

Original Research

Received 01 June 2026

Revised 21 July 2026

Accepted 03 August 2026

Natural Resources for Human
Health 2026, Vol. 6 (14s): 893–901

KEYWORDS:

HER2; L755S/T862A; Molecular
dynamics simulation;
Conformational dynamics; Kinase
domain

Computational Analysis of Structural and Conformational Dynamics of the HER2 L755S/T862A Double Mutation

Vignesh Sivaprakasam¹, Josephine Anthony^{1*}

¹Central Research Laboratory, Meenakshi Academy of Higher Education and Research (MAHER- Deemed to Be University), Chennai, 600078, Tamil Nadu, India

Corresponding Author* Dr. Josephine Anthony, M.Sc. Ph.D.,

E-mail ID: ajosephineanthony@gmail.com; josephine@maher.ac.in

ABSTRACT:

Background: Mutations that occur either by activation or acquired means in the HER2 kinase domain lead to aberrant signaling of the receptor and response in cancers caused by HER2. Double mutation in L755S/T862A is a condition that has been clinically reported, and this condition causes changes in HER2 signaling and resistance to treatment. However, the effects of such mutations are not well characterized at the molecular level.

Methods: In the current study, molecular dynamics (MD) simulations were used to explore the conformational properties of the HER2 L755S/T862A double mutants as well as the WT protein. The WT protein was subjected to a 200 ns MD simulation run, while two separate 200 ns trajectories were run for the L755S/T862A double mutants. Conformations of the systems were analyzed using the RMSD, RMSF, Rg, and SASA analysis methods.

Results: In comparison with the WT, the mutant L755S/T862A showed an increase in backbone conformation deviations from the WT, with an increase in RMSD in the latter part of the simulation period. An increase in residue level flexibility was seen for the mutant at certain parts of the protein. Higher values of Rg and SASA were seen for the mutant, reflecting a change in global compactness of the protein in addition to more solvent accessibility compared with the WT.

Conclusion: The current computational analysis elucidates the molecular structural details related to the HER2 L755S/T862A double mutation in terms of conformational changes and serves as an example for investigating this clinical mutation of HER2 protein.

INTRODUCTION

The human epidermal growth factor receptor 2 (HER2/ERBB2) is a type of transmembrane receptor tyrosine kinase that belongs to the ErbB family. HER2 is crucial in the process of regulating cellular proliferation, survival, differentiation, and tumor progression. Abnormal HER2 regulation is led by gene amplification or receptor overexpression, thus making HER2 an important oncological factor in all malignancies, including breast cancer. In addition to being caused by gene amplification, mutations in the coding region of HER2 may lead to constitutive activation of the receptor and downstream oncogenic signaling. It has to be noted that HER2 mutations may take place regardless of HER2 amplification; and they are considered clinically relevant now [1,2]. The intracellular TKD of HER2 plays a very important role as structural changes in this domain may affect the activation, substrate specificity, and even targeted therapy [1,3,4]. There have been several studies have

described the activating HER2 kinase mutations such as L755S, D769H/Y, V777L, V842I, and T862A that are known oncogenic and actionable mutations. These mutations affect structurally significant sites of the kinase domain, such as the α C-helix, activation loop, and neighboring regions. As a result, a small change in amino acids in these regions can have an effect on the conformational balance of the kinase [3]. Computational and experimental analysis of HER2 kinase revealed the existence of hot spots where an individual amino acid substitution affects the whole kinase behavior [5]. L755S is a clinical relevant mutant of HER2 kinase [6]. This mutation consists of replacing a leucine residue to serine at position 755 within the HER2 kinase domain, where a polar amino acid residue is introduced into the area responsible for kinase structure and inhibitor binding, causing changes in HER2 activity and response to therapy [6,7]. It has been found that mutation L755S changes the geometry and chemical properties of the drug binding site and influences the interaction between HER2 and various tyrosine kinase inhibitors (TKIs). Specifically, it has been shown through structural modeling that the L755S mutation can decrease the drug binding pocket volume and selectively alter inhibitor binding [8]. T862A is yet another mutation that is found to be functionally significant and present in the HER2 kinase domain. This particular residue is close to the activation loop region and has been classified as one of the recurrent HER2 mutations with transforming properties [1,5]. Studies have already proved T862A as a gain-of-function mutation, but computational analysis has categorized T862A as one of those HER2 kinase domain mutations that have the ability to affect the structure and functions of the receptor. Moreover, there is evidence of T862A from clinical sequencing research in patients suffering from HER2-mutated cancers. For instance, T862A has been identified in the clinical cohort of patients having HER2 mutation with an advanced case of breast cancer. The biological importance of such mutations is especially highlighted when the mutations arise in concert. Secondary HER2 mutations have been identified in patients where the tumor is progressing while being treated with HER2-specific therapy, and hence, the other mutations can modify the impact of a mutation already present in the cell [6,9]. Previous study showed that secondary mutations like T862A and L755S in HER2 can increase the activation of HER2 and make HER2 resistant to HER2-specific TKIs [10]. In addition, the study highlighted the role of double HER2 mutations in increasing HER2/MAPK signaling and making the cell resistant to various HER2 inhibitors. According to the computational modeling of the kinase structure, some secondary mutations could affect its conformation and inhibit the binding of inhibitors [9].

In this study, we explore the structural dynamics of the L755S/T862A double mutant. Experiments conducted on this double mutant showed increased signaling capacity and altered drug response compared to those on corresponding single mutants [9]. The molecular dynamics simulations results show that the L755S/T862A could adopt an activation-linked conformational state [4]. The researchers suggested that comprehensive conformational changes were not well represented by the collective variables used in the simulations. This observation is especially important because it indicates that the structural impact resulting from the combination of L755S/T862A mutations cannot be easily deduced from their separate effects. While experimental and computational studies have provided evidence of the functional significance of HER2 mutations, further detailed analysis of mutation-induced conformational dynamics is required [5]. Recent computational studies on HER2 mutations have used these parameters to analyze mutation-induced dynamics of proteins. Although the significance of L755S/T862A in a clinical and functional sense has been demonstrated, the effects of this double mutation on the structure of the protein need to be further elucidated, especially with regard to the conformations of the protein both globally and locally. It is important to note that the properties of the double mutant cannot be regarded as simply the sum of its component mutations because the mutations are located in different parts of the structure and thus can affect each other through conformational changes over distance [7,8]. Consequently, a description of the dynamic behavior of the complete mutant L755S/T862A as compared to the WT protein would yield more understanding of the effect that this particular mutation has on HER2 [11].

As a result, the present study used 200 ns MD simulations to comparatively analyze the structural and dynamical properties of WT HER2 and the clinically important double mutant L755S/T862A. The WT HER2 served as the structural reference, while two independent MD simulations have been conducted on the mutant form to examine its structural features. Structural deviation, residue flexibility, global compactness, and solvent accessibility were assessed via the application of backbone RMSD, RMSF, radius of gyration (R_g), and solvent-accessible surface area (SASA) analyses, respectively. PCA and FEL analyses were further performed to evaluate the collective motions and conformational states. Through the integration of complementary measures of protein dynamics and structure, the current work intends to offer a molecular level description of the structural consequences arising from the L755S/T862A double mutation. Overall, this analysis was designed to provide

a structural context for the dynamic nature of the L755S/T862A variant and to supplement the known experimental data on the biological behavior of this clinically important HER2 mutation pair.

MATERIALS AND METHODS

Protein Preparation

The amino acid sequence representing the sequence of the kinase domain of the human HER2 (ERBB2) protein was obtained from the UniProt database [12] and used as input for 3D structure prediction employing the SWISS-MODEL homology-based modeling server [13]. The generated HER2 kinase domain structure was assessed for structural integrity and further used as a template for the development of mutant proteins. The L755S/T862A double mutation was engineered in the WT model using the Swiss-PdbViewer [14] by replacing LEU755 residue with SER residue (L755S) and THR862 residue with ALA residue (T862A). The double mutant was assessed and optimized structurally to reduce any steric clashes and produce an ideal conformation.

Molecular dynamics simulation

MD simulations were carried out in GROMACS [15] to analyze the structural properties of the wild-type HER2 kinase domain and L755S/T862A double mutant of HER2. After protein preparation, the prepared structures were individually embedded in a periodic simulation box, solvated with explicit water molecules, and neutralized by adding counterions. Energy minimization was performed on each system to remove any unfavorable atomic interactions and to get energetically favorable structures. After energy minimization, the minimized systems were subjected to equilibration steps using NVT and NPT ensembles to ensure that temperature, pressure, and density are stabilized. Once equilibrated, 200 ns MD simulations were run for the wild-type system along with two independent trajectories of L755S/T862A double mutant under the same simulation conditions. Trajectories obtained from simulations were then post-processed to eliminate periodic boundary effects and molecular translation and rotation before further analysis. Equilibrated trajectories were analyzed for calculating backbone RMSD, residue-specific RMSF, radius of gyration (Rg), and SASA for characterizing the mutation-induced structural dynamics of HER2.

Results

RMSD analysis

The backbone RMSD was used to analyze the conformational stability of WT and the L755S/T862A double-mutated systems during the 200 ns MD simulation. The backbone of WT was found to have relatively stable dynamics, whereby its RMSD values remained stable in a range between 0.18 and 0.25 nm during the late stage of the simulation. The L755S/T862A mutant had an increased RMSD, starting around 60-70 ns of the simulation, whereby RMSD values reached a maximum value of almost 0.40 nm before fluctuating in an elevated RMSD value. From this result, it is clear that the double-mutation of L755S/T862A resulted in increased conformational rearrangements as compared to WT. However, the RMSD of the mutant did not exhibit a continuous increasing trend at the end of the simulation, implying that the structure of the system had reached relatively stable yet different conformations. The elevated RMSD values of L755S/T862A compared to wild type imply that there were mutations-induced conformational dynamics of the protein (**Fig 1A**).

RMSF analysis

Residue-based RMSF was calculated for the analysis of residue mobility in both WT and L755S/T862A double-mutant systems over a period of 200 ns of simulations. The RMSF analysis indicated that L755S/T862A mutant had larger fluctuations than the WT system, indicating that the L755S/T862A mutant possessed more flexibility in certain regions. However, in both the systems, there was no significant variation in fluctuation in many regions; but, in the mutant, increased fluctuation was seen around regions 730-770, 790-800, and 875-890. In particular, in the terminal region (~970-980), there was more fluctuation of almost 0.45 nm in the L755S/T862A mutant when compared to lower RMSF in the WT protein. These region-specific changes in residue mobility imply that the L755S/T862A mutations affect the dynamics of certain regions of the structure without leading to an overall change in protein dynamics. The overall picture by the RMSF plot suggests that the mutation impacts the dynamics of certain regions compared to the WT structure (**Fig 1B**).

Radius of gyration analysis

The radius of gyration (Rg) analysis revealed that the WT system has values mainly confined in the range of ~1.87–1.93 nm, while the double mutation system has higher Rg values especially after ~70 ns and reached around ~1.95–2.00 nm in the late simulation period. In addition, the Rg of the mutant trajectory stayed stable in this range without any further increment in Rg value towards the end of the simulation. Therefore, it can be concluded that the higher Rg values are due to a more expanded conformational state in the case of L755S/T862A double mutant (**Fig 1C**).

Solvent Accessible Surface Area (SASA) analysis

The SASA analysis of both the WT and the L755S/T862A double mutant system indicated differential solvent access patterns across the 200ns trajectory. While the WT exhibited changes only within ~135–150 nm², the SASA dropped to around 130–135 nm² at ~105–120 ns of the simulations. On the other hand, the L755S/T862A double mutant maintained relatively higher values of SASA for most of the trajectory, particularly within ~140–150 nm², with increased fluctuations in the middle to late part of the simulation run. The high SASA level implies that the mutant protein is more solvent exposed than the WT protein, which could be attributed to changes in the overall structure of the mutant protein due to the mutations. In general, the higher SASA values for the double mutant imply greater surface accessibility and hence a less compact conformational state than WT (**Fig 1D**). Average values of all parameters has been tabulated in **Table 1**.

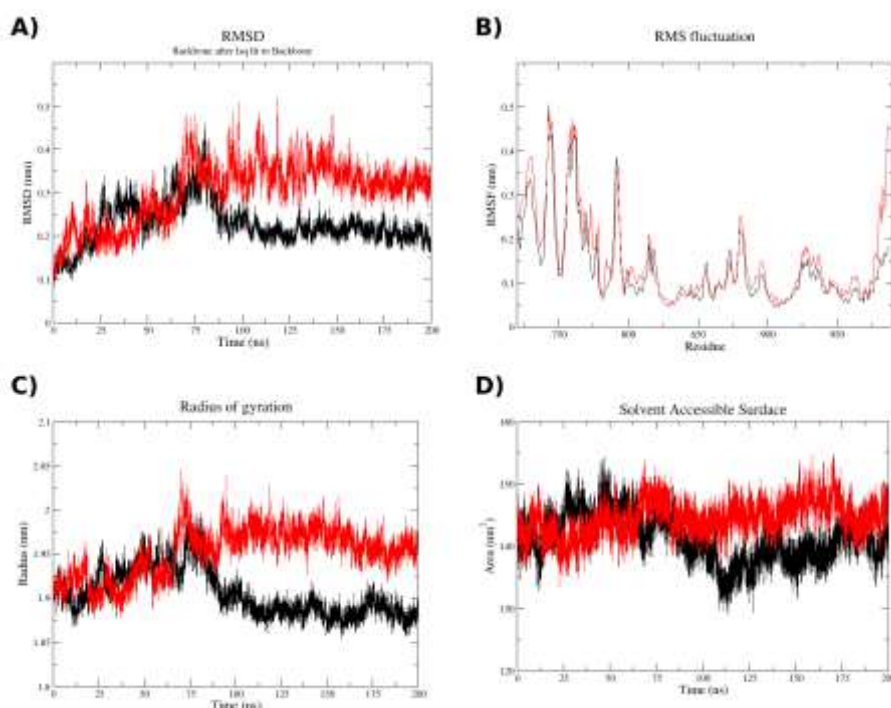


Figure 1. Molecular dynamics analysis of wild-type (WT) HER2 and L755S/T862A double-mutant HER2. (A) Backbone root mean square deviation (RMSD), (B) residue-wise root mean square fluctuation (RMSF), (C) radius of gyration (Rg), and (D) solvent-accessible surface area (SASA) calculated over the 200 ns MD simulation. Black represents WT HER2 and red represents the L755S/T862A double mutant.

MD Analysis	WT-Apo	L755S/T862A
RMSD	0.230 ± 0.046	0.305 ± 0.067
RMSF	0.167 ± 0.108	0.151 ± 0.100
Rg	1.888 ± 0.028	1.940 ± 0.029
SASA	140.50 ± 4.08	144.17 ± 2.50

Table 1. Average values (mean ± SD) of RMSD, RMSF, radius of gyration (Rg), and solvent-accessible surface area (SASA) for WT-APO and L755S/T862A HER2 systems during 200 ns molecular dynamics simulations.

PCA analysis

PCA analysis demonstrated differences in conformational sampling for the WT and L755S/T862A mutants. While the conformational space along PC1 was wider for the WT structure (~ -8 to $+4$ nm) and PC2 was also wide (~ -4 to $+7$ nm), the distribution of the conformations was almost continuous. Wide and connected distribution of the conformations implies a continuous sampling of major collective motions without separation of the conformational population. However, while the distribution along PC1 for the L755S/T862A double mutant was narrower (~ -4 to $+4$ nm) and the PC2 was also narrow (~ -6 to $+4$ nm), two major separated conformational populations were observed. Such a separated distribution implies the existence of the major collective motion sampling in separated ways. In summary, different distribution along PC1 and PC2 demonstrates a change in collective conformational dynamics in the HER2 kinase domain due to mutations (**Fig 2**).

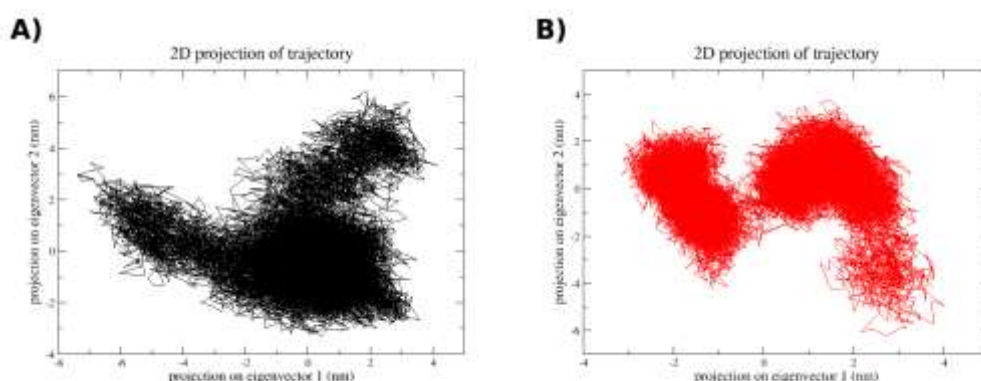


Figure 2. PCA of WT and L755S/T862A HER2. (A) WT and (B) double-mutant trajectory projections along PC1 and PC2.

Gibbs free energy landscape (FEL) analysis

Gibbs free energy landscapes were built on the basis of PC1 and PC2 for 2D plots, and these landscapes revealed different energy landscapes of conformations between the WT and L755S/T862A mutants. For the WT case (A), the landscape revealed a wide network of low energy regions, with values of free energy varying between ~ 0 and 15.2 kJ/mol. In comparison, the L755S/T862A double mutant (B) was found to have two separate low energy wells, with values of energy changing between ~ 0 and 14.7 kJ/mol. Thus, it may be concluded that the L755S/T862A mutant alters the energy landscape of HER2 kinase domains (**Fig 3**).

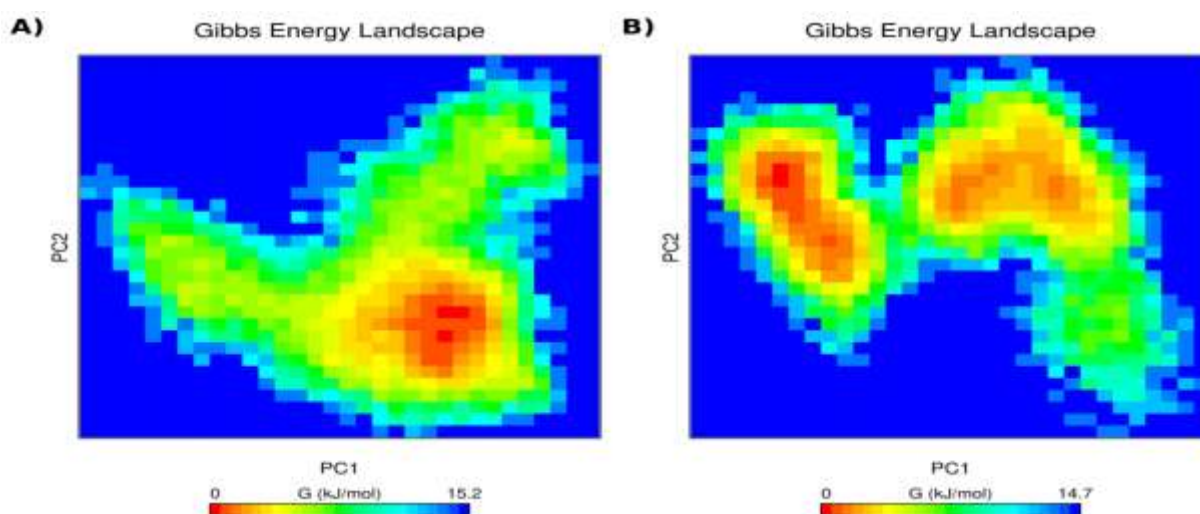


Figure 3. Gibbs free energy landscapes of WT and L755S/T862A HER2. (A) WT and (B) double-mutant conformational landscapes projected along PC1 and PC2.

DISCUSSION

Targeting HER2/ERBB2 is considered one of the most promising approaches in the treatment of breast cancer, with activating mutations of the HER2 kinase domain being able to significantly affect signaling and sensitivity of the receptors to targeted therapies [16,17]. Among those changes, L755S appears to be of great importance clinically, since this mutation was identified as the reason behind development of acquired resistance to HER2-targeted therapies [18]. Experimental evidence has shown that L755S may lead to reactivation of HER2 pathway and the development of lapatinib-based therapy resistance, whereas irreversible HER1/2 inhibitors like neratinib and afatinib would remain effective against L755S-induced cancer. This mutation has also been shown to increase downstream MAPK and PI3K/AKT/mTOR signaling, reinforcing its importance as an activating HER2 mutation related to acquired resistance. Further support on the significance of L755S/T862A mutation in combination was provided by functional and clinical studies of secondary HER2 mutations [6,19]. Indeed, Hanker et al. proved that acquired HER2 L755S and T862A mutations could be identified during HER2-targeted therapy [9]. The researchers found that although separate mutations could preserve sensitivity to neratinib in some cases, the double-mutated HER2 possessed increased signaling activity and much lower sensitivity to HER2 TKIs. Double-mutated HER2 systems with L755S/T862A mutation exhibited higher HER2/MAPK signaling and less sensitivity to neratinib, suggesting that there is biological relevance in studying of the combination of the mutation rather than each of L755S and T862A separately. Notably, the authors suggested that the secondary mutations stabilized the active form of HER2 and influenced the ligand binding, explaining the resistance phenotype [9].

In this current study, the conformational effects induced by L755S/T862A double mutant have been analyzed with the use of 200 ns MD simulation. The RMSD analysis results revealed that the WT system was able to maintain relative stable deviations in the later simulation stage while the double mutant experienced a significant increase in RMSD at the point of 60-70 ns and further fluctuations within the higher range of conformations. It means that the mutant is capable of adopting the different conformations which are more distant from its initial conformational state. Nevertheless, it is necessary to note that the higher RMSD value does not necessarily mean a greater structural damage of the protein [20]. On the contrary, the increased RMSD means more conformational change or deviation from the initial structure. Such an approach is consistent with the previous HER2-L755S MD studies where the difference in RMSD values between the WT and mutant systems was explained through the different conformational effects. RMSF analysis additionally confirmed mutations-based changes in the dynamical nature of the protein. The L755S/T862A mutant showed increased mobility in various parts of the protein, especially at regions 730-770, 790-800, 875-890, and the C-terminal region. RMSF shows the mobility of the particular residue, and hence increased RMSF values show increased flexibility and not structural damage. Increased flexibility in the mutant protein implies that the substitutions impact the dynamical nature of the regions found throughout the kinase domain. It is important to note that the function of the kinase is dependent on movements between various structural units and not on the rigidity of the protein. It was observed previously in the MD simulation of HER2-L755S mutant. Rg results provide further support on the existence of changed global architecture of the protein [8]. The wild type HER2 had an Rg value close to 1.87-1.93 nm while the L755S/T862A system exhibited increased Rg values especially towards the latter part of the simulation, amounting to about 1.95-2.00 nm. Increased Rg values mean that there is increased spread out of the protein mass compared to when the protein mass is highly compact [21]. Hence, an increase in the Rg value can support the increased RMSD and RMSF values, which suggests that the double mutation not only exhibits local fluctuations but also conformational changes. It is important to take note that the Rg value alone does not guarantee protein destabilization. In a similar way, the SASA analysis indicated a higher solvent accessibility in the double mutant. The SASA of the WT was observed to range from about 135 to 150 nm², while the mutant had comparatively higher SASA in several simulation steps. Increased SASA can be consistent with the fact that there is an exposed or an expanded structure and supports the Rg findings. In addition, in earlier MD analysis of HER2-L755S, it was found that there were mutation-induced variations in SASA and Rg, but in different directions depending on the simulation system and the conformations. That underscores that the interpretation of the MD parameters has to be done comparatively and as a whole, since a given direction cannot necessarily mean a pathogenic effect. The results from the PCA allowed for an overview of such conformational changes. The WT trajectory has displayed broad distribution along PC1 and PC2 coordinates, implying a significant number of sampling of related conformational states. On the other hand, the L755S/T862A trajectory displayed clearly separated conformational states. This observation implies that the mutations have resulted in alterations in the dominant collective movements of the protein kinase domain and lead to sampling of unique conformational states. This observation is quite significant biologically, since activation and interaction

with the inhibitor are dependent on conformational changes within the kinase domain. This was further supported by the FEL analysis. There were various energy landscapes in the WT structure while the L755S/T862A mutant had clear low energy basins. Presence of several basins implies that the mutant can adopt various conformational states which are energetically stable. This is consistent with earlier modeling studies on the effects of secondary HER2 mutations on the energetics of kinase conformations. Thus, the PCA and FEL analyses provide an explanation of the conformational changes in terms of the RMSD, RMSF, Rg and SASA. Overall, the results suggest that the L755S/T862A mutation does not induce any significant disruption in the structure of the HER2 kinase domain but leads to changes in the dynamics of its structural conformations. The higher RMSD, RMSF, Rg and SASA values, along with the changes in PCA and FEL landscapes, all point to a structurally more dynamic and conformational heterogeneous state for the mutant kinase domain. This interpretation is consistent with experiments that show the enhanced activity of the HER2 kinase domain which may lead to resistance toward HER2 TKIs due to the L755S/T862A mutation.

In summary, the current computation results offer an insight into the structurally dynamic nature of the biologically important L755S/T862A mutation. Although the present computations of the ligand-free systems are not able to measure the inhibitor binding affinity or experimentally confirm drug resistance, they show that the double mutation has a dramatic effect on the conformational dynamics of the HER2 receptor. This might help to understand why mutant HER2 tends to adopt signaling-competent states and why certain mutations like L755S and T862A are responsible for resistance to therapy. Further investigations involving mutant L755S and T862A alone and including ligand docking simulations are desirable to disentangle the role of each particular mutation.

CONCLUSION

The double mutation L755S/T862A has a significant effect on the inherent dynamics of the HER2 kinase domain compared to the WT. The L755S/T862A mutation was characterized by an increase in backbone RMSD and fluctuations at the residue level, radius of gyration, and solvent-accessible surface area, implying an increase in conformational flexibility and protein expansion. In addition, PCA showed a change in collective motions and conformational states and FEL showed that this mutation leads to a new energy landscape of the protein, with a presence of several energy wells. Overall, the results imply that L755S/T862A mutation destabilizes the HER2 kinase fold and changes its conformational heterogeneity. Considering the role of L755S mutation and other secondary mutations in the HER2 gene associated with changes in the kinase activity and drug resistance, these results present the molecular mechanism underlying this clinically significant double mutation. This study shows the significance of considering secondary mutations of the HER2 kinase and is the first step for further simulation of the protein-ligand interaction and experimental validation.

Acknowledgements:

The authors thank the Management of Meenakshi Academy of Higher Education and Research, Chennai, India, for the support provided in preparing this article.

Funding Declaration: The authors declare that there is no funding for this article.

Conflict of Interest: The authors declare that there are no conflicts of interest regarding the content of this article.

Author's contribution:

VS-Conceptualization, original draft writing and data analysis

JA-Editing and Reviewing

REFERENCE:

- [1] Bon G, Di Lisa FS, Filomeno L, Arcuri T, Krasniqi E, Pizzuti L, et al. *HER2* mutation as an emerging target in advanced breast cancer. *Cancer Sci*. 2024;115:2147–58. <https://doi.org/10.1111/cas.16148>
- [2] Galogre M, Rodin D, Pyatnitskiy M, Mackelprang M, Koman I. A review of *HER2* overexpression and somatic mutations in cancers. *Crit Rev Oncol Hematol*. 2023;186:103997. <https://doi.org/10.1016/j.critrevonc.2023.103997>
- [3] Bose R, Kavuri SM, Searleman AC, Shen W, Shen D, Koboldt DC, et al. Activating *HER2* Mutations in *HER2* Gene Amplification Negative Breast Cancer. *Cancer Discov*. 2013;3:224–37. <https://doi.org/10.1158/2159-8290.CD-12-0349>

- [4] Giacoletto CJ, Valente LJ, Brown L, Patterson S, Gokhale R, Mockus SM, et al. New Gain-of-Function Mutations Prioritize Mechanisms of HER2 Activation [Internet]. *Genetic and Genomic Medicine*; 2025 [cited 2026 Sept 10]. <https://doi.org/10.1101/2025.03.03.25323043>
- [5] Ishiyama N, O'Connor M, Salomatov A, Romashko D, Thakur S, Menten A, et al. Computational and Functional Analyses of HER2 Mutations Reveal Allosteric Activation Mechanisms and Altered Pharmacologic Effects. *Cancer Res.* 2023;83:1531–42. <https://doi.org/10.1158/0008-5472.CAN-21-0940>
- [6] Xu X, De Angelis C, Burke KA, Nardone A, Hu H, Qin L, et al. HER2 Reactivation through Acquisition of the HER2 L755S Mutation as a Mechanism of Acquired Resistance to HER2-targeted Therapy in HER2+ Breast Cancer. *Clin Cancer Res.* 2017;23:5123–34. <https://doi.org/10.1158/1078-0432.CCR-16-2191>
- [7] Verma S, Goyal S, Kumari A, Singh A, Jamal S, Grover A. Structural investigations on mechanism of lapatinib resistance caused by HER-2 mutants. Sapino A, editor. *PLOS ONE.* 2018;13:e0190942. <https://doi.org/10.1371/journal.pone.0190942>
- [8] Loganathan T, George Priya Doss C. Computational molecular insights into ibrutinib as a potent inhibitor of HER2-L755S mutant in breast cancer: gene expression studies, virtual screening, docking, and molecular dynamics analysis. *Front Mol Biosci.* 2025;12:1510896. <https://doi.org/10.3389/fmolb.2025.1510896>
- [9] Marín A, Mamun AA, Patel H, Akamatsu H, Ye D, Sudhan DR, et al. Acquired Secondary HER2 Mutations Enhance HER2/MAPK Signaling and Promote Resistance to HER2 Kinase Inhibition in Breast Cancer. *Cancer Res. American Association for Cancer Research (AACR)*; 2023;83:3145–58. <https://doi.org/10.1158/0008-5472.can-22-3617>
- [10] Kalra R, Chen CH, Wang J, Salam AB, Dobrolecki LE, Lewis A, et al. Pozotinib Inhibits HER2-Mutant–Driven Therapeutic Resistance and Multiorgan Metastasis in Breast Cancer. *Cancer Res.* 2022;82:2928–39. <https://doi.org/10.1158/0008-5472.CAN-21-3106>
- [11] Zabransky DJ, Yankaskas CL, Cochran RL, Wong HY, Croessmann S, Chu D, et al. *HER2* missense mutations have distinct effects on oncogenic signaling and migration. *Proc Natl Acad Sci [Internet]*. 2015 [cited 2026 Sept 17];112. <https://doi.org/10.1073/pnas.1516853112>
- [12] The UniProt Consortium, Bateman A, Martin M-J, Orchard S, Magrane M, Adesina A, et al. UniProt: the Universal Protein Knowledgebase in 2025. *Nucleic Acids Res.* 2025;53:D609–17. <https://doi.org/10.1093/nar/gkae1010>
- [13] Waterhouse A, Bertoni M, Bienert S, Studer G, Tauriello G, Gumienny R, et al. SWISS-MODEL: homology modelling of protein structures and complexes. *Nucleic Acids Res.* 2018;46:W296–303. <https://doi.org/10.1093/nar/gky427>
- [14] Guex N, Peitsch MC. SWISS-MODEL and the Swiss-Pdb Viewer: An environment for comparative protein modeling. *ELECTROPHORESIS.* 1997;18:2714–23. <https://doi.org/10.1002/elps.1150181505>
- [15] Abraham MJ, Murtola T, Schulz R, Páll S, Smith JC, Hess B, et al. GROMACS: High performance molecular simulations through multi-level parallelism from laptops to supercomputers. *SoftwareX.* 2015;1–2:19–25. <https://doi.org/10.1016/j.softx.2015.06.001>
- [16] Zhu W, Lu X. Recent advances in HER2-targeted inhibitors for cancer therapy. *Pharm Sci Adv.* 2026;4:100127. <https://doi.org/10.1016/j.pscia.2026.100127>
- [17] Swain SM, Shastry M, Hamilton E. Targeting HER2-positive breast cancer: advances and future directions. *Nat Rev Drug Discov.* 2023;22:101–26. <https://doi.org/10.1038/s41573-022-00579-0>
- [18] Hyman DM, Piha-Paul SA, Won H, Rodon J, Saura C, Shapiro GI, et al. HER kinase inhibition in patients with HER2- and HER3-mutant cancers. *Nature.* 2018;554:189–94. <https://doi.org/10.1038/nature25475>
- [19] Wu Z, Wang J, You F, Li X, Xiao C. The role of irreversible pan-HER tyrosine kinase inhibitors in the treatment of HER2-Positive metastatic breast cancer. *Front Pharmacol.* 2023;14:1142087. <https://doi.org/10.3389/fphar.2023.1142087>

- [20] Grossfield A, Patrone PN, Roe DR, Schultz AJ, Siderius D, Zuckerman DM. Best Practices for Quantification of Uncertainty and Sampling Quality in Molecular Simulations [Article v1.0]. Living J Comput Mol Sci [Internet]. 2019 [cited 2026 Sept 17];1. <https://doi.org/10.33011/livecoms.1.1.5067>
- [21] Lobanov MYu, Bogatyreva NS, Galzitskaya OV. Radius of gyration as an indicator of protein structure compactness. Mol Biol. 2008;42:623–8. <https://doi.org/10.1134/S0026893308040195>