Computational investigation of molecular dynamics simulations of siRNA/PEI complex stability

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Abstract

A systematic and rational approach is needed to develop nucleic acid-based nanocarrier systems, especially when using polycationic polymers such as Polyethyleneimine (PEI) to deliver small interfering RNA (siRNA). In the present study, we investigate an in silico approach to atomistic Molecular dynamics (MD) simulation of the interaction between siRNA and PEI, including complex formation and stability. Molecular modelling was performed for the generation of a 3-D structure of siRNA by the ModeRNA server, and for PEI, it was conducted by the Avogadro molecular editor tool and followed by molecular docking between siRNA and PEI to predict favourable binding interactions. The top docked complexes were further evaluated in the MD simulations to assess Root mean square deviations (RMSD), Root mean square fluctuations (RMSF), Radius of gyration (Rg), and Molecular Mechanics – Poisson-Boltzmann Surface Area (MMPBSA). The siRNA-PEI complex was primarily governed by secondary forces, including electrostatic interactions, the hydrophobic effect, and other non-covalent forces; all of these forces are influenced by the intrinsic properties of the polymer and nucleic acid, as well as environmental conditions. Branched PEI can form complexes with siRNA under physiological conditions. Computational findings were supported by thermodynamic parameters, underscoring the reliability of the in silico approach for siRNA-PEI complex formation, which can be further used in in vivo and in vitro studies.

Keywords: Polyethyleneimine, small interfering RNA, Root mean square deviations, Root mean square fluctuations, Radius of gyration, Molecular Mechanics – Poisson-Boltzmann Surface Area.

1.0 Introduction

Delivery of foreign genetic materials into host cells is termed gene therapy, and is frequently used for the treatment of various hereditary disorders, cancers, and viral infections. Gene therapy poses various challenges in gene delivery. Till now, both viral and non-viral methods have been used for the delivery of genetic materials. Use of synthetic polymers as non-viral gene delivery systems has gained popularity over the decades due to several advantages, including low toxicity and cost, as well as ease of industrial production (1, 2).

Recently, Polyethyleneimine (PEI), a polycationic polymer, has gained popularity for decades as a nonviral delivery method for genetic materials into host cells, surpassing viral methods such as lentiviral, retroviral, adeno-associated virus, adenoviral, and herpes simplex virus vectors (3). These viral methods have several disadvantages, including mutation and immunogenic responses, and are highly costly. On the other hand, cationic polymers like PEI have a superior safety profile, minimal or no off-target effects, and produce a proton sponge effect and high transition efficiency, and also have high affinity to small interfering RNA (siRNA) (4, 5).

RNA interference (RNAi) using siRNA is a promising therapeutic strategy for gene regulation and its expression (6). siRNA contains a phosphate group, which makes it an anionic molecule that cannot cross biological membranes, whereas PEI contains positively charged amine groups, thus favouring electrostatic interactions that promote complex formation. siRNA-PEI stable complex improves cellular internalization and provides protection for siRNA against enzymatic degradation (7).

In this study, we explore all-atom Molecular Dynamics (MD) simulations of siRNA and the PEI polymer, which enable quantitative predictions of binding patterns and dynamics. MD simulation is a tool for bridging the gap between X-ray crystallography or NMR structures and biological mechanisms. It also fixes steric clashes in computationally designed siRNA nanostructures, characterizes their dynamics, and investigates interactions between siRNA and other biomolecules, such as delivery agents and membranes (8). In the present study, we investigate the atomistic interaction of siRNA and PEI carrier and their stability, structural compactness, and rigidity of the complex over time in the presence of a force field.

2.0 Methods

2.1 siRNA Structure Preparation

The 3-D structure of HIF-1α siRNA was generated using the ModeRNA web server, which requires a pairwise sequence alignment and a structural template. Structural template have been taken from human argonaute-2 protein containing two strands of RNA i.e. guide strand and passenger strand, in which we take guide strand as a structural alignment and for sequence alignment we take siRNA sequences of HIF-1 α genes as previously reported in optimizing siRNA therapeutics targeting HIF-1α: computational design, screening, and molecular dynamics simulation studies (ACS, molecular pharmaceutics). We align the sequence and generate a .fasta file, then build a model using the template and alignment files. Afterwards, we got our 3-D structure of HIF-1α siRNA and saved it in PDB form (9).

2.2 3-D Structure Preparation of PEI

The 3D structure of branched PEI was generated using Avogadro. For this, we first drew ethyleneimine repeating units in a branched configuration and manually added primary, secondary, and tertiary amines, thereby constructing a low-molecular-weight PEI. To mimic physiological pH conditions, protonation of the amine groups is assigned, which resembles the polycationic nature of PEI. This structure was subjected to energy minimization using the in-built molecular mechanics force fields in Avogadro to remove steric clashes and optimize bond lengths and angles. The electrostatic interactions between siRNA and PEI are responsible for the formation of the complex. The optimized structure was exported to a .PDB file (10).

2.3 Molecular Docking and modeling of siRNA-PEI Complex

Molecular docking was performed between the designed siRNA and PEI via the HDOCK server (https://hdock.phys.hust.edu.cn) (accessed on 22/03/2025 at 11:00 AM). On the basis of the docking score and electrostatic interactions between the molecules, a selection was made. Further, the best docked complex was imported into LEaP (AmberTools v22) for system preparation (11).

2.4 Molecular Dynamics Simulation Studies

The 3D structure of siRNA was prepared and parameterized using the ff14SB force field within the AMBER molecular dynamics environment. Topology and coordinate files for siRNA were generated using the LEaP module of AmberTools v22, with standard RNA residues and phosphate parameters assigned automatically (12). PEI structures were prepared and downloaded from Avogadro tools in PDB format,

which was further processed using Antechamber (AmberTools v22), in which atoms present in PEI were assigned a force field, namely General Amber Force Field 2 (GAFF2), and then partial atomic charges were calculated using Austin Model 1 – Bond Charge Correction (AM1-BCC) method (13). To generate the PEI mol2 file, we used another tool, Antechamber, which provides force-field integration. To generate the .frcmod file, we used the parmchk2 module to identify any missing force-field parameters in the mol2 file (14). To generate the amber topology (.prmtop) and coordinate (.inpcrd) files, we combined the two force fields, ff14SB and GAFF2, using the LEaP module of Amber. To generate GROMACS-compatible topology (.top) and coordinate (.gro) files, we used the AnteChamber Python Parser interface (ACPYPE) for subsequent energy minimization, validation, and ease of MD simulation (15). Next, the siRNA-PEI complex was placed in a triclinic simulation box with a minimum distance of 10 Å from the box edge and solvated with TIP3P water. To mimic physiological ionic conditions and neutralize the system conditions with counter ions like (Na+/CL-) ions, followed by the addition of 0.15 M NaCl to mimic physiological salt concentration. To remove steric clashes, the system was minimized for 5000 steps, then heated from 0 K to 300 K over 20 ps. Equilibration of the heated system was performed for up to 6 ns at 300 K and 1 bar to stabilize temperature, pressure, density, and potential energy. At equilibration phase, the system adapts to physiological ionic conditions, ensuring solvent molecules and ions are properly distributed around the siRNA-PEI complex before proceeding to the MD simulations and analysis behaviour of various parameters include Root mean square deviation (RMSD), Root mean square fluctuations (RMSF), Radius of gyration (Rg) and Molecular Mechanics Poisson- Boltzmann Surface area (MMPBSA) analysis (16, 17, 18).

3.0 Results

3.1 Visualization of siRNA structure

3-D structure of HIF-1α siRNA was generated using the ModeRNA web server based on sequence-template homology modelling. Using PyMol, the geometry and coordinate file of siRNA were analyzed to assess base-pairing consistency and backbone continuity, thereby confirming suitability for *in silico* interaction analysis (Figure 1).

Figure 1: Pictorial representation of siRNA

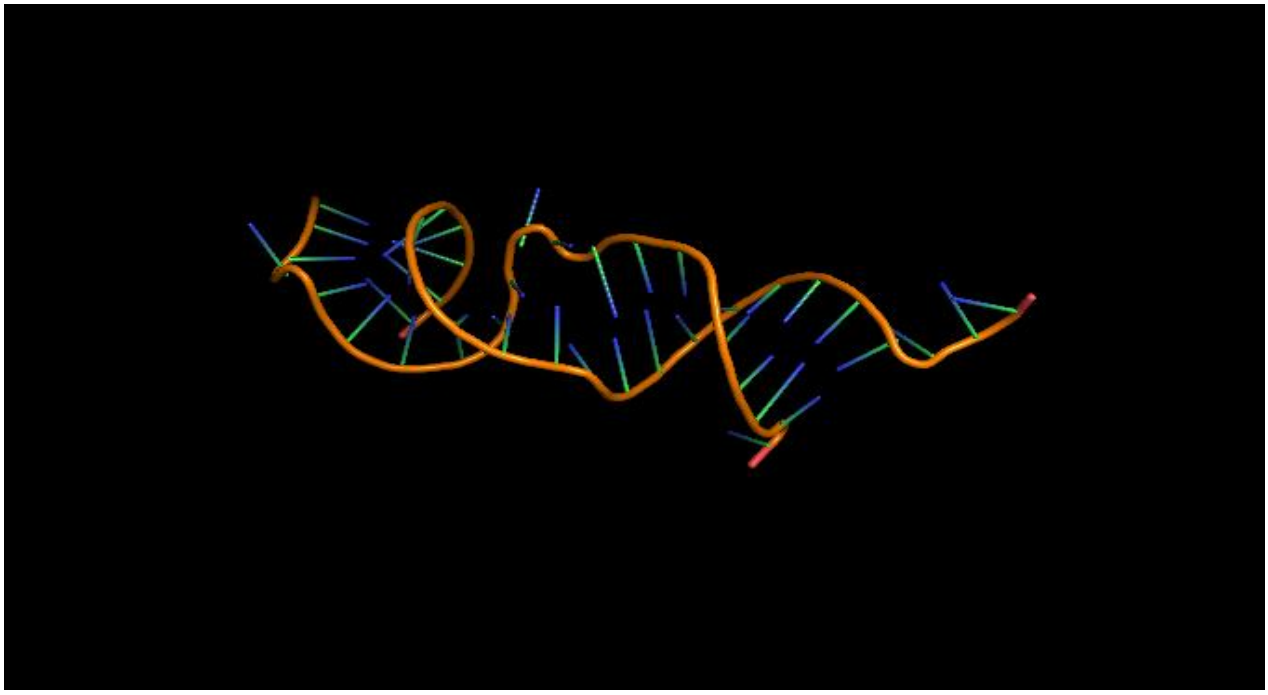

Figure showing 3-D depiction of siRNA having both sense and antisense strand arranged in double-stranded helical conformations.

3.2 Visualization of PEI structure

Avogadro molecular editor was used to construct the 3D structure of branched PEI, and the force field MMFF94 was used to remove constraint bonds and optimize the structure, energy minimization, bond length, and bond angles. Once energy minimization was done, there are no abnormal bond distortions and atomic overlaps, and there is a uniform charge distribution along with the flexibility of PEI. For detailed visualization of PEI, Pymol was used to inspect the structure, geometry, and coordinate file to ensure the molecular bonding and other aspects (Figure 2)

Figure 2: Pictorial representation of PEI

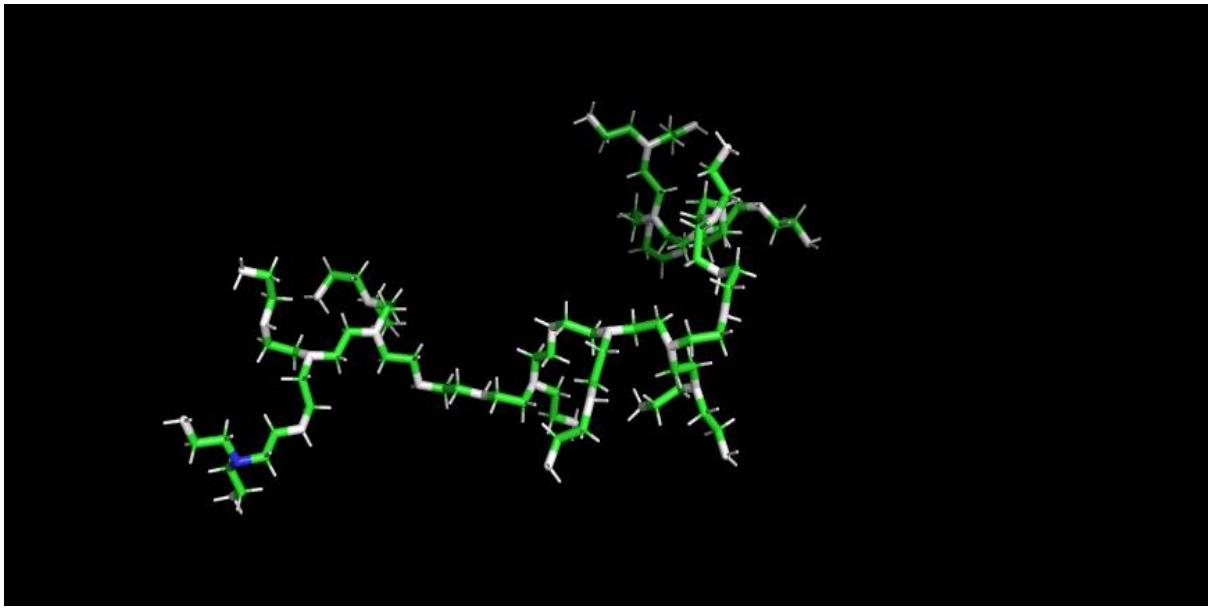

Figure depicts branched PEI highlighting the branched architecture and amine-rich backbone of PEI used as a cationic carrier for siRNA complextaion.

3.3 Molecular Docking and modeling of siRNA-PEI Complex

Molecular docking shows a binding score of siRNA, and PEI was -330.43k/cal and a confidence score of around 0.9735, along with RMSD deviations of around 21.70, as shown in Figure 3. More negative energy values indicate stronger binding affinity, which correlates with higher system stability (Figure 3).

Figure 3: Three-dimensional structural representation of the siRNA–PEI complex.

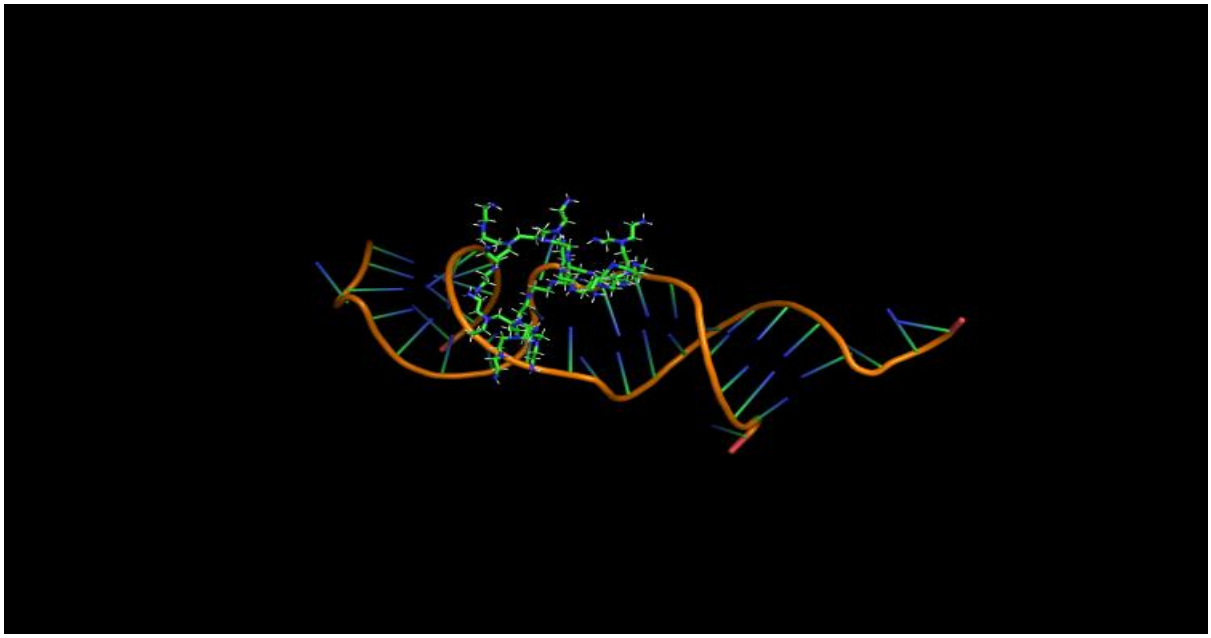

Figure depicts of 3D molecular visualization of the siRNA–PEI complex obtained after Molecular docking. The siRNA is represented in a ribbon format highlighting the phosphate backbone, while the branched PEI polymer is shown in stick representation.

3.4 Molecular Dynamics Simulation Studies

In our study, we conducted 25000ps MD simulations studies between siRNA and PEI polymer under standard conditions (Figure 4). Further system is equilibrated shows and potential energy confirms the structural integrity and thermodynamic stability of the system. To assess the behaviour of the siRNA-PEI complex in the presence of force field, we calculated the Rg over time. Rg is a measure of the average distance between any point on the particle and its centre of mass. The equation used to compute Rg is

$$Rg == \sqrt{((1/N) \sum\_(i = 1)\^N | r\_i - r\_com |^2)},$$

Here, N represent the total number of atoms in the system, r_i denotes the position vector of atom I, and r_{com} represents the position vector of the centre of mass of the particle.

Figure 4: Structural representation of siRNA-PEI complex during MD simulation.

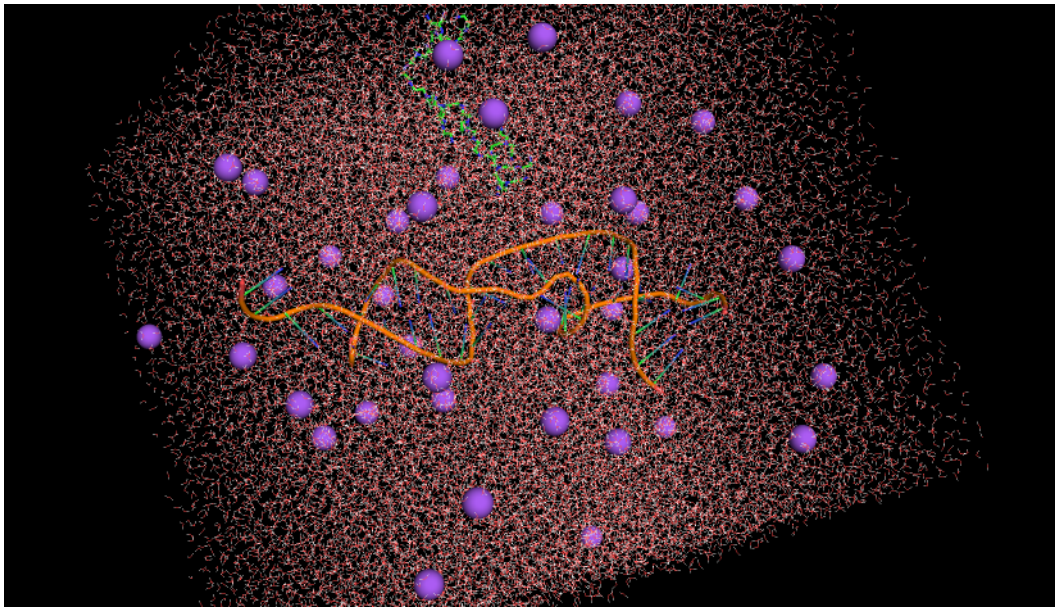

Figure shows snapshot of the siRNA-PEI complex obtained during MD simulation, illustrate the spatial arrangement and interaction of siRNA with the PEI matrix.

Rg graph indicates initial relaxation upto 1,000ps, after which it decline from 1.1-1.2nm to 0.65 -0.7nm, which reflects rapid structural rearrangement and compaction of the siRNA-PEI complex, followed by equilibration phase from 1,000-25,000ps remains constant and very small fluctuation is noted (Figure 5).

Figure 5: Depicts both trajectories of siRNA-PEI complex

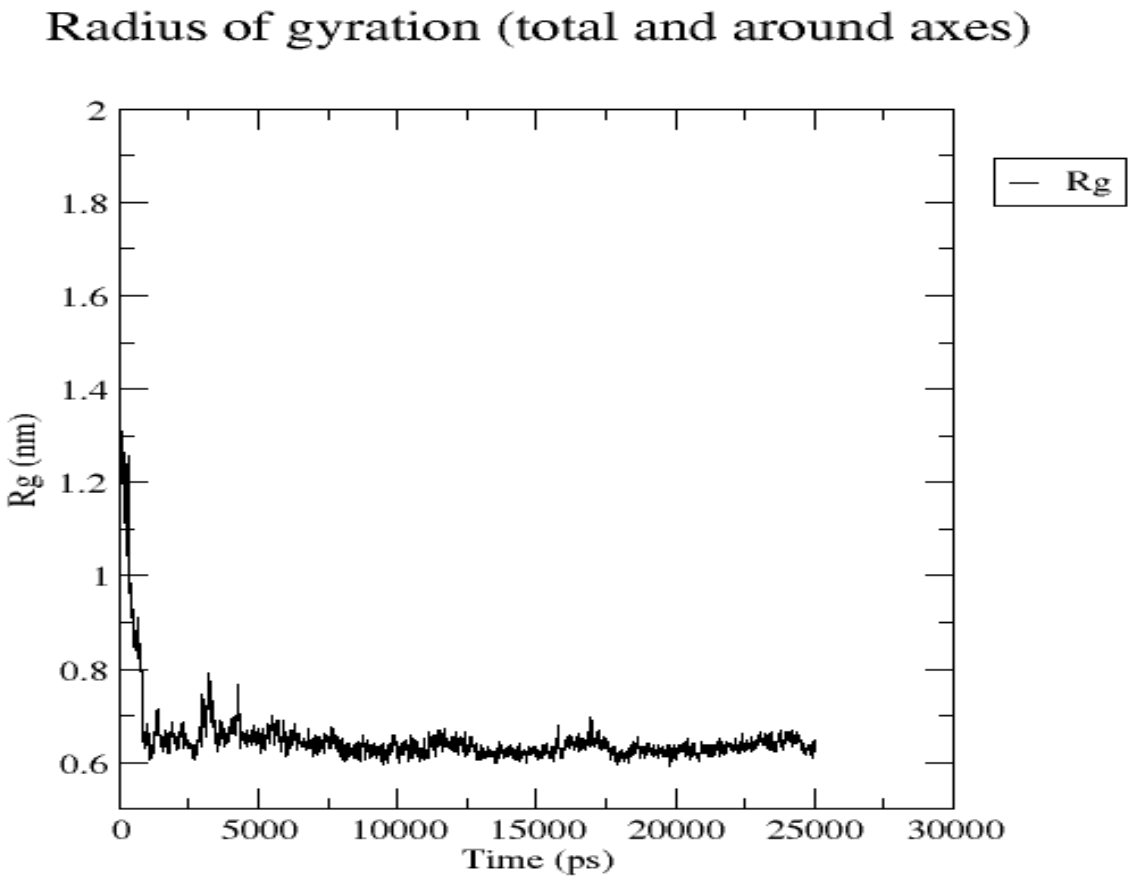

Figure depicts Rg of the siRNA-PEI complex system during MD simulation upto 25ns, shows initial fluctuation and remains consistent throughout the period.

To investigate the conformational changes occurring during MD simulations, we employed the RMSD as a quantitative measure. The RMSD analysis offers valuable insights into the average spatial separation between two distinct groups of atoms within the system. By calculating the RMSD, we can gauge the extent of atomic fluctuations and ascertain whether the system has reached equilibrium conditions. This analysis yields a consistent value reflect the overall displacement of atoms

over time, thereby providing information about the stability and dynamics of the molecular system. RMSD complex of the analysis shows ligand behaviour in which there is initial equilibration phase from 0 to 2,000ps and relative movement of molecule around 0.9-1.0nm, which indicates adjustment of the initial structure from the starting conformations after minimization and equilibration. Following equilibration phase, there is a stabilization phase from 2,000 to 25,000ps in which fluctuation around 0.9-1.0nm with very little fluctuations, which indicates system reaches the equilibration phase and maintains stable conformations throughout the run (Figure 6).

Figure 6: RMSD analysis over 25ns MD simulation of siRNA-PEI complex**.**

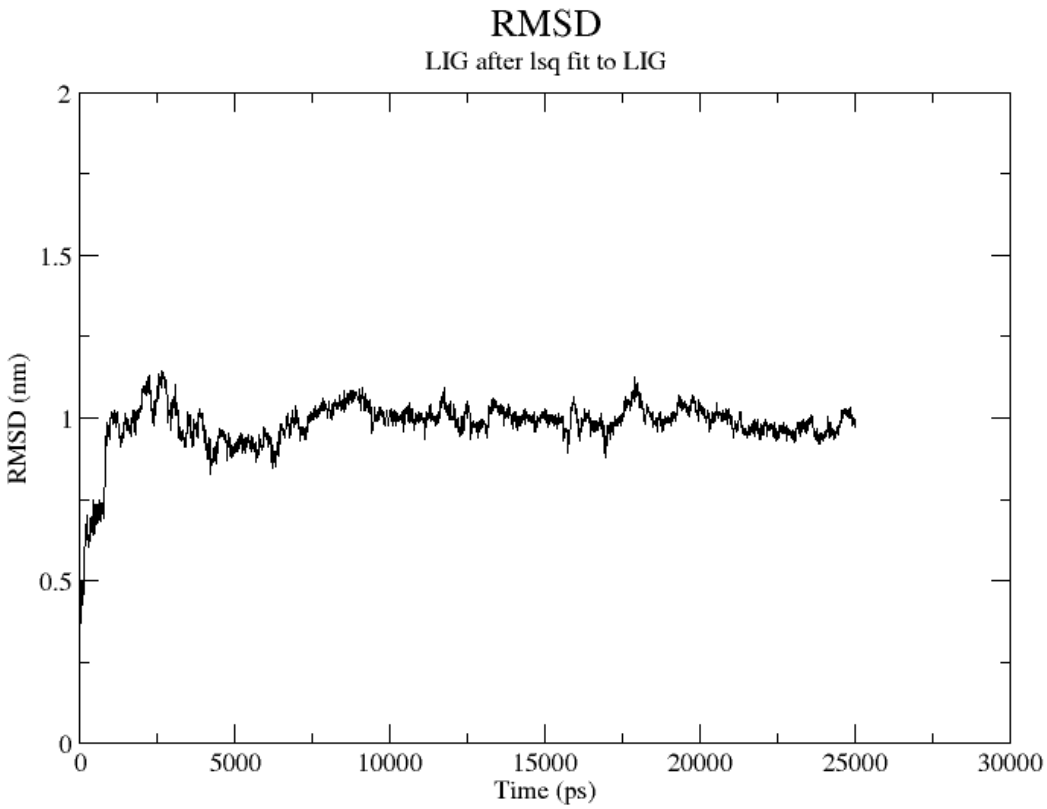

Figure indicates the system attains a stable conformation with RMSD values fluctuating around 1.0nm.

RMSF analysis shows most atom exhibits a very low RMSF values (0.3-0.5 nm), which indicate atom have constrained mobility and rigidity of the complex, some fluctuation were seen around (0.6-0.8 nm) in specific regions which corresponds to terminal segments of siRNA-PEI complex (Figure 7).

Figure 7: RMSF analysis of siRNA-PEI complex during MD simulation

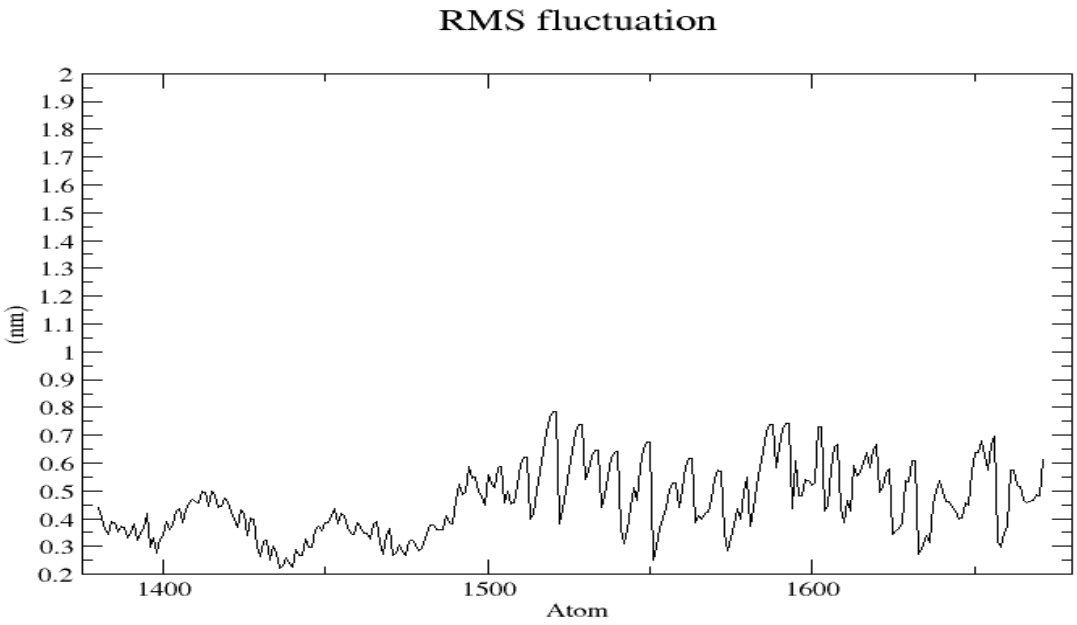

Figure shows siRNA-PEI complex trajectories during 25ns, reflects RMSF analysis highlighting regions of different flexibility within the complex.

Post MD-analysis includes MMPBSA approach as implemented in GROMACS, primarily focus on energy interactions between siRNA-PEI complexes, this tools allowed to explore vander walls (vDw) interaction, electrostatic, and total resultant energy of the complex. vDw interactions provides attractive and repulsive forces among non-bonded atoms. In electrostatic terms of energy indicates interaction among charged atom or groups. Thus, allowed us to calculate long range electrostatic effects, discern regions of positive and negative charge distributions, and uncover electrostatic attractions or repulsion between different molecular components. Combinational approach of electrostatic, vDw energy and total resultant energies (Table 1).

Table 1: Binding free energy (ΔG bind) of siRNA-PEI complex over 25,000ps MD simulation.

Complex	ΔG bind	Δ VdW	ΔEEL	ΔEGB	ΔESURF	ΔGGAS	ΔGSOLV
siRNA-PEI Complex	-137.82	-97.45	-563.46	649.97	-15.05	-660.91	634.65

The table summarizes the binding free energy (ΔG_bind) and its individual energy components calculated using the MM-PBSA method. ΔG_bind represents the total binding free energy of the complex. ΔVdW denotes van der Waals interaction energy, while ΔEEL corresponds to electrostatic interaction energy. ΔEGB represents the polar solvation energy calculated using the Generalized Born solvation energy model, and ΔESURF indicates the nonpolar solvation energy contribution. ΔGgas is the total gas-phase interaction energy, calculated as the sum of ΔVdW and ΔEEL, whereas ΔG_solv is the total solvation free energy, obtained from the sum of ΔE_GB and ΔE_surf. All energy values are reported in kcal/mol.

This results indicates PEI is a suitable and effective carrier for siRNA delivery, providing every aspects into interactions energetics, conformational stability, and complex behaviour that support its further development for therapeutic applications.

4.0 Discussion

Gene therapy is an evolving technology in which nucleic acids are delivered into host cells to treat various diseases, such as cancer and hereditary diseases. RNAi is an essential method for delivering non-coding RNAs, such as 19-25 nucleotide-long siRNA, shRNA, and miRNA (19). These non-coding RNAs bind to their specific mRNAs, thus inducing post-transcriptional gene silencing by degrading mRNA via the RNA-induced silencing complex (RISC). For efficient delivery of these non-coding RNAs, it requires vehicles; in this study, PEI is a non-viral vector used to deliver siRNA into cells (20). Present studies include an in-silico approach to the siRNA-PEI complex and its atomistic interactions in the presence of a force field over a time of 25,000 ps. For this first we prepared the 3D structure of siRNA from ModeRna web server, this server work on principle of homology modelling and sequence alignment. Further, Molecular docking was performed by HDOCK server to asses binding interactions of siRNA and PEI, results of molecular docking states a stable and strong interactions of siRNA and PEI complex (21). For elucidation of atomistic interaction of siRNA and PEI under physiological ionic concentration, MD

simulation was carried out for 25ns. For performing MD simulations, first force field of siRNA and PEI were generated in AMBER using Leap module, then these amber files were converted into gromacs file using ACPYPE module. RMSD analysis shows initially fluctuations at initial phase of the simulations (10ns). This can be ascribed to structural rearrangements and conformational adjustments of PEI around the siRNA molecules during equilibration phase. After this phase, there is stabilization due to strong electrostatic interactions between siRNA and PEI complex. *Rg* analysis of the siRNA-PEI complex over 25ns states initial compaction phase followed by equilibrated phase with very less fluctuation, which shows siRNA-PEI complex remains compact and structurally stable throughout the simulation period. To investigate residue level flexibility within the complex we assessed RMSF analysis in which we observed siRNA regions involved in direct interactions with PEI. However, some fluctuations were seen due to terminal regions of siRNA, which is consistent and does not adversely affect overall complex stability (22, 23). To asses compactness of the structure we investigate Rg, during initial phase it shows fluctuation then relatively constant throughout the simulation. This shows this complex are stable enough to cross biological membranes, thus highlighting PEI mediated condensation of siRNA. Post-MD analysis includes MMPBSA, which shows favourable binding free energy. All in all, the results show PEI forms a stable, compact, and energetically favourable complex with siRNA. This in-silico approach shows mechanistic support for the use of PEI as a non-viral vehicle in siRNA-based gene therapy. It offers a rational basis for further experimental validation and formulation optimization.

5.O Conclusion

The present study was undertaken to evaluate *in silico* molecular interactions of PEI, a cationic polymer carrier for siRNA delivery. For this, we used molecular modelling, molecular docking, molecular dynamics simulation, and post-MD-simulation free energy analysis. The binding free energy between siRNA and PEI was computed using molecular docking studies, showing favourable interactions between the negatively charged siRNA and the positively charged PEI. To evaluate the atomistic interactions of the siRNA-PEI complex, we performed an MD simulation for 25,000 ps, and calculate RMSD, RMSF and Rg showing structural compactness and stability over time. To assess electrostatic interaction, we conducted an MMPBSA analysis, which shows an energetically favourable complex over time. An in-silico approach shows a strong electrostatic interaction between

siRNA-PEI complexes. The observed structure stability, fav orable interactions dynamics, and strong binding energetics gave valuable insights for further experimental validations and optimization of PEI-based siRNA delivery system for therapeutic applications.

6.0 Acknowledgement

Author's are thankful and showing gratitude to Babasaheb Bhimrao Ambedkar University for providing the necessary facilities and support to carry out this research.

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List of abbreviation's: **Polyethyleneimine:** PEI, **small interfering RNA**: siRNA, Molecular dynamics: MD, **Root mean square deviations**: RMSD, **Root mean square fluctuations:** RMSF, **Radius of gyration**: Rg, **Molecular Mechanics – Poisson- Boltzmann Surface Area;** MMPBSA, **General Amber Force Field 2:** GAFF2, **Austin Model 1 – Bond Charge Correction:** AM1-BCC, **AnteChamber PYthon Parser interface:** ACPYPE, **RNA-induced silencing complex:** RISC.