International Journal of Zoology and Applied Biosciences
Volume 10, Issue 6, pp: 443-446, 2025 (Special Issue)
https://doi.org/10.55126/ijzab.2025.v10.i06.SP088
ISSN: 2455-9571
http://www.ijzab.com
Research Article

RMSD-BASED MOLECULAR DYNAMICS INVESTIGATION OF PARKINSON'S DISEASE THERAPEUTIC TARGETS USING GROMACS

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Article History: Received 25th September 2025; Accepted 22nd November 2025; Published 1st December 2025

ABSTRACT

Understanding conformational stability and structural deviations of Parkinson's disease (PD)–related proteins is essential for rational drug design. We performed atomistic molecular dynamics (MD) simulations using GROMACS to investigate the conformational dynamics of two therapeutically relevant PD proteins α-synuclein and the kinase domain of LRRK2. Simulations (200 ns × triplicate for each system) were analyzed by root-mean-square deviation (RMSD) and complementary metrics (RMSF, radius of gyration, secondary structure evolution). RMSD trajectories revealed distinct stability profiles: LRRK2 kinase domain stabilized within ~20-50 ns showing RMSD plateaus ~1.5–2.5 Å, while α-synuclein showed higher RMSD fluctuations consistent with its intrinsically disordered nature. These results provide structural insight into dynamic regions that could be targeted in drug discovery pipelines. The manuscript includes full methods, sample GROMACS commands, and recommendations for reproducible RMSD analyses in PD protein MD studies.

Keywords: Parkinson's disease, α-synuclein, LRRK2, molecular dynamics, RMSD, GROMACS, Protein dynamics.

INTRODUCTION

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by motor and non-motor deficits; protein misfolding and aggregation, especially of α-synuclein (α-syn), are central to PD pathology. α-syn is intrinsically disordered and samples a diverse conformational ensemble, complicating structural characterization and therapeutic targeting. Another genetically linked PD protein, leucine-rich repeat kinase 2 (LRRK2), has disease-associated mutations and a kinase domain that is a prominent drug target. Structural studies of LRRK2 (cryo-EM) and biophysical characterization of α-syn inform MD simulations that probe conformational dynamics for drug discovery. MD simulations with GROMACS remain a standard, efficient approach to study atomic-level protein motions and compute RMSD as a principal metric for structural deviation over time. Perform atomistic MD simulations (GROMACS) of α-synuclein and the catalytic/kinase portion of LRRK2 to examine conformational dynamics. Compute and compare RMSD profiles to identify stable and flexible regions. Provide reproducible GROMACS workflows and interpretation guidelines for RMSD analysis relevant to PD therapeutic targeting.

Parkinson's disease (PD) is a progressive neurodegenerative disorder in which aberrant protein conformations and aggregation play central pathogenic roles. Two proteins have emerged repeatedly as mechanistic and therapeutic foci: α-synuclein, an intrinsically disordered protein whose misfolding and aggregation underlie Lewy body formation, and leucine-rich repeat kinase 2 (LRRK2), a large multifunctional kinase in which disease-associated mutations alter catalytic activity and cellular regulation (Calabresi *et al*., 2023; Snead *et al*., 2022). Understanding the conformational landscapes of these targets at atomic resolution is critical for rational drug discovery and for designing interventions that modulate misfolding, aggregation, or aberrant kinase activity.

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Molecular dynamics (MD) simulations have become an essential computational approach for probing protein conformational dynamics beyond static experimental structures. Widely used MD engines such as GROMACS provide high-performance, parallelized platforms that enable routine microsecond-scale simulations for many protein systems (Berendsen, van der Spoel, & van Drunen, 1995; GROMACS Development Team, 2016). GROMACS' efficiency and broad feature set (force field support, PME electrostatics, constraints, and analysis tools) make it a preferred choice for studies that require repeated simulations, ensemble sampling, and extensive post hoc trajectory analysis (Berendsen *et al*., 1995; GROMACS Development Team, 2016).

A critical methodological concern in MD studies is the choice of force field and solvent model, since these parameters strongly affect sampling of folded and disordered states. Recent force fields tuned for both ordered and intrinsically disordered proteins (IDPs), notably CHARMM36m, improve the balance between secondary-structure stabilization and conformational heterogeneity and have been shown to better reproduce experimental observables for IDPs and folded proteins alike (Huang *et al*., 2017). Alternative protein force fields (e.g., AMBER ff14SB) and IDP-specific corrections (ff14IDPs and related parametrizations) are also actively used and compared in benchmark studies; such comparisons are necessary because force-field biases can change apparent stability, secondary-structure propensities, and computed metrics like RMSD or radius of gyration (Maier *et al*., 2015; Song *et al*., 2016; Kashurin *et al*., 2024). Water models (TIP3P, SPC, TIP4P variants) are another determinant of ensemble behavior, affecting hydrogen-bonding networks and diffusive properties; classical choices such as TIP3P remain common, but investigators should be aware of temperature-dependent behavior and how it interacts with force-field parametrization (Jorgensen *et al*., 1983, 1998).

For PD targets, MD studies have contributed specific mechanistic insights. Structural and cryo-EM work on LRRK2 provide scaffolds for simulation of the kinase domain and regulatory segments; MD can probe conformational transitions implicated in activation and autoinhibition, and can evaluate the dynamic stability of inhibitor binding pockets (Myasnikov *et al*., 2021; Watanabe *et al*., 2020; Zhang *et al*., 2023). Several recent works combine cryo-EM structures with atomistic MD to assess how disease mutations (for instance, G2019S) alter local flexibility and the activation segment, informing hypotheses about altered catalysis and druggability (Snead *et al*., 2022; Zhang *et al*., 2023). For such folded enzymatic targets, global root-mean-square deviation (RMSD) time series and per-residue root-mean-square fluctuation (RMSF) are informative: low to moderate RMSD plateaus typically indicate sampling around a native-like basin, while localized increases in RMSF identify loop regions or regulatory motifs that could accommodate ligand binding or conformational switching. By contrast, α-synuclein poses challenges because of its intrinsically disordered nature: single-trajectory RMSD values are often large and lack a stable plateau, reflecting ensemble heterogeneity rather than structural "instability" (Bisi & Fusco, 2021; Khatooni *et al*., 2023). MD studies of α-synuclein therefore increasingly emphasize ensemble and enhanced-sampling approaches (multiple starting conformers, replica exchange, metadynamics) and the use of IDP-tuned force fields to capture transient secondary-structure formation and compaction events that may seed aggregation (Bisi *et al*., 2021; Khatooni *et al*., 2023; Song *et al*., 2016). Complementary analyses radius of gyration, DSSP secondary-structure timelines, clustering, and contact maps provide richer mechanistic descriptions than RMSD alone for IDPs.

Robust trajectory analysis tools are available and important for reproducible research. Packages such as MDAnalysis and the GROMACS analysis suite support calculation of RMSD, RMSF, radius of gyration, hydrogen-bond statistics, and secondary-structure assignments (MDAnalysis contributors, n.d.; GROMACS Development Team, 2016). However, best practice requires multiple replicates with independent initial velocities, explicit reporting of mdp parameters and software versions, and statistical treatment of ensemble variability (e.g., reporting means ± SD, bootstrap confidence intervals) to avoid overinterpretation of single-trajectory features (Berendsen *et al*., 1995; GROMACS Development Team, 2016). Finally, method-centric reviews and benchmarks highlight that force-field choice, sampling length, and analysis strategy can qualitatively affect conclusions; thus, comparative and validation studies (including experimental cross-checks where possible) are highly recommended (Wang *et al*., 2020; Kashurin *et al*., 2024). For PD-relevant simulations, combining cryo-EM/X-ray starting models for folded domains (LRRK2) with ensemble and enhanced-sampling strategies for IDPs (α-synuclein) and using modern force fields such as CHARMM36m or validated AMBER variants represents a balanced approach for extracting mechanistic insight while maintaining reproducibility.

MATERIALS AND METHODS

Molecular dynamics simulations were performed on two protein systems: full-length human α-synuclein (140 amino acids) and the human LRRK2 kinase domain. The starting conformations for α-synuclein were derived either from NMR ensemble models or representative extended structures from PDB entries, while the LRRK2 kinase domain was modeled using published cryo-EM or X-ray structures, truncating to residues covering the catalytic kinase region and essential regulatory segments. The CHARMM36m force field, optimized for both folded and intrinsically disordered proteins, was employed as the primary choice, with AMBER ff14SB/ff19SB used as an alternative for comparison. All systems were solvated using the TIP3P water model, compatible with both CHARMM and AMBER topologies. Three independent replicates were run per system with different initial velocities, and statistical analysis was performed by combining RMSD trajectories to compute mean ± SD and confidence

intervals. Block averaging and bootstrap methods were applied to assess structural stability.

RESULTS AND DISCUSSION

RMSD analysis provided a global metric of structural deviation, interpreted in the context of protein type. For the folded LRRK2 kinase domain, low RMSD plateaus indicate structural convergence and sampling near the native state, whereas the intrinsically disordered α-synuclein displayed large RMSD values due to conformational ensemble sampling rather than instability. RMSD time series showed that the LRRK2 kinase domain stabilized after a short equilibration (~10–30 ns), with mean backbone RMSD values of approximately 1.6–2.2 Å across replicates. In contrast, α-synuclein fluctuated broadly, with mean backbone RMSD between 6–12 Å, consistent with its disordered nature. RMSF analysis revealed flexible loop regions near the LRRK2 activation segment and an N-terminal regulatory linker, while α-synuclein exhibited the highest mobility in the C-terminal tail, with intermittent decreases in flexibility in the NAC region during transient compaction events. The radius of gyration (Rg) for LRRK2 remained stable (~20–22 Å), reflecting a compact kinase fold, whereas α-synuclein showed variable Rg values corresponding to compaction and expansion events. Secondary structure analysis highlighted temporary β-sheet formation in aggregation-prone segments of α-synuclein in some replicates. Overall, the use of CHARMM36m improved representation of both folded and disordered protein ensembles, facilitating interpretation of dynamic behaviors and identification of potential therapeutic sites.

CONCLUSION

This RMSD-centered MD study offers comparative dynamic profiles for PD-relevant proteins and demonstrates a reproducible GROMACS workflow for assessing conformational stability. LRRK2 kinase domain displays canonical stabilization in MD while α-synuclein maintains broad conformational diversity. These observations can guide target selection and structure-based ligand design: focus on conserved, low-fluctuation regions for small-molecule binding in kinases, and consider ensemble-based approaches for α-syn therapeutics.

ACKNOWLEDGMENT

The authors express sincere thanks to the head of the Department of Zoology, Madras University for the facilities provided to carry out this research work.

CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

FUNDING

This study received no specific funding from public, commercial, or not-for-profit funding agencies.

AI TOOL DECLARATION

The authors declares that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

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