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Combining Molecular Docking and Molecular Dynamics Simulations to Explore the Binding Affinity and Interactions of Berberine with Wild-Type and Mutated Phosphatase and Tensin Homolog (*PTEN*)

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ABSTRACT

Background: We aimed to find the role and effect of berberine on wild type Phosphatase and Tensin Homolog (*PTEN*) and mutated type *PTEN* by utilizing molecular techniques.

Methods: This investigation executed in bioinformatics center of Ghalib University, Kabul, Afghanistan in 2024. To evaluate the binding interactions and affinity of berberine with both wild-type and mutated *PTEN*s, molecular docking was carried out using the Autodock 4.2.2 software. This analysis was followed by molecular dynamics (MD) simulations, which provided insights into the structural dynamics of the complexes. The simulations were performed utilizing the AMBER99SB force field and using the GROMACS 2019.6 software.

Results: Our research on berberine and Wild-type *PTEN* and mutant *PTEN*, by utilizing molecular docking and molecular dynamics simulation approaches showed adequate binding affinity and binding energy between both complexes. Docking result parameters shows -7.37 binding energy for wild *PTEN*, and -6.28 for mutated *PTEN*. Which means that berberine has more binding affinity with wild *PTEN* compared with mutated *PTEN*.

Conclusion: Using advanced computational techniques such as molecular docking and molecular dynamics simulations, this study highlights the potential of Berberine—a natural compound known for its diverse health-promoting properties—as a promising therapeutic candidate for treating cancers linked to *PTEN* dysfunction.

Keywords: Wild-type *PTEN*, Mutated-type *PTEN*, Berberine, Molecular docking, Molecular dynamics simulation

Introduction

Cancer remains one of the most severe health challenges worldwide, characterized by uncontrolled cell growth, invasion, and the potential for metastasis, making it a leading cause of death globally (1). Tumor suppressor genes are essential elements of cellular systems that control cell growth, division, and programmed death, ensuring cellular balance. These genes encode proteins that act as regulators, halting excessive cell proliferation, repairing DNA damage, and triggering apoptosis when damage is beyond repair (2). By maintaining these control mechanisms, they prevent the uncontrolled cell growth that characterizes cancer development. Mutations or dysfunction in

critical tumor suppressor genes like *PTEN* significantly contributes to cancer development and progression (3).

PTEN (Phosphatase and Tensin Homolog) is essential for regulating cell proliferation, growth, and survival by negatively controlling the PI3K/AKT signaling pathway. Loss of *PTEN* function due to mutations, deletions, or epigenetic modifications disrupts these regulatory mechanisms, leading to unchecked cell division and resistance to programmed cell death (4). This dysfunction is observed in several aggressive cancers, including breast, prostate, endometrial, and glioblastomas (5). *PTEN* mutations are also linked to increased tumor invasiveness and metastasis, further worsening the prognosis. *PTEN* is a vital tumor suppressor gene integral to maintaining cellular balance and regulating numerous biological processes. *PTEN* is situated on chromosome 10q23 and produces a lipid phosphatase enzyme essential for controlling cell growth, division, survival, and mobility. Its tumor-suppressive function is primarily mediated through inhibition of the PI3K/AKT signaling pathway, which is central to cell proliferation and survival (6). *PTEN* counteracts this pathway by dephosphorylating phosphatidylinositol 3,4,5-trisphosphate (PIP3) back to phosphatidylinositol 4,5-bisphosphate (PIP2), effectively halting AKT activation and downstream tumor-promoting effects. In addition to regulating the PI3K/AKT pathway, *PTEN* contributes to other crucial cellular functions, such as maintaining genomic stability, orchestrating cell cycle progression, and inducing apoptosis (7). By interacting with proteins involved in DNA repair, *PTEN* helps avert the accumulation of genetic mutations (8). Furthermore, it plays a role in cell migration and invasion by modulating the cytoskeleton, processes essential for normal tissue repair but detrimental when dysregulated, as seen in cancer metastasis. Several mutations in the *PTEN* gene have been identified, such as Val119Ile (V119I), Val158Ile (V158I), and Arg234Gln (R234Q), linked to various types of cancer (9).

Berberine is a potent bioactive compound found in various plants, including Berberis vulgaris (barberry), Coptis chinensis (golden thread), Phellodendron amurense, and Hydrastis canadensis (goldenseal) (10). This alkaloid is widely studied for its potential synergistic effects when combined with other therapeutic compounds (11). A notable example is its combination with curcumin, derived from turmeric, to enhance therapeutic outcomes, especially in treating cancer and inflammatory conditions. Berberine and curcumin work together to inhibit tumor growth, promote apoptosis (programmed cell death), and reduce inflammation. Additionally, berberine is investigated for its ability to improve the bioavailability of other drugs, particularly chemotherapy agents. It does so by inhibiting enzymes like cytochrome P450 and modulating intestinal transporters, which may boost the effectiveness of these drugs when used in combination therapies. Berberine also has its positive impact on the cardiovascular system (12), antidiabetic action (13), anti-obesity action (14), neurodegenerative and neuropsychiatric disorders (15), as well as sensitization and drug resistance (16).

Considering the importance of identifying *PTEN* activators in the treatment of various types of cancers and the importance of natural compounds of berberine, we aimed to investigate the activating role and function properties of berberine inside the binding site of *PTEN*s by using computational methods of molecular docking and molecular dynamics simulation.

Materials and Methods

Computational methods

The combination of computational biology and molecular modeling has proven to be a valuable and effective strategy in drug discovery and design. By employing virtual assessment techniques, researchers can efficiently search and screen vast structural libraries within virtual and computer environments. As a result, the use of computational biology and molecular modeling presents promising opportunities for advancing drug discovery and design processes (17). This crucial investigation took place at the Bioinformatics Center of Ghalib University, Kabul, Afghanistan in 2024.

Molecular docking

The affinity and interactions of berberine with Wild-type *PTEN* and Mutated *PTEN* were investigated through the utilization of Autodock 4.2.2 software in molecular docking analysis (18). Access to the x-ray crystallographic structures of *PTEN*, identified by pdb code (1D5R), was made possible through the RCSB protein data bank (19). As no specific mutated *PTEN*s required were available in protein data bank, VMD software was used to make mutations to the wild-type *PTEN* pdb file by introducing three mutation points, V119I, V158I, and R234Q. In order to enhance docking calculations, non-polar hydrogen atoms were incorporated into the PDB file instead of water molecules and the original ligands. The PubChem server was utilized to download Berberin's 3D structure with CID 2353 in SDF format, which was then converted to PDB format using the OpenBabel program (18). The AMBER99SB force field was employed to minimize the energy of the enzyme using the GROMACS 2019.6 package (20). Docking calculations were performed with the macromolecules held rigid, while Berberin was allowed to rotate freely. By utilizing co-crystallized ligands from the enzyme's pdb files, the active site of the macromolecule was determined. Following the identification of the active site, a grid map consisting of 50 points and a grid point spacing of 0.375 was selected. To conduct the docking calculations, the Lamarckian genetic algorithm (LGA) approach was utilized, running a total of 200 calculations with 25 million energy evaluations for each complex. Ultimately, the conformation in the cluster with the lowest binding energy was chosen as the optimal docking mode.

Molecular dynamic simulation

Molecular dynamic simulations were performed on the enzyme in both its free state and in complex with Berberine, contained within a cubic box solvated by the tip3p water molecule model using the GROMACS 2019.6 program on the Kubuntu 2020.4 Linux operating system (20). Systems force field parameters were generated utilizing the Python-based ACPYPE tool (21). To maintain system neutrality, ions were introduced, and the dissolved systems were initially minimized using the steepest descent method to alleviate excessive forces. Subsequent stabilization of the systems at 310 K and 1 bar was achieved through 1 ns simulation in nvt and npt ensembles. Following thorough system equilibration, a molecular dynamics simulation was conducted with a time step of 2 fs over a duration of 200 ns. The molecular structures of enzymes, ligands, and intermolecular interactions were then analyzed based on the simulated trajectories.

Results

Molecular docking

The molecular docking performed for Wild-type and mutant *PTEN* suggests that the ligand has well positioned within the active

site of the Proteins. The key residue in active site of *PTEN* and its interaction with atoms of berberine exhibited in Figure 1. Key residues are Ala 126, His 93, Gly129, Lys128, Lys 125, Arg 130, Asp 92, Ile 168, Gin 171, Cys 124Gly 165, Lys 130 and Thr 167.The carboxyl group of berberine formed hydrogen bond with Wild-type *PTEN* carboxyl atoms of Thr 167 and carbonyl atoms of Lys 330 and Arg 130. Additionally, carboxyl group of berberine formed bivalent H-bond with Lys 332 cabonyl group of Mutated *PTEN*.

Binding energies and inhibition constants of berberine with Wild-type and mutant *PTEN* exhibited in Table 1. Notably, the Wild-type and mutant *PTEN* system exhibits the suitable binding energy, suggesting high affinity between berberine and the wild-type *PTEN*.

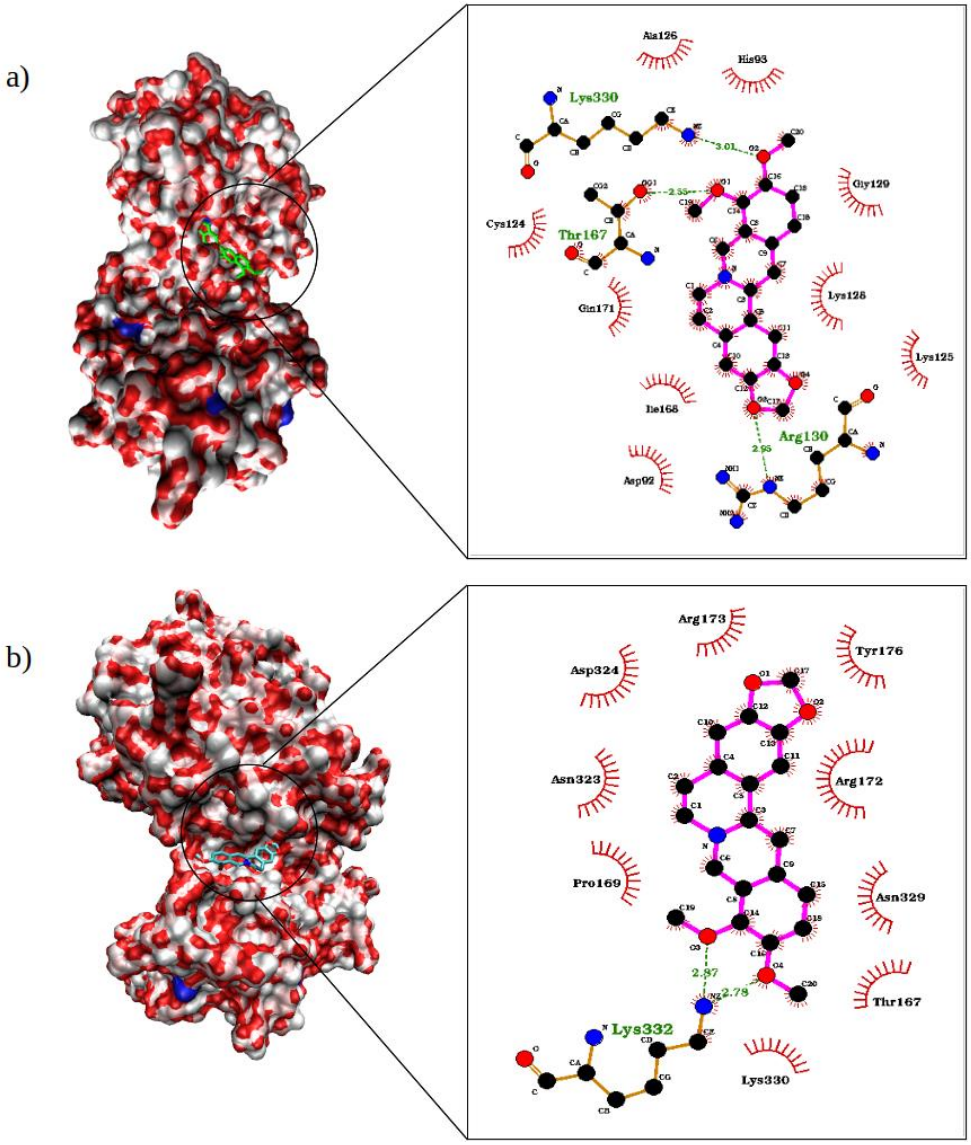


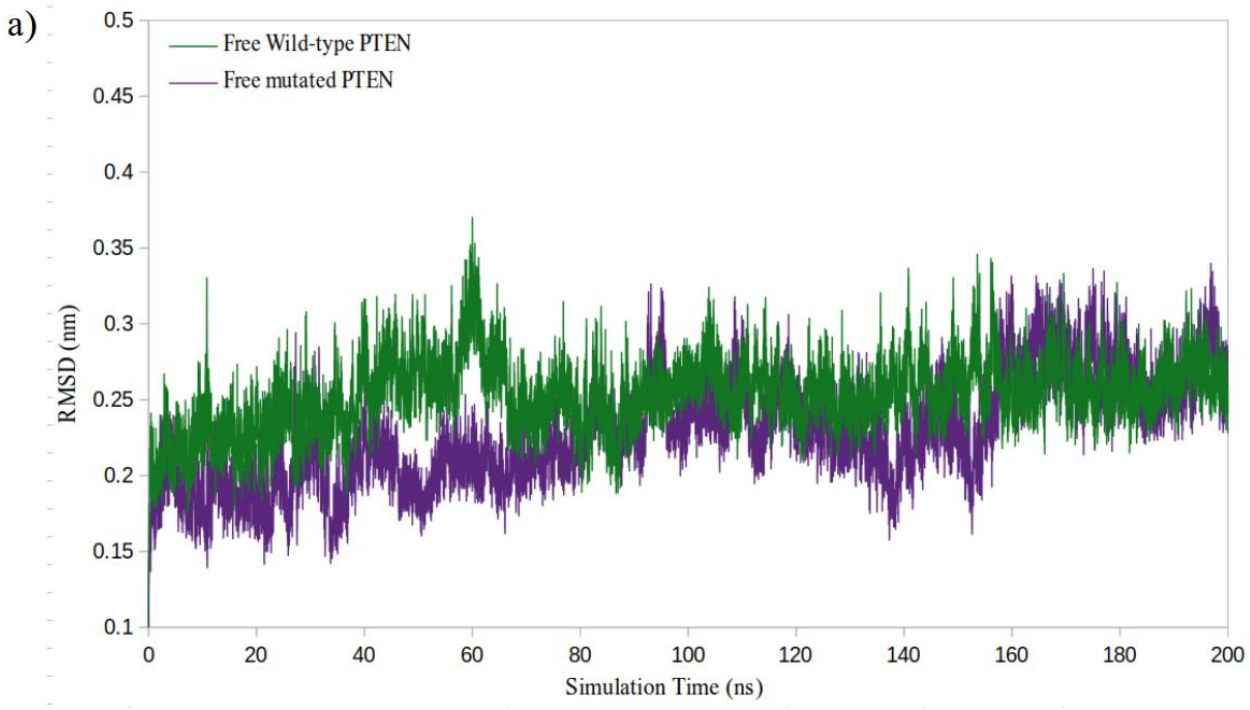
Figure 1a and b: The best docking pose and molecular interactions of the Beberine and the residues of the Wild-type *PTEN* and Mutant *PTEN* respectively. The C, N and O atoms are indicated in black, blue and red respectively. Hydrogen bonds are identified by green drops and hydrophobic interactions are shown by red curves with spokes radiating towards the ligand atoms they interact. The atoms in contact are shown with spokes radiating back. Figures provided by VMD1.9.3 and Ligplot+ programs.

Table 1: The obtained docking results, binding energies and inhibition constants predicted by AutoDock program.

System	*ΔG binding (KCal/mol)*	*Ki (μM)*
Wild-type *PTEN*/ Berberine	-7.37	3.97
Mutated *PTEN*/ Berberine	-6.28	25.07

Analysis of the Root Mean Square Deviation (RMSD)

RMSD used to find the structural changes and stability of all four system. Free wild *PTEN* has higher stability than mutated *PTEN* shown in Figure 2a. Moreover, wild-type *PTEN*/ berberine complex has slightly higher stability during simulation compared to Free wild-type *PTEN*, achieving equilibrium within 20 ns exhibited in Figure 2b. Furthermore, mutated *PTEN* with berberine also showed unstable equilibrium and high structural fluctuation during simulation time than free mutated *PTEN* represented in Figure 2c. Significantly, the average RMSD values for the last 20 ns exhibited in Table 2, referring that wild-type *PTEN* with berberine forms a stable system.



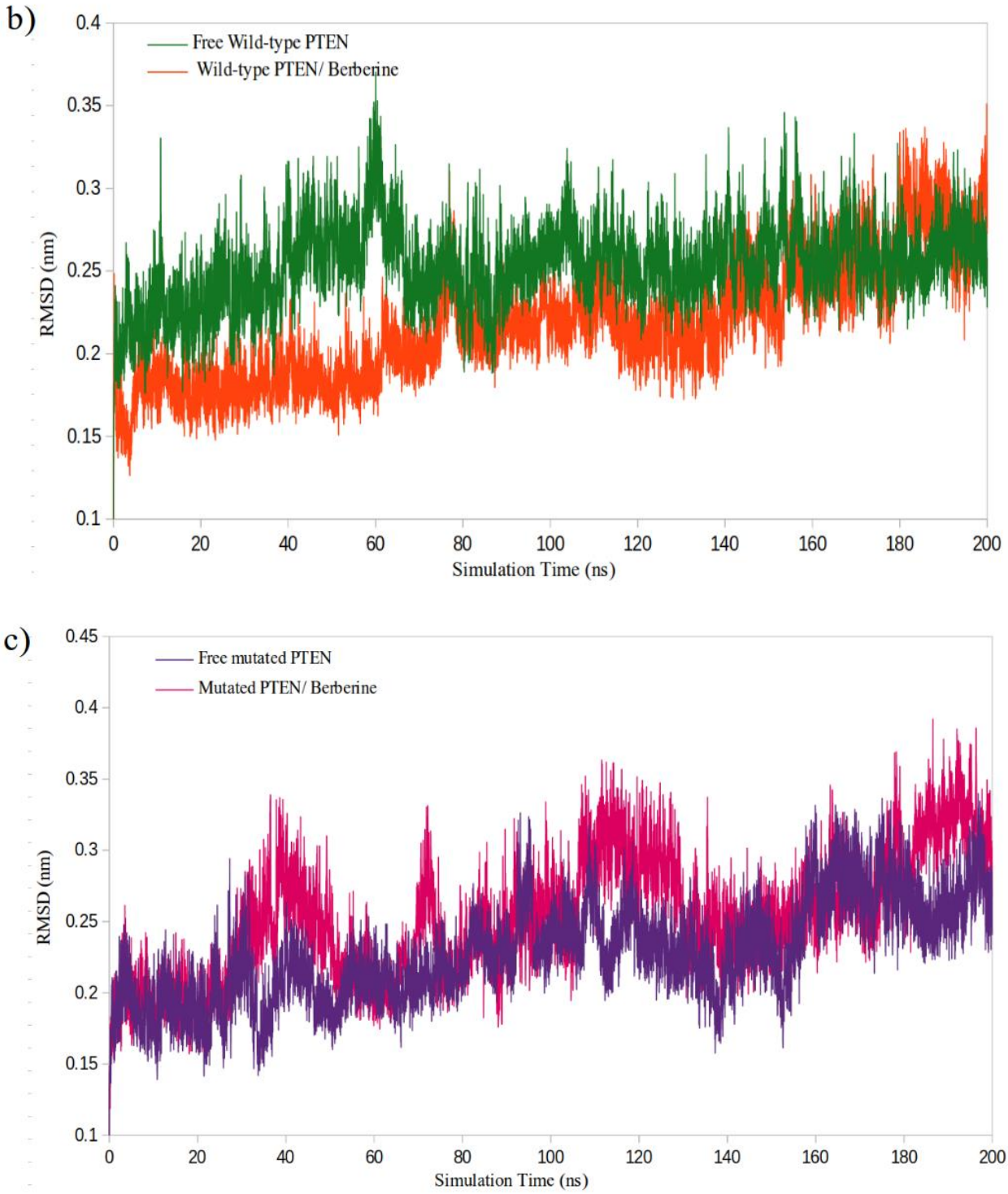


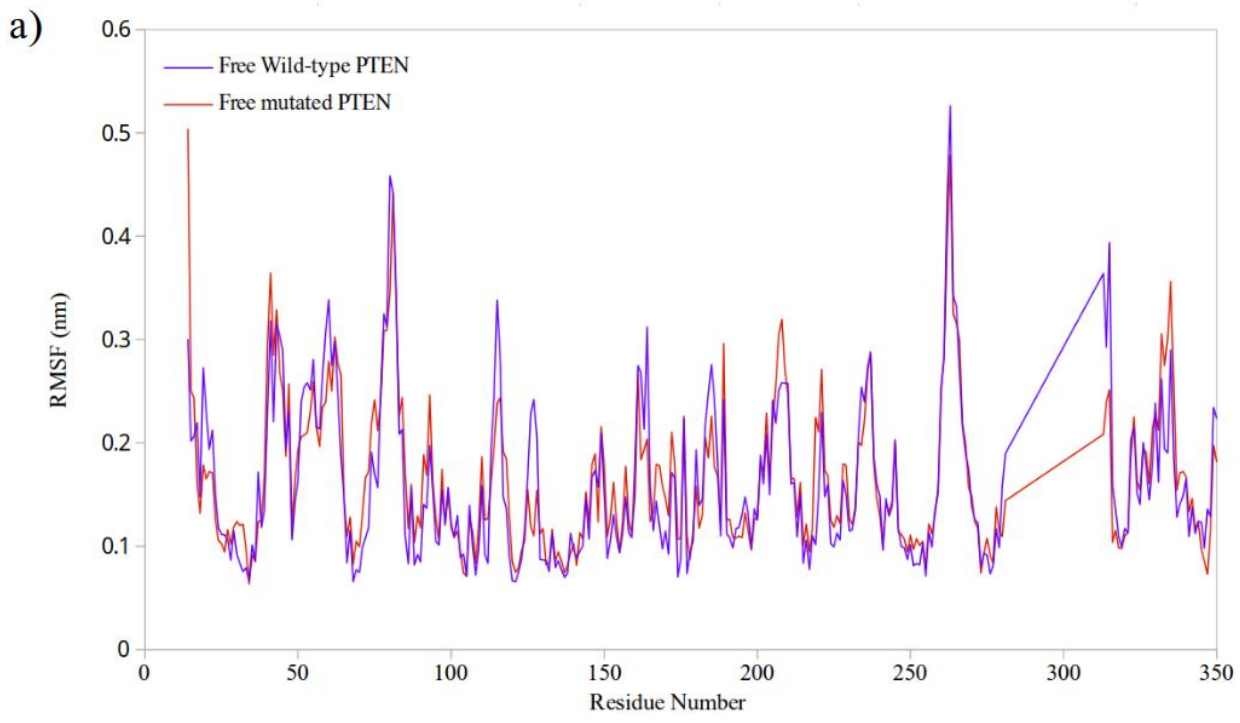
Figure 2a,b,c: RMSD plots of free and complex systems as a function of time.

Table 2: The average and standard deviations of RMSD, Rg, RMSF and SASA for free and complex enzyme during the last 20ns

System	*Mean RMSD (nm)*	*Mean Rg (nm)*	*Mean RMSF (nm)*	*Mean SASA (nm)*
Free Wild-type *PTEN*	0.261±0.018	2.196±0.007	0.183±0.079	170.006±1.524
Wild-type *PTEN*/ Berberine	0.262±0.024	2.199±0.008	0.130±0.033	165.641±1.732
Free mutated *PTEN*	0.263±0.0271	2.227±0.010	0.180±0.071	165.350±1.680
Mutated *PTEN*/ Berberine	0.285±0.033	2.226±0.009	0.157±0.036	168.0134±2.053

Analysis of the root mean square fluctuation (RMSF)

RMSD analysis used to calculate the average deviation of a residue, over time. Figure 3 depicts the RMSF values for all free enzymes and when it is complexed with Berberine. According to Figure3a free mutated PETN offered low residual fluctuations rather than free Wild-type *PTEN*. When berberine binds to Wild-type *PTEN*, there's a noticeable decline in the system's fluctuations compared to free Wild-type *PTEN* displayed on Figure 2b. In addition, when berberine binds to Mutated *PTEN*, there's also major decline in the system's fluctuations compared to free Mutated shown in Figure 2c. Additionally, Table 2, confirming above interpretation about residual integrity.



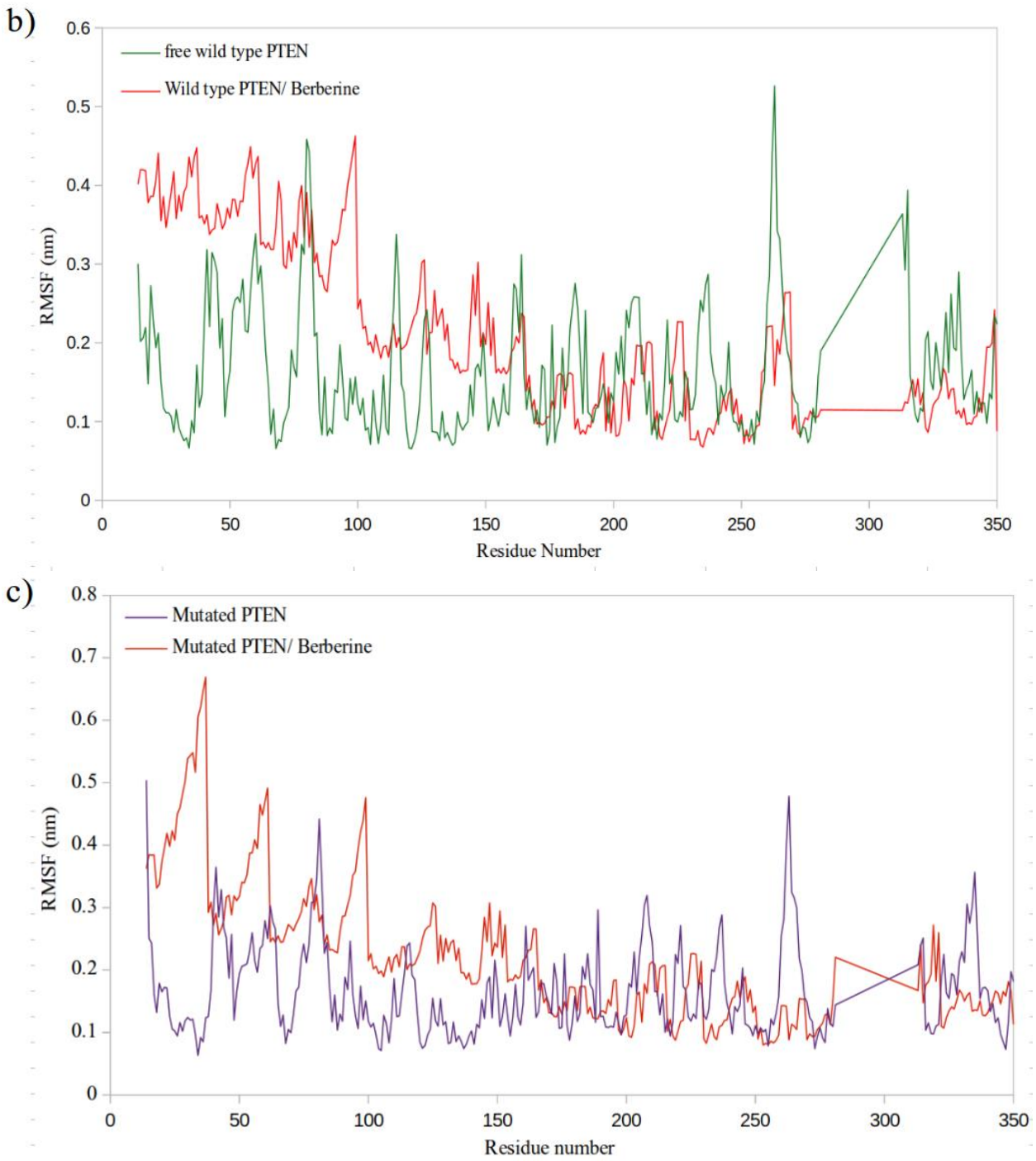


Figure 3a, b, c: RMSF plots of free and bound enzyme.

Analysis of the radius of gyration (Rg)

The Rg measurement is utilized to evaluate the structural compactness. Findings stated Rg of the free Wild-type *PTEN* has smaller RG compared to other three system displayed in Table 2. The graphic representation of rg for Free mutated *PTEN* showed up less fluctuation of structural integrity rather than Free wild-type *PTEN* displayed in Figure4a.In presence of berberine the RG for Wild-type *PTEN* has decreased compared to free Wild-type *PTEN* and the presence of

berberine with Mutated *PTEN* showed small decrease average value for RG compared to Free mutated *PTEN* represented in Figure4b and c. All systems shown to be equilibrated in 180 ns except for Mutated *PTEN*/ berberine complex which reached within 160 ns. As a result, in absence of berberine, mutated *PTEN* shown to be more compactness less than wild-type *PTEN*.

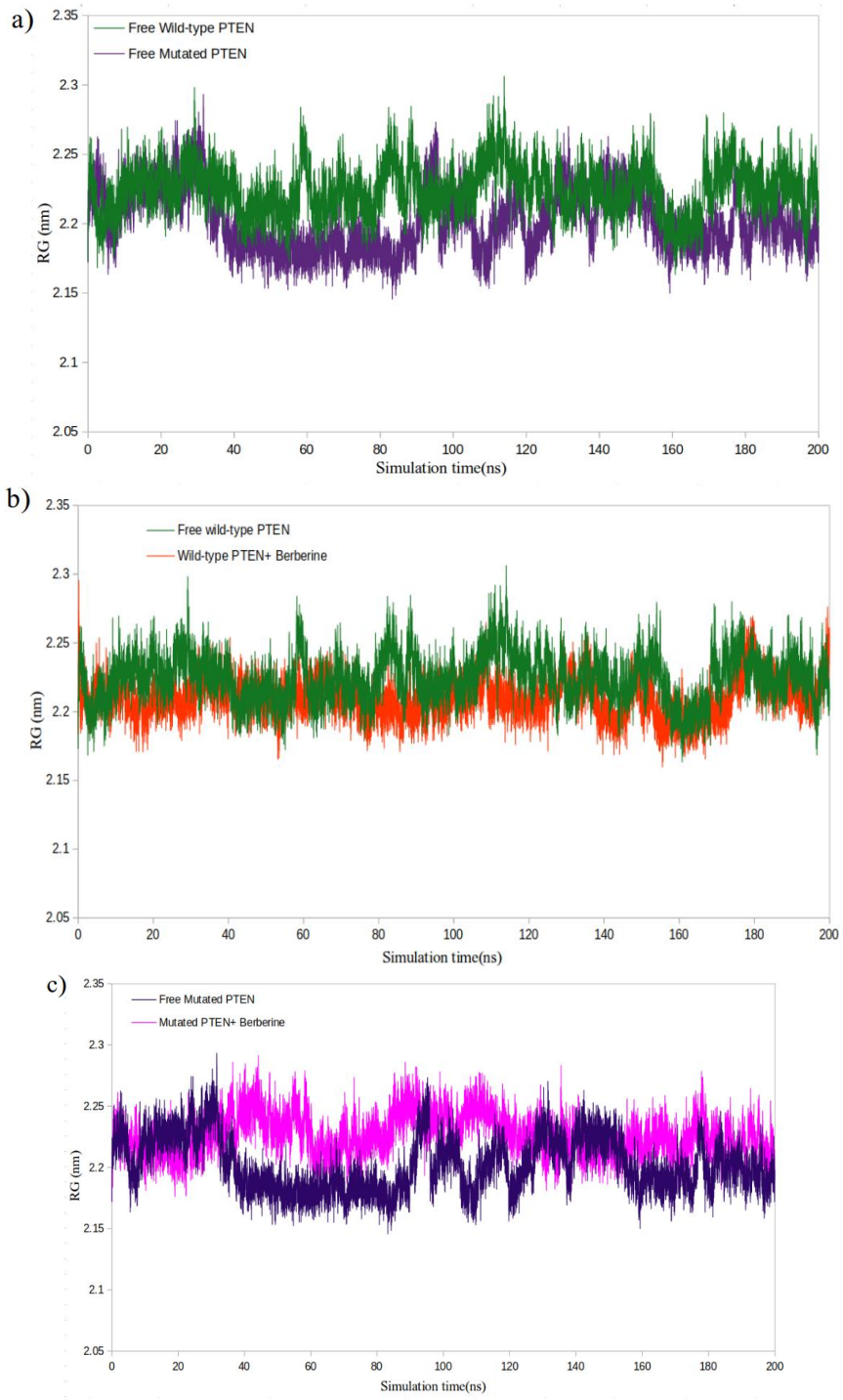


Figure 4: A-C: RG plots of free and bound enzyme as a function of time

Analysis of the solvent accessible surface area (SASA)

SASA ease to understand surface area of a biomolecule that is accessible to a solvent and used to find molecular interactions, stability, and function in duration of the simulation. The SASA diagrams is illustrated in Figure 5. The average SASA for the Free mutated had diminished compared to free Wild-type *PTEN* shown in Figure5a. The SASA plot for Wild and Mutated complexes seem to be in same insane displayed Figure 5b and c. The average SASA value for wild-type *PTEN* has diminished when berberine binds to it, because the ligand replaces the active site surrounded by solvent (Table 2). That also seem to be same in case of Mutated complexes.

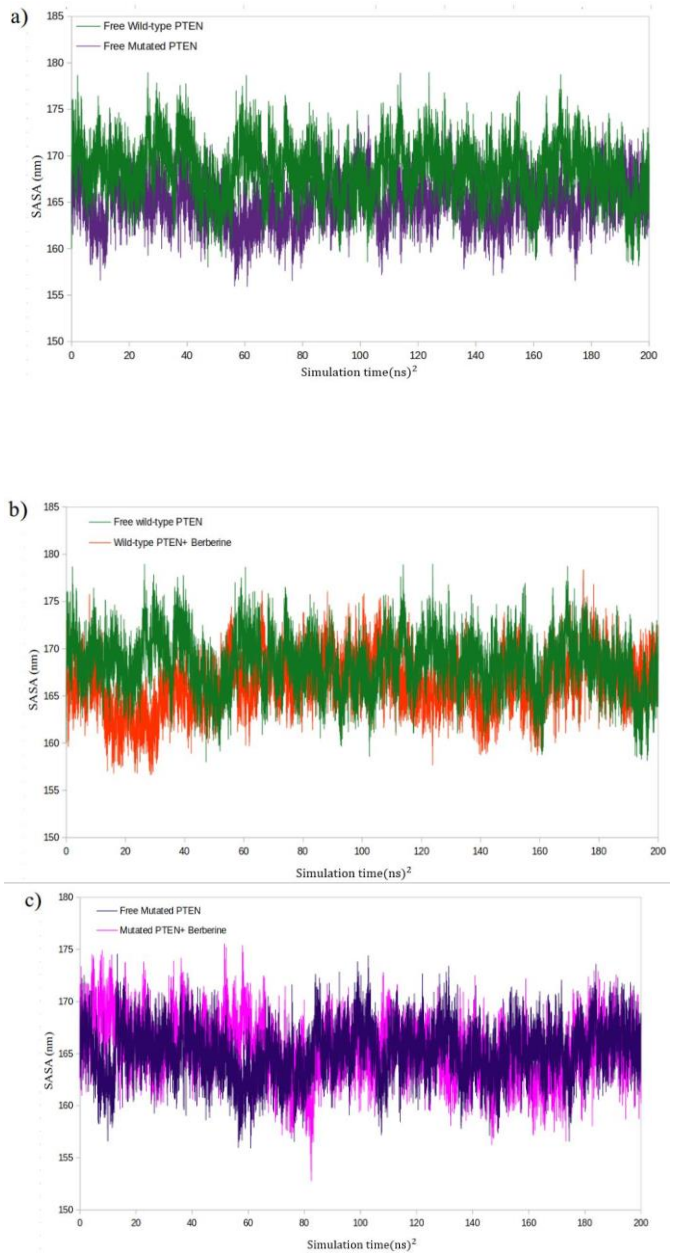


Figure 5: SASA plots of free and bound enzyme as a function of time.

Hydrogen bonds analysis

Hydrogen bond analysis is essential for investigating molecular interactions, specifically in the study of molecular dynamics simulations. The mutated *PTEN* combined with berberine formed more stable hydrogen bonds with a higher number's bonds compared to wild-type *PTEN*/ Berberine. Additionally, the mutated *PTEN* with berberine exhibited a maximum of six hydrogen bonds over the simulation period as shown in Figure 6. Furthermore, the presence of berberine led to an increase in the number of hydrogen bonds between Enzyme-Enzyme interactions of Wild-type *PTEN*, while the number of hydrogen bonds between Enzyme-solvent interactions of Wild-type *PTEN* decreased. The average number of hydrogen bonds between protein-protein and protein-solvent can be found in Table 3. The peak number of hydrogen bonds observed between berberine and Wild-type *PTEN* was four, confirming molecular docking outcomes and indicating the structural stability of complexes. The average hydrogen bond count among the enzyme's atoms of both *PTEN*s has increased in the presence of berberine represented in Figure 7. In contrast, there was a fall in hydrogen bonding between enzyme and solvent molecules for both *PTEN*s when berberine was bounds as shown in Figure 8.

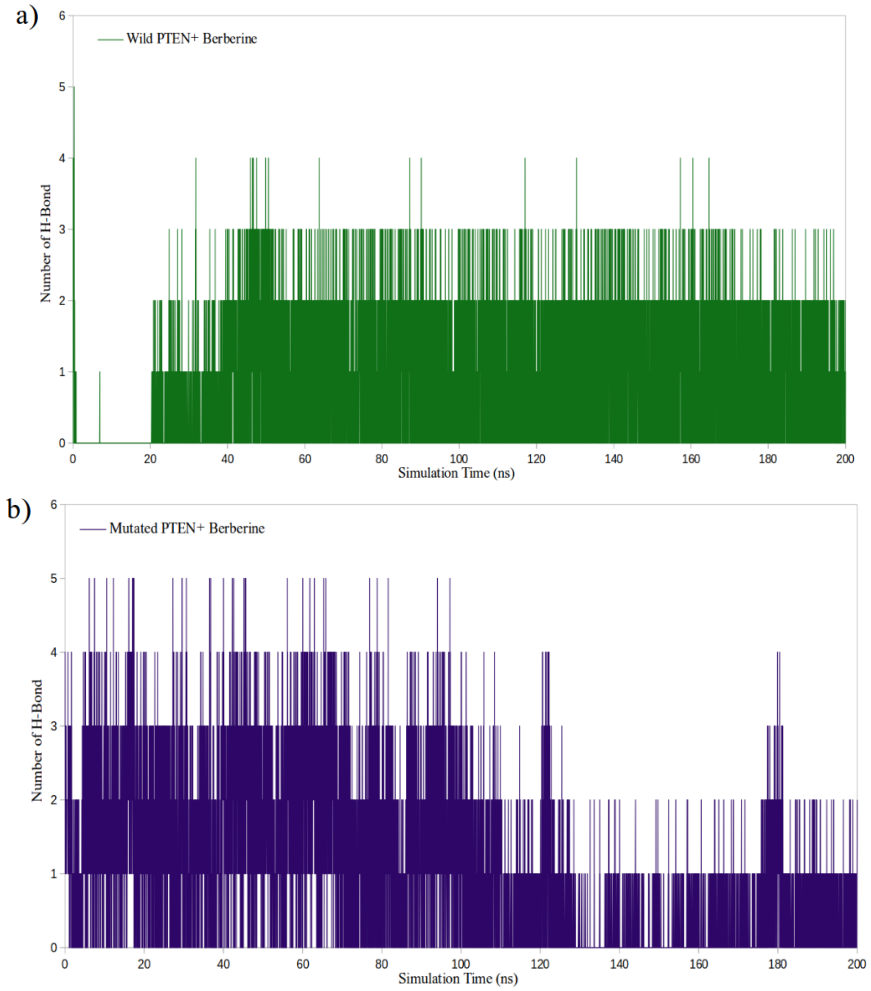


Figure 6a and b: Time dependence of the number of hydrogen bonds between berberine and enzyme during the simulation time

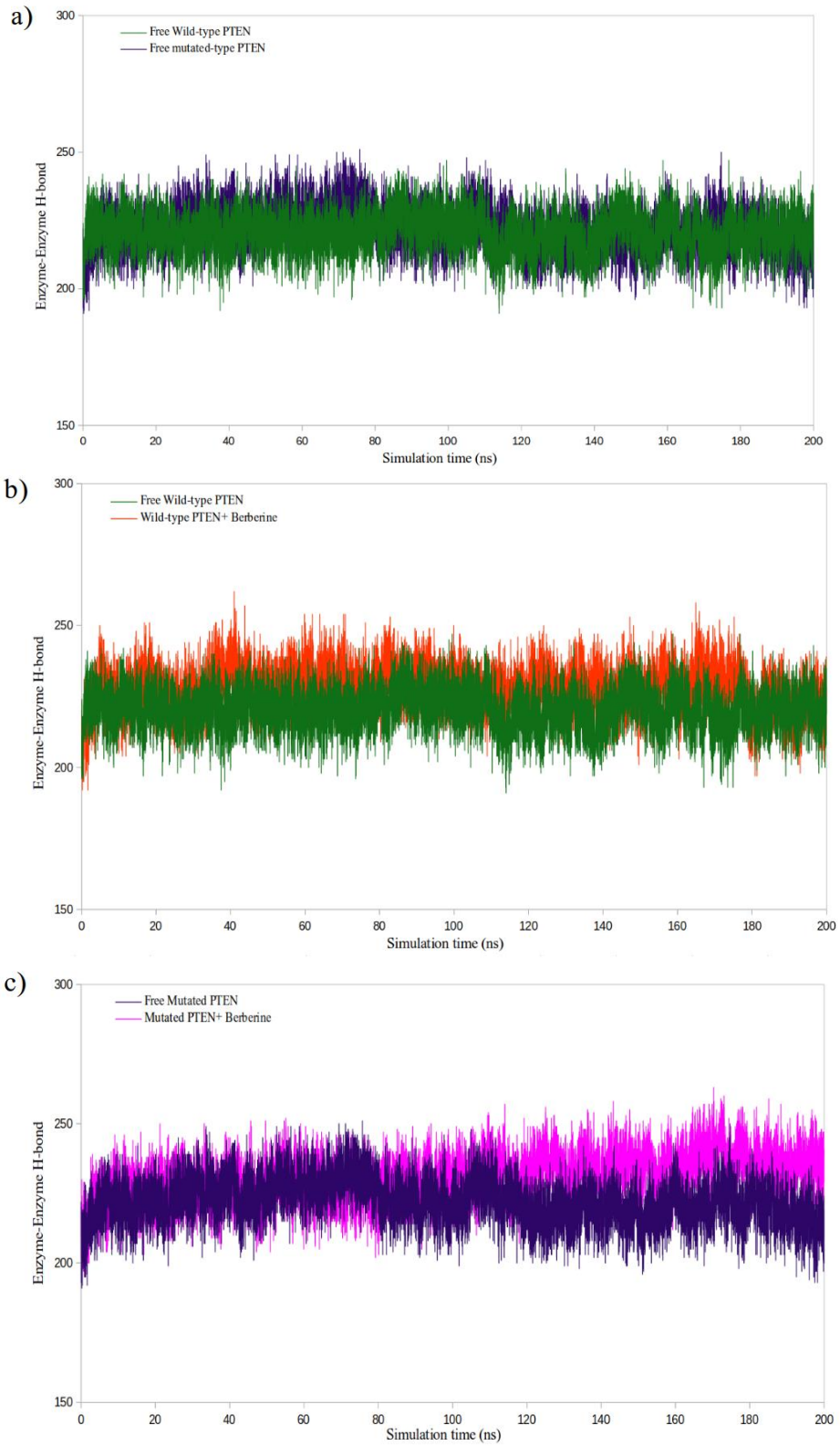


Figure 7a, b and c: Enzyme - Enzyme hydrogen-bond plots of free and bound enzyme as a function of time

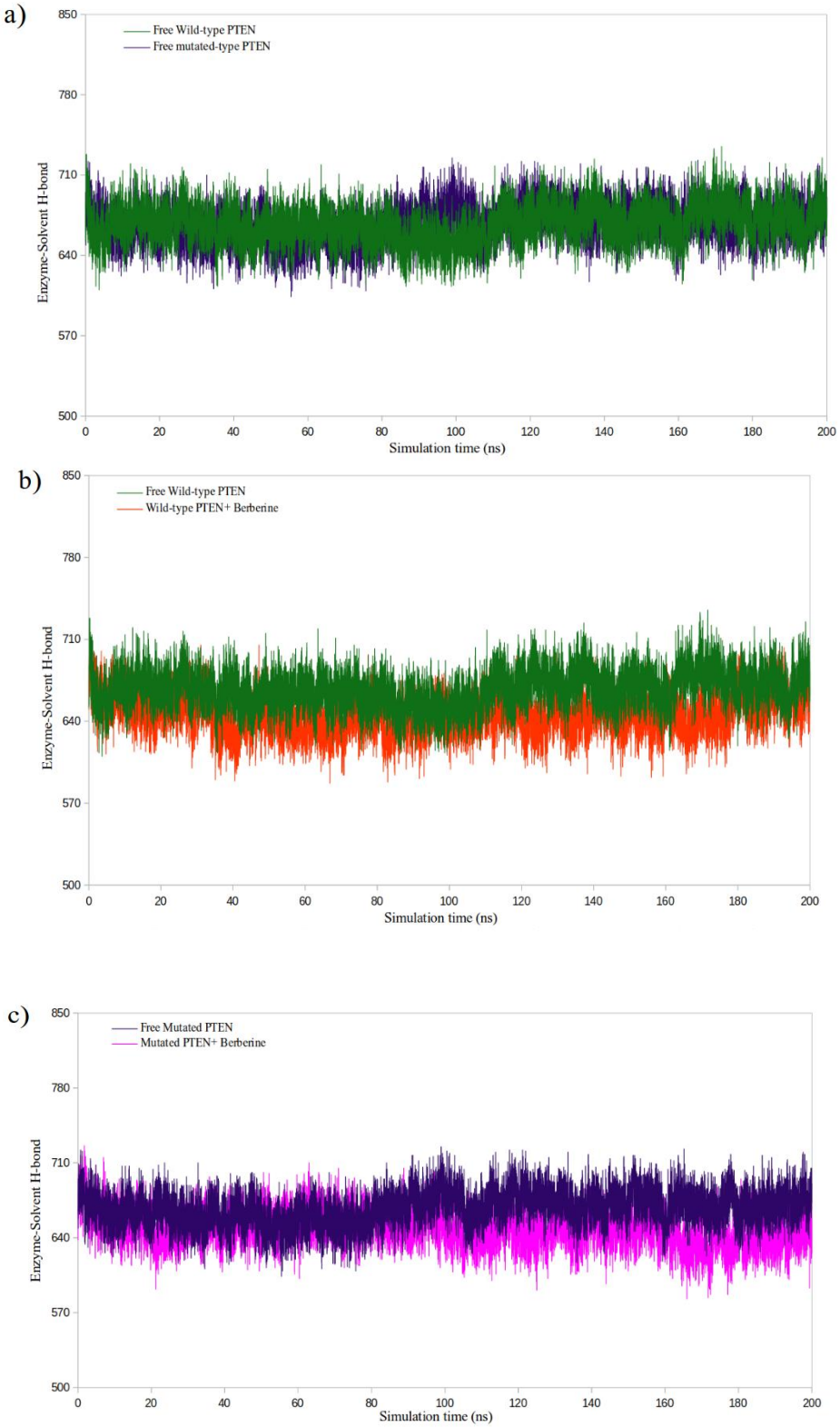


Figure 8a, b and c: Enzyme-Solvent hydrogen bond plots of free and bound enzyme as a function of time

Table 3: The average and standard deviations of intra molecular enzyme and enzyme-solvent hydrogen bonds during last 20 ns

System	***Enzyme-Enzyme***	***Enzyme-Solvent***
Free Wild-type *PTEN*	220.512 ±7.409	667.404±16.579
Wild-type *PTEN*/ Berberine	227±7.837	646.127±16.470
Free mutated *PTEN*	222.105±8.081	667.422±16.442
Mutated *PTEN*/ Berberine	230.730±8.351	649.070±16.417

Discussion

This research highlights the significant potential of berberine as a therapeutic agent, particularly in its interaction with both wild-type and mutated *PTEN*. Through molecular docking and molecular dynamics simulations, we observed that berberine exhibits strong binding affinity to both forms of *PTEN*. Docking result showed high binding affinity of berberine with wild *PTEN*, with a marked increase of its bonding with amino acids of wild *PTEN*.

The research findings suggest that piperine may serve as a potential activator of wild-type *PTEN*, due to its low binding energy and strong affinity. Other computational investigations have studied the effects of natural compounds, such as Thymoquinone analogs and Naringin, on *PTEN* (22, 23). These studies revealed that *PTEN* exhibited favorable molecular interactions and binding stability with Naringin, while Thymoquinone was found to upregulate *PTEN* gene expression. Moreover, several studies have shown that *PTEN* reactivation could be achieved using compounds derived from cruciferous vegetables or synthetic transcription factors like dCas9-VPR (24, 25). Conversely, recent research examining the impact of curcumin on both wild-type and mutated *PTEN* using molecular docking and molecular dynamics simulations showed that curcumin interacted more strongly with mutated *PTEN* compared to the wild-type, indicating that curcumin might inhibit mutated *PTEN* (26).

Nonetheless, our findings underscore the promising activation of wild-type *PTEN* by piperine

Conclusion

Berberine could effectively bind to *PTEN*, and potentially restore its tumor-suppressive functions. The molecular dynamics simulations further confirmed the stability of the *PTEN*–berberine complex, with critical parameters such as RMSD, RMSF, SASA and RG showing favorable results. Berberine not only stabilizes wild *PTEN* but also may activate it, which is especially promising in cases where *PTEN* is mutated or dysfunctional, such as in various cancers. By activating *PTEN*, berberine could potentially regulate the cell cycle and prevent uncontrolled cell proliferation, a hallmark of cancer. Berberine may act as a novel therapeutic agent, capable of inhibiting cancer growth by activating the *PTEN*/PI3K/AKT signaling pathway, which plays a key role in regulating cell survival, growth, and metabolism. This could provide a safe and effective approach to treating cancers associated with *PTEN* dysfunction, highlighting berberine's therapeutic potential in cancer therapy.

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Conflict of interest

The authors declare that there is no conflict of interests.

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