A DISSERTATION ON

An insight into the resistance mechanism of p53 having mutation on DNA binding domain causing ovarian cancer

SUBMITTED TO THE
DEPARTMENT OF BIOENGINEERING
FACULTY OF ENGINEERING & INFORMATION TECHNOLOGY
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IN PARTIAL FULFILMENT
FOR THE
DEGREE OF MASTERS OF TECHNOLOGY
IN BIOINFORMATICS

BY
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M. Tech Bioinformatics (IV Semester)
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UNDER THE SUPERVISION OF

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DECLARATION FORM

I, **Mantasha Khan**, a student of **M. Tech Bioinformatics** (II Year/ IV semester), Integral University have completed my six months dissertation work entitled **"**An insight into the resistance mechanism of p53 having mutation on DNA binding domain causing ovarian cancer**"** successfully from the **CSIR- Central Drug Research Institute, Lucknow** under the able guidance of **Dr. Mohammad Imran Siddiqi.**

I, hereby, affirm that the work has been done by me in all aspects. I have sincerely prepared this project report and the results reported in this study are genuine and authentic.

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09/08/2023

TO WHOMSOEVER IT MAY CONCERN

This is to certify that **Ms. MANTASHA KHAN** (Enrollment Number 1700100173) has carried out the research work presented in this thesis entitled "An Insight into the resistance mechanism of P53 having mutation on DNA binding domain causing Ovarian Cancer" for the award of M.Tech Bioinformatics from Centre of Bioinformatics & Computational Biology, Biochemistry & Structural Biology Division,CSIR – Central Drug Research Institute, Lucknow under my supervision. The thesis embodies results of original work and studies carried out by the student herself and the contents of the thesis do not form the basis for the award of any other degree to the candidate or to anybody else from this or any other University/Institution. The dissertation was a compulsory part of M.Tech Bioinformatics.

I wish her good luck and bright future.

Sincerely,

Imran Siddiqi

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I wish her good luck and bright future.

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I wish her good luck and bright future.

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Last but not least, my warm and heartfelt thanks to my family for the tremendous support and hope they have given me.

MANTASHA KHAN

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LIST OF ABBREVIATIONS

S.NO.	NAME	ABBREVIATIONS
1	Breast cancer gene	**BRCA**
2	High grade serous ovarian carcinoma	**HGSOC**
3	Epithelial ovarian carcinoma	**EOC**
4	BRAF (V-raf murine sarcoma viral oncogene homolog B	**BRAF**
5	Ki-ras2 Kirsten rat sarcoma viral oncogene homolog	**KRAS**
6	Phosphate and TENsin homolog deleted on chromosome 10	**PTEN**
7	Deoxyribonucleic acid	**DNA**
8	Tumor protein 53	**TP53**
9	B-cell lymphoma-extra large	**Bcl-XL**
10	Murine double minute 2	**MDM2**
11	Transactivation domain	**TAD**
12	DNA binding domain	**DBD**
13	Proline- rich domain	**PRD**
14	Linker region	**LR**
15	Carboxyl terminal domain	**CTD**
16	International Agency for Research on Cancer	**IARC**
17	Loss-of-function	**LOF**
18	Dominant-negative	**DN**
19	Gain-of-function	**GOF**
20	Transformed mouse 3T3 cell double minute 4	**MDM4**
21	Research Collaboratory for Structural Bioinformatics	**RCSB**
22	Protein Data Bank	**PDB**
23	Canonical ensemble	**NVT**
24	Assisted Model Building with Energy Refinement	**AMBER**
25	Constant-temperature, constant-pressure ensemble	**NPT**
26	Root mean square deviation	**RMSD**
27	Root mean square fluctuation	**RMSF**
28	Accessible Surface Area	**SASA**

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1. INTRODUCTION

Ovarian cancer is a leading cause of death and the fifth most common cancer linked to p53 mutations. Ovaries, the female reproductive organs, produce ova, progesterone, and oestrogen. Ovarian cancer results from the growth of malignant cells in the ovaries, which rapidly proliferate and can destroy healthy body tissue.

Ovarian cancer has four phases, starting from phase I and progressing to phase IV, the most severe. Initially asymptomatic, signs of ovarian cancer can be confused with other disorders. Common symptoms include bloating, abdominal swelling, quick satisfaction, weight loss, pelvic pain, fatigue-related backache, and urinary incontinence. Ovarian cancer is caused by DNA mutations in cells near the ovaries, which direct cancer cells to proliferate and develop rapidly, resulting in a mass of cancer cells called a tumor. The exact cause remains unknown.

Epithelial ovarian cancer is a diverse disease with five major subtypes, with high-grade serous carcinoma (HGSC) being the most common. It is highly associated with four gene mutations: TP53, BRCA1/BRCA2, PIK3CA, and KRAS. The P53 mutant is the most prevalent in HGSC, with mutations found in 96% of serous ovarian, 85% of small cell lung, 75% of pancreatic, 60% of head and neck squamous cell carcinomas, and 54% of invasive breast cancers.

Molecular docking and molecular dynamics simulation will help us understand TP53's DNA mechanism by focusing on mutational hotspots like R175H, R175G, R248Q, R248W, R273H, R273C, R273L, and R273P. This research will aid in developing cancer treatments by analysing DNA-binding behaviour and the affinity of DNA-induced mutations to amino acid changes in human tumours.

OBJECTIVES

This study focuses on:

- Assembly of p53 complete structure and preparation of different mutants of p53 protein.
- Molecular Docking study of p53 (wild-type and mutants) with DNA molecule.
- MD Simulation study of DNA bound p53 complexes.

2. REVIEW OF LITERATURE

2.1 Origin of Ovarian Cancer

The classification of ovarian cancer types, their following treatments, and their response rates are still up for debate, making the diagnosis and detection of HGSOC problematic. In the evolution of HGSOC, there have historically been two schools of thought. The surface ovarian epithelium experiences one, while the fallopian tube epithelium experiences the other [N Auersperg *et al*., 2013]. According to one idea, p53 expression in the inclusion cysts and fimbriae of the ovary causes HGSOC to start in the fallopian tube [K Lawrenson *et al*., 2009]. On the other hand, it is believed that HGSOC is brought on by alterations in the ovarian surface epithelium brought on by repeated ovulatory problems [N Auersperg *et al*., 2013]. The hunt for prospective biomarker candidates is underway to determine the cause of HGSOC. For the development of HGSOC, it is crucial to comprehend the genetic setting and biomarkers.

2.2 The Biology of Epithelial ovarian cancer

The most lethal type of gynecologic cancer and the fifth most common reason for cancer-related deaths in women is epithelial ovarian carcinoma (EOC). A total of 152,000 deaths are anticipated in 2012 from the disease, which has a 239,000-year incidence worldwide [Lindsey A Torre *et al.*, 2012]. EOC is one of the top 10 malignancies that Chinese women are most likely to develop. Ovarian cancer accounts for 3.11% of all female cancer patients in China, where there were 45,233 cases in 2011, with a prevalence of 6.89 per 105 [Wanqing Chen *et al.*, 2011]. With age comes an increased risk of ovarian cancer. Women of all ages can be afflicted by EOC, but postmenopausal women (often those over the age of 50) have the highest prevalence.

Recent decades have seen a drop in the incidence of ovarian cancer as well as age-adjusted cancer mortality rates. Ovarian cancer's short-term prognosis has been significantly improved thanks to significant advancements in our knowledge of the disease's natural history, strict early staging, and aggressive surgical and chemotherapy treatments. EOC mortality fell by 14% in accordance with the most recent data on cancer incidence and mortality.

Ovarian cancer 5-year overall survival has increased from 36% to 44%. However, EOC is the only cancer where morbidity has decreased below death. Ovarian cancer research is significantly behind the other three [Barbara Goff *et al*., 2015]. Despite these advancements, EOC, particularly high-grade serous ovarian cancer (HGSOC), is more challenging to treat because the majority of EOC patients experience relapses following initial therapy and pass away as a result of the disease's development. It is among the most prevalent illnesses. However, over the past ten years, tremendous progress has been made in the identification and treatment of EOC.

Type I EOC (low-grade tumours with BRAF, K-RAS, and PTEN mutations) and type II EOC (high-grade tumours with TP53 mutations) have been classified according to their histology and genetic abnormalities. Classified [Ie-Ming Shih and Robert J. Kurman, 2004]. Since HGSOC is the most aggressive subtype, it accounts for 67% of all instances of ovarian cancer. P53 is mutated in all HGSOC tumours [Russell Vang *et al*., 2016].

2.3 Cancer and the p53 protein

When p53 is mutated, it can lose function or gain carcinogenic activity, and it is the most changed gene in most cancers (50%) [Evan H Baugh *et al*., 2018] Cell cycle halt, apoptosis, senescent growth arrest, chromosomal instability, and poor DNA base excision repair can all result from loss-of-function p53 tumour suppressor mutations [Michael B Kastan and Elijahu Berkovich, 2007]. These alterations allow for the avoidance of critical cell cycle pathways, encouraging cancer development in the long run. Oncogenic p53 gain-of-function mutations, on the other hand, can hasten tumour progression, alter transcriptional activation of target genes, suppress apoptosis, and improve resistance to chemotherapy [Michael B Kastan and Elijahu Berkovich, 2007]. alterations in the p53 gene have been detected in up to 96% of HGSOC patients, with both gain-of-function (oncogenic) and loss-of-function (p53 activity) alterations occurring.TP53 has been a popular study topic since it was discovered in 1979. It is currently the gene with the greatest relationship with human cancers, and the understanding of TP53 has evolved from oncogene to tumour suppressor gene [Arnold J. Levine and Moshe Oren, 2009]. TP53 has been dubbed the "guardian of the genome" due to its role in responding to a variety of external and internal stressors, including DNA damage, oncogene activation, food deprivation, and hypoxia [Anthony M. Boutelle and Laura D. Attardi, 2021]. Unfortunately, TP53 inactivation is frequent in carcinogenesis, with mutations seen in more than half of all human primary tumours [Pawana Lakshmi Vaddavalli, Bjorn Schumacher, 2022].

2.4 P53 structure

When acting as a transcription factor in the nucleus, p53 is usually found as a homotetramer (4 monomers that form a dimer of dimers) [O Laptenko and C Prives, 2006]. p53 can also be present in the cytoplasm and mitochondria, where it is involved in the regulation of apoptosis and autophagy. According to new research, mitochondrial p53 interacts with the anti-apoptotic protein Bcl-XL and the apoptotic regulator Bak in dimeric and monomeric forms. An N-terminal ubiquitin ligase (MDM2) binding site, amino-terminal transactivation domains TAD1 and TAD2, a proline-rich domain (PRD), a DNA-binding domain (DBD), a linker region (LR), a tetramerization domain (TD), and a carboxyl terminal domain (CTD) comprise the structure of the p53 domain. Sixty-two percent of all cancer-inducing p53 mutations are missense variants in the DBD (eighty-two percent for HGSOC) [Colleen A Brady and Laura D Attardi, 2010].

2.5 Functions and mutations of p53

P53 binds to DNA to maintain homeostasis and genomic integrity while controlling the expression of a variety of target features. In addition to allowing DNA repair and inducing apoptosis when DNA damage occurs, P53 can activate DNA repair proteins, inhibit cell development by arresting the cell cycle at the G1/S transition, and cease cell growth altogether. A DNA binding domain specific to core sequences, a tetramerization domain, and a regulatory domain at the C-terminus are the four functional domains of the p53 protein. The cellular environment ensures that once activated, p53 triggers cell cycle capture, senescence, division, apoptosis, or ferroptosis by boosting the expression of a group of traits found inside the already-established cellular morphologies. P53 activation has been linked to a number of factors, such as nuclear or ribosomal stress, hypoxia, mitotic signalling, erroneous proto-oncogene activation, DNA damage from UV or gamma radiation, and proto-oncogene activation. By boosting the expression of several genes involved in the aforementioned cellular processes, p53, if activated, causes cell cycle arrest, senescence, differentiation, apoptosis, or ferroptosis, depending on the cellular context. Due to the crucial role, they play in tumour suppression, TP53 mutations have been identified in more than 50% of human malignancies. 2,329 unique TP53 mutations were found in human ovarian cancer by the IARC TP53 database, 70% of which were missense mutations nearly proportionate to their wild-type counterparts.

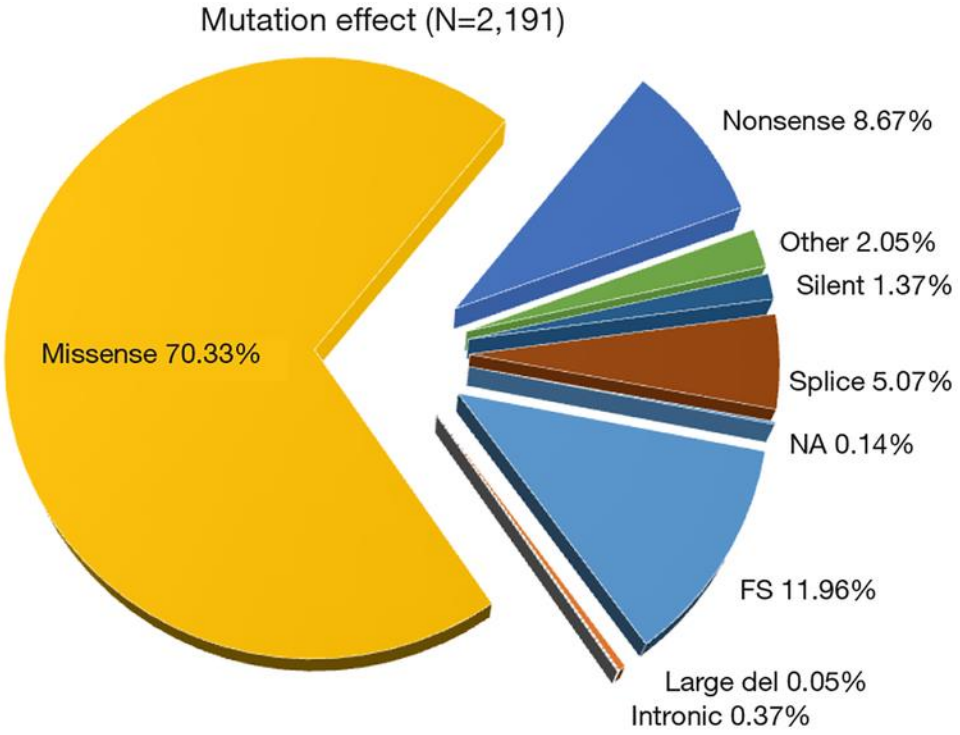


Figure 1: *Percentage of the different types of TP53 somatic mutations in human ovarian cancers. Data from the IARC TP53 Database (http://www-p53.iarc.fr/).*

Even though 80% of the amino-corrosion codons in this quality, which are located between exons 5 and 8, encode the exceedingly rare DNA authority space of the moderate p53 protein.

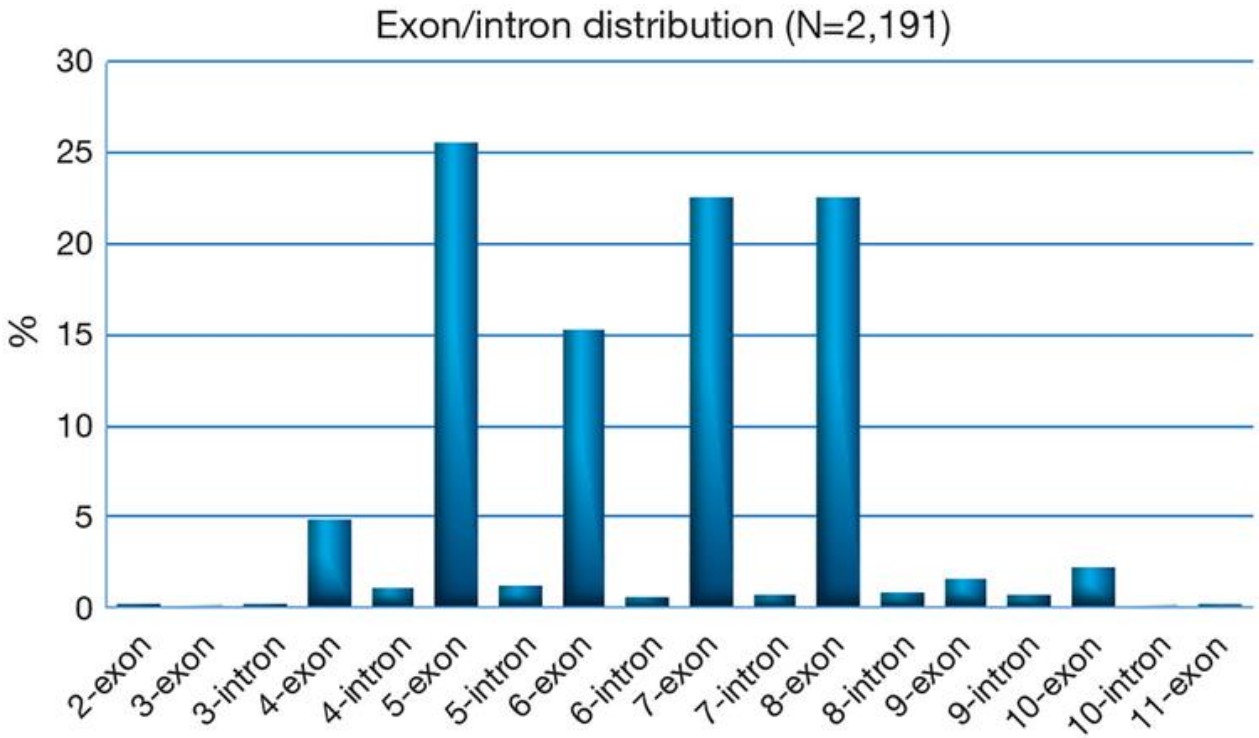


Figure 2: *Percentage of somatic mutations in each exon or intron of TP53 in human ovarian cancers. Data obtained from the IARC TP53 Database (http://www-p53.iarc.fr/).*

TP53 point variation has been recognised in the amino-corrosion codons of this quality. Transformations result in either a loss-of-function (LOF), dominant-negative (DN), or gain-of-function (GOF) phenotype because p53 has the potential to be a homotetrameric transcriptional figure. One of the most significant consequences of all p53 modifications is loss of function (LOF), which is caused by the adverse characteristics that WT p53 possesses [Y Haupt *et al*., 1997]. These altered p53 proteins typically work nonstop in cancer cells due to MDM2's innate E3 ubiquitin ligase activity. Additionally, missense p53 mutations typically inhibit the activity of WT p53 because the former and latter may combine to form a heterotetramer, which would impair the former's WT transcriptional mobility [Karolina Edlund *et al*., 2012]. GOF mutations are p53 missense variants that usually secure unexpected carcinogenic capabilities and are referred to as hot spot mutations, such as R175H, G245S, R248W, R249S, R273C, R273H or R282W [Jiajun Zhu *et al.*, 2015].

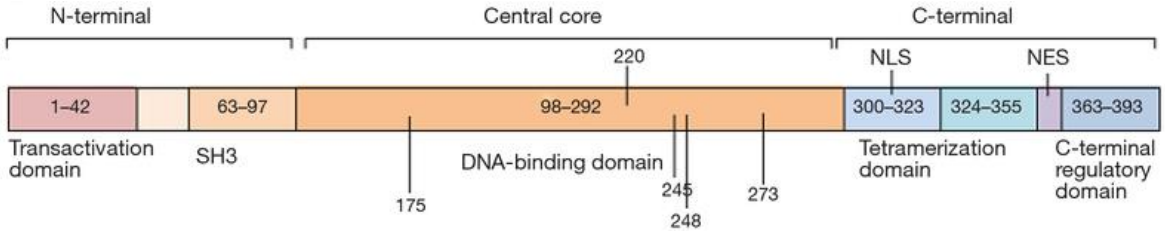


Figure 3: *Functional domains of p53 and the locations of the 5 most frequent mutations found in human ovarian cancers. Data from the IARC TP53 Database (http://www-p53.iarc.fr/). Numbers represent the positions of amino acids in p53.*

2.6 The role of p53 mutations in the aetiology and progression of EOC

However, genetic abnormalities that disrupt the cell cycle and encourage cell death are unavoidable and play a major role in the development of human cancer. Rearrangements, translocations, and changes in the gene's usual chromosomal placement are examples of genetic modifications linked to carcinogenesis. Gene mutations are changes in the sequence of a gene. Growth genes (oncogenes) are activated by these alterations, and growth genes (tumour suppressor genes) are repressed. During the process of malignant transformation, DNA repair pathways are regularly interfered with, which could hasten the accumulation of genetic abnormalities. As a result of their ability to repair genetic damage, DNA repair genes are also referred to as tumour suppressors. All of these genetic changes—some of which were briefly addressed above—may be linked to the emergence of EOC, although the TP53 mutation is the most frequent genetic change in the disease. Based on cellular phenotype, EOC is divided into a number of subtypes, such as serous (the most prevalent), mucinous, endometrioid-like, clear cell, undifferentiated, or unclassifiable cells. Today, regardless of histology, everyone is treated equally. EOC was formerly believed to solely develop from ovarian epithelial cells. The

secretory epithelial cells of the fimbriae, which make up the fallopian tubes, have been shown to be a possible source of EOCs, particularly HGSOCs, over the past ten years. It is crucial to know that serous precursor lesions of the fallopian tube epithelium might cause HGSOC [Ruth Perets *et al.,* 2013]. This is because restricting our understanding of the early tumorigenic mechanisms that control HGSOC to ovarian epithelial cells. However, it is still unknown what the aetiology and risk factors for EOC are. When a woman is young, her risk of acquiring ovarian cancer is minimal, but as she gets older, that risk rises. Women over 50 years old account for more than 80% of EOC diagnoses. Inheritable genetic changes (germline mutations) are estimated to be the root cause of 10% of EOCs. 97% of patients with HGSOC had TP53 mutations that were harmful. TP53 mutations have accumulated, according to an analysis of somatic HGSOC mutations. But the ratios changed depending on the ethnic group [Takahide Hayano *et al.,* 2014].

The most frequent TP53 mutations in ovarian cancer are missense mutations, and early-stage ovarian cancer is known to have much more null mutations than late-stage ovarian cancer. Over 25 years have passed since it was discovered that MDM2 and its homolog, MDMX (sometimes referred to as MDM4), cause cancer. However, these two proteins may also have p53-independent purposes. They are best described as p53's negative regulators. It is possible to develop more accurate detection and prognosis methods by better understanding how MDM2 and MDMX dysregulation in human malignancies contribute to carcinogenesis. A potentially effective therapeutic approach for the treatment of some cancers may involve the targeted use of proteins or their modulators.The transcription factor p53 is inactivated in almost all cancers, either directly or as a result of a flaw in one of its numerous regulatory pathways. In order to combat cancer, various strategies have been developed, ranging from the structure-directed design of chemical chaperones to the restoration of function in structurally labile p53 tumours. These strategies were made possible by screening for p53 activators and gaining a better understanding of the molecular mechanisms of oncogenic disruption of p53 activity.

The tumor suppressor protein p53 regulates numerous target genes involved in cell cycle regulation, senescence and death by activating or inactivating in response to oncogenic or other cellular stress signals. It protects DNA through a complex interplay of independently folded and intrinsically disordered functional domains. Many stages that make up the cell cycle can be negatively or positively affected by a variety of variables. One of the most important antagonistic regulators is the protein p53. Mutation, inactivation, or interaction of p53 with oncogene products of DNA tumor viruses can cause cancer.

3. MATERIALS AND METHODS

3.1 Retrieval/Selection of P53 protein.

In this study, we used the RCSB PDB Database to retrieve P53-related proteins. Seven PDBs had been retrieved. Among them, we chose the 2ac0 protein structure to study the mechanism since it had the complete sequence.

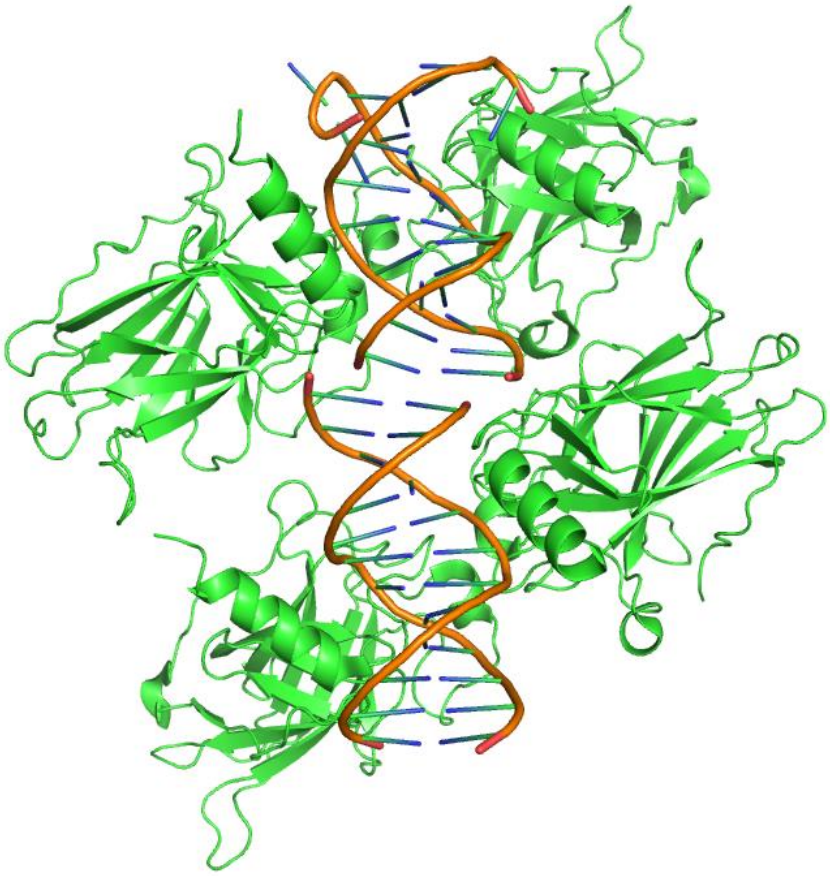


Figure 4: *2ac0 [Structural Basis of DNA Recognition by p53 Tetramers (complex I)]*

3.2 Preparation of complete structure assembly with DNA.

(a)

(b)

(c)

Figure 5: *Preparation of complete structure assembly (a) Apo from 2ac0, (b) DNA from PDB: 1TSR, (c) Complex of 2ac0 with 1TSR DNA*

The protein that we obtained from the PDB was a tetramer with damaged DNA. We therefore extracted DNA from the trimer-like core domain P53 (1TSR) and the tetramer from 2ac0 in order to assemble a stable, full structure. In addition, we prepared the entire p53 molecule assembly, which forms a tetramer by self-assembling on two DNA half-sites, by performing docking using the pyDockDNA web server.

3.3 Preparation of mutants and interaction with DNA

Studies show that half of all human cancers contain *TP53* mutations. Therefore, we reviewed the highly prevalent mutations that are mentioned in the literature. Further, we generated mutants using PyMOL software.

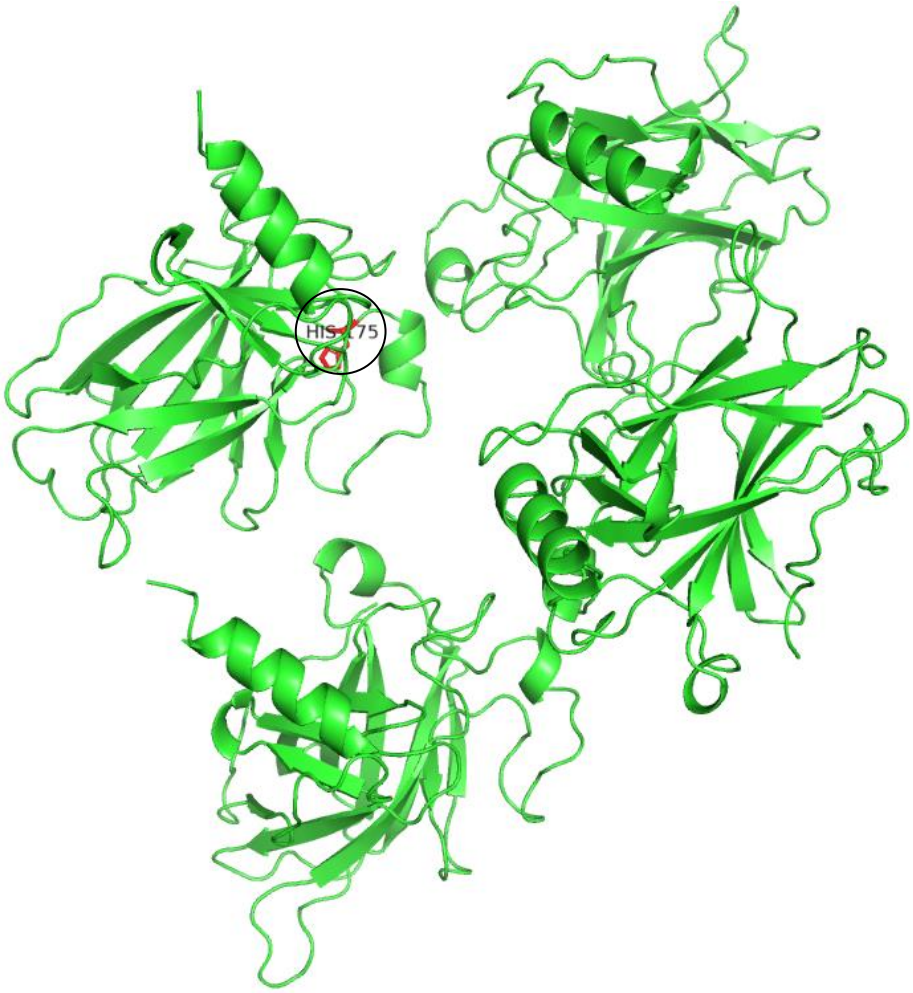


Figure 6: *The tetramer of P53 showing mutation R175H is highlighted in red.*

Mutants that were generated are as follows:

R175H, R175G, R248W, R248Q, R273H, R273L, R273C, R273P, HWP (R175H, R248W, R273C) and, GQL (R175G, R248W, R273L).

3.4 Molecular Docking using pyDockDNA web server

4.4.1 pyDockDNA web server

To determine the optimal interaction between our protein and DNA, we performed molecular docking using the pyDockDNA web server.

Molecular docking techniques have played a significant role in the advancement of current drug discovery. Computational techniques not only provide insight into the structure and dynamics of p53, but they also help in the identification of potential therapeutic compounds (Lauria *et al*., 2010). These in silico approaches provide insights into the mechanisms, binding energetics, and impact of ligand/DNA binding on the structure and dynamics of p53.

The pyDockDNA web server has been released as a new docking web server for energy-based protein DNA docking and scoring. where we can easily and accurately dock protein-DNA complexes. The server calculations are divided into three steps: FTDOCK sampling, scoring with new energy-based parameters, and the possibility of applying external limitations. The final results are a 3D representation of each of the top 10 models, along with a table selecting the model based on the previously chosen scoring function. Furthermore, for the top 100 models, we can extract the output files predicted by the server.

As a control, the DNA of 1TSR and the protein 2ac0 (Structural Basis of DNA Recognition by p53 Tetramers) were docked in the study. Further, we docked the mutants R175H, R175G, R248W, R248Q, R273H, R273L, R273C, R273P, and HWP (R175H, R248W, and R273C) with the DNA of 1TSR.

3.5 Molecular Dynamics Simulation

Gromacs was used to run Molecular Dynamics simulations in order to determine the radius of Gyration, Root Mean Square Deviation, Root Mean Square Fluctuation, Solvent Accessible Surface Areas, and Hydrogen bond interactions. We have performed molecular docking and molecular dynamics simulations of Apo protein, Wild Protein, R175H, R248Q, R248W, R273C, and HWP, the most common p53 mutants. A force field of the amber99SB-ILDN protein, nucleic AMBER94, has been used to generate topologies. To solvate the system with accurate periodic boundary conditions, a cubic box of water molecules was used. By using a.tpr file, we then generated an ions.mdp file. After assembling the system, energy was minimised by following 50,000 steps. After following the energy minimization step, Molecular dynamics simulations of both mutants and the apo protein were run at 10 ns, while NVT and NPT were run at 100 ps, respectively.

4. RESULTS AND DISCUSSION

4.1 Docking Analysis

Tetramer 2ac0 was docked with DNA of 1TSR. The studies with the highest pyDockDNA docking scores are displayed in Table 1 along with their best-fitting models in Table 2. With the highest docking score and rank 1, 2ac0-DNA was determined to be the best model. The rank will, however, alter if mutations are added. The mutation alignment, which has been adapted as the best model, is the most preferred alignment.

Table 1: pyDockDNA results with highest docking score

Conf	pyDockDNA	Rank
2ac0_DNA	**-229.99**	**1**
R175H	-295.117	1
R175G	-297.014	1
R248Q	-301.218	1
R248W	-301.386	1
R273H	-300.327	1
R273C	-300.254	1
R175L	-300.866	1
R175P	-300.779	1
RHWP	-292.631	1
RGQL	-291.347	1

The docked representation of the highest docking score and best-fit models

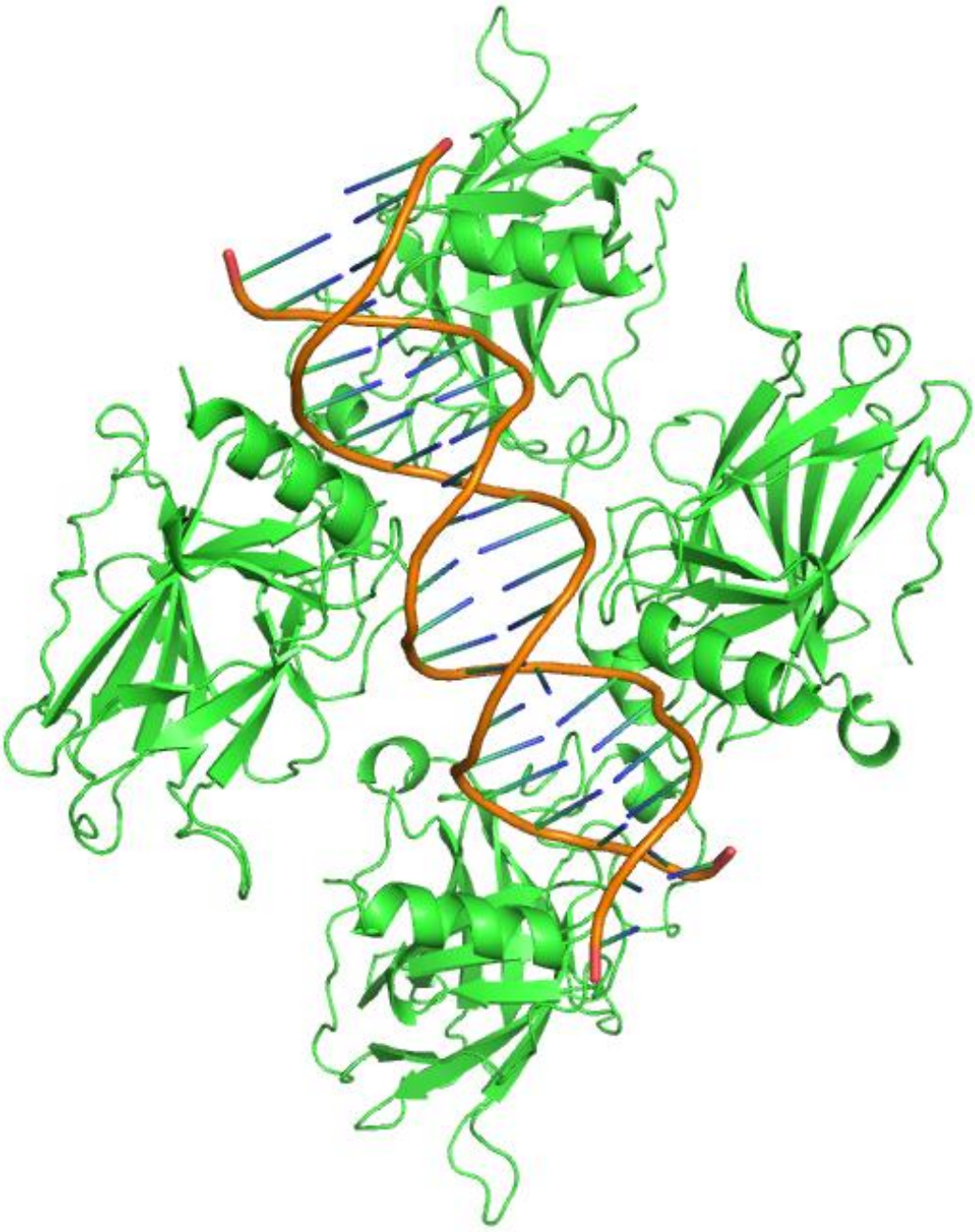


Figure 7: *The docked representation of the highest-ranked (rank -1) and best-fit model (rank-1) of 2ac0_DNA*

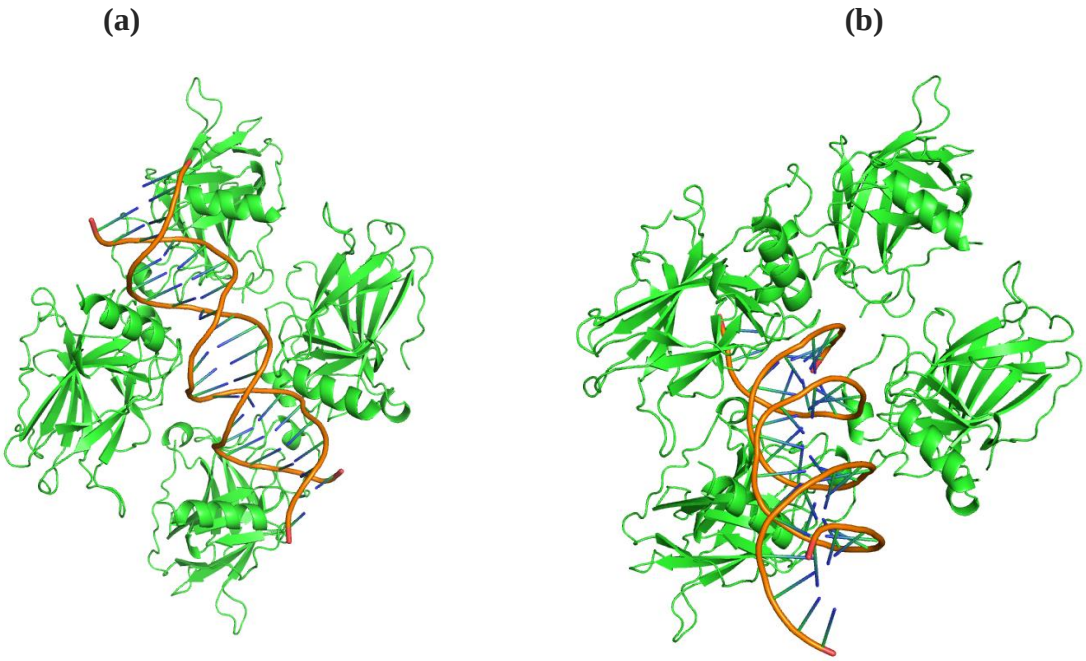


Figure 8: *The docked representation of (a) the highest-ranked (rank-1) and (b) best-fit model (rank-8) of R175H*

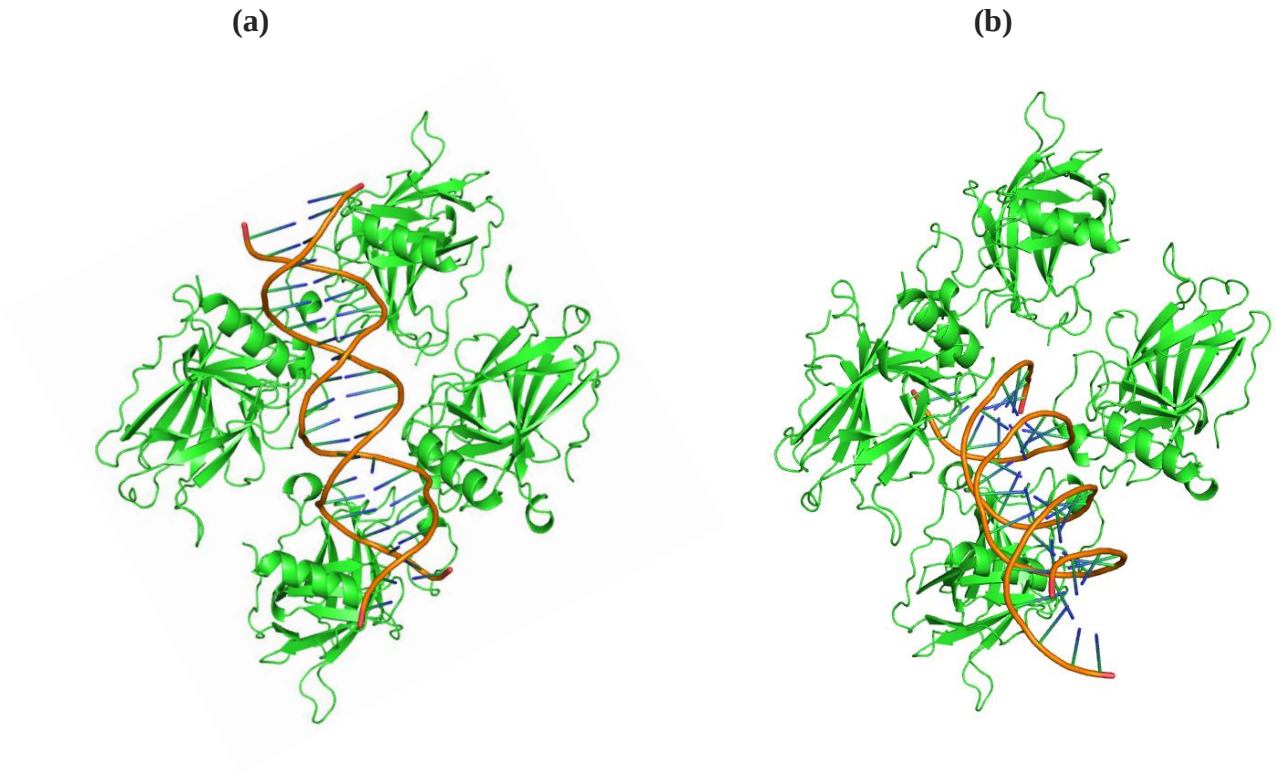


Figure 9: *The docked representation of (a) the highest-ranked (rank-1) and (b) best-fit model (rank-6) of R175G*

(a) **(b)**

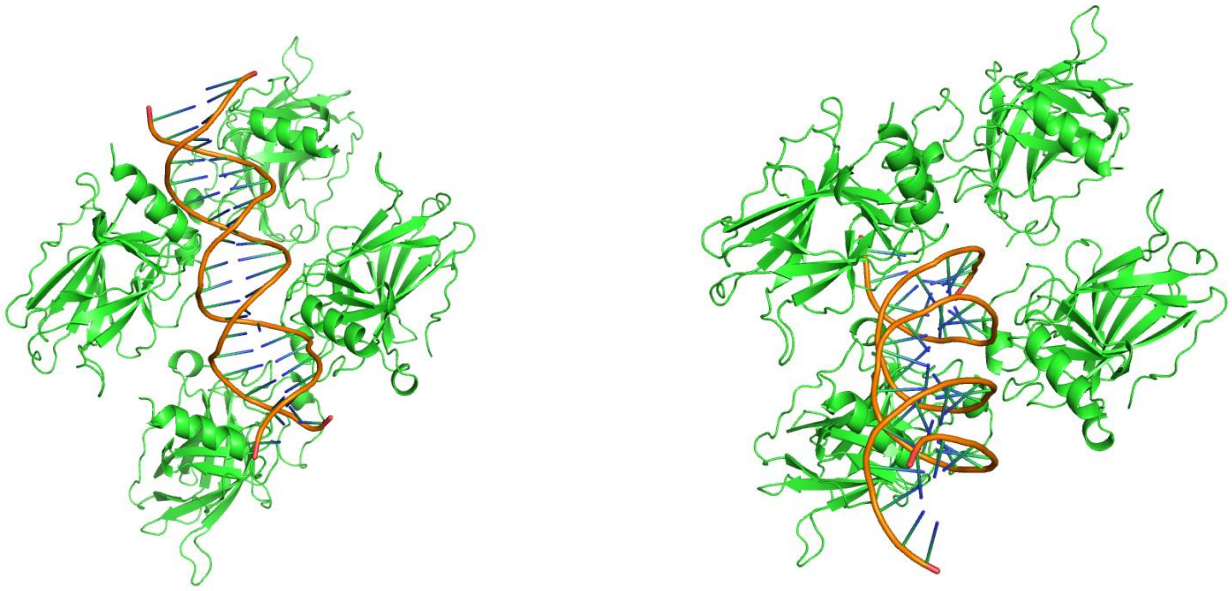


Figure 10: *The docked representation of (a) the highest-ranked (rank-1) and (b) best-fit model (rank-17) of R248Q*

(a) **(b)**

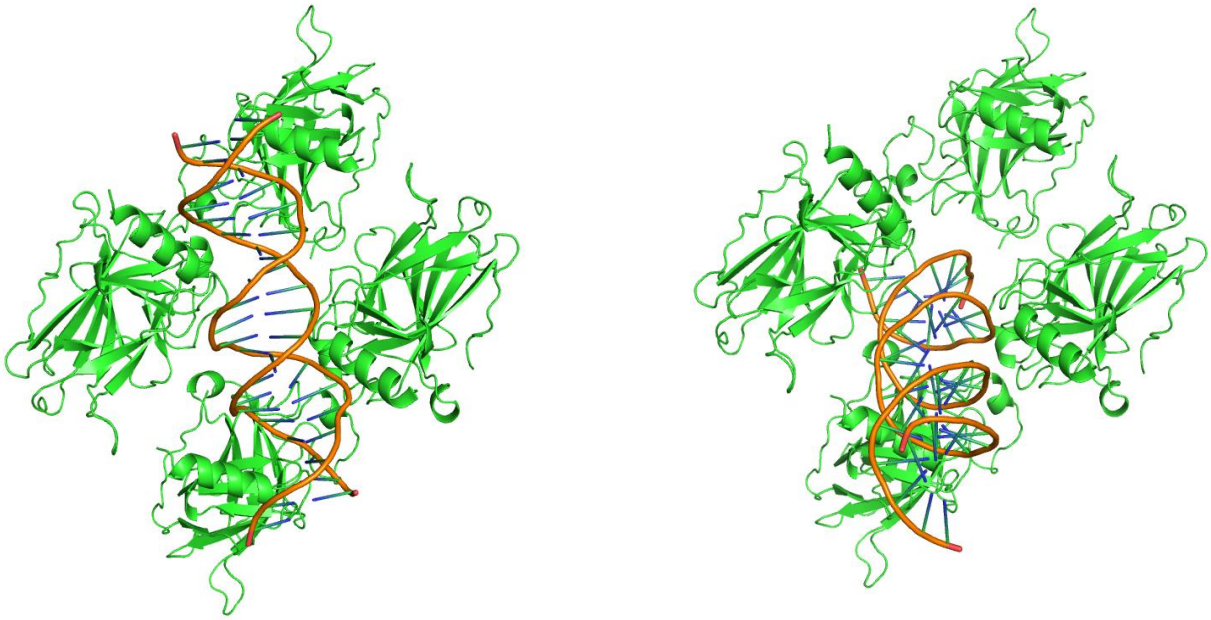


Figure 11: *The docked representation of (a) the highest-ranked (rank-1) and (b) best-fit model (rank-24) of R248W*

(a) **(b)**

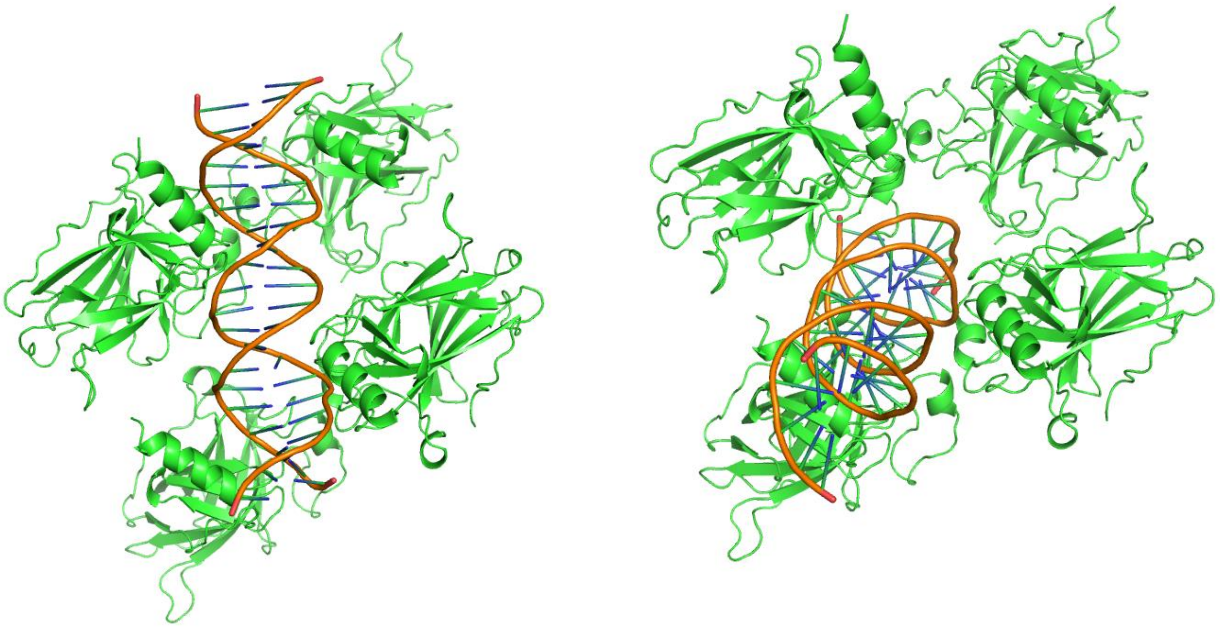


Figure 12*: The docked representation of (a) the highest-ranked (rank1) and (b) best-fit model (rank-5) of R273H*

(a) **(b)**

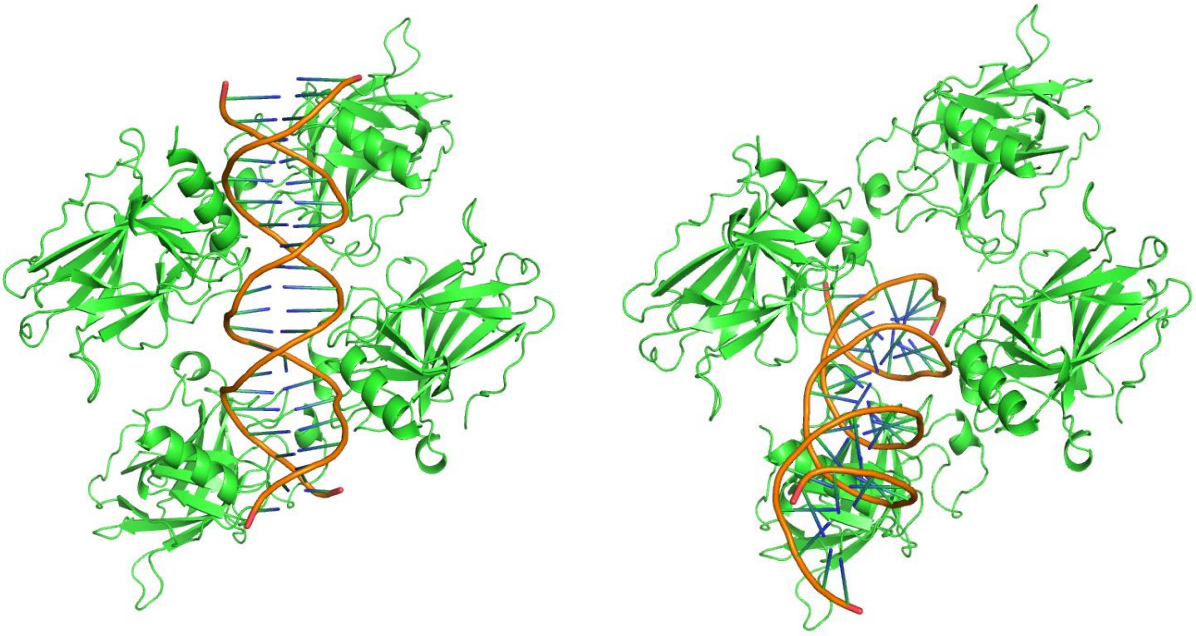


Figure 13: *The docked representation of (a) the highest-ranked (rank1) and (b) best-fit model (rank-4) of R273C*

(a) **(b)**

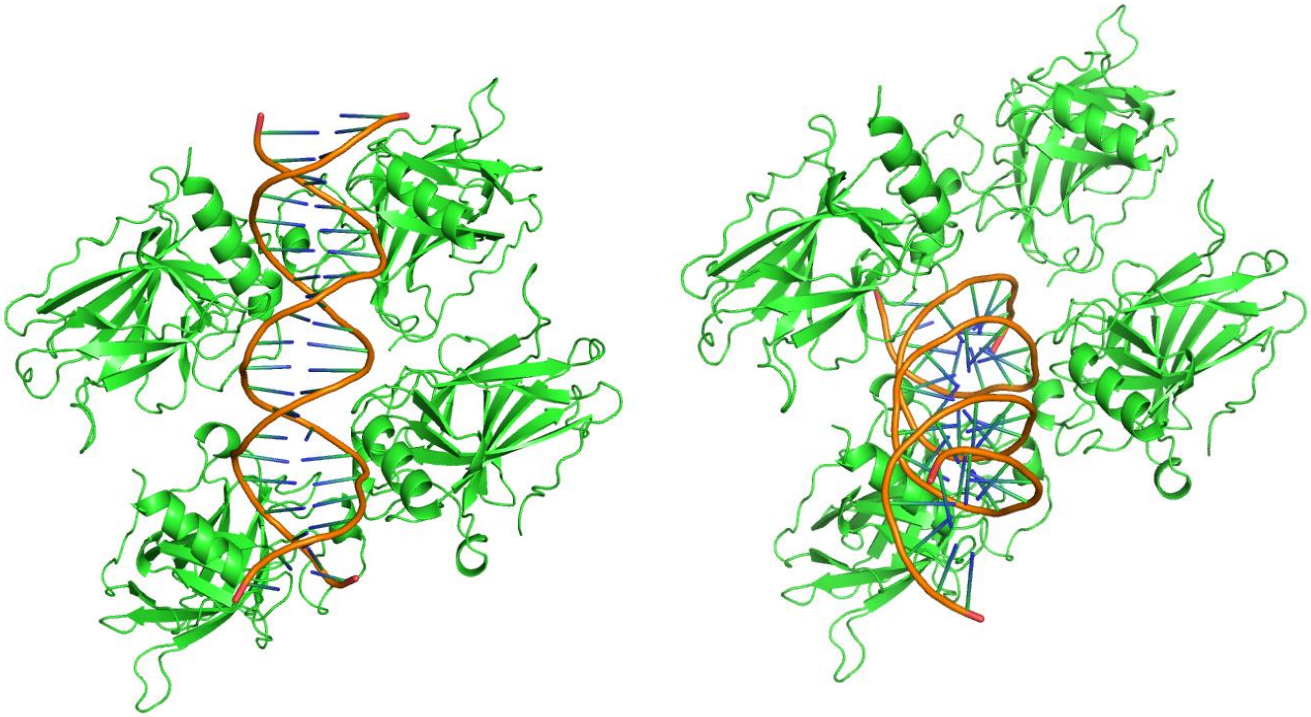


Figure 14*: The docked representation of (a) the highest-ranked (rank1) and (b) best-fit model (rank-5) of R273L*

(a) **(b)**

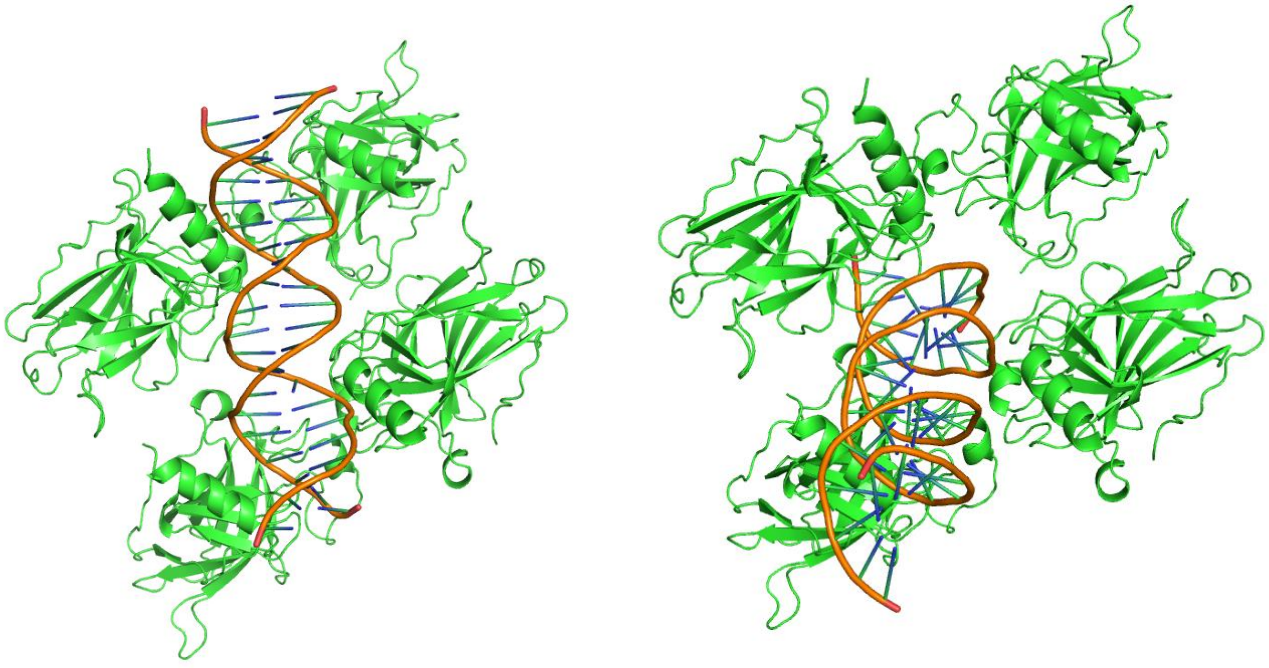


Figure 15: *The docked representation of (a) the highest-ranked (rank1) and (b) best-fit model (rank-5) of R273P*

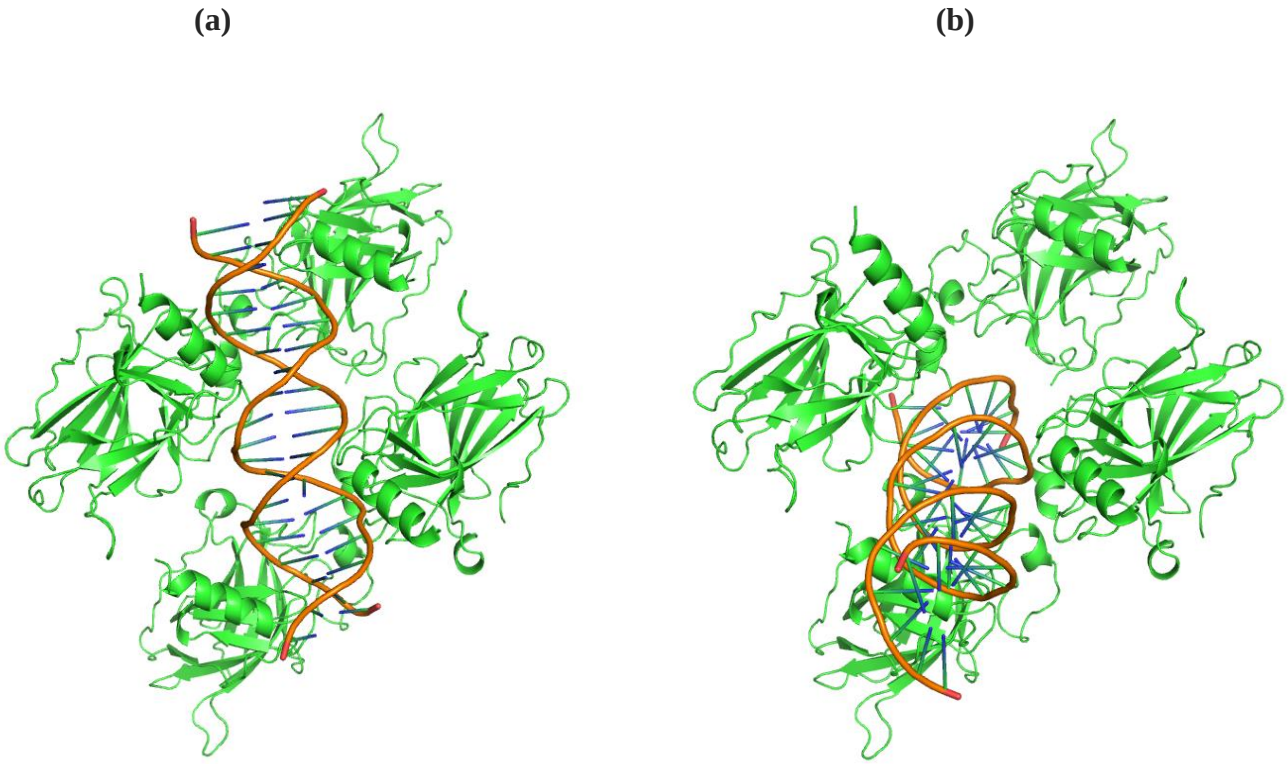


Figure 16: *The docked representation of (a) the highest-ranked (rank-1) and (b) best-fit model (rank-8) of HWP*

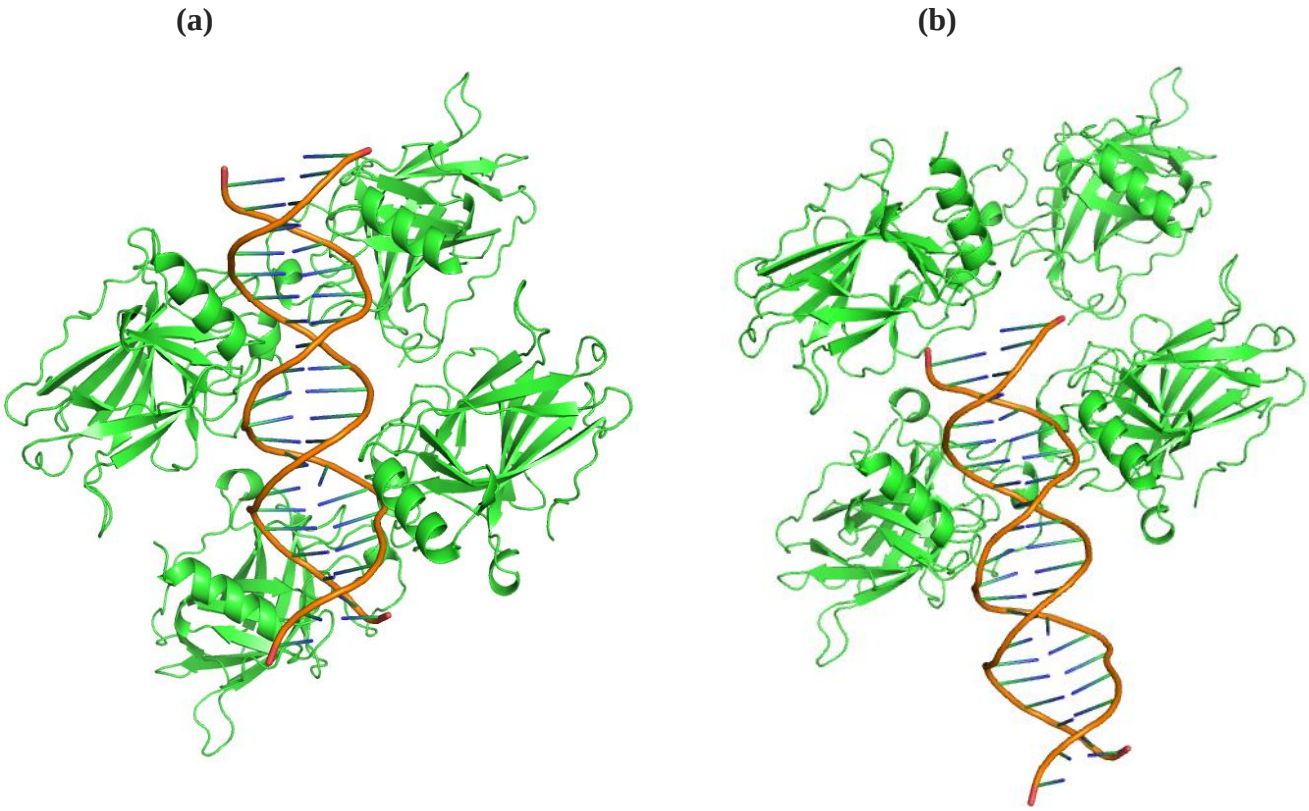


Figure 17: *The docked representation of (a) the highest-ranked (rank-1) and (b) best-fit model (rank-7) of GQL*

Table 2: pyDockDNA results for best-fit models

Conf	pyDockDNA	Rank
2ac0_DNA	**-229.99**	**1**
R175H	-268.395	8
R175G	-276.623	6
R248Q	-251.776	17
R248W	-240.016	24
R273H	-279.048	5
R273C	-283.989	4
R273L	-285.988	5
R273P	-284.602	5
RHWP	-257.78	8
RGQL	-258.545	7

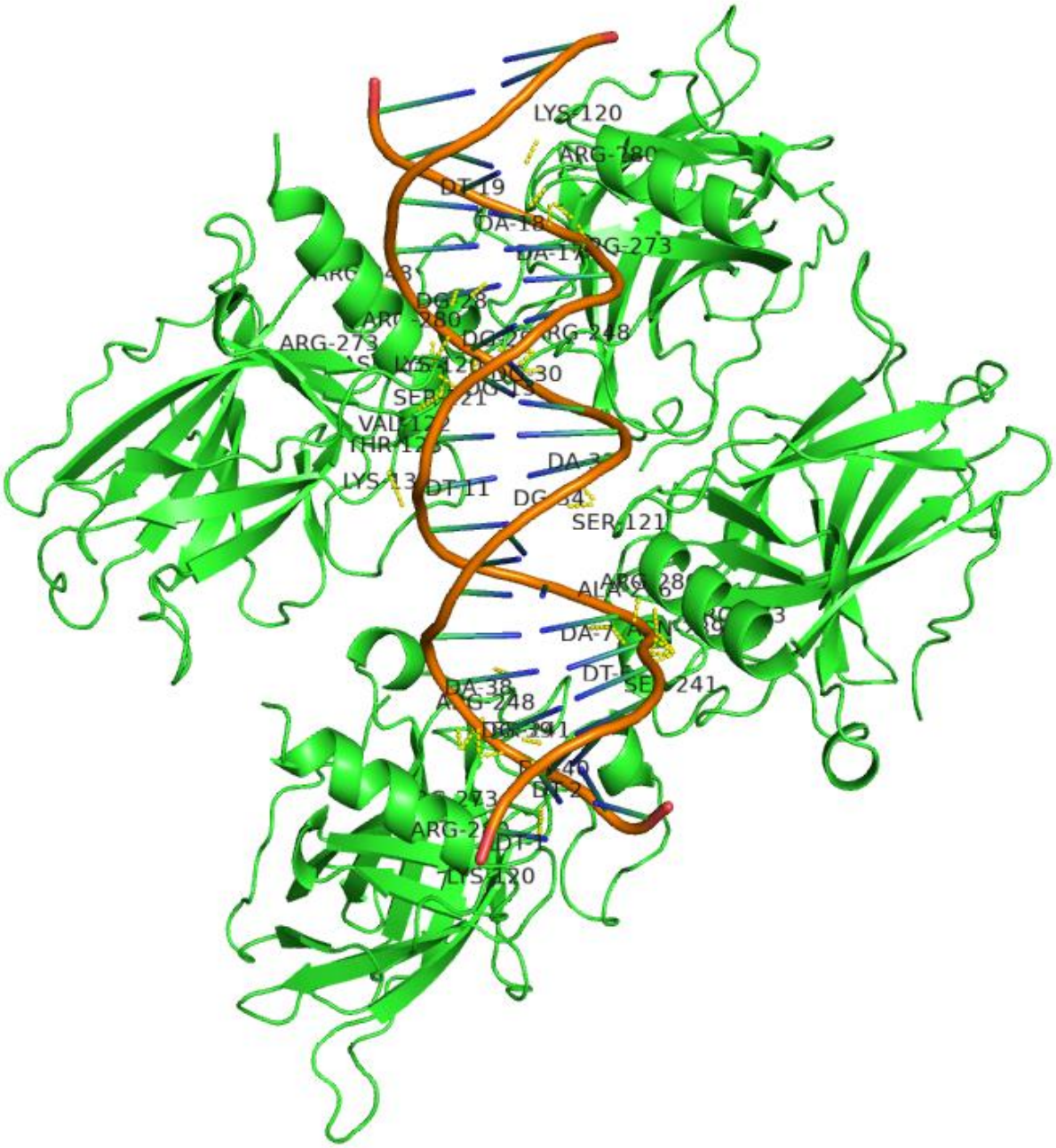


***Figure 18**: H-Bond interaction between 2ac0 tetramer and DNA*

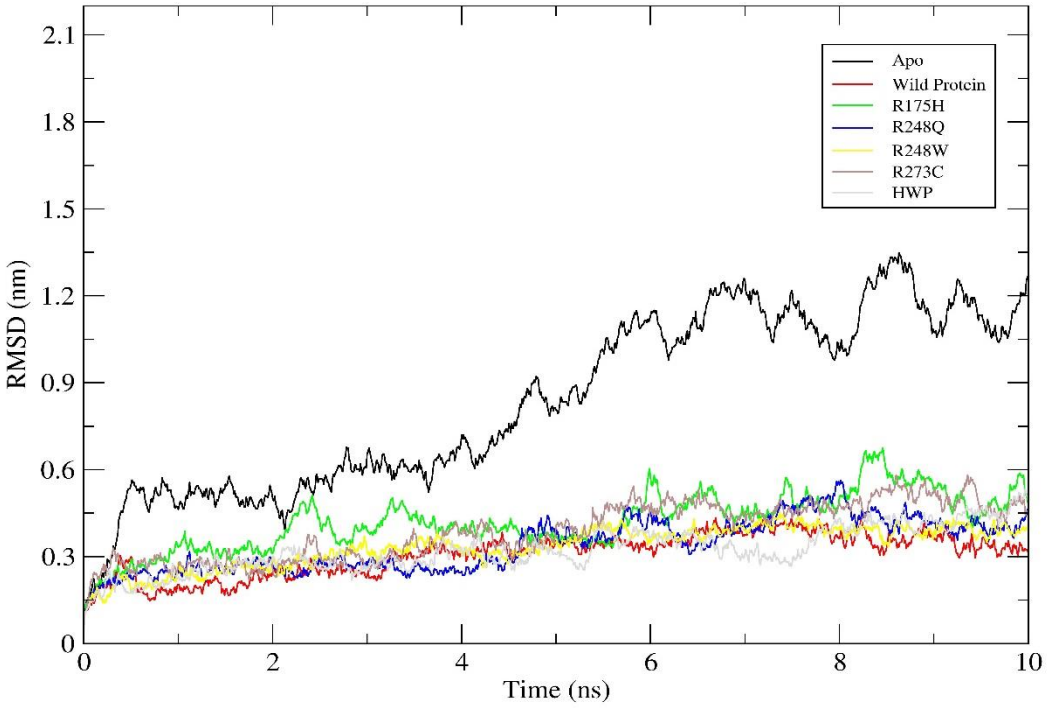
Table 3: H-Bond Interaction between DNA and Protein

Conf	Chain A	Chain B	Chain C	Chain D
2ac0_DNA	Lys-120, Ser-121, Val-122, Thr-123, Arg-273, Arg-280	Lys-120, Arg-248, Arg-273, Arg-280	Ser-121, Asn-239, Ser-241, Arg-278, Arg-280	Lys-120, Arg-248, Arg-273, Arg-280
R175H	Lys-120, Val-122, Lys-139, Asn-239, Arg-273, Arg-280	Lys-120, Asn-239, Arg-248, Arg-280	Ser-121, Asn-239, Ser-241, Ala-276, Arg-280	Lys-120, Arg-273, Arg-248, Arg-280
R175G	Ser-121, Val-122, Lys-139, Arg-273, Arg-280	Arg-248, Arg-273, Arg-280	Lys-120, Ser-121, Ser-241, Arg-273, Ala-276	Lys-120, Ser-241, Arg-248, Ala-276, Arg-280
R248Q	Lys-120, Val-122, Asn-239, Gln-248, Arg-280	Lys-120, Asn-247, Arg-248, Arg-280	Lys-120, Ser-121, Asn-239, Ser-241, Ala-276	Lys-120, Arg-248, Arg-273, Arg-280
R248W	Lys-120, Ser-121, Lys-139, Arg-280, Thr-284	Arg-280	Lys-120, Asn-239, Arg-280, Arg-283	Lys-120, Ser-241, Arg-273, Ala-276, Arg-280
R273H	Lys-120, Ser-121, Va-122, Thr-124, Asn-239, Arg-248, Arg-280	Arg-248, Arg-273, Arg-280	Ser-121, Ser-241, Arg-273, Ala-276, Arg-280	Lys-120, Ser-241, Arg-248, Arg-273, Arg-280
R273C	Ser-121, Thr-284	Lys-120, Arg-248, Arg-280	Ser-121, Ser-241, Arg-273	Lys-120, Arg-273, Ala-276
R273L	Ser-121, Asn-239, Thr-284	Lys-120, Arg-248, Arg-280	Ser-121, Ser-241, Arg-273, Arg-280	Lys-120, Ser-241, Arg-273, Arg-276
R273P	Ser-121, Asn-239, Ser-241, Thr-284	Lys-120, Arg-248, Arg-280	Ser-121, Arg-273, Arg-280	Lys-120, Ser-241, Arg-273, Arg-276
HWP	Ser-121, Lys-139, Asn-239, Ser-241	Lys-120, Asn-247, Arg-248, Arg-280	Ser-121, Ser-241, Ala-276, Arg-280	Lys-120, Ser-241, Arg-273, Ala-276, Arg-280
RGQL	Lys-120, Ser-121, Val-122, Arg-280	Lys-120, Arg-248	Ser-121, Asn-239, Ser-241, Arg-273, Arg-280	Lys-120, Arg-273, Arg-248, Arg-280

4.2 Molecular Dynamics Simulation Analysis:

4.2.1 Root Mean Square Deviation

(a) Protein



(b) DNA

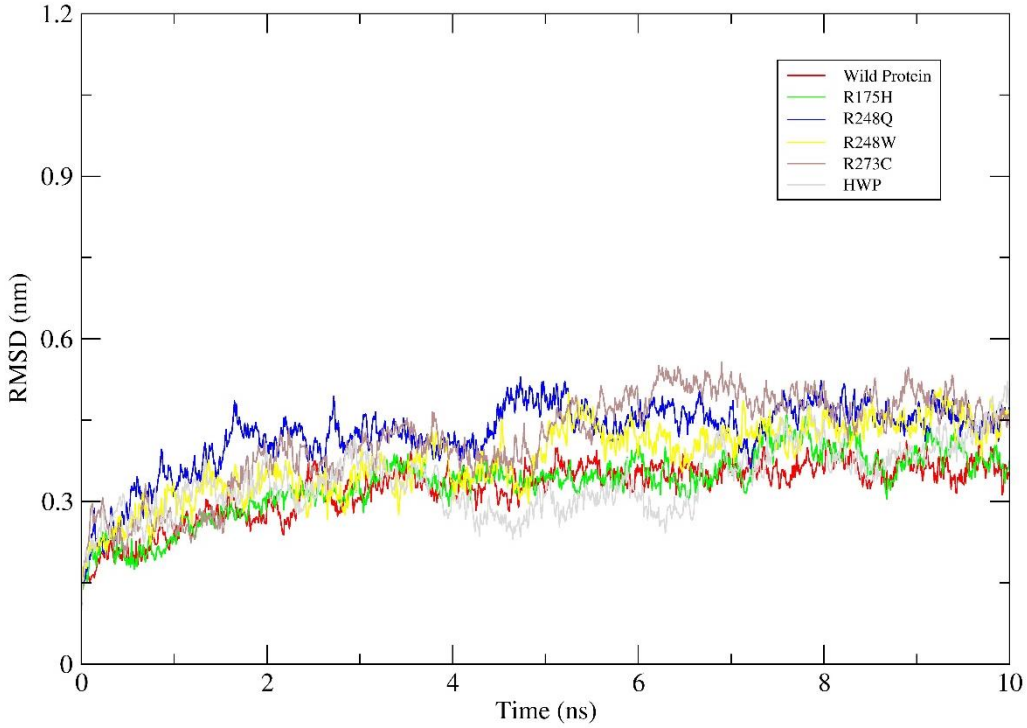


Figure 19: *RMSD plot of (a) Protein (b) DNA*

Table 4: RMSD Protein

S.No	System	Mean (nm)
1	Apo	0.840 +/- 0.301
2	Wild Protein	0.308 +/- 0.071
3	R175H	0.420 +/- 0.098
4	R248Q	0.339 +/- 0.885
5	R248W	0.334 +/- 0.701
6	R273C	0.387 +/- 0.097
7	HWP	0.322 +/- 0.077

Table 5: RMSD DNA

S.No	System	Mean
1	Wild Protein	0.324 +/- 0.534
2	R175H	0.333 +/- 0.059
3	R248Q	0.423 +/- 0.063
4	R248W	0.378 +/- 0.068
5	R273C	0.415 +/- 0.086
6	HWP	0.337 +/- 0.060

The RMSD was calculated for studying the protein systems convergence. In the protein RMSD plot, mutants possessed a decreased RMSD value compared to the Apo protein structure. As a result of the mutation, the mutant R175H eventually outperformed the other mutants. This data suggests that p53 lacks stability as a result of the R175H mutation and may affect DNA binding. Mutants R248Q and R273C exhibit a small level of deviation in the DNA RMSD plot, indicating that their RMSD value is higher than that of other mutant structures. While both the wild protein and the mutants (R175H and HWP) exhibit a similar degree of RMSD values.

4.2.2 Radius of gyration

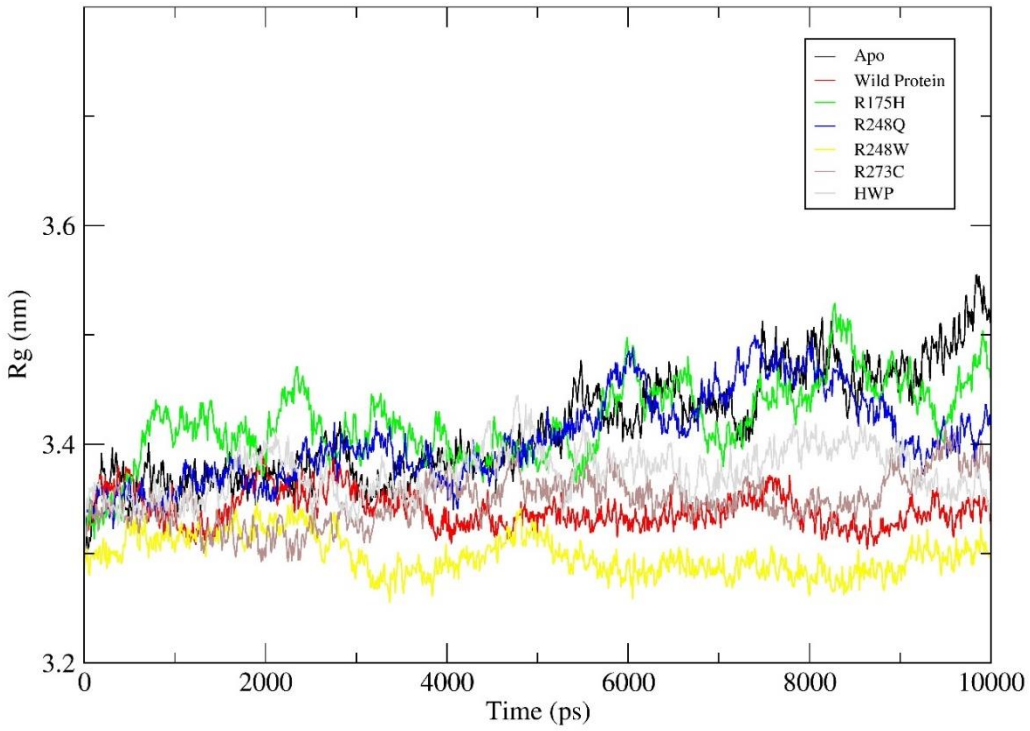


Figure 20: *Time evolution of the Radius of Gyration*

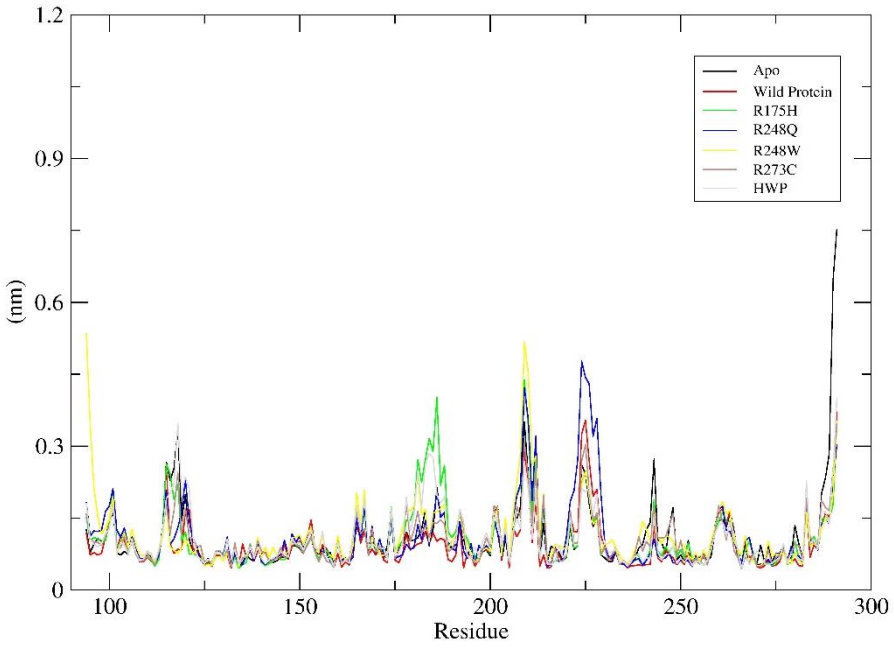
Table 6: Radius of Gyration

S.No	System	Mean (nm)
1	Apo	3.415 +/- 0.049
2	Wild Protein	3.340 +/- 0.016
3	R175H	3.421 +/- 0.036
4	R248Q	3.405 +/- 0.401
5	R248W	3.298 +/- 0.017
6	R273C	3.347 +/- 0.021
7	HWP	3.374 +/- 0.022

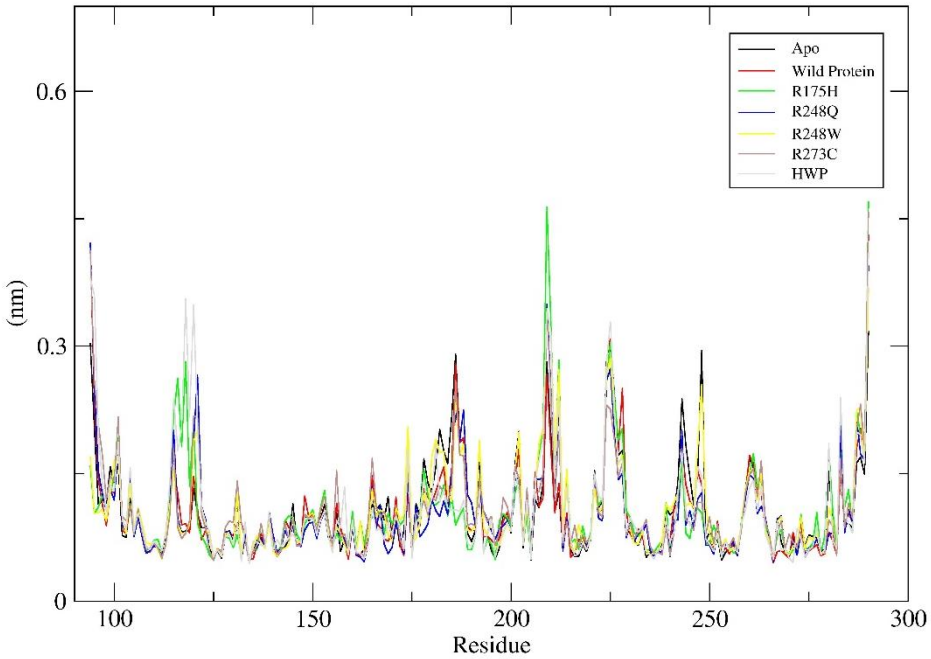
The Rg parameter describes the amount of protein structural compaction. In this Rg plot, the mutants R175H and R248Q have a greater Rg value than the Apo structure. Other than that, the Mutant HWP gradually increased afterwards. While the wild-type Protein and the remaining mutants R248W and R273C have a lower Rg value than the mutants R175H and R248Q. These findings suggest that the Rg value of the Wild protein is lower than that of the Apo protein. Whereas mutants (R248W) have the lowest Rg value.

4.2.3 Root Mean Square Fluctuation

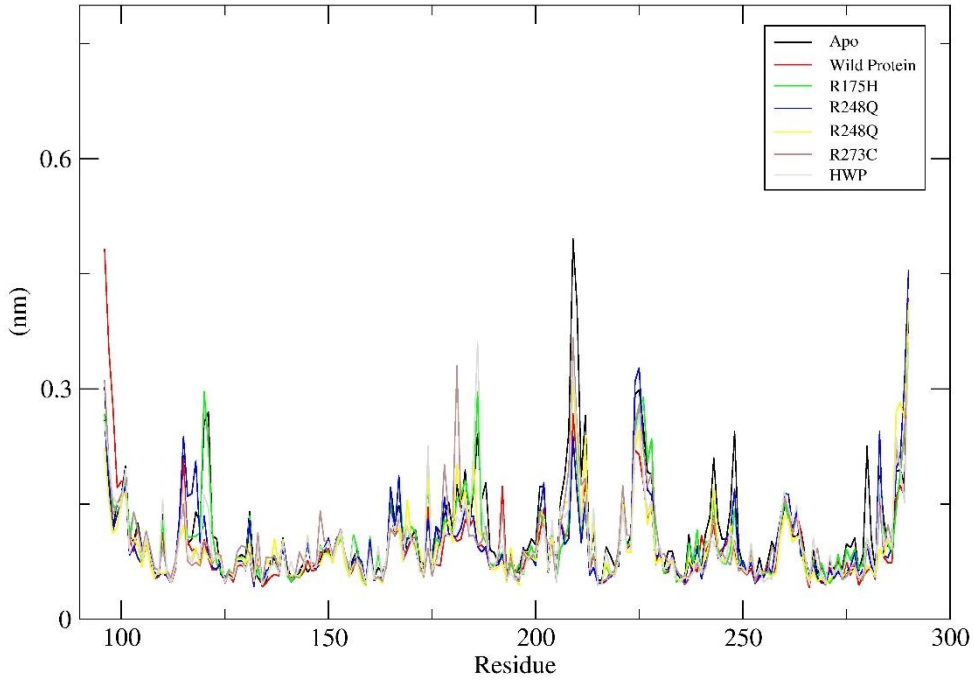
(a)



(b)



(c)



(d)

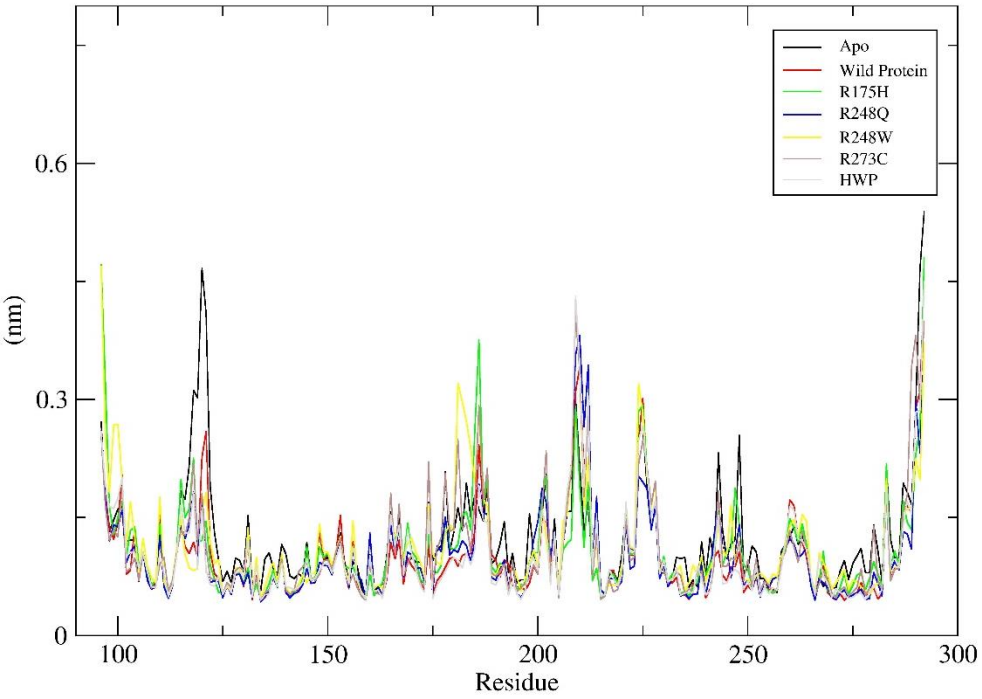


Figure 21: *RMSF plot of (a) Chain A, (b) Chain B, (c) Chain C, (d) Chain D*

The RMSF of the Apo protein, the structure of the wild-type protein, and the structures of the mutant proteins were all interpreted in order to determine the dynamic behaviour of the systems. In the plot of RMSF 1 (chain A of the p53 protein), mutant R175H shows higher fluctuation between the residues 181-186 and at 283, while mutant R248Q shows higher fluctuation between the residues 224-228 and at 167. The mutant R273C shows higher fluctuation between the residues 224-225 and at residue 178, 212, 225, 243, and 283. Also, the mutant R248W shows higher fluctuations at residue 165, 167, 181 and 209. And the mutant HWP shows higher fluctuation at the residue 165, 167, 178, 184, and 283. In the plot of RMSF 2 (chain B of the p53 protein), the mutant R248W show a similar flexibility as Apo structure of p53. While The other DNA-contact mutations (R175H, R248Q, R273C and HWP) show a higher degree of flexibility than the Apo structure. The mutant R175H shows higher fluctuation at the residue 209. In the plot of RMSF 3, mutants R175H, R273C, and HWP shows higher fluctuation between the residues 120, 186, 167, 224, 225, 283, and 174. In the plot of RMSF 4, mutants R175H, R248Q, R248W, and R273C, shows higher fluctuation between the residue 181, 186, 209, and 224.

4.2.4 Solvent Accessible Surface Area

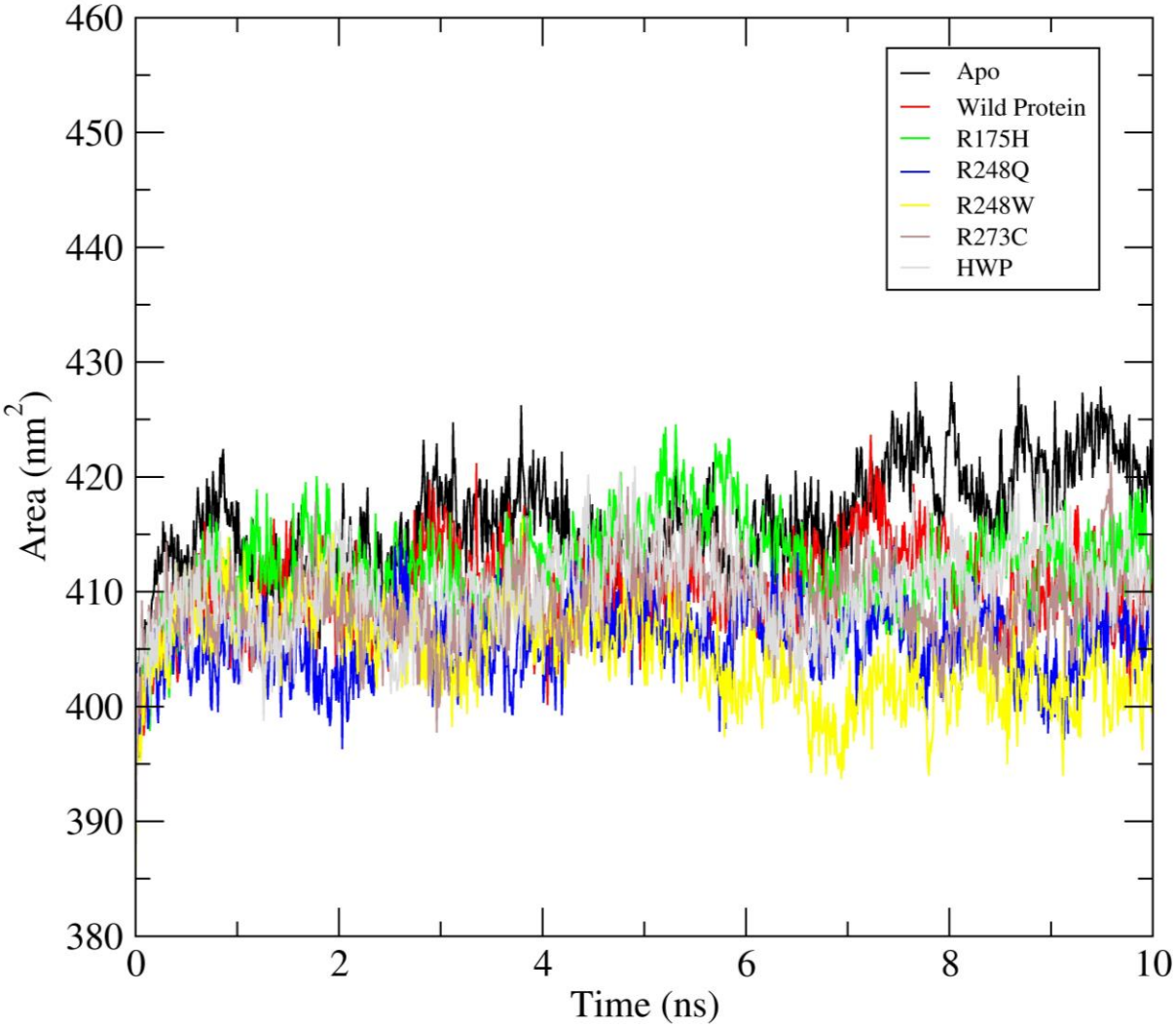


Figure 22: *Solvent Accessible Surface Area*

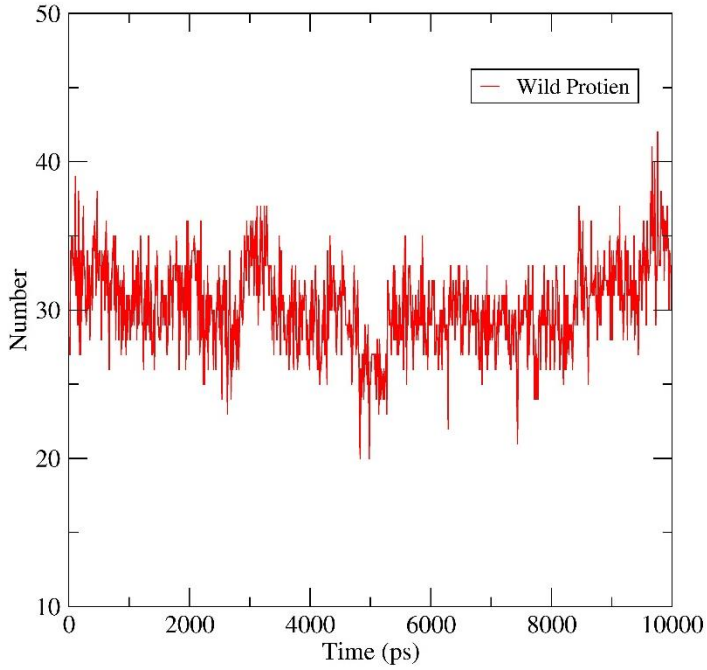
Table 7: SASA

S.No	System	Mean (nm 2)
1	Apo	416.072 +/- 4.689
2	Wild Protein	410.194 +/- 3.757
3	R175H	412.256 +/- 3.966
4	R248Q	405.585 +/- 3.211
5	R248W	404.424 +/- 4.061
6	R273C	408.852 +/- 3.492
7	HWP	410.173 +/- 3.803

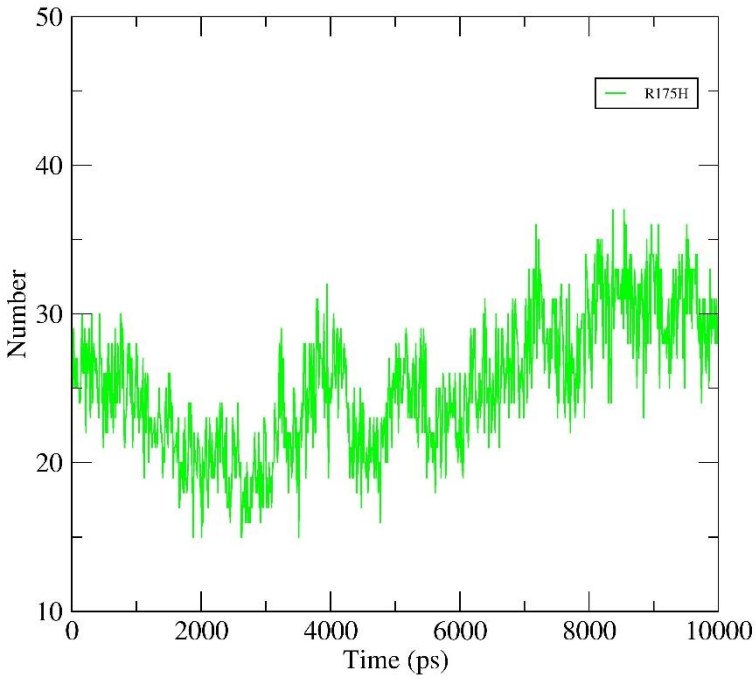
This SASA figure depicts that all the mutants and the wild protein structure have lower SASA values as compared to the APO structure. And the mutant R175H shows a greater SASA value, while rest of the mutants show lower SASA values as compared to the wild protein.

4.2.5 Intramolecular Hydrogen Bonds

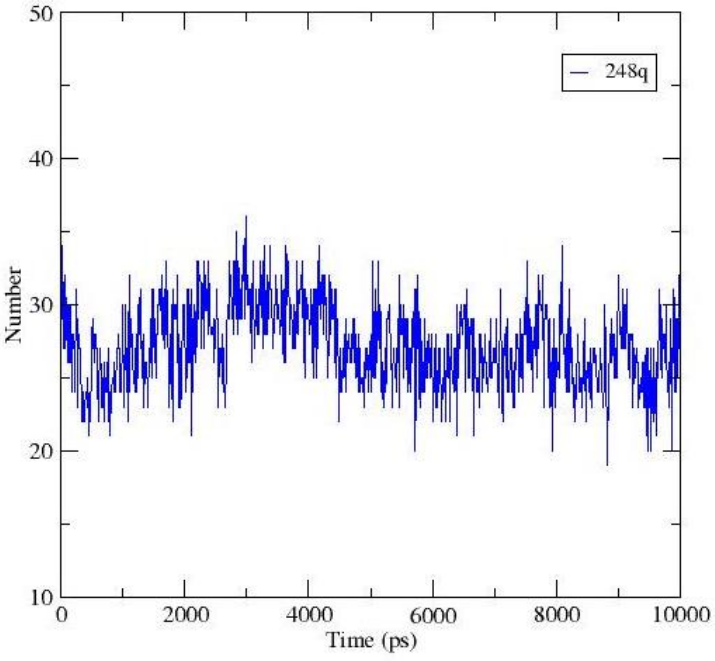
(a)



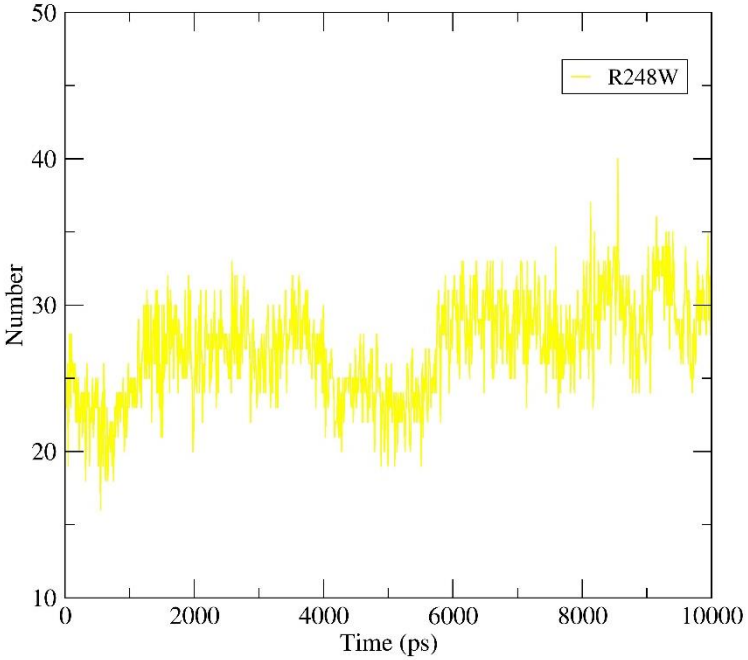
(b)



(c)



(d)



(e) (f)

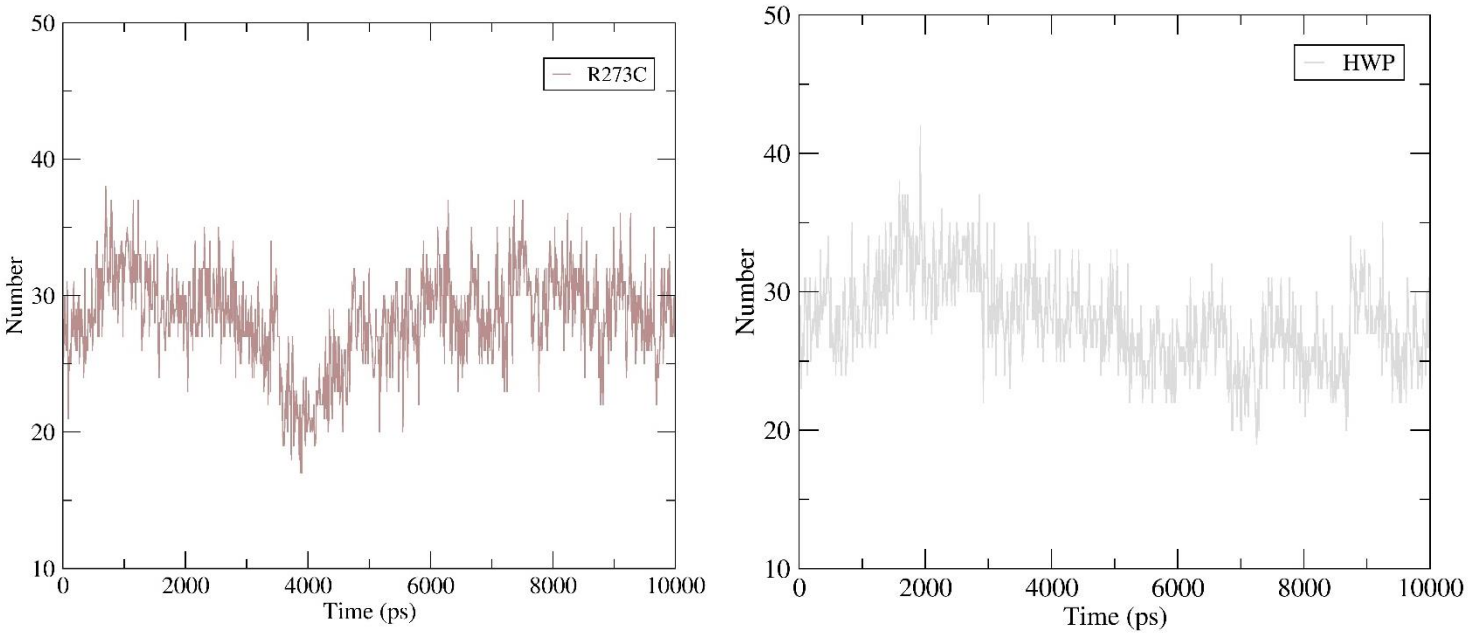


Figure 23: *Intramolecular Hydrogen Bond (a) Wild Protein (b) R175H, (c) R248Q, (d) R248W, (e) R273C, (f) HWP*

Table 8: Intramolecular Hydrogen Bonds

S. No	System	Mean
1	Wild Protein	30.2 +/- 2.91
2	R175H	25.2 +/- 4.46
3	R248Q	27.1 +/- 2.78
4	R248W	27.0 +/- 3.42
5	R273C	28.3 +/- 3.51
6	HWP	27.8 +/- 3.27

During the simulation, we have observed noticeable changes in the hydrogen bond pattern. To better understand the relationship between flexibility and hydrogen bond formation, hydrogen bond analysis of wild-type proteins and DNA-contact mutations of the p53 proteins was performed. The wild-type protein shows a slightly larger number of hydrogen bonds formed during the simulation than the mutants. The total number of hydrogen bonds formed in the wild-type protein is 35. The total number of hydrogen bonds formed in the mutant R175H was 24. The total number of hydrogen bonds formed in the mutant R248Q was 28. The total number of hydrogen bonds formed in the mutant R248W was 26. The total number of hydrogen bonds formed in the mutant R273C was 29. And the mutant HWP formed a total of 29 hydrogen bonds.

5. CONCLUSION

In this study, we looked into the p53 protein's structure, functionality, or mechanism, as well as how it binds to DNA. We found that the mutants showed fluctuations, while the control showed stability. From the literature we reviewed, that R175H and R248W are the most common highly occurring p53 mutants, which lead to structural instability and loss of function. Also from our study, we concluded that R175H, R248Q, and R273C showed higher fluctuations than the wild type, so these mutations might disturb or affect the binding of DNA, which could be a cause of cancer activity. Further we will investigate these systems at atomic level. Findings from the study will be used to investigate the residual involvement which may further utilised to derive a new lead molecule for the DNA-protein complex stabilization.

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