased variability in the energy profile may imply some instability within the mutant binding interaction, possibly due to dynamic adjustments in response to new contacts formed between specific amino acids as a result of the H376Y and G435R mutations. Overall, while the mutant exhibits a stronger interaction, the higher fluctuations suggest a more complex, less rigid binding arrangement.

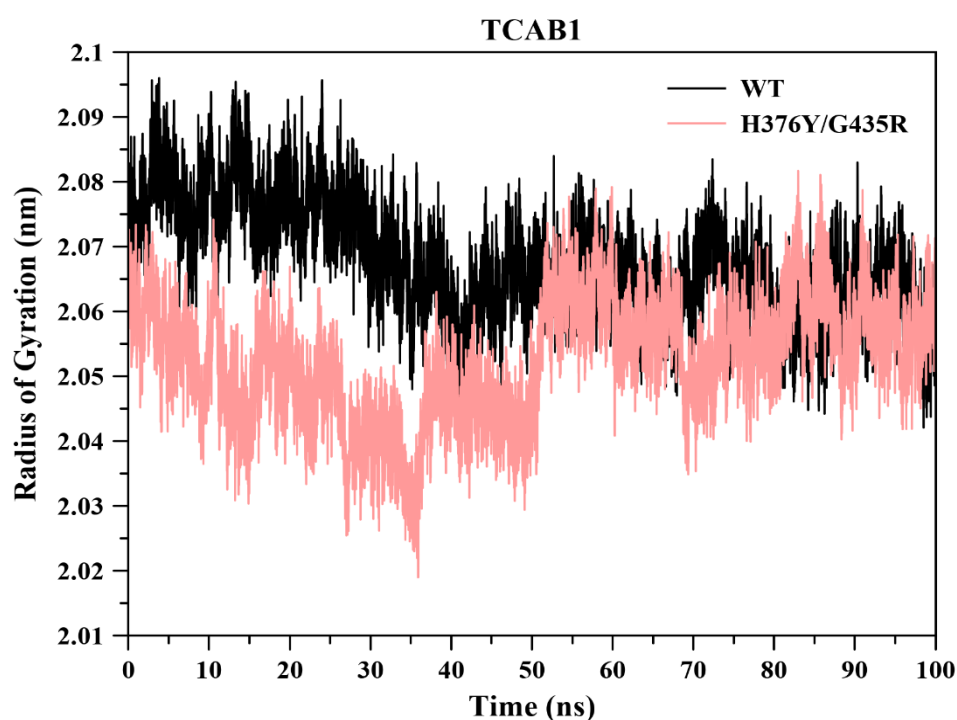


Figure 4.117. Radius of gyration graph of TCAB1 protein in WT complex and H376Y/G435R mutant complex.

According to Figure 4.117., in the Rg plot for TCAB1, the WT exhibits consistent fluctuations within a narrow range around 2.07–2.09 nm, suggesting a stable structural conformation. In contrast, the H376Y/G435R double mutant displays a generally lower Rg, indicating a more compact structure. Over time, the Rg values for the double mutant settle around 2.03–2.06 nm, with reduced fluctuations compared to the WT.

The reduction in Rg for the H376Y/G435R mutant relative to the WT implies that these mutations may induce a tightening of the protein structure, potentially affecting its flexibility or accessibility. This compaction could impact TCAB1's function or interactions, particularly if a more rigid structure hinders necessary conformational changes for its biological roles. Further analysis may be necessary to determine if this compacted structure alters functional capabilities or binding efficiency.

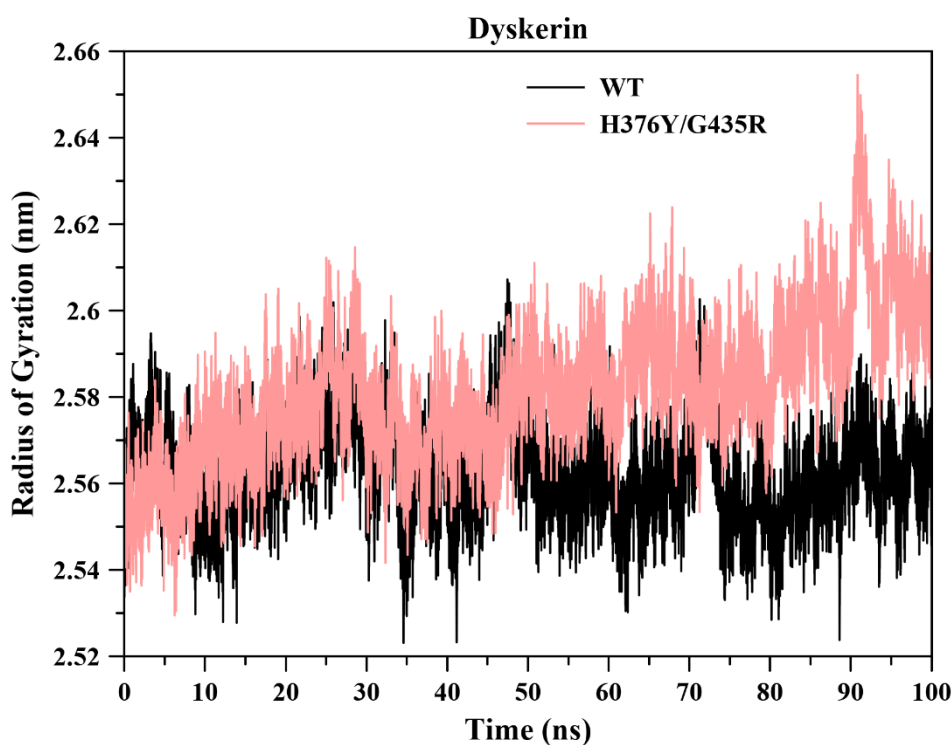


Figure 4.118. Radius of gyration graph of dyskerin protein in WT complex and H376Y/G435R mutant complex.

In the Rg plot in Figure 4.118 for dyskerin, the WT fluctuates around 2.55–2.59 nm, showing relatively consistent behaviour. The H376Y/G435R double mutant, however, demonstrates an upward shift in Rg values, averaging around 2.58–2.63 nm, with an overall increase in fluctuation range.

This increase in Rg for the H376Y/G435R mutant suggests a tendency towards a more expanded structure compared to the WT, indicating potential changes in structural dynamics. The greater Rg and wider fluctuations may imply reduced compactness or increased flexibility in dyskerin due to the double mutation, which could affect its stability and interaction with other proteins or nucleic acids. Further exploration would be necessary to understand how these structural alterations impact dyskerin's function in the context of these mutations.

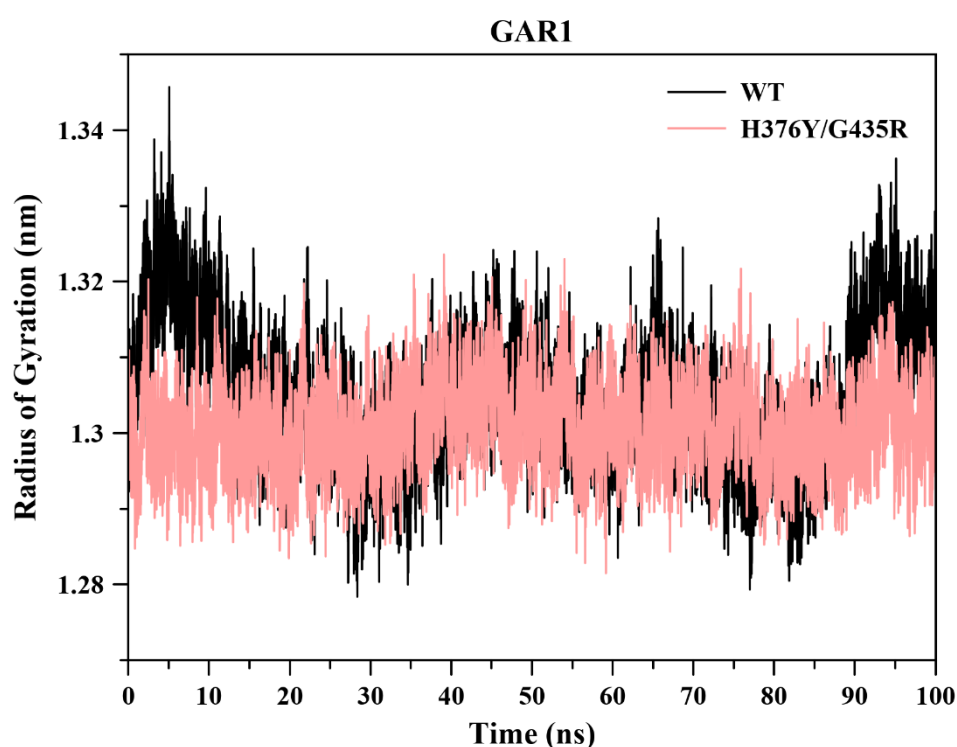


Figure 4.119. Radius of gyration graph of GAR1 protein in WT complex and H376Y/G435R mutant complex.

According to Figure 4.119., in the Rg plot for GAR1, the WT shows consistent fluctuations around 1.29–1.33 nm, maintaining a compact structure. The H376Y/G435R double mutant, on the other hand, demonstrates a slight decrease in Rg, fluctuating around 1.28–1.32 nm.

This reduction in Rg suggests a potential shift towards a more compact conformation for the H376Y/G435R mutant compared to the WT. The narrowing in fluctuation range indicates a possibly stabilized structure, potentially due to changes in interaction dynamics within GAR1. This compaction may influence GAR1's functional

interactions or flexibility, though additional structural analysis would be required to fully understand the impact of these mutations.

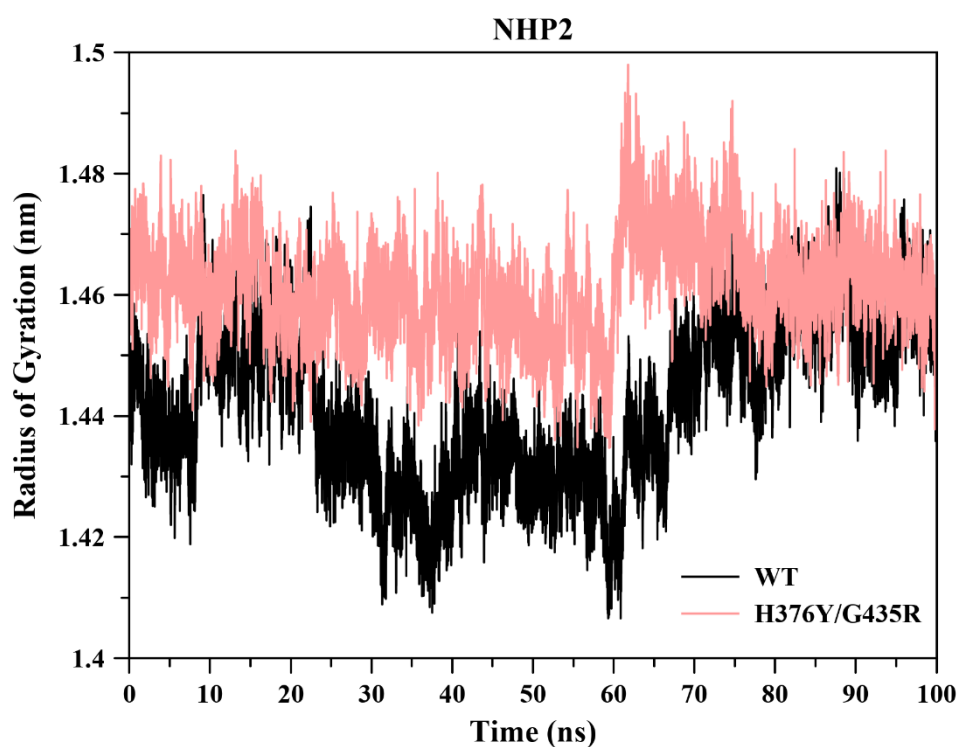


Figure 4.120. Radius of gyration graph of NHP2 protein in WT complex and H376Y/G435R mutant complex.

In the Rg plot in Figure 4.120. for NHP2, the WT fluctuates around 1.43–1.46 nm, showing a stable and compact structure throughout the simulation. The H376Y/G435R mutant, however, exhibits slightly higher Rg values, mostly fluctuating around 1.45–1.48 nm, with occasional spikes above this range.

This increase in Rg suggests that the H376Y/G435R mutation may lead to a more expanded or flexible structure in NHP2, potentially affecting its structural stability and interactions with other molecular components. The shift in Rg and increased fluctuation range could imply that this mutation affects the conformational dynamics of NHP2, potentially altering its functional properties.

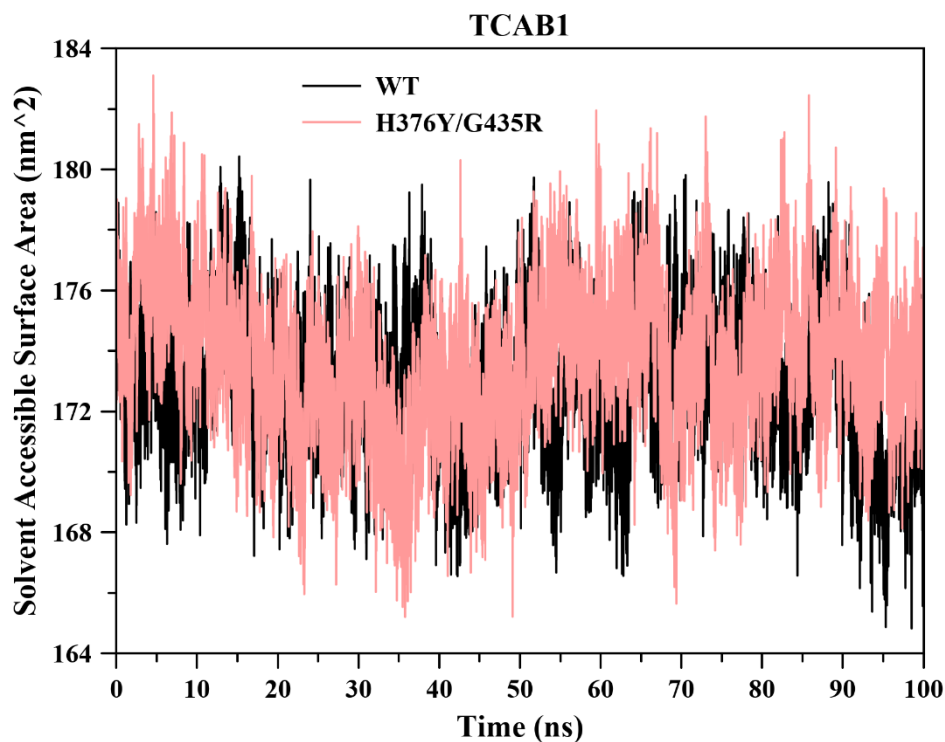


Figure 4.121. Solvent-accessible surface area graph of TCAB1 protein in WT complex and H376Y/G435R mutant complex.

According to Figure 4.121., in the SASA plot for TCAB1, the WT exhibits fluctuations around 172–176 nm², indicating a relatively stable solvent exposure over time. The H376Y/G435R mutant, on the other hand, shows an increase in SASA values, fluctuating more around 175–180 nm².

This shift suggests that the H376Y/G435R mutation might expose more of TCAB1 to the solvent, possibly due to structural rearrangements or a more open conformation. Increased SASA could indicate alterations in the protein's surface properties, potentially impacting interactions with other molecular partners or affecting its overall stability.

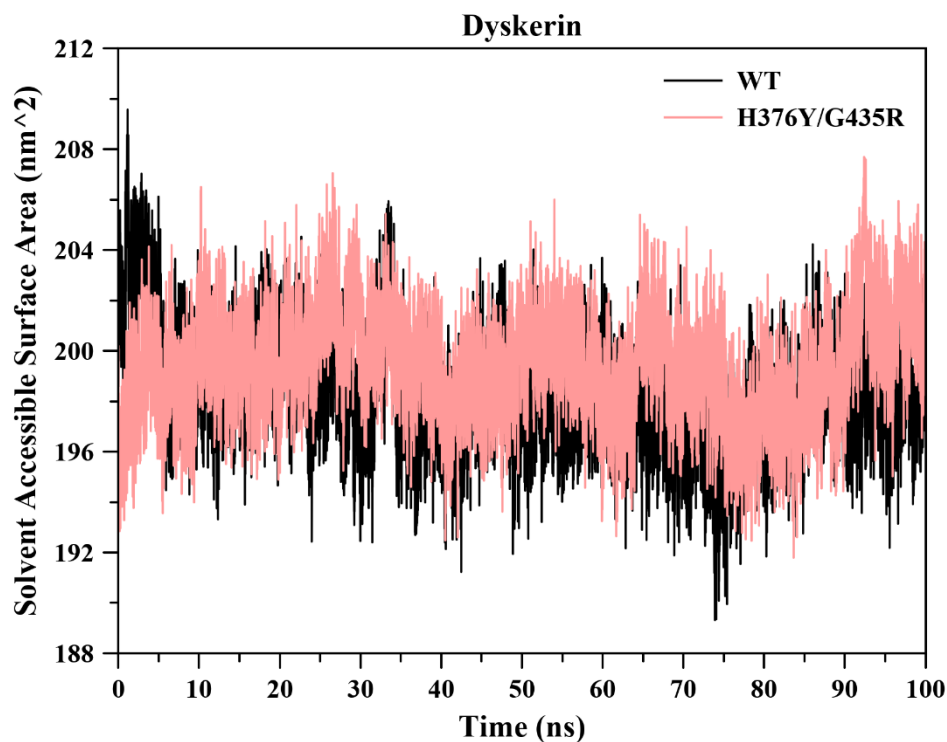


Figure 4.122 Solvent-accessible surface area graph of dyskerin protein in WT complex and H376Y/G435R mutant complex.

According to Figure 4.122., in the SASA plot for dyskerin, the WT maintains a steady solvent-accessible surface area around 196–202 nm², indicating a consistent exposure profile over time. The H376Y/G435R mutant, however, displays slightly elevated SASA values, predominantly fluctuating between 198–204 nm².

This increase in SASA for the H376Y/G435R variant could suggest that the mutation induces subtle conformational changes in dyskerin, making the protein surface more accessible to the solvent. This altered surface exposure might affect protein stability, interaction capability, or overall structural dynamics.

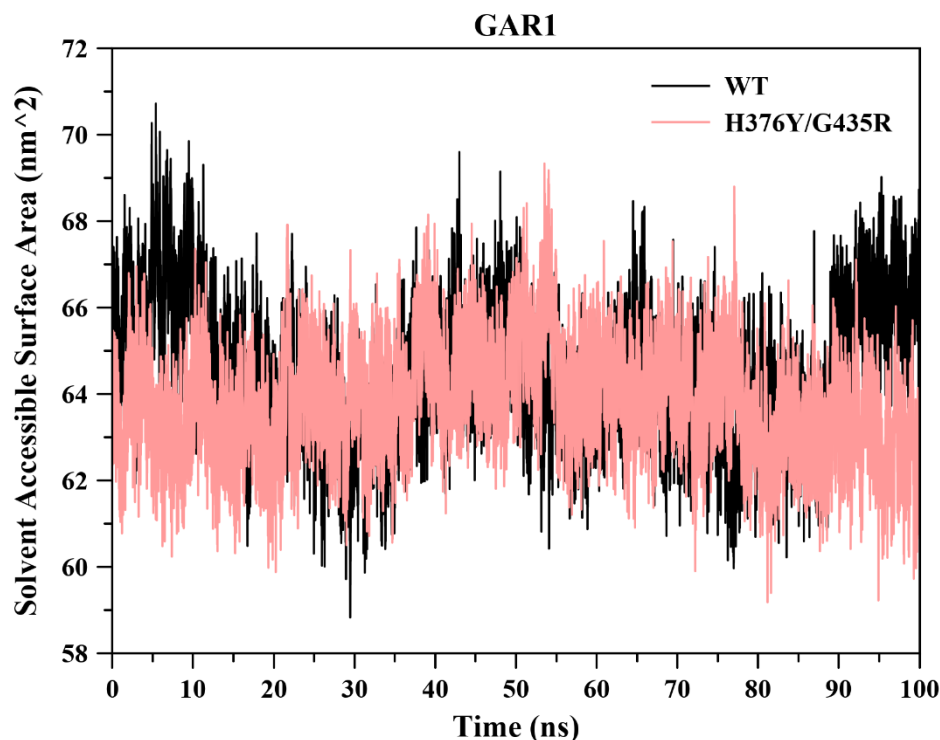


Figure 4.123. Solvent-accessible surface area graph of GAR1 protein in WT complex and H376Y/G435R mutant complex.

According to Figure 4.123, in the SASA plot for GAR1, the WT shows a fairly consistent solvent-accessible surface area, primarily fluctuating between 62–68 nm² throughout the simulation. However, the H376Y/G435R mutant exhibits a slightly lower SASA range, generally between 60–66 nm².

This reduction in SASA for the H376Y/G435R variant suggests that the mutation may induce a more compact structure, leading to decreased surface exposure to the solvent. This structural alteration could potentially influence GAR1's interactions with other molecules, affecting its functional properties in the cellular context.

According to Figure 4.124., in the SASA plot for NHP2, the WT shows SASA fluctuations mainly between 80–86 nm², while the H376Y/G435R mutant displays a slightly elevated SASA, fluctuating between 82–88 nm² throughout the simulation.

This increased SASA for the H376Y/G435R variant suggests a more exposed structure, potentially impacting NHP2's interactions within the protein complex or with other biomolecules. The structural changes implied by the increased SASA may influence the protein's stability and functional interactions in a cellular context.

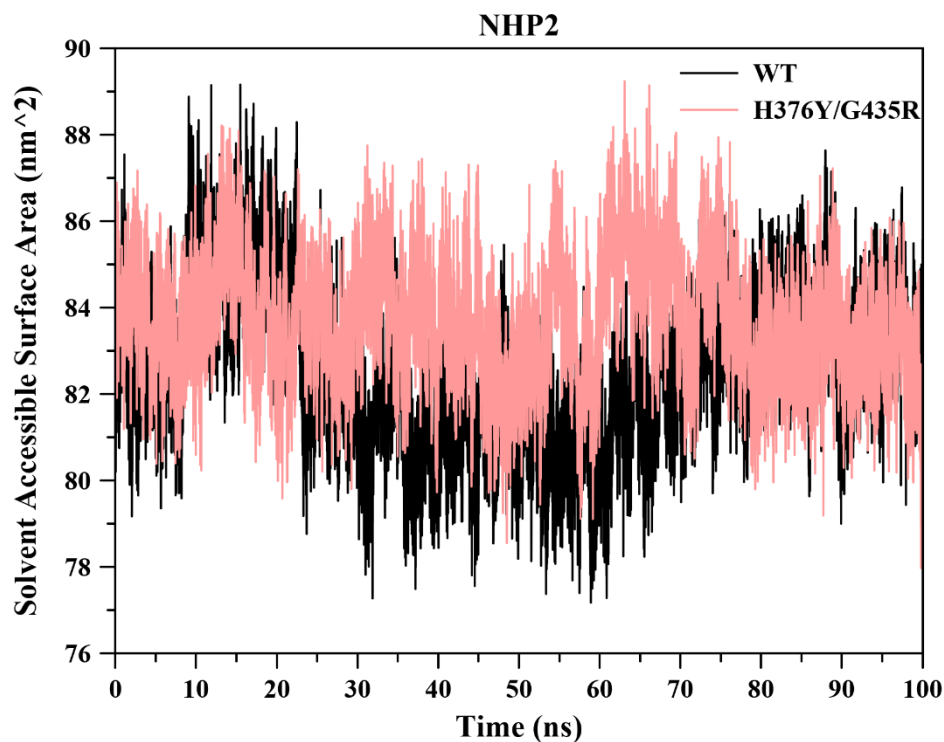


Figure 4.124 Solvent-accessible surface area graph of NHP2 protein in WT complex and H376Y/G435R mutant complex.

According to Figure 4.125., in the interaction energy profile between TCAB1 and RNA, the WT exhibits a mean interaction energy of -5 kcal/mol, indicating stronger binding to RNA. In contrast, the H376Y/G435R mutant has a higher average energy of -2.5 kcal/mol, reflecting a weaker interaction with RNA. The mutant's binding profile is consistently less negative throughout the simulation, suggesting a substantial reduction in binding strength compared to the WT.

Moreover, the WT displays deeper fluctuations, indicating moments of stronger binding stability during the simulation. In contrast, the mutant profile remains relatively higher, showing less variability and suggesting a less dynamic interaction. This pattern implies that the H376Y and G435R mutations together may disrupt optimal RNA binding, likely affecting TCAB1's binding affinity and functional interaction with RNA. The reduced binding strength in the mutant might impair TCAB1's role in stabilizing RNA or facilitating RNA processing, potentially impacting its biological function.

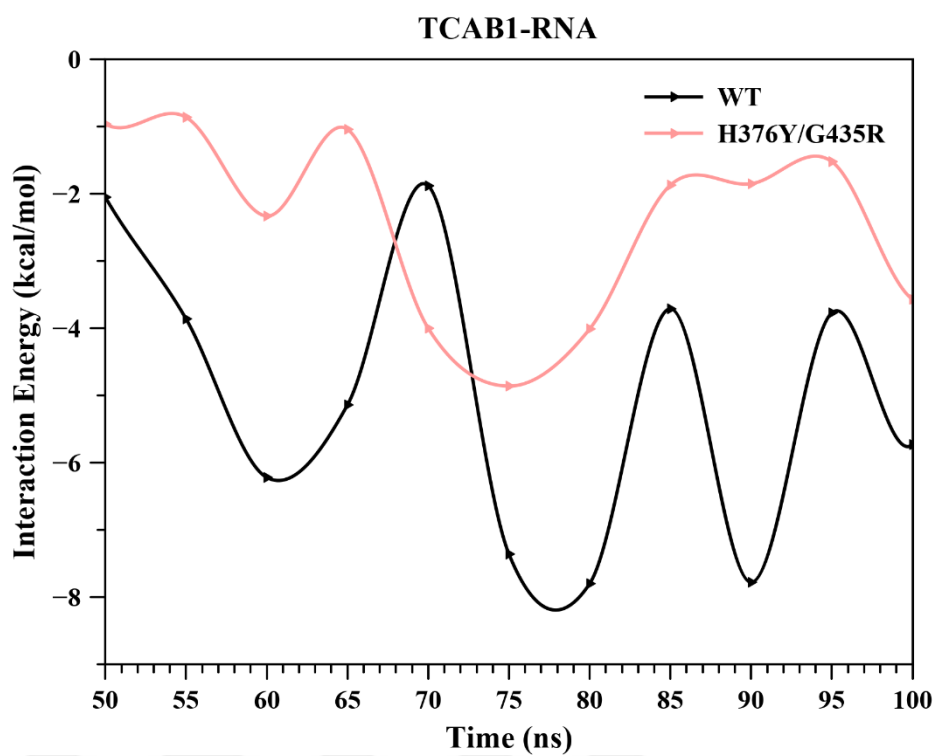


Figure 4.125. Interaction energy profile of WT and H376Y/G435R mutant TCAB1-RNA complexes over time.

5. DISCUSSION

Root Mean Square Deviation (RMSD) is a critical metric in structural biology used to assess the deviation of protein structure during molecular dynamics simulations from a reference structure. This measure is crucial in understanding the stability and conformational dynamics of proteins, particularly in evaluating the impact of mutations on a protein's function and structural integrity (**Arnittali et al., 2019**).

In Figure 4.1, comparing the WT and the F164L mutant over 150 nanoseconds, the F164L mutant consistently exhibits higher RMSD values than the WT. This suggests that the F164L mutation may induce increased flexibility or conformational instability in the protein structure. The RMSD plot suggests that the WT protein stabilizes relatively quickly, maintaining a lower and more consistent RMSD after about 25 ns. In contrast, the F164L mutant exhibits prolonged fluctuations, suggesting that it reaches equilibration later in the simulation. This could indicate that the F164L mutation introduces structural changes that require longer to accommodate within the protein's dynamics, potentially affecting its functional stability.

Figure 4.22., which presents the RMSD of the WT and the H376Y mutant over 100 nanoseconds, highlights that the H376Y mutation alone increases reaching the plateau time relative to the WT. This indicates that even a single point mutation like H376Y can have a profound impact on the protein's dynamics, suggesting a destabilizing effect that could alter its biological activity.

Figure 4.43 compares the WT with the R398W mutant over 200 nanoseconds, where the R398W mutation generally shows higher RMSD values than the WT, and the mutant does not equilibrate within the 200 ns simulation. This suggests that the R398W mutation alone significantly affects the protein's structural stability, leading to greater deviations from the initial conformation.

The Figure 4.63. features the RMSD comparison between the WT complex and the G435R mutant complex over 100 nanoseconds. Although both complexes exhibit similar RMSD values at various intervals, the G435R complex occasionally reaches higher RMSD peaks than the WT complex. This could imply that the G435R mutation introduces changes that affect the overall stability and structure of the protein complex, possibly influencing its functional interactions and assembly.

In Figure 4.84., the RMSD values of the WT and the F164L/R398W double mutant over 200 nanoseconds are compared. Both the WT and the double mutant display similar RMSD values intermittently, but the overall trend for the mutant shows greater fluctuations. In this simulation, there appears to be a gradual equilibration process, with fluctuations decreasing over time. By around 100 ns, both the WT and the mutant show signs of reaching a more stable state. The observation of gradual stabilization suggests that the interactions between the F164L and R398W mutations may induce complex conformational adjustments that take longer to stabilize.

Finally, the Figure 4.110. shows the RMSD trajectories of the WT and the H376Y-G435R double mutant over 100 nanoseconds. Here, the double mutant's RMSD values are consistently higher than those of the WT, indicating that the combined mutations contribute to a more significant deviation from the reference structure, potentially altering the protein's functional properties due to increased structural instability. Also, both the WT and the double mutant display ongoing fluctuations throughout the 100 ns simulation, with no clear plateau indicating full equilibration. This prolonged equilibration period could be due to the combined effect of the mutations, creating a more complex dynamic environment that requires more time to stabilize.

When examining all graphs collectively, it becomes evident that mutations distinctly influence RMSD, reflecting their impact on protein dynamics and structural stability. Single mutations like F164L and R398W show significant alterations in RMSD, suggesting they can independently affect protein behaviour. The observations from double mutants (H376Y-G435R, F164L/R398W) provide insights into how combinations of mutations might interact to further destabilize the protein or synergistically alter its dynamics.

This analysis underscores the importance of considering individual and combined effects of mutations on protein dynamics as assessed by RMSD. Such insights are vital for predicting the functional consequences of mutations and can guide the design of therapeutic interventions that target these altered protein states. This discussion also highlights the utility of RMSD as a robust tool in molecular dynamics simulations for investigating the detailed impacts of genetic variations on protein structure and function.

In MD simulations, the RMSF value measures how much the atoms or groups of atoms in a protein or other molecular structure deviate from their average positions during the simulation. This value is used to indicate how flexible or dynamic certain regions of the protein are. RMSF is an important tool for understanding the dynamic properties of protein's active sites, binding sites, or other functional domains (**Benson & Daggett, 2012**).

The comprehensive analysis of RMSF values in TCAB1 across various mutations provides an in-depth look at the molecular dynamics and structural consequences of these genetic alterations. This extensive examination will delve into each mutation individually, detailing the affected regions and their implications on protein functionality and stability.

As can be seen in Figure 4.3, the F164L mutation exhibits a mixed effect on the RMSF values, with some regions showing increased flexibility and others decreasing. The areas with reduced flexibility (161-183 and 484-510) indicate an increase in structural stability, which might enhance the protein's functional integrity and interaction consistency within the telomerase complex.

Conversely, the areas with increased flexibility (331-373, 443-461) might introduce some level of destabilization, which could either disrupt normal function or introduce new dynamic capabilities to the protein, depending on the context of the interaction and cellular conditions. In the study of the F164L mutation in the TCAB1 protein, an analysis of RMSF values revealed distinct stability dynamics. Specifically, a decrease in RMSF values was noted in the region interfacing with NHP2, indicative of enhanced stability, whereas an increase in RMSF values was observed in regions interacting with RNA, suggesting increased instability. As demonstrated in the graphical representations such as Figure 4.11. and 4.12. the interaction between the TCAB1 protein and the NHP2 protein not only proved energetically favourable but also exhibited a significant improvement in both the quantity and quality of bond/interaction formations. Conversely, the interaction energy between the TCAB1 protein and RNA displayed no notable differences, indicating a neutral effect on binding affinity in the context of this mutation (Figure 4.21.).

In the context of the H376Y mutation within the TCAB1 protein, a comprehensive analysis of RMSF values highlighted a pronounced increase across regions that

interface with all other members of the complex. This destabilizing effect, primarily noted in the interaction landscape with dyskerin, is thoroughly documented in Figure 4.27. Despite these perturbations in RMSF values suggesting altered dynamics, the interaction energy between TCAB1 and dyskerin proteins remains remarkably unchanged, indicating a complex interplay of stabilizing and destabilizing forces at the molecular level.

Further analysis, as depicted in Figures 4.28-31, reveals an enhancement in the interactions between TCAB1 and the proteins NHP2 and GAR1. These interactions not only increased in frequency but also demonstrated a tendency towards greater stabilization in their interaction energies. This suggests a compensatory mechanism that may be offsetting the destabilizing effects observed elsewhere within the protein complex.

The observed discrepancies between the RMSF graph, interaction maps, and interaction energy charts may potentially be attributed to errors in the selection or delineation of regions where RMSF values increased. Such inaccuracies could skew the perceived extent of instability across the protein complex. Additionally, while Figure 4.24 indicates significant spikes in RMSF values at individual amino acid residues, a holistic view across broader regions reveals that these increments are not substantial enough to assert a significant overall increase. This nuanced understanding underscores the complexity of molecular interactions and the critical need for precise analytical methods in interpreting dynamic changes within protein structures.

According to Figure 4.62, the R398W mutation in the TCAB1 protein has predominantly exhibited a stabilizing effect, as evidenced by a general decrease in RMSF values across the protein structure, except for the region interacting with RNA. This decrease in RMSF values across the majority of the protein domains has led to enhanced stability, which has significantly influenced its interaction dynamics with surrounding molecules.

Further detailed analysis, as presented in Figures 4.65-68, demonstrates that the interaction of the R398W mutant TCAB1 protein with dyskerin and GAR1 proteins has markedly improved. This improvement is characterized by an increase in both the number and types of interactions formed, as well as a noticeable enhancement in interaction energies. These findings suggest a refined molecular adaptation that

enhances the overall stability and functional performance of the protein complex in the mutated state.

Conversely, the already minimal interactions of the R398W mutant TCAB1 with the NHP2 protein appear to have been adversely affected by the observed reductions in RMSF. This suggests a selective destabilization in specific protein-protein interactions, which could have implications for the functional integration within the protein complex.

Additionally, despite a slight increase in RMSF values observed in the RNA-binding region of the TCAB1 protein, there has been no significant change detected in its interaction with RNA, as evidenced by Figure 4.72. This indicates that the mutation-induced alterations in RMSF values do not uniformly translate to functional changes in protein-RNA interactions, highlighting the complex and nuanced nature of molecular dynamics in response to mutational impacts.

According to the data presented in Figure 4.83, the G435R mutation predominantly exerts a destabilizing effect on the TCAB1 protein, as indicated by the general increase in RMSF values across various regions of the protein compared to the wild type (WT). Notably, this mutation has led to a slight increase in RMSF values, specifically in regions that are crucial for the interaction of the TCAB1 protein with dyskerin, GAR1, and RNA. These areas correspond to the key functional domains involved in molecular interactions essential for the protein's activity.

In contrast, the interaction domain of the TCAB1 protein with the NHP2 protein has shown a decrease in RMSF values due to the mutation, suggesting a stabilizing influence in this particular area. This selective stabilization may reflect a mutation-induced conformational adaptation that enhances protein-protein interactions within the complex.

Furthermore, as detailed in Figures 4.85-90, the interactions of the G435R mutant TCAB1 protein with dyskerin, GAR1, and NHP2 proteins have not only increased in the number and diversity of interactions but also shown significant improvements in interaction energies. This enhancement indicates a beneficial modulation of the protein's structural and interactional dynamics, likely contributing to a more robust molecular assembly.

However, as depicted in Figure 4.86, there has been a noticeable decrease in interaction energy between the TCAB1 protein and the RNA molecule, with a reduction of 2 kcal/mol as a result of the mutation. This decrease is significant and points to a potential impairment in the protein-RNA interaction efficiency, which could have profound implications for the functional capabilities of the TCAB1 protein within the cellular environment.

This comprehensive analysis underscores the complex and nuanced effects of the G435R mutation on the TCAB1 protein's structure and its molecular interactions. It highlights the importance of considering both destabilizing and stabilizing influences when assessing the overall impact of genetic variations on protein functionality.

According to the data illustrated in Figure 4.102, the F164L/R398W double mutation exhibits both stabilizing and destabilizing effects on the TCAB1 protein, as evidenced by the RMSF values. Notably, the destabilizing effects, characterized by increases in RMSF, predominantly occur in regions where TCAB1 interacts with RNA and the dyskerin protein. Conversely, stabilizing effects are observed as decreases in RMSF values in the region associated with binding to the NHP2 protein.

Detailed analysis of the interaction dynamics, as depicted in Figures 4.103-106 and Figure 4.119, reveals that the interactions between TCAB1 and both RNA and dyskerin are compromised under the influence of the destabilizing effects of the mutations, resulting in diminished interaction strengths. While the dyskerin-TCAB1 interaction energy graph (Figure 4.112) suggests that the mutant protein interactions are generally more favourable, fluctuations within the graph and occasional peaks reaching positive values indicate that the WT TCAB1 protein binds more stably with dyskerin.

Furthermore, the interactions between TCAB1 and the NHP2 protein have shown significant improvements both in terms of interaction energy and the number of interacting residues, indicating a shift towards more favourable conditions. Although partial increases in RMSF values are observed in the region where TCAB1 interacts with the GAR1 protein, a reduction in RMSF values within GAR1 has facilitated the formation of more effective qualitative and quantitative interactions between these two proteins.

Thus, the F164L/R398W double mutation, while disrupting interactions between TCAB1 and its partner's dyskerin and RNA, has enhanced its interactions with GAR1 and NHP2 proteins. This dual effect of the mutations illustrates a complex balance between destabilizing and stabilizing influences, revealing intricate molecular adaptations that modulate protein interaction networks within the cellular framework.

As depicted in Figure 4.124, the H376Y/G435R double mutation has exhibited both stabilizing and destabilizing effects on the TCAB1 protein, according to RMSF values. The destabilizing impacts are confined to regions responsible for RNA binding, leading to a significant reduction in the interaction strength with RNA, as evidenced by a 50% decrease shown in Figure 4.116. On the other hand, Figures 4.118-125 indicate no notable changes in binding energy or interactions formation/loss between TCAB1 and dyskerin proteins, suggesting that the mutations do not influence the interaction dynamics with dyskerin.

Intriguingly, the stabilizing influence of the mutations is apparent in the interactions involving the GAR1 and NHP2 proteins. As Figures 4.118-121 demonstrate, the mutations have substantially enhanced the interactions of TCAB1 with GAR1 and NHP2, both in terms of the quality and quantity of interactions, as well as the interaction energies. This improvement indicates a mutation-driven optimization in the binding affinities and interactional stability within the protein complex.

Consequently, the H376Y/G435R double mutation has been determined to not affect the interaction with dyskerin while positively augmenting the interactions with GAR1 and NHP2. Furthermore, it has significantly compromised the interaction capabilities of TCAB1 with RNA. This comprehensive analysis highlights the nuanced impact of specific mutations on protein interaction networks, demonstrating a complex balance between enhancing and diminishing interactions within different domains of the TCAB1 protein. These findings underscore the critical importance of considering the broader implications of mutations on protein functionality and their potential effects on cellular processes.

The overall interaction energy between complex proteins and TCAB1 can be seen in Table 5.1.

Table 5.1. Overall interaction energies between complex proteins and TCAB1.

Mutant	Interaction Energy with TCAB1 (kcal/mol)		
	Dyskerin	GAR1	NHP2
WT	-1.5	-0.5	0.7
F164L	-2.7	-1.5	-4
H376Y	-2	-0.4	-2
R398W	-3	-2.5	0.5
G435R	-2.5	-1.2	-2.2
F164L/R398W	-3	-3	-6
H376Y/G435R	-1.2	-3	-2

Below, overall interaction energy graphs between RNA and TCAB1 can be seen in Figure 5.1.

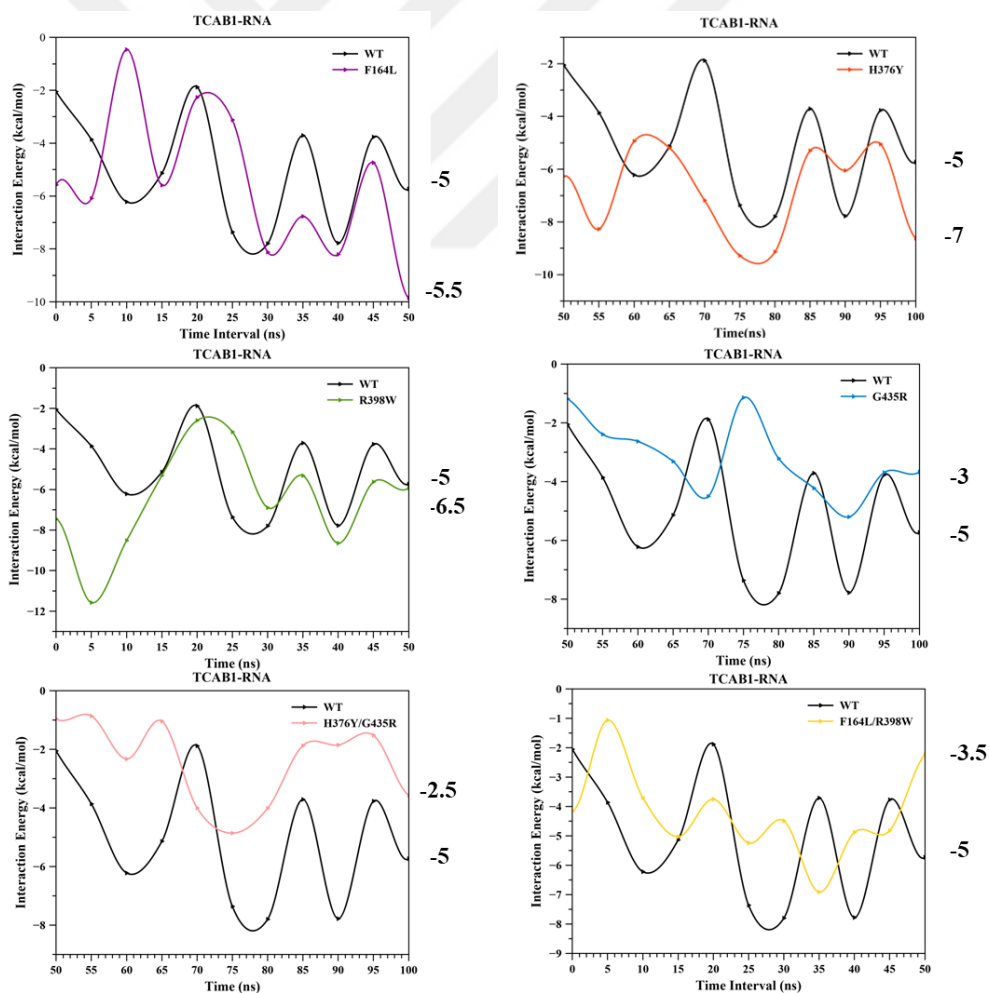


Figure 5.1: The overall interaction energy graphs between RNA and TCAB1.

In the interaction between WT TCAB1 and Dyskerin, hydrophilic interactions play a crucial role, primarily established through hydrogen bonds and ionic interactions between polar and charged residues. These interactions stabilize the binding interface, ensuring strong affinity due to the hydrophilic nature of the residues involved.

In the WT TCAB1 protein, the interaction interface with Dyskerin is strongly hydrophilic, primarily mediated by residues such as Arg158 and Glu251. These residues, being charged and polar, facilitate ionic and hydrogen bonding interactions, which enhance the hydrophilic nature of the interface. Additional contributions from residues such as His273 and Asp275 further stabilize this hydrophilic environment, creating a favourable binding interface for Dyskerin in aqueous conditions.

The F164L mutation alters this landscape by introducing a leucine, a nonpolar and hydrophobic residue. This change reduces the overall polarity at the interface, disrupting some of the hydrophilic interactions present in the WT. Although Arg158 and Glu251 still participate in interactions, the reduction in polar contacts with Dyskerin suggests a shift toward a slightly more hydrophobic interface. This could affect the stability and affinity of the TCAB1-Dyskerin complex in hydrophilic environments.

In the double mutant F164L/R398W, the reduction in hydrophilic interactions becomes even more pronounced. The presence of additional nonpolar residues diminishes the number of polar contacts, leading to a more hydrophobic interface compared to the WT. Although the Arg158-Glu251 ionic interaction remains, the overall interaction profile is less hydrophilic, potentially weakening the interaction between TCAB1 and Dyskerin in aqueous conditions and affecting complex stability.

The G435R mutation introduces a new arginine at the binding interface, adding a highly hydrophilic and positively charged residue. This modification partially restores the hydrophilic character of the interface by increasing the potential for polar interactions. The presence of additional arginine, with its capacity for hydrogen bonding and ionic interactions, enhances the hydrophilic interactions, which could counterbalance some of the hydrophobic tendencies introduced by other mutations. This suggests that G435R maintains or even strengthens the hydrophilic environment, which could positively influence binding stability with Dyskerin.

In the H376Y mutant, tyrosine replaces a residue, potentially altering the balance of hydrophilic and hydrophobic interactions. Tyrosine, though polar, has a less pronounced hydrophilic character compared to arginine or glutamate. This change likely reduces the hydrophilicity of the interface slightly, though not to the same extent as in F164L. The remaining Arg158-Glu251 ionic interaction continues to provide hydrophilic support, but the overall reduction in polar contacts may result in a more hydrophobic binding environment than WT.

The double mutant H376Y/G435R combines the hydrophilic arginine (introduced by G435R) with the relatively hydrophobic tyrosine (from H376Y). This combination creates a mixed profile at the interface. While arginine helps retain some hydrophilic character, the presence of tyrosine tempers this effect. Thus, the interface in this mutant exhibits both hydrophilic and hydrophobic qualities, likely resulting in a moderately altered binding affinity with Dyskerin compared to the WT.

The R398W mutation, which introduces tryptophan, adds a significant hydrophobic character to the interface, replacing a charged residue with a nonpolar one. This substitution decreases the hydrophilicity of the interaction site, diminishing the ability of TCAB1 to form polar contacts with Dyskerin. The Arg158-Glu251 interaction is preserved, but the overall environment becomes less hydrophilic, potentially destabilizing the interaction in aqueous environments.

In the context of the double mutant F164L/R398W, the combined introduction of leucine and tryptophan leads to a marked increase in hydrophobicity at the binding interface. This shift away from hydrophilic interactions significantly reduces the polarity of the interface, potentially weakening the affinity for Dyskerin due to the loss of stabilizing hydrophilic interactions.

In summary, the mutations F164L and R398W and their combination shift the interface toward a more hydrophobic nature, reducing the stability of TCAB1's interaction with Dyskerin. Conversely, the G435R mutation enhances hydrophilicity, helping to preserve or even strengthen binding affinity. H376Y and H376Y/G435R introduce mixed effects, combining both hydrophilic and hydrophobic properties. These shifts in hydrophilicity and hydrophobicity directly impact the interaction dynamics, influencing the stability and affinity of the TCAB1-Dyskerin complex.

Analysis of SASA (Solvent Accessible Surface Area) reveals distinct effects of each mutation on the TCAB1 and Dyskerin proteins, impacting their hydrophilic interactions and solvent exposure.

The F164L mutation in TCAB1 leads to a decrease in SASA, indicating a reduction in hydrophilic surface area and suggesting that the protein has adopted a more compact conformation. Dyskerin shows a similar decrease in SASA, implying reduced solvent interaction on the protein surface.

In contrast, the R398W mutation results in an increase in SASA for TCAB1, signifying greater accessibility of hydrophilic regions to the solvent. Similarly, Dyskerin exhibits an increase in SASA, which suggests that its hydrophilic regions are more exposed to the solvent environment.

For the H376Y mutation, TCAB1 experiences a reduction in SASA, indicating a decrease in hydrophilic regions or a more compact structure overall. Dyskerin also shows a decline in SASA with this mutation, supporting the interpretation of a more compact protein surface with reduced solvent accessibility.

The G435R mutation causes a marked increase in SASA in TCAB1, suggesting an opening of the protein structure or enhanced exposure of hydrophilic areas to the solvent. Dyskerin similarly exhibits an increase in SASA, indicating greater solvent interaction with the protein surface.

The F164L/R398W double mutation leads to a significant rise in SASA in TCAB1, indicating that hydrophilic regions on the protein surface have become more accessible to the solvent. Dyskerin also displays an increase in SASA with this double mutation, suggesting a more open conformation with increased solvent exposure.

Lastly, the H376Y/G435R double mutation similarly induces an increase in SASA for TCAB1, reflecting heightened accessibility of hydrophilic areas to the solvent. Dyskerin's SASA also increases, implying enhanced solvent interaction and a more open structure.

In summary, each mutation induces notable changes in SASA for both TCAB1 and Dyskerin, affecting the hydrophilic surface area and, consequently, the solvent exposure of the proteins. These alterations are indicative of structural modifications that potentially influence the proteins' interactions with their molecular environment.

In the WT TCAB1-GAR1 interaction, the binding interface is predominantly characterized by polar interactions, specifically between Glu81 from GAR1 and Arg269 from TCAB1. This interaction is highly hydrophilic due to the charged nature of these residues, with Glu81 being negatively charged and Arg269 positively charged, facilitating strong ionic and hydrogen bonding, which contributes to stability and specificity at the interface.

For the F164L single mutant, an additional interaction is observed involving Arg310 from TCAB1, which further interacts with Glu81. This modification enhances the hydrophilic nature of the interaction surface by introducing another positively charged residue (Arg310) in close proximity to Glu81. This likely strengthens the interaction by providing additional ionic bonding potential, thus amplifying the polar character of the binding interface. Consequently, the hydrophilicity at the binding site increases with F164L.

In the H376Y mutation, histidine at position 376 is replaced by tyrosine. Both amino acids are polar, but tyrosine contains a hydroxyl group capable of forming hydrogen bonds, while histidine's imidazole ring offers versatility in bonding. This mutation may alter the hydrogen bonding pattern without drastically changing the hydrophilic nature of the binding interface. Tyrosine can still participate in hydrophilic interactions with GAR1, likely maintaining the stability and affinity of the TCAB1-GAR1 complex similar to the WT.

In the R398W mutation, arginine at position 398, a positively charged hydrophilic residue, is replaced by tryptophan, which is hydrophobic and bulky. This substitution reduces the number of hydrophilic interactions at the binding interface by eliminating ionic and hydrogen bonding opportunities provided by arginine. The loss of these interactions can weaken the overall affinity between TCAB1 and GAR1. However, other hydrophilic residues, such as Arg269 and Arg310, may compensate partially by maintaining their interactions with GAR1, mitigating the detrimental effects of the mutation.

The G435R mutation further strengthens the hydrophilic environment at the interface by adding Arg310 alongside Arg269, which engages with GAR1 residues Asp82 and Asp83, respectively. This configuration amplifies the hydrophilic interactions through additional charged residues, particularly with the dual Asp residues in GAR1, which

are highly compatible with the positively charged arginine residues. Consequently, the G435R mutation results in a highly polar and hydrophilic binding interface, increasing the stability of interaction due to the prevalence of ionic interactions.

In the double mutant F164L/R398W, the interaction pattern expands with contributions from Arg310 and Arg269, and additional polar and charged residues such as Asp82, Ser130, and Glu131 from GAR1. This mutant configuration increases both the number and diversity of hydrophilic contacts through several charged and polar residues, maintaining a highly polar interface. Therefore, the hydrophilic character of the interface is further enhanced in F164L/R398W, potentially increasing binding affinity due to the expanded network of electrostatic and hydrogen bonds.

Finally, the H376Y/G435R double mutation introduces substantial alterations at the GAR1-TCAB1 interface. Key residues include Arg310 and Arg269 from TCAB1, which interact with hydrophilic and charged residues Glu81, Asp82, and Asp83 from GAR1. The substitution of Tyr in this double mutant configuration also potentially impacts the interaction dynamics due to the introduction of additional hydrophobic character near Arg residues, which might slightly modulate the overall hydrophilicity without substantially diminishing the hydrophilic and charged nature of the interface. This double mutation sustains a hydrophilic interaction interface, although with potentially less uniformity than observed in previous mutants due to the mixed character of Tyr.

In summary, mutations introducing hydrophobic residues, such as R398W and the double mutation F164L/R398W, tend to reduce the hydrophilic interactions at the TCAB1-GAR1 interface, potentially weakening the binding affinity. Conversely, mutations that introduce or involve hydrophilic residues, such as G435R and the double mutation H376Y/G435R, enhance the hydrophilic interactions, potentially strengthening the binding. The H376Y mutation maintains the hydrophilic nature of the interface with minimal changes, while the overall effect on binding affinity depends on the balance between lost and gained hydrophilic interactions and any compensatory structural rearrangements that occur to preserve binding stability.

The SASA analysis for the GAR1 protein across all mutations indicates variations in hydrophilic surface exposure, reflecting structural changes that may influence its interactions with surrounding molecules.

In the case of the F164L mutation, GAR1 shows a slight decrease in SASA. This suggests that the mutation may induce a more compact conformation, with reduced exposure of hydrophilic regions to the solvent. This decrease in SASA could indicate limited solvent accessibility, potentially impacting GAR1's capacity for intermolecular interactions.

With the R398W mutation, GAR1 experiences an increase in SASA. This suggests that the hydrophilic regions are more exposed, possibly due to a loosening of the protein structure. The increased SASA implies enhanced solvent accessibility, which may affect GAR1's interactions by allowing more extensive molecular engagement in hydrophilic environments.

For the H376Y mutation, GAR1 exhibits a slight reduction in SASA, indicating a more compact structural arrangement with diminished solvent exposure. This compaction implies that the protein's hydrophilic regions are less accessible to the solvent, potentially limiting interaction sites available for hydrophilic bonding.

The G435R mutation causes an increase in GAR1's SASA, suggesting an expanded structure with greater solvent interaction. This expansion might facilitate more hydrophilic interactions, as more of GAR1's surface is accessible for molecular engagement with its environment.

When considering the double mutation F164L/R398W, GAR1 shows a noticeable increase in SASA, implying a more open conformation. This elevated SASA indicates enhanced solvent exposure, suggesting that the mutation pair significantly affects the protein structure, potentially altering GAR1's interaction dynamics in hydrophilic conditions.

Similarly, the H376Y/G435R double mutation leads to an increase in SASA for GAR1. This suggests that hydrophilic areas on the protein surface are more accessible, reflecting a more relaxed structural conformation. This heightened solvent exposure may enhance GAR1's capacity to interact with other molecules in hydrophilic contexts, potentially influencing its functional properties.

In summary, the SASA variations for GAR1 across the different mutations demonstrate distinct impacts on the protein's hydrophilic surface exposure. Mutations such as R398W, G435R, and both double mutations tend to increase SASA, implying a more open structure, while F164L and H376Y slightly reduce SASA, suggesting a

more compact form. These structural changes likely modulate GAR1's ability to engage in hydrophilic interactions, which could have implications for its functional roles in molecular processes.

The wild-type TCAB1 interacts with NHP2 primarily through several hydrophilic residues, creating a robust network of hydrogen bonds and ionic interactions. This hydrophilic interface is essential for stabilizing the protein-protein interaction, with notable contributions from Lys56 and Lys388 on NHP2, which form ionic interactions with acidic residues on TCAB1.

In the F164L mutation, we observe significant changes in the interaction interface. The mutant introduces a range of interactions with residues such as Glu487 and Glu494, which enhance the hydrophilic character of the binding surface. This modification potentially strengthens the interaction with NHP2 by adding more polar and charged residues to the interface, suggesting an increase in binding affinity due to enhanced electrostatic attraction.

The H376Y mutation also alters the hydrophilic balance, replacing some of the original residues involved in binding. The hydrophilic interaction network is modified by introducing Ser491 and Glu490, which continue to contribute to the polar character of the interface. This mutation appears to maintain a similar hydrophilic interaction pattern, possibly preserving binding affinity.

In the R398W mutation, the absence of interaction between TCAB1 and NHP2 highlights a major disruption in the hydrophilic interface. The substitution of arginine, a positively charged and hydrophilic residue, with tryptophan, a bulky and hydrophobic residue, likely removes critical electrostatic and hydrogen bonding interactions essential for stabilizing the TCAB1-NHP2 complex. This change reduces the hydrophilic compatibility at the interface and introduces steric hindrance, ultimately preventing effective binding between TCAB1 and NHP2. This underscores the importance of hydrophilic interactions, particularly involving charged residues, in maintaining the structural stability of this protein-protein interaction.

For the G435R mutation, the binding interface shifts with the addition of new interactions involving Ser491 and Asp493. The presence of Asp493 enhances the hydrophilic nature of the interface due to its negative charge, potentially reinforcing

electrostatic interactions with positively charged residues on NHP2. This shift may result in a stronger hydrophilic interface compared to the wild type.

In the double mutant F164L/R398W, the loss of the interaction between TCAB1 and NHP2 suggests a significant alteration in the hydrophilic profile. This mutation combination disrupts the binding interface, reducing the hydrophilic interactions necessary for stabilizing the complex. The absence of these interactions likely weakens the overall binding affinity and stability between TCAB1 and NHP2.

Finally, the H376Y/G435R double mutant maintains several hydrophilic interactions through residues such as Ser491, Gly492, and Asp493. This combination retains a moderately hydrophilic interface, though the specific interactions differ from those in the wild type. While some hydrophilic interactions are preserved, the binding affinity might still be slightly altered due to the reconfiguration of interacting residues.

In summary, each mutation or combination affects the hydrophilic balance at the TCAB1-NHP2 interface differently. While single mutations generally preserve or enhance hydrophilic interactions, the double mutations, particularly F164L/R398W, significantly disrupt the interaction interface, leading to a loss of hydrophilic contacts critical for binding stability.

The SASA analysis for the NHP2 protein across all mutations reveals significant alterations in solvent-exposed surface areas, which may impact the protein's hydrophilic interaction potential and overall functionality.

For the F164L mutation, there is a moderate decrease in SASA for NHP2, suggesting a more compact structural arrangement. This reduced surface exposure could limit the availability of hydrophilic regions, potentially decreasing NHP2's ability to form interactions that rely on solvent accessibility.

With the H376Y mutation, NHP2's SASA slightly decreases, pointing to a more constrained structure with reduced solvent accessibility. This compaction suggests that hydrophilic interaction sites may be less accessible, possibly limiting the protein's functional versatility in binding contexts.

The R398W mutation, on the other hand, induces an increase in NHP2's SASA, indicating a structural relaxation or expansion. This higher SASA implies greater exposure to hydrophilic regions, which could enhance NHP2's capacity for interactions in aqueous environments and potentially alter its binding dynamics.

The G435R mutation leads to a notable increase in SASA for NHP2. This expanded surface area implies that the protein's hydrophilic regions are more solvent-accessible, which may enhance its ability to engage in interactions with other molecules in hydrophilic settings.

When examining the double mutation F164L/R398W, NHP2 exhibits a substantial increase in SASA, indicating a marked expansion of the protein structure. This increase in solvent-exposed surface area suggests that the mutation pair may significantly alter NHP2's structural dynamics, potentially enhancing its interaction capabilities in hydrophilic environments.

Similarly, the H376Y/G435R double mutation results in an increase in SASA for NHP2, reflecting a relaxed structural state with greater hydrophilic region exposure. This heightened solvent accessibility may improve NHP2's ability to interact with surrounding molecules, potentially influencing its functional properties.

In conclusion, the SASA analyses for NHP2 across various mutations highlight distinct structural shifts that alter the protein's hydrophilic surface exposure. Mutations such as R398W, G435R, and both double mutations tend to increase SASA, promoting a more open conformation with enhanced solvent accessibility, while mutations like F164L and H376Y slightly decrease SASA, suggesting a more compact structure. These structural adaptations are likely to influence NHP2's interaction potential and may have functional implications for its role in cellular processes.

The Rg analysis for TCAB1 across all mutations reveals distinct shifts in compactness relative to the WT, with each mutation producing varying degrees of structural condensation. The F164L mutation demonstrates a considerable reduction in Rg, indicating that this single substitution promotes a more compact structure compared to the WT. This effect suggests alterations in intramolecular interactions, potentially leading to closer domain packing within TCAB1.

The R398W mutation, when analysed alone, also shows a decrease in Rg relative to the WT, though the compaction is less pronounced than in the F164L mutant. This compactness points to stabilising effects from the R398W substitution, perhaps due to hydrophobic interactions introduced by the new residue. In the double mutation F164L/R398W, however, the Rg reduction is more substantial than in either single mutation alone, suggesting a synergistic effect that drives TCAB1 into an even more

compact conformation. This result implies that the two mutations together significantly enhance the folding and compactness of the protein.

Similarly, the G435R mutation, while initially exhibiting Rg values close to those of the WT, demonstrates a moderate trend towards compaction over time. The arginine substitution at this position could introduce stabilising electrostatic interactions, leading to a slightly more condensed structure. The H376Y mutation, however, presents only minimal deviations from the WT in terms of Rg, suggesting that this mutation does not significantly impact the compactness of TCAB1 under the observed conditions.

The combined H376Y/G435R double mutation exhibits a more evident reduction in Rg compared to each mutation alone, indicating enhanced compactness. This result implies that the interaction between these two substitutions promotes further structural condensation, potentially through complementary stabilising interactions. The double mutant's behaviour indicates that TCAB1's structure can be significantly altered when multiple mutations interact synergistically.

In summary, each mutation contributes differently to TCAB1's overall compactness. While single mutations like F164L and R398W individually promote moderate compaction, their effects are amplified in double mutants such as F164L/R398W and H376Y/G435R. These findings reveal that TCAB1's structural integrity is sensitive to specific mutations, particularly in combination, which likely influences its functional interactions with binding partners.

For the F164L mutation in Dyskerin, the observed decrease in the radius of gyration (Rg) compared to the WT suggests a more compact structure. This implies that the F164L substitution may induce conformational changes that enhance the internal packing of Dyskerin, potentially stabilising the protein structure or altering its flexibility.

With the H376Y mutation, the Rg values exhibit a noticeable decline compared to the WT, indicating a trend toward compaction. This mutation replaces a histidine with a tyrosine, which could introduce additional hydrogen bonding or π - π interactions, thereby stabilising certain regions of the protein. This change might impact Dyskerin's overall conformation and its potential to interact with other molecules.

The R398W mutation, on the other hand, also demonstrates a reduction in Rg relative to the WT, albeit with unique fluctuation patterns. This trend towards compaction may reflect increased hydrophobic interactions due to the presence of the tryptophan residue, which is larger and more hydrophobic than the original arginine. Such structural changes could influence Dyskerin's interaction with its binding partners.

The G435R mutation maintains a similar Rg to the WT initially but shows notable deviations over time, particularly with higher fluctuations. This suggests that the introduction of arginine at this position may lead to localised structural perturbations, potentially disrupting specific interactions and increasing the overall flexibility of Dyskerin's structure.

The F164L/R398W double mutation in Dyskerin reveals a marked reduction in the radius of gyration (Rg) compared to the wild type (WT). This pronounced decrease suggests that the combined effect of the F164L and R398W mutations results in a significantly more compact structure. The hydrophobic leucine in place of phenylalanine at position 164, along with the large hydrophobic tryptophan at position 398, likely contribute to enhanced hydrophobic interactions within the protein's core. This compactness induced by the double mutation could influence Dyskerin's stability and its capacity for interactions with binding partners, potentially altering its functional dynamics.

Finally, the double mutation H376Y/G435R shows a distinct pattern in Rg behaviour, where the structure becomes more compact overall. This dual substitution appears to cumulatively enhance the compactness of Dyskerin, suggesting possible cooperative effects between these two mutations that reinforce the internal interactions within the protein, resulting in an altered structural equilibrium compared to the WT.

For the F164L mutation, the Rg of GAR1 appears to be slightly reduced compared to the WT, suggesting a marginal increase in compactness of the GAR1 structure. This minor compactness could imply a subtle alteration in the structural integrity of GAR1, possibly due to a change in hydrophobic interactions that restrict its overall expansion.

In the F164L/R398W double mutation, there is a notable increase in the Rg of GAR1 over time, which indicates a more expanded structure relative to the WT. This increase in structural flexibility or looseness could be attributed to the synergistic effects of

both mutations, which might disrupt the native interaction patterns and contribute to destabilizing the protein's compactness.

The G435R mutation results in an Rg profile that is quite comparable to the WT, with only minimal variations observed. This suggests that the G435R mutation does not significantly impact the overall compactness of GAR1, possibly indicating that this mutation does not majorly disrupt the structural integrity or stability of the complex.

With the H376Y mutation, there is a slight increase in Rg relative to WT, which points to a mild reduction in compactness. This loosening may result from specific alterations in hydrogen bonding or hydrophobic interactions, possibly induced by the mutation, leading to an expanded conformation.

The H376Y/G435R double mutation exhibits a more pronounced reduction in Rg, suggesting an increased compactness of the GAR1 structure compared to the WT. This combined effect might imply that the double mutation fosters interactions that constrain GAR1 more than either mutation alone, leading to a tighter structure.

Finally, the R398W mutation shows an increase in the Rg of GAR1 compared to WT, suggesting that this mutation promotes a more expanded structure. The increased Rg might be due to disruptions in stabilizing interactions, resulting in a structural configuration that allows for greater flexibility or expansion.

Each mutation uniquely influences the structural compactness of GAR1, indicating that specific amino acid changes in TCAB1 can modulate the interaction dynamics and stability of GAR1 within the complex.

In examining the Radius of Gyration (Rg) of the NHP2 protein in the context of various mutations, distinct structural compactions and conformational effects emerge. Each mutation exhibits unique Rg patterns when compared to the wild type (WT), suggesting differential impacts on the compactness and overall structure of NHP2.

The F164L mutation in NHP2 shows a trend toward a slightly reduced Rg compared to WT, indicating a more compact structure. This suggests that the F164L mutation stabilizes certain intramolecular interactions, leading to a tighter conformation. The data reflect that F164L maintains a consistently lower Rg across the simulation, reinforcing the notion of increased structural compactness in comparison to WT.

The H376Y mutation also displays a modestly increased Rg relative to WT, though less prominently than G435R. This indicates a slight destabilization effect, likely due to altered hydrophobic or hydrophilic interactions within the protein structure, resulting in a looser conformation.

The R398W mutation in NHP2 shows an increase in Rg values relative to WT, indicating a trend towards a less compact structure. This suggests that the R398W mutation may disrupt certain stabilizing interactions within NHP2, potentially due to the introduction of the bulky and hydrophobic tryptophan residue in place of arginine. This structural alteration likely increases the overall flexibility of the protein, leading to a more expanded conformation compared to WT.

For the G435R mutation, the Rg values are marginally higher than those of the WT, suggesting a tendency towards a less compact structure. This increase in Rg may imply that G435R destabilises certain interactions within NHP2, leading to a less compact configuration and increased flexibility in the protein's conformation relative to WT.

The double mutation F164L/R398W demonstrates an even more pronounced decrease in Rg values, further amplifying the compactness observed with the single F164L mutation. This indicates a possible cumulative effect of the mutations, which potentially enhances interactions that promote compactness or reduce steric hindrance within the protein's structure, thus stabilizing a more compact form.

Finally, the double mutation H376Y/G435R exhibits a significantly higher Rg compared to WT, indicating the most substantial deviation from compactness among the tested mutations. This double mutation appears to amplify the destabilizing effects of each mutation, promoting a more expanded structure that may lead to greater flexibility or conformational variability in the protein.

In summary, the analysis of Rg for NHP2 across these mutations reveals that mutations F164L and F164L/R398W contribute to a more compact structure, whereas G435R, H376Y, and especially H376Y/G435R and R398W lead to less compact conformations. This suggests differing impacts of hydrophobic and hydrophilic interactions on NHP2's structural integrity, with potential implications for its functional stability.

6. CONCLUSION & FUTURE PERSPECTIVES

Analysis of individual mutations within the TCAB1 protein revealed that while there were notable increases in root mean square fluctuation (RMSF) across specific regions, these alterations did not significantly impact the protein-protein interactions within the complex. In fact, the overall interaction of TCAB1 with adjacent proteins exhibited an enhancement. The effects of these mutations on TCAB1-RNA interactions were more variable; some mutations led to increased interaction strengths, others resulted in a decrease, and a few appeared to have minimal impact.

In the case of double mutations, the interaction dynamics between TCAB1 and surrounding proteins consistently demonstrated stronger associations compared to the wild type. However, it was observed that TCAB1-RNA interactions were energetically destabilized under the influence of both double mutations. This destabilization suggests that the interaction domain between TCAB1 and RNA within the H/ACA complex is particularly sensitive to mutational disruptions.

Consequently, it has been elucidated that the TCAB1-RNA interaction region emerges as a critical target for modulating the overall stability and functional integrity of the H/ACA complex. Strategies aiming to disrupt the TCAB1 interactions should, therefore, focus on this region to potentially regulate the complex's activity and its involvement in cellular mechanisms.

In this context, future research could focus on the targeted disruption of telomerase activity by inhibiting the Cajal body protein TCAB1. Specifically, it is proposed that targeting the interaction region between TCAB1 and RNA could be a strategic approach. This could involve systematically mutating amino acids within this binding region to identify the most potent mutation(s) that could effectively inhibit the interaction. The inhibition strategy would then be designed based on the effects of these key mutations, potentially disrupting the functional interface between TCAB1 and RNA.

Additionally, to further understand how a mutation in one protein could influence mutations in another, Normal Mode Analysis (NMA) methods can be employed. These techniques would allow researchers to explore the dynamic effects of mutations on protein structures, offering insights into the interdependent nature of protein

interactions within complex molecular systems. Such studies would not only deepen our understanding of protein interaction networks but also enhance the development of targeted therapeutic strategies.



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