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Mechanistic Insights into Tacrine Binding to Acetylcholinesterase in Alzheimer's Disease from Molecular Docking and Molecular Dynamics Simulations

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Abstract

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that leads to memory loss and cognitive decline. Acetylcholinesterase (AChE) is an important therapeutic target in the treatment of AD, and tacrine is one of the earliest drugs used as an AChE inhibitor. In the present study, computational methods were used to investigate interaction of tacrine with acetylcholinesterase (PDB ID: 1EVE). The tacrine molecule (1,2,3,4-tetrahydroacridin-9-amine; $C_{13}H_{14}N_2$) was optimized using Gaussian software, followed by molecular docking to study its binding behavior within the active site of the protein. Docking analysis showed that tacrine binds effectively with acetylcholinesterase, with a binding energy of -7.17 kcal/mol and the formation of hydrogen bond interactions, indicating favorable binding within the enzyme cavity. To further examine the stability of the complex, the best-docked structure was subjected to a 100 ns molecular dynamics (MD) simulation using GROMACS. The RMSD, RMSF, and radius of gyration analyses indicated that the protein remained structurally stable and compact throughout the simulation, while the ligand stayed firmly bound within the active site. Interaction analysis revealed that the complex is mainly stabilized by strong π - π stacking interactions with important aromatic residues such as Trp84, Tyr121, Tyr334, and Phe330. Hydrophobic interactions and transient hydrogen bonds also contributed to the stability of the ligand-protein complex. The study shows that tacrine forms a stable interaction with acetylcholinesterase and highlights the importance of aromatic interactions in ligand binding. These findings may help in the future design and optimization of improved acetylcholinesterase inhibitors for Alzheimer's disease treatment.

Keywords: Alzheimer, Docking, Simulations, Inhibition, GROMACS, Tacrine

Introduction:

Alzheimer's disease (AD) is the most prevalent form of dementia and represents one of the leading causes of cognitive impairment among the elderly. It is a progressive neurodegenerative disorder characterized by the gradual deterioration of memory, learning ability, language, reasoning, and behavioural functions, ultimately compromising an individual's capacity to perform routine daily activities. Beyond its profound impact on patients, AD places a substantial emotional, social, and economic burden on caregivers and healthcare systems worldwide. According to recent estimates, approximately 50 million people are living with dementia globally, of which 60–70% of cases are attributed to Alzheimer's disease [1, 2]. With increasing life expectancy and an ageing population, the prevalence of AD is expected to rise considerably, underscoring the need for more effective therapeutic interventions. The pathological progression of AD is primarily associated with the extracellular accumulation of amyloid- β plaques arising from the abnormal processing of amyloid precursor protein (APP) [3], together with the intracellular aggregation of hyperphosphorylated tau protein into neurofibrillary tangles [4]. These pathological events impair synaptic communication, promote neuroinflammation, and ultimately result in neuronal degeneration and progressive brain atrophy [5]. Although several therapeutic agents are currently available for symptomatic management, none effectively halt or reverse disease progression. Consequently, the identification and development of improved therapeutic candidates remain a major objective in Alzheimer's disease research. Computational approaches have become indispensable tools in modern drug discovery because they enable the rapid evaluation of molecular interactions while reducing the time and cost associated with experimental screening. Techniques such as density functional theory (DFT), molecular docking, and molecular dynamics (MD) simulations provide valuable insights into ligand geometry, binding affinity, conformational stability, and the dynamic behaviour of biomolecular complexes. These approaches are particularly useful for understanding the molecular basis of protein–ligand recognition and for guiding the rational design of novel therapeutic agents.

Acetylcholinesterase (AChE) is one of the most extensively investigated molecular targets for the treatment of Alzheimer's disease. The enzyme rapidly hydrolyses acetylcholine at cholinergic synapses, and inhibition of its catalytic activity increases acetylcholine levels in the brain, thereby improving neurotransmission and temporarily alleviating cognitive symptoms. The crystal structure of human acetylcholinesterase (PDB ID: 1EVE) [6] has therefore served

as a valuable structural model for investigating inhibitor binding and designing new anti-Alzheimer agents. Tacrine (1,2,3,4-tetrahydroacridin-9-amine) was the first acetylcholinesterase inhibitor approved for the clinical treatment of Alzheimer's disease [7-9]. Although its clinical use was discontinued because of hepatotoxicity, tacrine continues to serve as an important reference compound in medicinal chemistry owing to its well-established binding mode and high affinity towards acetylcholinesterase. Considerable efforts have therefore been directed towards developing tacrine derivatives with improved efficacy and reduced toxicity while retaining favourable pharmacological characteristics [10,11]. A detailed understanding of the molecular interactions responsible for tacrine binding remains valuable for the rational design of next-generation acetylcholinesterase inhibitors.

In the present study, DFT calculations were employed to optimize the molecular geometry of tacrine prior to investigating its interaction with acetylcholinesterase. Molecular docking was performed to predict the preferred binding orientation of the ligand within the enzyme, and the most favourable docking complex was subsequently subjected to a 100 ns molecular dynamics simulation to evaluate its structural stability and interaction dynamics. Analyses of binding affinity, residue-level interactions, root mean square deviation (RMSD), root mean square fluctuation (RMSF), and radius of gyration (R_g) were carried out to characterize the behaviour of the tacrine–AChE complex throughout the simulation. The findings provide molecular-level insights into the binding mechanism of tacrine and contribute to the rational development of improved acetylcholinesterase inhibitors for the treatment of Alzheimer's disease.

Computational details

Structure

GAUSSIAN 09 [12] program package is employed to carry out density functional theory (DFT) calculation to find out the optimized structure of tacrine molecule. We have used B3LYP [13-15] functional with 3-11G basis set [16] for geometry optimization. There are many drugs that are available for the treatment of Alzheimer disease in which Tacrine ($C_{13}H_{14}N_2$) is widely used. Bond length and bond angle are observed for the optimized Tacrine molecule, as a result it's found that the bond length between C_3 - C_4 and C_8 - C_9 has the highest bond length of 1.44 pm and C_4 - N_{22} has the lowest bond length of 1.351pm, While C_7 C_3 C_2 has the highest bond angle of 122.89 degree and $H_{21}C_{14}C_8$ has the lowest bond angle of 116.21 degree.

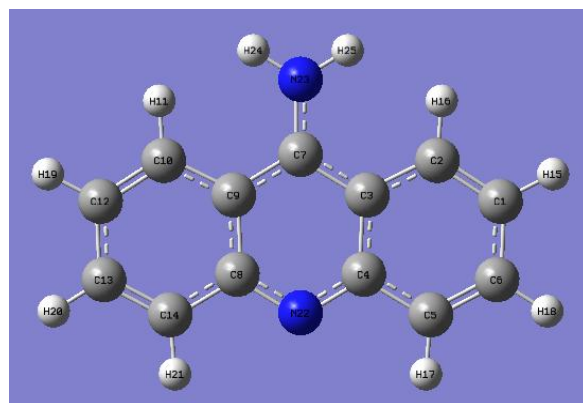


Figure 1: Optimised structure of Tacrine



Figure 2: 3D structure of 1EVE protein

Molecular Docking

The DFT-optimized structure of tacrine was employed as the ligand for molecular docking studies. The crystal structure of acetylcholinesterase (AChE) was retrieved from the Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank and used as the receptor (Figure 2). Prior to docking, the protein structure was prepared with all crystallographic water molecules removed, polar hydrogen atoms added, Gasteiger partial charges assigned, and non-polar hydrogen atoms merged. Blind molecular docking was carried out using AutoDock 4.2 [17] to identify the preferred binding site of tacrine on AChE. A three-dimensional grid box encompassing the entire receptor was generated with dimensions of 126 x 126 x 126 grid points and a grid spacing of 0.514 Å. The grid centre was positioned at coordinates (3.040, 68.428, 56.077), allowing unrestricted exploration of the protein surface by the ligand during docking. The Lamarckian Genetic Algorithm implemented in AutoDock was used for conformational sampling. Ten independent docking runs were performed with a maximum of 2,500,000 energy evaluations and 27,000 genetic algorithm operations for each

run. The initial population size was fixed at 150, while the mutation and crossover rates were maintained at 0.02 and 0.80, respectively. The resulting docked conformations were ranked according to their predicted binding energies, and the most favourable binding pose was selected for subsequent molecular dynamics simulations.

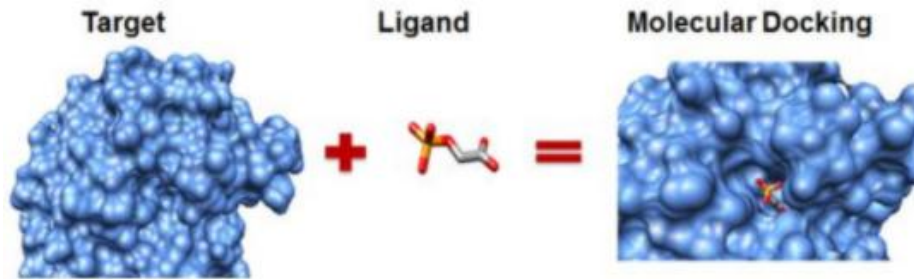


Figure 3: Docking of ligand and receptor

Molecular Dynamics Simulation

The stability of the docked tacrine-AChE complex was investigated through 100 ns molecular dynamics (MD) simulations using GROMACS version 5.1.2 [18] with the OPLS-AA all-atom force field [19,20]. The protein-ligand complex was placed in a triclinic periodic simulation box and solvated using the explicit SPC/E water model [21]. Approximately 18,000 water molecules were introduced into the simulation box, and appropriate counterions were added to neutralize the total charge of the system. Following system construction, energy minimization was performed to remove unfavorable steric contacts. The minimized system was subsequently equilibrated at 298 K and 1 bar using the Berendsen pressure coupling algorithm [22]. After equilibration, a 100 ns production simulation was carried out under periodic boundary conditions. The total potential energy of the system was described by the OPLS-AA force field, which accounts for bonded interactions, including bond stretching, angle bending, and torsional contributions, together with non-bonded electrostatic and Lennard-Jones interactions. The corresponding energy terms are represented by Equations (1)-(4).

$$E_{nb} = \sum_i^a \sum_j^b \left[\frac{q_i q_j e^2}{r_{ij}} + 4 \epsilon_{ij} \left(\frac{\sigma_{ij}^{12}}{r_{ij}^{12}} - \frac{\sigma_{ij}^6}{r_{ij}^6} \right) \right] f_{ij} \quad (1)$$

$$E_{angle} = \sum_{angles} K_{\theta} (\theta - \theta_{eq})^2 \quad (2)$$

$$E_{bond} = \sum_{bonds} K_r (r - r_{eq})^2 \quad (3)$$

$$E_{torsion} = \sum_i \frac{V_1^i}{2} [1 + \cos(\phi_i + f_i 1)] + \frac{V_2^i}{2} [1 - \cos(2\phi + f_i 2)] + \frac{V_3^i}{2} [1 + \cos(3\phi + f_i 3)] \quad (4)$$

Trajectory analyses were performed using the built-in GROMACS analysis utilities, while graphical representations were generated using XMGrace [23]. The structural stability of the protein–ligand complex was evaluated by monitoring the root mean square deviation (RMSD), root mean square fluctuation (RMSF), and radius of gyration (R_g) throughout the simulation. Protein–ligand interactions were further examined and visualized using BIOVIA Discovery Studio and UCSF CHIMERA [24, 25].

Results & Discussion:

Docking Study

Molecular docking was performed to investigate the binding behaviour of tacrine within the active site of acetylcholinesterase. Ten independent docking runs were carried out using AutoDock 4.2, and the resulting binding poses showed a high degree of consistency, indicating reliable prediction of the ligand–protein interaction. The three lowest-energy conformations exhibited binding energies of -7.17, -7.16, and -7.00 kcal mol⁻¹, suggesting that tacrine adopts energetically favourable orientations within the catalytic pocket of the enzyme. Among the generated conformations, the lowest-energy docking pose displayed a binding energy of -7.17 kcal mol⁻¹ with an estimated inhibition constant (K_i) of 5.59 μ M, indicating a favourable binding affinity towards acetylcholinesterase. The corresponding docking parameters for the best-ranked complex are summarized in Table 1. The calculated intermolecular energy was dominated by van der Waals interactions together with hydrogen-bonding and desolvation contributions (-7.40 kcal mol⁻¹), whereas the electrostatic component (-0.06 kcal mol⁻¹) made only a minor contribution to the overall binding energy. These results indicate that non-covalent interactions, particularly hydrophobic contacts and hydrogen bonding, play the principal role in stabilizing the tacrine–AChE complex.

Table 1 presents the docking parameters obtained for the most favourable binding pose.

Parameter	Value
Best Binding Energy	-7.17 kcal mol ⁻¹
Estimated K_i	5.59 μ M
vdW + H-bond + Desolvation Energy	-7.40 kcal/mol

Electrostatic Energy	-0.06 kcal/mol
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The negative binding energy obtained for the docked complex indicates that the interaction between tacrine and acetylcholinesterase is thermodynamically favourable under the docking conditions. Analysis of the predicted binding mode further revealed that tacrine occupies the active-site gorge of the enzyme, where it establishes multiple favourable contacts with surrounding amino acid residues. The interaction pattern suggests that the ligand is appropriately accommodated within the binding pocket, supporting the observed binding affinity.

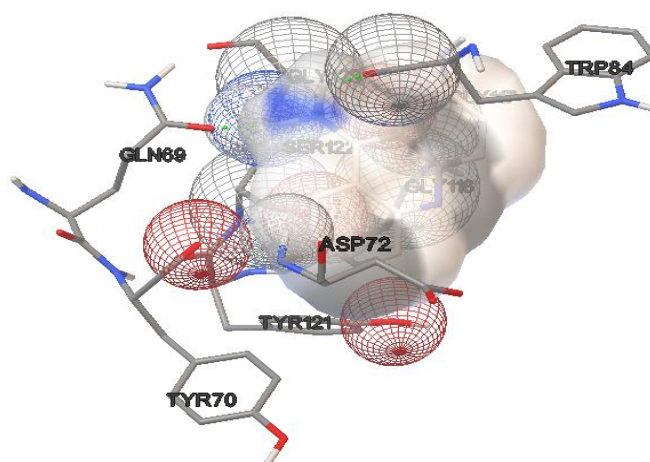


Figure 4: Ligand binding site post docking calculations at active site showing interactions of Tacrine at 1EVE active site

Cluster analysis of the docking solutions provided additional evidence for the reliability of the predicted binding mode. Ten docking runs generated three distinct conformational clusters using an RMSD tolerance of 2.0 Å, with all three clusters containing multiple conformations. The calculated clustering entropy was 0.41, indicating limited structural variation among the docked poses and suggesting convergence of the docking algorithm towards similar low-energy conformations. Such clustering behaviour supports the reproducibility of the docking calculations and reinforces the stability of the predicted binding orientation.

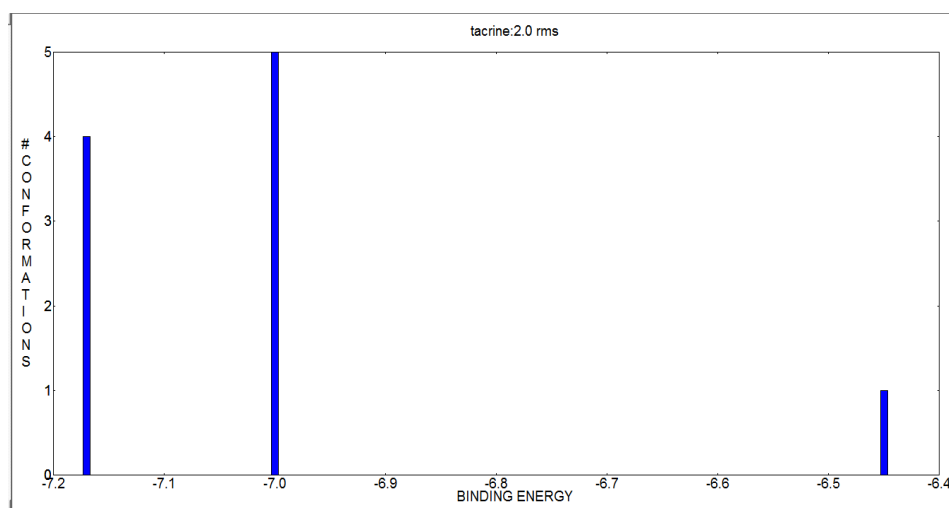


Figure 5: Clustering data after docking study

It has been observed that the docking results demonstrate that tacrine exhibits favourable affinity towards acetylcholinesterase, with the binding process being predominantly stabilized by van der Waals interactions, hydrogen bonding, and hydrophobic contacts. The consistent binding energies together with the clustering analysis indicate that the ligand adopts a stable and reproducible binding orientation within the active site, making the selected docked complex suitable for subsequent molecular dynamics simulations.

Molecular Dynamics Study

To evaluate the stability of the docked tacrine–acetylcholinesterase complex under dynamic conditions, a 100 ns molecular dynamics simulation was performed. Throughout the simulation, the protein preserved its overall structural architecture, with no evidence of significant conformational distortion or unfolding. The β -sheet core remained well conserved, whereas minor fluctuations were mainly confined to loop regions and peripheral helices, reflecting the intrinsic flexibility of these segments rather than any loss of structural integrity. These observations indicate that the protein rapidly attained equilibrium and remained stable during the simulation. Analysis of the ligand-protein interactions demonstrated that tacrine remained favourably positioned within the active-site gorge throughout the simulation. The binding mode was predominantly maintained through persistent π - π stacking interactions involving aromatic residues such as Trp84, Tyr70, Tyr121, Phe330, and Tyr334, which consistently contributed to ligand stabilization. Hydrogen-bond interactions with residues including Gln69, Ser122, and Tyr334 were also observed. Although these polar interactions

exhibited some temporal variation during the simulation, they played an important role in maintaining the orientation of the ligand within the binding pocket. In addition to aromatic stacking and hydrogen bonding, numerous van der Waals contacts were established with neighbouring residues, including Asp72, Val71, Gln74, Ser81, Asn85, Pro86, Gly118, Gly123, Leu127, Tyr130, Phe331, and several surrounding amino acids. The collective contribution of these non-covalent interactions generated a favourable microenvironment that supported stable ligand binding throughout the simulation. The snapshots of the simulations revealed that the overall binding orientation of tacrine remained largely unchanged during the 100 ns trajectory. The aromatic residues Trp84, Tyr121, Tyr334, and Phe330 consistently formed the principal interaction network responsible for anchoring the ligand within the catalytic gorge of acetylcholinesterase. Although hydrogen-bond partners varied at different stages of the simulation, the persistence of aromatic interactions ensured that the ligand remained securely accommodated within the active site.

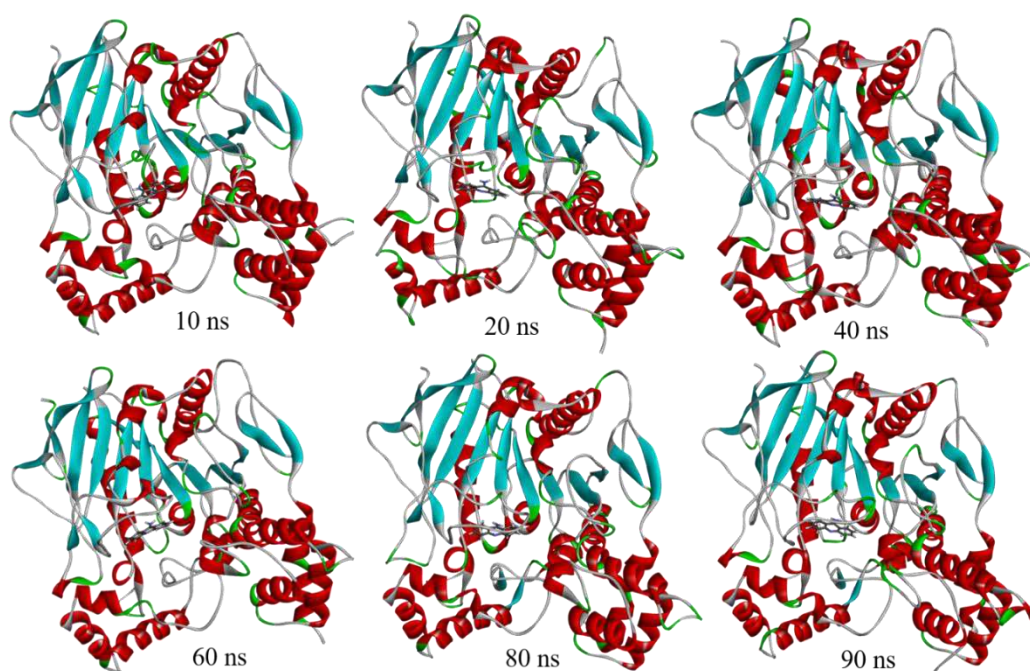


Figure 6: Structural superposition of representative MD snapshots at different timestamps

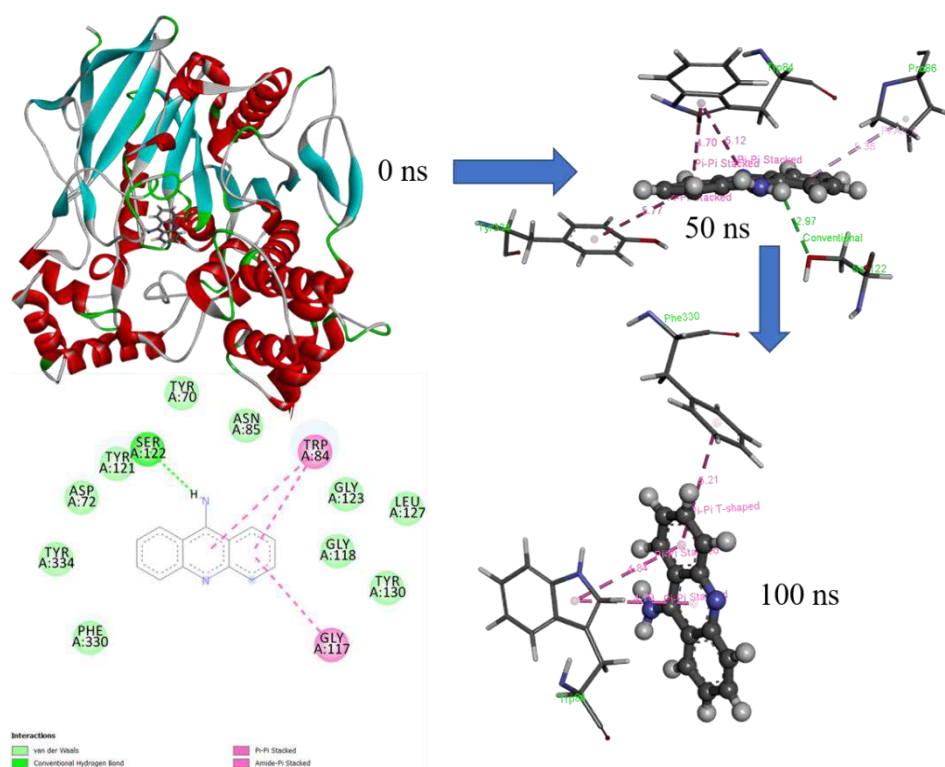


Figure 8: From 0 to 100 ns MD simulation results

Molecular dynamics simulation demonstrates that tacrine remains firmly associated with the active site of acetylcholinesterase over the entire 100 ns simulation period. The persistence of key aromatic interactions, together with favourable hydrophobic and polar contacts, indicates that the docked complex is dynamically stable and that the predicted binding mode obtained from molecular docking is maintained under simulated physiological conditions.

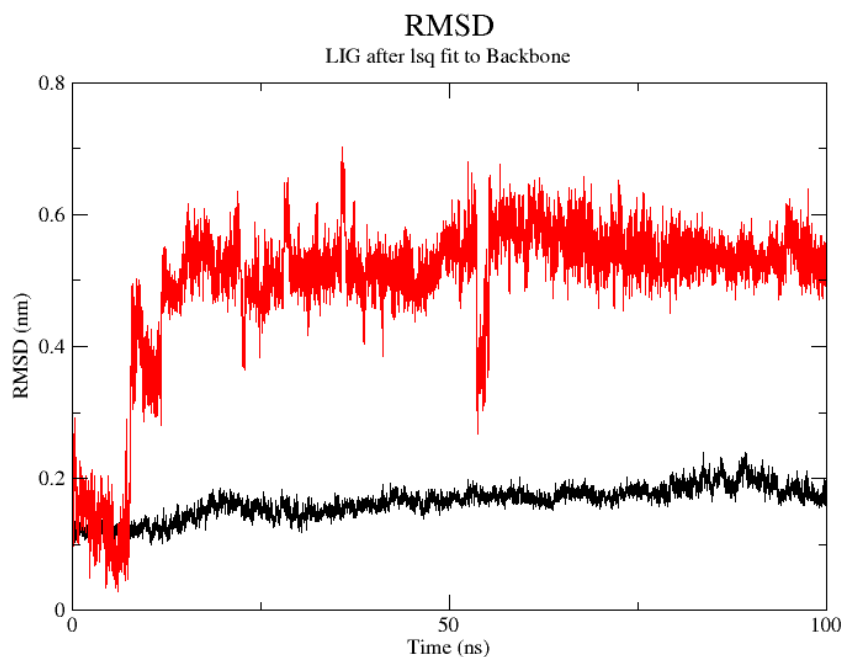


Figure 9: RMSD plot for 100 ns

The root mean square deviation (RMSD) profile was analysed to assess the structural stability of the tacrine–acetylcholinesterase complex throughout the 100 ns molecular dynamics simulation. The protein backbone exhibited consistently low RMSD values, fluctuating between approximately 0.15 and 0.22 nm, indicating that the overall conformation of acetylcholinesterase remained stable during the simulation. The absence of large structural deviations suggests that ligand binding does not induce significant conformational changes in the protein and that the system remains well equilibrated over the simulation period. In contrast, the ligand displayed comparatively higher RMSD values, reaching approximately 0.50–0.60 nm during the initial stage of the simulation. These fluctuations are indicative of conformational adjustment of tacrine within the binding cavity before the complex attained equilibrium. Following this equilibration phase, the ligand RMSD remained relatively constant without any continuous increase, demonstrating that tacrine remained confined within the active-site pocket throughout the simulation. The RMSD behaviour is consistent with the interaction analysis, which revealed persistent π – π stacking interactions involving Trp84, Tyr121, Tyr334, and Phe330. These aromatic contacts provide a stable framework for the ligand, while transient hydrogen bonds and surrounding hydrophobic interactions permit limited local rearrangements within the binding pocket. Minor fluctuations observed around the middle of the simulation (~ 50 ns) are therefore attributed to subtle changes in ligand orientation rather than dissociation from the active site. The RMSD analysis confirms the

dynamic stability of the tacrine–acetylcholinesterase complex and supports the maintenance of the docking-derived binding mode during molecular dynamics simulation.

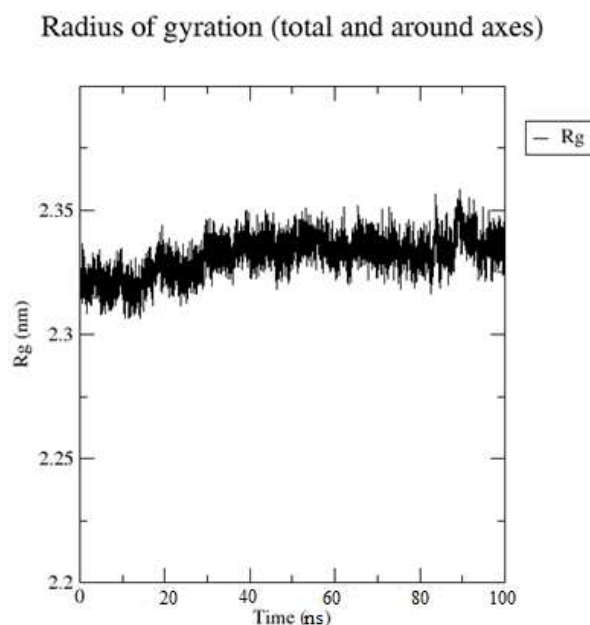


Figure 10: Radius of gyration (R_g) Plot for 100 ns

The radius of gyration (R_g) was monitored to evaluate the compactness of the protein structure during the simulation. The R_g profile remained essentially constant over the entire 100 ns trajectory, indicating that acetylcholinesterase preserved its overall structural compactness without undergoing noticeable expansion or unfolding. The absence of significant variation in R_g suggests that the tertiary structure of the protein remained stable following ligand binding. The stable R_g profile is in agreement with the RMSD results and demonstrates that the integrity of the ligand-binding pocket was maintained throughout the simulation. The compact protein structure provides a favourable environment for sustained ligand accommodation, allowing tacrine to remain associated with the active site under dynamic conditions. These observations further support the conclusion that the protein–ligand complex remained structurally stable during the simulation.

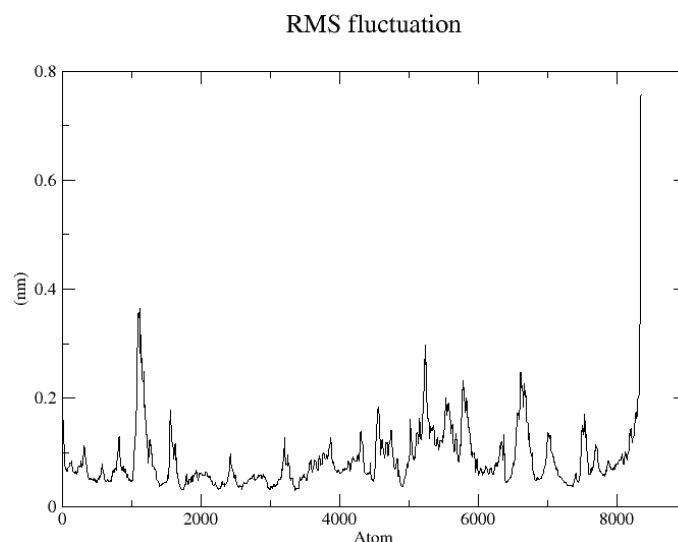


Figure 11: RMSF plot for 100 ns

The residue-wise flexibility of acetylcholinesterase was investigated using root mean square fluctuation (RMSF) analysis. Most amino acid residues exhibited relatively low fluctuations, typically within the range of 0.05–0.15 nm, indicating that the majority of the protein maintained a rigid and well-defined conformation during the simulation. Higher fluctuations were primarily observed in loop regions and terminal residues, reflecting their inherent flexibility rather than any global structural instability. Residues constituting the ligand-binding pocket showed comparatively low fluctuations, demonstrating that the active-site architecture remained well preserved throughout the simulation. The limited mobility of these residues is consistent with the persistent interaction network identified during the molecular dynamics analysis, where aromatic residues such as Trp84, Tyr121, Tyr334, and Phe330 continuously stabilized tacrine through π – π stacking interactions. The localized flexibility observed in peripheral regions therefore had little influence on the structural integrity of the binding cavity. RMSF profile indicates that although flexible loop and terminal regions undergo normal thermal motion, the catalytic pocket retains its structural rigidity during the simulation. The combined results of RMSD, R_g , RMSF, and interaction analyses consistently demonstrate that the tacrine-acetylcholinesterase complex remains structurally stable throughout the 100 ns simulation with ligand binding primarily maintained through persistent aromatic interactions supported by hydrophobic contacts and transient hydrogen bonding.

Conclusion

In the present study, an integrated computational approach combining density functional theory (DFT), molecular docking, and molecular dynamics (MD) simulations was employed to investigate the interaction of tacrine with human acetylcholinesterase (PDB ID: 1EVE). Geometry optimization using DFT provided the optimized ligand structure for subsequent docking and simulation studies, while molecular docking predicted a favourable binding orientation of tacrine within the catalytic gorge of the enzyme. The docking analysis revealed a binding energy of $-7.17 \text{ kcal mol}^{-1}$ with an estimated inhibition constant of $5.59 \text{ }\mu\text{M}$, indicating favourable binding affinity between tacrine and acetylcholinesterase. Interaction analysis demonstrated that ligand recognition is primarily governed by aromatic π - π stacking interactions involving residues such as Trp84, Tyr121, Phe330, and Tyr334, together with hydrophobic contacts and hydrogen-bond interactions that collectively stabilize the protein–ligand complex. The 100 ns molecular dynamics simulation further confirmed that the docked complex remained structurally stable under dynamic conditions. The RMSD, RMSF, and radius of gyration analyses showed that the overall protein structure retained its integrity throughout the simulation, while tacrine remained confined within the active-site gorge without inducing significant conformational changes in the enzyme. The persistence of key intermolecular interactions throughout the simulation supports the stability of the predicted binding mode obtained from molecular docking.

The combined computational results demonstrate that tacrine forms a stable complex with acetylcholinesterase through a cooperative network of aromatic, hydrophobic, and hydrogen-bonding interactions. These findings provide molecular-level insights into the binding mechanism of tacrine and contribute to a better understanding of ligand recognition by acetylcholinesterase. The present study also highlights the usefulness of integrated computational approaches in investigating protein–ligand interactions and provides a structural framework that may support the future design and optimization of tacrine-based acetylcholinesterase inhibitors with improved therapeutic potential for Alzheimer's disease.

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Ethics, Consent to Participate, and Consent to Publish declarations

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Compliance with Ethical Standards

The authors declare no competing financial interests.

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