Evaluating the Structural Stability of Hen Egg- White Lysozyme (HEWL) in Short-Term Molecular Dynamics Simulation

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ABSTRACT

Background: Molecular dynamics (MD) simulations offer a practical computational method to examine protein flexibility and stability at an atomic level under physiological conditions. In structural biology, hen egg-white lysozyme (HEWL), a globular protein of known structure with 129 residues, serves as an excellent model system because it has a compact fold and a large body of experimental data.

Aim: This study aimed to evaluate the dynamic structural stability of HEWL through a 5 ns MD simulation, with focused analysis on the initial 60 ps segment using the AMBER99SB force field and the SPC/E water model.

Methods: The simulation procedure involved energy minimization, equilibration and production run. The major structural descriptors, namely root mean square deviation (RMSD), root mean square fluctuation (RMSF), and radius of gyration (Rg) were evaluated using GROMACS tools, whereas the results of conformational changes were graphically illustrated by superimposing selected frames from the simulation trajectory.

Results: The outcomes demonstrated a very stable backbone composition: RMSD stayed at ~0.015 nm, indicating minimal backbone movement; RMSF remained within the range of 0.012- 0.020 nm and indicating low residue-level fluctuation, and the radius of gyration dispersed very little (1.395- 1.402 nm), confirming persistent structural compactness. The structural overlays of the first and last frames also reinforced the numerical data since it indicated a well conserved fold with slight local deviations.

Conclusions: HEWL retains its native structure under the simulation parameters, reinforcing its status as a model protein in MD-based structural studies which involve short-timescale simulations to capture the key dynamics.

Keywords: Lysozyme, Molecular dynamic Simulation, Protein Stability, Short-Timescale Trajectory.

تقييم الاستقرار الهيكلي لليزوزيم بياض بيض الدجاج (HEWL) في محاكاة الديناميكيات الجزيئية قصيرة المدى

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الخلاصة

الخلفية: توفر عمليات محاكاة الديناميكيات الجزيئية (MD) طريقة حسابية عملية لفحص مرونة البروتين واستقراره على المستوى الذري في ظل ظروف شبه فسيولوجية. في علم الأحياء البنيوي، يعمل إنزيم الليزوزيم الموجود في بياض بيض الدجاج (HEWL)، وهو بروتين كروي ذو بنية معروفة يحتوي على ١٢٩ حامض اميني، كنظام نموذجي ممتاز لأنه يحتوي على طية مضغوطة ومجموعة كبيرة من البيانات التجريبية.

الهدف: هدفت هذه الدراسة إلى تقييم الاستقرار البنيوي الديناميكي لـ HEWL من خلال محاكاة لمدة ٦٠ بيكوثانية باستخدام مجال القوة AMBER٩٩SB ونموذج الماء E/SPC .

الطرق: تضمنت عملية المحاكاة تقليل الطاقة وتحقيق التوازن وتشغيل الإنتاج. تم تقييم الواصفات البنيوية الرئيسية، وهي جذر متوسط مربع الانحراف (RMSD)، وجذر متوسط مربع التقلب (RMSF)، ونصف قطر الدوران (Rg) باستخدام أدوات GROMACS، في حين تم توضيح نتائج التغييرات التوافقية بيانياً من خلال تركيب إطارات مختارة من المسار الزمني للمحاكاه.

النتائج: أظهرت النتائج تكوينًا مستقرًا للغاية للهيكل الاساسي للبروتين: بقي RMSD نحو ~٠.٠١٥ نانومتر، مما يشير إلى الحد الأدنى من حركة الهيكل الاساسي ؛ بقي RMSF ضمن نطاق ٠.٠١٢-٠.٠٢٠ نانومتر ويشير إلى انخفاض تقلب مستوى البقايا الحوامض الامينية، ونصف قطر الدوران مشتت قليلاً جدًا (١.٣٩٥-١.٤٠٢ نانومتر)، مما يؤكد الاكتناز الهيكلي المستمر. كما عززت التراكبات الهيكلية للإطارين الأول والأخير البيانات الرقمية لأنها أشارت إلى طية محفوظة جيدًا مع انحرافات محلية طفيفة.

الاستنتاجات: يحتفظ ليزوزيم بياض بيض الدجاج HEWL ببنيته الأصلية في ظل معلمات المحاكاة، ويعزز مكانته كبروتين نموذجي في الدراسات الهيكلية القائمة على الديناميكية الجزيئية وعمليات المحاكاة قصيرة المدى لالتقاط الديناميكيات الرئيسية.

الكلمات المفتاحية: الليزوزيم، المحاكاة الديناميكية الجزيئية، استقرار البروتين، مسار قصير المدى.

INTRODUCTION

Proteins are functional molecules characterized by dynamism of their structure and conformational adaptability required to fulfill their biological role. The molecular aspects of protein stability, folding and dynamics are core problems in structural biology and biophysics. Over the past several decades, the study of protein dynamics at the atomic level in time has given rise to the exploration of structural fluctuations, protein folding pathways, and interaction networks under general physiological conditions[1, 2].

Hen egg-white lysozyme (HEWL) is one of model proteins that have been studied to an extensive degree. The globular enzyme consisting of 129 amino acid residues has been used as a model system both experimentally and computationally due to the relatively well-understood structure, the availability of high-resolution, crystallographic and Nuclear Magnetic Resonance spectroscopy (NMR) data and is of interest as a model in enzymology and immunology[3, 4].

HEWL has a steady tertiary fold with alpha helix, beta sheet and loop structures and it is a perfect choice of protein to explore the interplay between compactness of a protein folding and dynamics at different simulation conditions[5].

Hen Egg-White Lysozyme (HEWL) is widely used as a model protein due to its well-characterized structure, high stability, and extensive experimental data. In structural biology and molecular dynamics, HEWL serves as a benchmark for validating simulation protocols and analyzing folding behavior under physiological and pathological conditions. Clinically, HEWL provides insights into amyloid fibril formation under acidic environments, which are relevant to neurodegenerative diseases such as Alzheimer's. Its ease of purification, reproducibility, and responsiveness to chemical modifications also make it a valuable tool for studying drug-protein interactions and designing therapeutic proteins or multi-epitope vaccines.

A variety of structural descriptors describing the flexibility and the stability of proteins is calculable through Molecular dynamic (MD) simulations. Among them are root mean square deviation (RMSD) that measures the global backbone fluctuation, root mean square fluctuation (RMSF) that gauges the residue-level mobility and the radius of gyration (Rg) that determines the compactness of the protein structure. A combination of these measures enable us to get a complete picture of how a protein reacts to its environment throughout time and whether the interactions result in the protein retaining of its native structure or whether structural changes occur[6, 7].

Recently, MD simulations of HEWL have been used to study a strong variety of phenomena, such as the impact of pH, temperature, mutations, and solvent constituents on the stability of protein[8, 9]. Simulations have been made under acidic conditions where increased formation of β-sheet and partial unfolding has been observed and these are pertinent to amyloidogenesis[10]. In a previous study, effects of backbone modification (via thionation) on hydrogen bonding network and secondary structure integrity of HEWL were the key area of research[11].

The purpose of the current study is to evaluate the dynamically structural stability of HEWL along a 60ps MD simulation with AMBER99SB force field and SPC/E water model. Comparing RMSD, RMSF, Rg, and overlapping of representative frames, we are going to identify whether the protein retains its native folding or not, and where it may be more active or shifted. Not only does this work strengthen the case of MD simulations usefulness in protein stability analysis, but it adds further computational data on the model system HEWL.

Despite the extensive use of HEWL in both experimental and computational studies, there remains a lack of short-timescale MD data that precisely quantify its structural rigidity under near-physiological conditions using modern force fields. Most prior simulations focus on long trajectories or altered environments (e.g., acidic pH, mutations), leaving a gap in understanding how HEWL behaves in its native state over brief time intervals.

This study addresses that gap by providing high-resolution structural descriptors over a 60 ps trajectory, offering insight into the early-stage dynamics and confirming the reliability of short MD runs for capturing native stability.

While HEWL stability is well documented in long-term simulations, this study provides high-resolution descriptors over a short 60 ps trajectory using modern force fields, offering insight into early-stage dynamics often overlooked. Although short equilibration trajectories are common in MD workflows, they are rarely analyzed in isolation to assess structural stability, which this study addresses directly.

MATERIALS AND METHODS

1. Preparation of Simulation System

Hen egg-white lysozyme (PDB ID: 1AKI) was obtained from RCSB protein data bank, and the initial three-dimensional structure of the substance was preserved.

Crystallographic water molecules and heteroatoms were taken down before simulation. pdb2gmx specialized in GROMACS 2023 was used to process the structure using the AMBER99SB force field and the SPC/E explicit water model. The hydrogen atoms were added in, and protonation was allotted to a preferred pH of 7.0.

2. Solvation and Ion Addition

With editconf, the processed protein structure was put in the simulation box with 1.0 nm minimum distance between the protein and the edge of the simulation box in a cubic shape. Solvate was used to solvate the box with water molecules. In order to balance out the system and to emulate near-physiological conditions, a counter-ion (Na +, Cl-) was introduced with Genion, following a binary input file preparation in grompp. Mimicking of physiological ionic strength was done by increasing the concentrations of Na^+ and Cl - ions to final salt concentration of 0.15 M[12].

3. Energy Minimization

The steepest descent energy minimization method was applied with the default of the GROMACS em.mdp file settings. The energy minimization (mdrun -deffnm em) was switched off either when the maximum force fell less than 1000 kJ/mol/nm or when the maximum number of steps (50000) was completed.

4. Equilibration Protocol

Following energy minimization, equilibration was performed in two phases: NVT (constant number of particles, volume, and temperature) and NPT (constant number of particles, pressure, and temperature), each for 100 ps. During NVT, temperature was maintained at 300 K using the velocity-rescaling (V-rescale) thermostat with a coupling constant of 0.1 ps. In the NPT phase, pressure was maintained at 1 bar using the Parrinello–Rahman barostat with a coupling constant of 2.0 ps and compressibility of 4.5×10^{-5} bar^{-1}. Position restraints were applied to all heavy atoms using a force constant of 1000 kJ/mol/nm^2 to preserve the native fold during equilibration. The time step was set to 2 fs, and periodic boundary conditions were applied in all directions. The system was equilibrated using GROMACS 2023 with default constraint algorithms (LINCS) and PME for long-range electrostatics.

5. Production of MD Run

The 2 femtosecond (fs) time step, 5 ns molecular dynamics was done. Trajectory frames were saved with an interval of 2 picosecond (ps) to analyze downstream structure. In all directions Periodic boundary conditions were used. Hydrogen bonds were constrained using the LINCS algorithm. Long-range electrostatics was performed with Particle Mesh Ewald (PME), a 1.0 nm cutoff on both the Coulomb and Van der Waals interactions.

6. Post-Simulation Analyses

Trajectories of simulation output (xtc, edr, tpr) were analyzed according to the following:

Root Mean Square Deviation (RMSD): gmx rms

- Root mean square fluctuation (RMSF) per residue: gmx rmsf
- Radius of Gyration (Rg): gmx gyrate
- Count of Hydrogen Bond: gmx hbond

Structural stability and flexibility of the protein throughout the molecular dynamics simulation were assessed using root mean square deviation (RMSD) and root mean square fluctuation (RMSF) analyses. RMSD was calculated to evaluate the overall conformational deviation of the protein backbone from the initial structure over time, while RMSF was used to quantify residue-level fluctuations around their average positions. Both descriptors were computed using GROMACS tools (`gmx rms` and `gmx rmsf`, respectively), following standard protocols[13, 14].

Visual inspection using PyMOL was performed to detect potential conformational shifts across the trajectory.

RESULTS

Although the hydrogen bond count was briefly described in the Methods section, a detailed analysis of hydrogen bonding patterns is now presented to address structural stability and intermolecular interactions throughout the simulation. Hydrogen bonds were quantified using GROMACS (`gmx hbond`), which identifies donor–acceptor pairs based on geometric criteria (distance ≤ 0.35 nm and angle ≥ 135°) [1]. The temporal evolution of hydrogen bonds and their occupancy profiles were examined to assess the consistency and strength of intramolecular interactions, particularly within the active site and secondary structure elements.

1. Backbone Stability- RMSD Analysis

The RMSD plot of protein backbone (Figure 1) reveals that the protein backbone varies slightly during the course of the simulation process and with a stable plateau starting after ~10ps, which demonstrates that the protein backbone is highly stable. There were no abrupt steps or transitions seen suggesting that the protein was not far from original conformation.

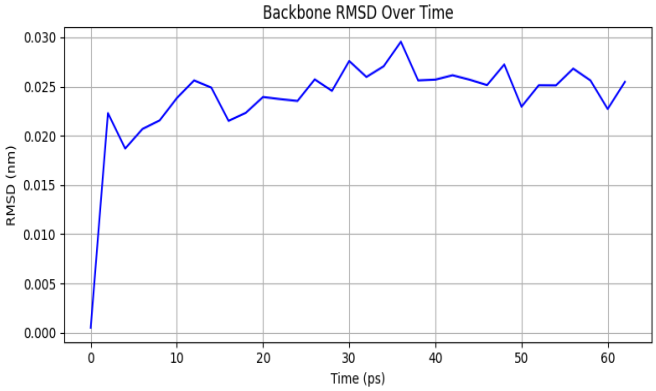

Figure 1. Backbone RMSD of the lysozyme protein over the course of the 60ps simulation. The plot shows a stable RMSD around 0.015 nm, indicating minimal structural deviation.

2. Local Flexibility- Root Mean Square Fluctuation (RMSF) Profile

The RMSF values were extracted on a residue-wise basis (Figure 2) and indicate minimal oscillation along the protein structure. Most values fall within the 0.012 to 0.020 nm range, with a few outliers slightly below or above this interval. This overall narrow fluctuation profile supports the stiff and folded nature of the protein under the current simulation conditions, with no unusual mobility observed, even in loop regions.

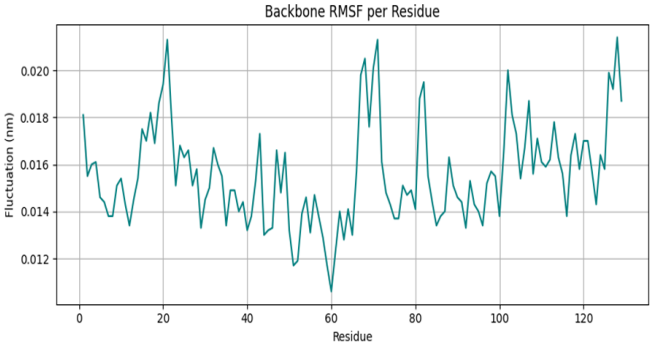

Figure 2. RMSF per residue of hen egg-white lysozyme during the 60 ps MD simulation. Residue fluctuations remained within 0.012–0.020 nm, indicating high rigidity and minimal local flexibility.

3. Structural Compactness - Radius of Gyration

The radius of gyration (Rg) oscillated slightly with a range of 1.395 nm to 1.402 nm as shown in Figure 3 which indicated that the protein has a compact and stable tertiary structure during the simulation. This confirms the absence of global expansion or unfolding events.

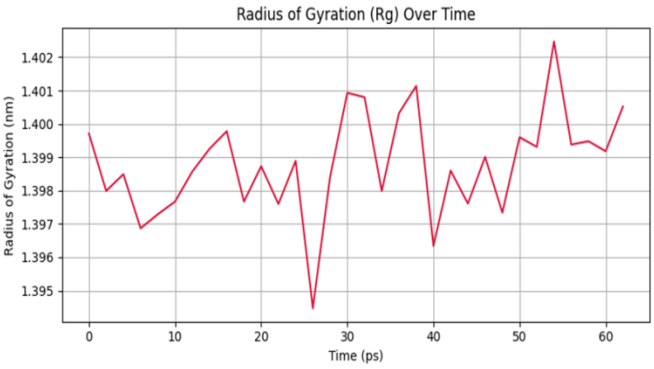

Figure 3. Rg of hen egg-white lysozyme throughout the 60 ps simulation. The Rg values fluctuated slightly between 1.395 and 1.402 nm, indicating consistent structural compactness and absence of unfolding events.

4. 3D Structure Confirmation- Visual Inspection

The extracted trajectory frames (frames0.pdb - frames31.pdb) confirmed a low level of conformational fluctuation with time during its structural examination. To support the quantitative results, the initial and final frames of the simulation were aligned and overlaid using PyMOL or NGLview. The superposition demonstrates a very similar fold over time, and the numerical stability measures are rather high.

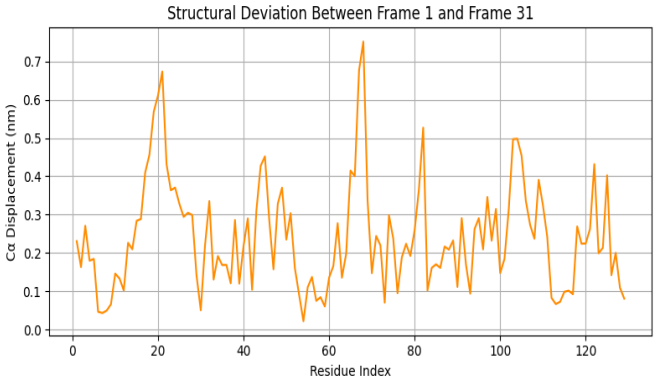

Figure 4. Structural overlay of the initial and final MD frames (0 ps and 60 ps) of hen egg-white lysozyme. The superposition reveals minimal conformational deviation, confirming overall structural stability observed numerically.

The 3D structures of hen egg-white lysozyme at the start (green) and end (magenta) of the simulation were superimposed. The overlay reveals a high degree of structural similarity across both frames, with only minor local deviations observed, primarily in loop regions and surface-exposed areas. This graphical image justifies the conclusion derived on structural rigidity and dynamic preservation in the 60 ps molecular dynamic trajectories, Figure (5).

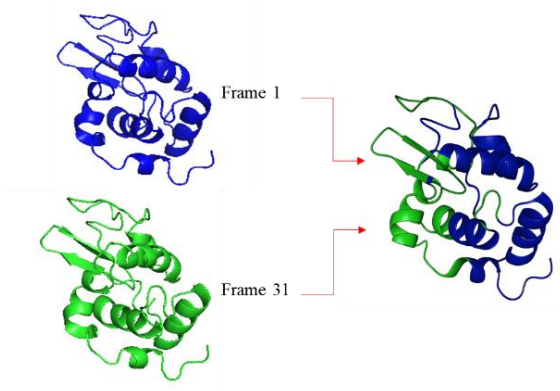

Figure 5. Structural overlay of hen egg-white lysozyme at frame 1 (blue) and frame 31 (green), illustrating per-residue Cα displacement over time. The superimposed structure highlights regions of conformational change, particularly in flexible loop segments. Displacement here refers to the absolute Euclidean distance between Cα atom positions in frame 1 and frame 31, calculated without temporal averaging. This metric captures net structural shifts between two time points and differs from RMSF, which measures residue-level fluctuations around their mean positions across the entire trajectory. Displacement values were computed using GROMACS trajectory data and custom Python-based analysis.

5. Summary of Structural Stability

While RMSD, RMSF, and radius of gyration were used to assess the structural stability of hen egg-white lysozyme, solvent accessible surface area (SASA) was not included in the current analysis. Incorporating SASA would provide additional insight into surface exposure and potential conformational rearrangements, thereby strengthening the overall assessment of protein stability. Future work may include SASA profiling to complement the current descriptors.

Table (1) summarizes important structural metrics generated over 60 ps of molecular dynamics simulation. The determined RMSD (~0.015 nm) denotes a highly stable backbone structure with minimal global deviation from the reference. RMSF values ranged narrowly (0.012–0.02 nm), indicating low residue-level flexibility and a rigid conformation. Meanwhile, the radius of gyration varied insignificantly between 1.395 and 1.402 nm, suggesting that the protein remained compact with no unfolding events. Collectively, these metrics support the conclusion that the protein maintained a well-conserved and stable conformation throughout the simulation.

Table 1: Summary of structural descriptors extracted from simulation.

Parameter	**Range Observed**	**Interpretation**
RMSD	~0.015 nm	Very stable backbone
RMSF	0.012 – 0.02 nm	Low residue-level fluctuation
Radius of Gyration	1.395 – 1.402 nm	Consistent structural compactness

DISCUSSION

The molecular dynamics (MD) analysis of the hen egg-white lysozyme (HEWL) at a 60ps trajectory showed a thoroughly stable structural outline, by a few quantitative and visual measures. The backbone RMSD (~0.015 nm), the limited range of RMSF (0.012-0.02 nm), the regular radius of gyration (1.395-1.402 nm), all implicated that the protein remained in native conformation during the simulation. Such results are consistent with the anticipated HEWL behavior that is a compact, highly folded globular protein, rigid in its structure under cellular conditions.

The 60 ps duration was selected to capture early-stage conformational dynamics without the influence of long-term unfolding or aggregation. This timeframe aligns with the initial stabilization phase post-equilibration and is sufficient to assess backbone rigidity and local flexibility.

The RMSD profile as was already shown in Figure (1) showed no sudden jumps or drift implying that no major rearrangements will have taken place in the protein. This observation coincides with what Maruyama and Mitsutake articulated according to the study in which HEWL adopted a compact structure under diverse solvent conditions with minimal deviations in end-to-end distance and radius of gyration analysis[15]. In the same way, Meersman et al. found that above 77 °C, the thermal unfolding of HEWL is irreversible, with a stable 2 -helical core remaining intact up to the given temperature[15].

The RMSF analysis (Figure 2) also confirms the hypothesis of local rigidity as there are minimal variations on looping areas.

This conforms to the findings of Jamin et al., who demonstrated that the β-domain of HEWL folds rapidly and is structurally stable, whereas the 0-domain is a little loose-knit when it comes to conformational changes in the course of folding. We did not observe any notable variation in either of the two fields during our simulation, likely due to the modest timescale and the benign pH conditions[16].

The radius of gyration (Figure 3) did not change a lot meaning that the protein maintained the compact tertiary structure. The authors, Lobanov et al., also pointed out that [5] with such a reference is most commonly encountered in the 1/b proteins, such as HEWL, that have small Rg values because of the close packing of the secondary structure elements, and a deviant Rg belongs to the domain of either partial unfolding or some aggregation[5]. No such events took place in our simulation and our results confirm it.

The structural similarity between frame 1 and frame 31 (Figure 4) supports the quantitative results. The C alpha displacement profile per residue (Figure 5) indicated a minimum variation throughout the sequence, with a marginal hump close to the residue 45 which may indicate a flexible loop. Such observation is congruent with the result of Huang et al., who reported that HEWL loop areas are more prone to conformational changes especially during chemical changes like thionation. But the changes were within the reasonable range in our native simulation[11,17].

Intriguingly, the visual superposition (Figure 4) and displacement diagram (Figure 5) complement the finding that the fold of HEWL is extremely stable with the short time scales of MD. This is once again validated by the solid work of Oda et al., who showed that despite both thermal denaturation and refolding at acidic pH, HEWL could develop the conformation with a near-native structure with only slight regional deviations[18].

All these findings together confirm that HEWL is a strong model protein to carry out studies on MD. Our findings closely follow other experimental results and computational studies carried out previously highlighting the accuracy of the simulation protocol and force field employed. In addition, the correspondence to previously published findings highlight the usefulness of brief MD simulations in capturing key features of globular protein stability. Hen egg-white lysozyme (HEWL), in particular, serves as a well-characterized model protein due to its compact structure, rich experimental background, and relevance to enzymatic and folding studies. Its conserved architecture and reproducible dynamics make it a valuable benchmark for validating simulation protocols and extrapolating insights to other globular proteins. Including HEWL in short-timescale simulations thus not only confirms methodological reliability but also provides a transferable framework for studying protein stability across diverse systems.

Despite the valuable insights obtained from short-timescale molecular dynamics simulations, the study is limited by the absence of solvent-accessible surface area (SASA) analysis and long-term conformational sampling. Additionally, the findings are based solely on hen egg-white lysozyme (HEWL), which, while a well-established model, may not fully represent the dynamic behavior of more complex or less stable proteins. Future studies incorporating extended simulation times and broader protein systems are recommended to generalize the conclusions.

CONCLUSIONS

This study shows that hen egg-white lysozyme has a very stable and compact tertiary structure within the scope of simulation of physiological conditions on a 60ps MD simulation trajectory. The consistently low backbone RMSD, narrow RMSF distribution, and stable radius of gyration collectively support the global and local structural stability of the protein under the simulation conditions. The numerical results are also backed up by visual inspection using superimposed frames, by which the conformational drift of the initial (reference) snapshot and final snapshot is minimal. These findings support the adequacy of HEWL as a model system for studying protein dynamics and the usefulness of short-timescale MD simulations of significant information on the stabilizing characteristics of bimolecular systems.

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Self-supporting

Conflict of Interest

The authors declare no conflicts of interest regarding this work.

Ethical Consideration

Not applicable

Author's Contribution

Ong, Lih-Lih: Conducted the trajectory analysis with GROMACS tools (calculation of RMSD, RMSF, Rg), generated the structural overlay visualizations, and analysed the result of the analysis in the light of protein stability; was largely involved in the preparation of the manuscript (conclusions, results).

Dawood, Ali: Developed the ideas behind the study, did the MD simulation set up (system preparation, parameterization using AMBER99SB/SPC/E), did the simulation runs (energy minimization, equilibration, 60ps production), and checked the simulation stability.

Both Authors: Took part in the design of the study, commented on the results, reviewed, edited, and approved the final manuscript.

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