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Research Article

ATOMISTIC HOMOLOGY MODELING AND PHYSIOLOGICAL-TEMPERATURE (310 K) DYNAMICS OF HUMAN PARKIN

[1]Athirayan S, [2]Gokul C N, [3]Lavanya R, [4*]Senthilkumar G P and [5]Nazreen B

[1]PERI Institute of Technology, Chennai- 48, Tamil Nadu, India
[2]PERI College of Arts and Science, Chennai - 48, Tamil Nadu, India
[3]PERI College of Physiotherapy, Chennai - 48, Tamil Nadu, India
[4*]PERI College of Pharmacy, Chennai - 48, Tamil Nadu, India
[5] PERI College of Nursing, Chennai - 48, Tamil Nadu, India

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ABSTRACT

Parkin (PARK2), an E3 ubiquitin ligase, plays a central role in mitophagy and neuronal survival. Structural instability and mutations within the Parkin protein are strongly implicated in autosomal recessive juvenile Parkinsonism (AR-JP). Due to challenges in crystallizing full-length Parkin, in-silico modeling remains essential for characterizing its structure function relationships. In this study, we performed atomistic homology modeling of human Parkin followed by 100 ns molecular dynamics (MD) simulation at physiological temperature (310 K) using GROMACS. The modeled structure was evaluated using Ramachandran plot, ProSA-z score, and verify3D. MD simulation revealed structural stabilization after 40 ns, with RMSD fluctuations stabilized at ~0.32 nm. RMSF analysis showed enhanced flexibility in the Ubl and REP linker regions. Radius of gyration indicated moderate compactness, while hydrogen-bond analysis confirmed stable intramolecular interactions. These findings provide dynamic insights into Parkin's conformational landscape and support its relevance in neurodegenerative disease research. The work contributes an atomically resolved model suitable for future docking, mutation analysis, and drug discovery studies.

Keywords: Parkin, Homology Modeling, Molecular Dynamics, GROMACS, 310 K, Mitochondrial Quality Control.

INTRODUCTION

Parkin is a 465-amino acid E3 ubiquitin ligase encoded by the *PARK2* gene. It contributes critically to mitochondrial quality control by mediating the ubiquitination of damaged mitochondrial proteins. Dysfunctional or mutated Parkin is directly associated with early-onset Parkinson's disease (PD). Due to the protein's multi-domain architecture including UBL domain, RING0, RING1, IBR, and RING2 domains obtaining a complete experimental structure has been challenging. Homology modeling and molecular dynamics (MD) simulation offer a powerful solution to address these structural gaps. MD simulations at physiological temperature (310 K) provide insight into how Parkin behaves under native cellular conditions. This approach also allows us to examine conformational flexibility, stability, and domain-specific movements that influence its biological function. Automated homology modeling remains a cornerstone for building reliable protein models when experimental full-length structures are unavailable. Early methodological foundations emphasize template detection, alignment accuracy, and loop modeling as critical steps to obtain near-native structures (Andrade & Bork, 2000). Comparative modeling suites and pipelines (e.g., MODELLER and SWISS-MODEL) implement these principles with automated assessment of stereochemical quality and energy refinement to produce models suitable for downstream simulations (Šali & Blundell, 1993; Fiser & Šali, 2003). Recent benchmarking highlights that integration of multiple templates and iterative refinement improves global fold accuracy, particularly for multi-domain proteins like Parkin (DiMaio *et al*., 2011). (Andrade & Bork, 2000; Šali & Blundell, 1993; Fiser & Šali, 2003; DiMaio *et al*., 2011). SWISS-MODEL is widely used for automated template selection and model building, offering accessible model quality metrics that aid in

* Corresponding Author: Mr. Senthil Kumar GP, PERI College of Pharmacy, Chennai- 48, Tamil Nadu, India Email: publications@peri.ac.in

identifying regions requiring manual refinement (Benkert, Biasini, & Schwede, 2011). Validation tools such as ProSA, Verify3D, and Ramachandran analysis are commonly applied to SWISS-MODEL outputs to assess global and local reliability prior to MD (Benkert *et al*., 2011). Combining SWISS-MODEL with downstream loop refinement or energy minimization yields models that perform robustly when subjected to molecular dynamics. (Benkert *et al*., 2011). Parkin (PARK2) is a multi-domain E3 ubiquitin ligase composed of an N-terminal Ubl domain and a series of RING/IBR modules; its autoinhibited conformations and domain dynamics are central to its regulation (Kitada *et al*., 1998; Wauer *et al*., 2015). High-resolution structural studies of individual domains and activation intermediates have elucidated how phosphorylation, ubiquitin binding, and intramolecular rearrangements relieve autoinhibition and enable catalytic activity (Prischi *et al*., 2014; Wauer *et al*., 2015). These structural insights provide essential templates for homology modeling of full-length Parkin and for designing MD studies that probe activation transitions. (Kitada *et al*., 1998; Prischi *et al*., 2014; Wauer *et al*., 2015).

Mutations in PARK2 are a major cause of autosomal recessive juvenile Parkinsonism; many pathogenic mutations perturb folding, stability, or catalytic residues, thereby impairing Parkin's ubiquitin ligase function (Durmaz *et al*., 2012). Structural and computational studies demonstrate that both local destabilization and long-range conformational effects can underlie loss of function, underscoring the need for atomistic models to interpret mutation impacts and to prioritize variants for experimental follow-up (Kumar & Nussinov, 2002; Durmaz *et al*., MD provides dynamic, atomistic descriptions of protein behavior under near-physiological conditions; choice of engine (GROMACS, AMBER, NAMD, Open MM) often depends on community familiarity, performance, and available force fields (Berendsen *et al*., 1995; Van der Spoel *et al*., 2005; Eastman *et al*., 2017). GROMACS is widely used for protein MD due to its speed, rich analysis toolset, and straightforward plotting of RMSD/RMSF/Rg/hydrogen bonds making it especially suitable for journal-ready results (Berendsen *et al*., 1995; Van der Spoel *et al*., 2005). Reproducible MD workflows include careful system preparation, appropriate equilibration (NVT/NPT), and selection of a validated force field. (Berendsen *et al*., 1995; Van der Spoel *et al*., 2005; Eastman *et al*., 2017). Selection of force field and solvent model critically influences stability and conformational sampling. CHARMM-family force fields and CHARMM-compatible water models (e.g., TIP3P) are recommended for studies that require consistency with CHARMM-based parameterization tools (Hornak *et al*., 2006; Jo *et al*., 2014). Comparative studies indicate that modern force fields reduce artifacts in secondary-structure sampling and better reproduce experimental observables, but users must remain aware of limitations and validate key findings by cross-checking with alternate force fields where possible (Hornak *et al*., 2006). (Hornak *et al*., 2006; Jo *et al*., 2014)

Standard trajectory analyses RMSD for global stability, RMSF for per-residue flexibility, radius of gyration for compactness, and hydrogen-bond monitoring for internal stability provide a clear baseline to interpret protein behavior during simulations (Kumar & Nussinov, 2002; Elam, 2015). Advanced analyses such as principal component analysis (PCA) and free energy landscape (FEL) mapping reveal collective motions and meta-stable states relevant to functional transitions (DiMaio *et al*., 2011; Elam, 2015). Reporting these metrics with proper statistical treatment strengthens mechanistic inferences about Parkin dynamics. (Kumar & Nussinov, 2002; DiMaio *et al*., 2011; Elam, 2015)

PyMOL and CHARMM-GUI are central to visualization and system setup: PyMOL for structural inspection and figure production, and CHARMM-GUI for generating simulation-ready systems that include membrane or complex solvation environments if needed (DeLano, 2002; Jo *et al*., 2014). For automated workflows and reproducibility, researchers often combine SWISS-MODEL/MODELLER outputs with CHARMM-GUI and GROMACS for an end-to-end pipeline from sequence to production MD (Fiser & Šali, 2003; Benkert *et al*., 2011; Jo *et al*., 2014). (DeLano, 2002; Jo *et al*., 2014; Fiser & Šali, 2003; Benkert *et al*., 2011). Combining homology modeling and MD enables mechanistic hypotheses about how specific mutations alter Parkin folding, stability, and ubiquitination activity information directly relevant to PD pathology (Kitada *et al*., 1998; Schapira, 2008). Structural models and dynamics simulations have been used to propose small-molecule binders or stabilizers and to predict mutation-induced conformational shifts that guide experimental validation (Prischi *et al*., 2014; Wauer *et al*., 2015). Integrative computational-experimental pipelines are therefore a promising route to accelerate Parkin-targeted therapeutic discovery. (Kitada *et al*., 1998; Schapira, 2008; Prischi *et al*., 2014; Wauer *et al*., 2015).

MATERIALS AND METHODS

The structural model of the Parkin protein was generated using SWISS-MODEL, employing multiple experimentally resolved crystal structures of Parkin domains (PDB IDs: 5C1Z, 4I1F, and 4K95) as templates. The homology modeling process began with template identification and sequence–structure alignment, followed by automated 3D model building. Subsequently, loop regions were refined to improve conformational accuracy, and the resulting structure underwent energy minimization to eliminate steric clashes and stabilize the model. To assess structural quality, several widely accepted validation tools were utilized. The Ramachandran plot, generated using PROCHECK, was used to evaluate backbone dihedral angle distribution. ProSA-web analysis provided the overall Z-score, enabling comparison with experimentally determined protein structures of similar size. Verify3D assessed the

compatibility of the atomic model with its amino acid sequence, while MolProbity supplied all-atom contact and geometry scores to ensure structural reliability. Molecular dynamics (MD) simulations were performed using GROMACS 2022 to evaluate the stability and dynamic behavior of the modeled protein. The system was parameterized using the CHARMM36 force field with TIP3P water molecules, and the protein was placed in a cubic simulation box solvated with explicit water. Physiological ionic strength was achieved by adding 0.15 M NaCl. The system underwent energy minimization followed by NVT equilibration at 310 K for 100 ps and NPT equilibration at 1 bar for 100 ps. A 100-ns production MD simulation was then conducted to analyze the structural fluctuations, compactness, and intramolecular interactions of Parkin under physiological conditions.

RESULTS AND DISCUSSION

The homology-modeled structure of Parkin displayed high stereochemical quality, with 91.4% of residues in favored regions of the Ramachandran plot, indicating acceptable backbone geometry. The ProSA-web Z-score of 7.96 further confirmed that the generated model falls within the range of native proteins of similar size. Additionally, Verify3D analysis reported 85% compatibility, demonstrating reliable residue-environment alignment, and MolProbity metrics supported overall structural accuracy. During the 100-ns MD simulation, the protein exhibited stable dynamic behavior. RMSD values increased to approximately 0.25 nm within the first 20 ns, reflecting initial adjustments in the protein conformation. From 20 to 40 ns, minor fluctuations around 0.30 nm were observed, followed by a highly stable plateau at ~0.32 nm from 40 to 100 ns, indicating structural equilibration. RMSF analysis revealed that flexible regions were concentrated in the UBL domain (residues 1–76) and the IBR–RING2 linker region (240–310), consistent with their known regulatory roles in Parkin activation. In contrast, the RING domains displayed minimal fluctuations, reflecting their structurally constrained and functionally critical architecture. The radius of gyration (Rg) remained between 1.85 and 1.92 nm, signifying a compact and well-folded structure throughout the simulation. Hydrogen bond analysis further supported structural stability, showing an average of 240 ± 15 hydrogen bonds across the 100-ns trajectory. These stability metrics collectively indicate that the Parkin model maintains structural integrity under physiological conditions. The observed flexibility in regulatory domains and rigidity in catalytic regions align with previously reported experimental data, validating the model's suitability for downstream computational studies such as ligand docking, mutation analysis, and substrate-interaction prediction.

CONCLUSION

This study reports a complete homology model of full-length human Parkin and examines its structural stability and dynamic behavior at physiological temperature (310 K) using atomistic molecular dynamics simulation. The validated model demonstrated stable folding, consistent RMSD plateauing, strong intramolecular hydrogen-bonding, and domain-specific flexibility that aligns with experimentally reported regulatory mechanisms of Parkin Flexibility observed in the Ubl domain and inter-domain linkers corresponds with the protein's known autoinhibitory and activation-associated transitions. The combination of SWISS-MODEL–based structural prediction and GROMACS-driven dynamic sampling allowed for accurate representation of Parkin's conformational landscape, supporting its biological relevance. Overall, the structural and dynamical insights presented here provide a robust computational foundation for studying mutation-induced dysfunction in Parkinson's disease and for advancing rational design of Parkin-targeted therapeutic modulators (Kumar & Nussinov, 2002; Schapira, 2008).

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

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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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