Compatible Coarse-Grained Caprylic Acid-substituted Polyethylenimine and siRNA for Simulations in Gene Delivery

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Introduction

Gene therapy

- Introduces a therapeutic gene to treat cellular dysfunctions caused by genetic mutation [1].
- Short interfering RNA (siRNA) can selectively cleave messenger RNA (mRNA) that produces harmful proteins. This process is known as RNA interference (RNAi) [2].

Polyethylenimine (PEI) as a potent siRNA carrier

- Native siRNA is susceptible to degradation, and has limited ability to translocate across cell membrane.
- PEI neutralizes, condenses, and protects siRNAs.
- High molecular weight PEIs result in high toxicity and low biodegradability.
- Low molecular weight PEIs exhibit tolerable toxicity but lack efficient siRNA delivery.

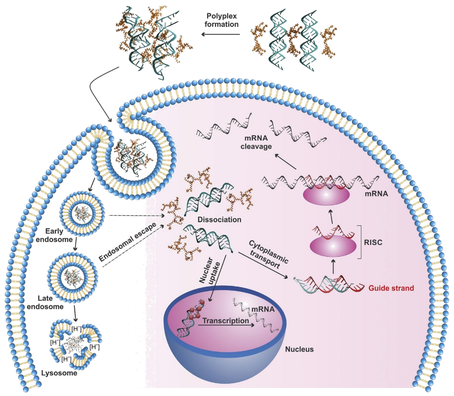

Figure 1: Schematic of gene delivery process [3].

Enhancing PEI performance with lipids

- Improves biocompatibility
- Enhances hydrophobic interactions with lipid membranes such as cell membrane and lung surfactant, increasing cellular uptake [4].
- Facilitates endosomal escape [4].
- Provides better protection from degradation [4]

Coarse-grained (CG) molecular dynamics (MD) simulations

- Improves computational efficiency to extend simulation time and length scales.
- Allows study of large biomolecular systems comparable to experiments.
- New CG forcefield needs validation with all-atom (AA) simulations to ensure accuracy.

Methods

Coarse-graining:

- Martini CG forcefield was used [5], where nonbonded interaction parameters are determined from solvation and partitioning free energy of atomistic analogue of beads.
- CCN, CCN^+, $C(=O)C_{2.5}$ and $C_{4.5}$ atom groups were mapped into beads with bead type P_1, Q_d, , N_a, and C_1, respectively (**Figure 2**).
- Bonded interaction parameters are determined by matching probability distributions of bonded interactions (length, angle, dihedral) from CG and AA simulations.

Simulations:

PEI-CA in water

- 2 kDa PEI substituted with one or three caprylic acids (PEI-CA; **Figure 2**) was simulated in water at 300 K and 1 bar with neutralizing Cl^- ions in a 7.1 x 7.1 x 7.1 nm^3 cuboidal box.
- TIP3P water used for AA and polarizable Martini water for CG simulations.

Potential of mean force (PMF) calculation

- To validated CG-PEI/PEI-CA and siRNA interactions, PMFs were calculated using AA and CG simulations with distance between their centers of mass as the reaction coordinate. (**Figure 3**)
- The PEI used in PMF simulations was a smaller 500 Da fragment of 2 kDa PEI (**Figure 2**) with two protonation states: fully unprotonated or 40% protonated, and two lipid modification states: 0 or 1 CA substitution (site 2 in **Figure 2**).
- The siRNA were used in a PMF simulations was fully deprotonated 18-nucleotide $(UGUGGAUGA)_2$.
- PMF calculations were conducted in a 6.3 x 6.3 x 20 nm^3 cuboidal box with water and neutralizing NaCl salt at 300 K and 1 bar.

Tuning of nonbonded interactions:

- Default Lennard-Jones (LJ) parameters (interaction strength and van der Waal's radius) in Martini were adjusted to improve the accuracy of CG PEI-siRNA interactions.
- Nonbonded parameters were adjusted by trial-and-error to match CG and AA PMF.
- Different adjustments were performed for siRNA backbone and sidechain beads.

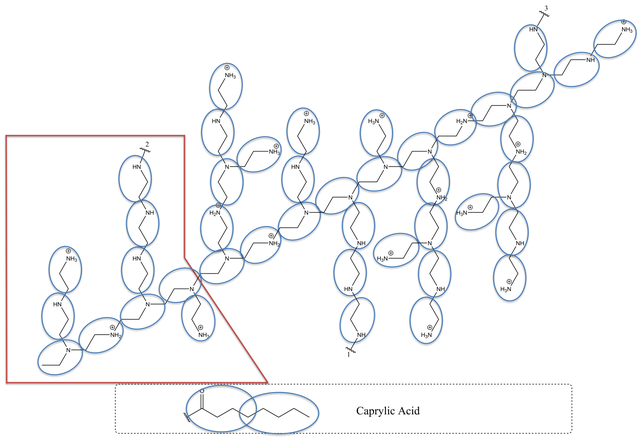

Figure 2: Molecular structure, protonation sites, and lipid substitution sites of 2 kDa PEI, with red enclosure depicting a 500 Da portion. The blue enclosures show the CG mapping. The substitution sites for caprylic acid (CA) are indicated by 1, 2, 3. For one CA substitution, site 2 and 1 is chosen for 500 Da and 2 kDa PEI, respectively. The nitrogen connected to substitution sites are protonated if they were not substituted.

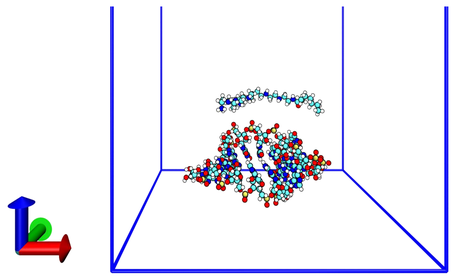

Figure 3: PEI-CA is moved along the z-axis (blue axis) to span various reaction coordinate values.

Results & Discussions

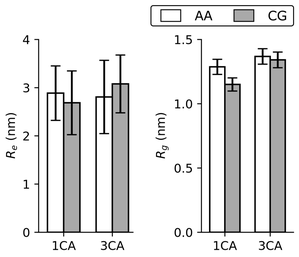

Figure 4: End-to-end distances (left) and radius of gyration (right) for 2 kDa PEI with one or three caprylic acid substitutions in both AA and CG simulations.

- CG 2 kDa PEI with caprylic acid substitutions accurately reproduces the structural properties of its AA counterpart (**Figure 4**).
- CG PMF between 500 Da PEI and siRNA with default nonbonded parameters does not match the AA-PMF (**Figure 5a**); subsequent adjustments to nonbonded parameters improve PMF matching (**Figure 5b**).
- AA and CG PMF between 500 Da PEI substituted with 1 CA and siRNA were comparable without additional adjustments of nonbonded parameters (**Figure 5c**).

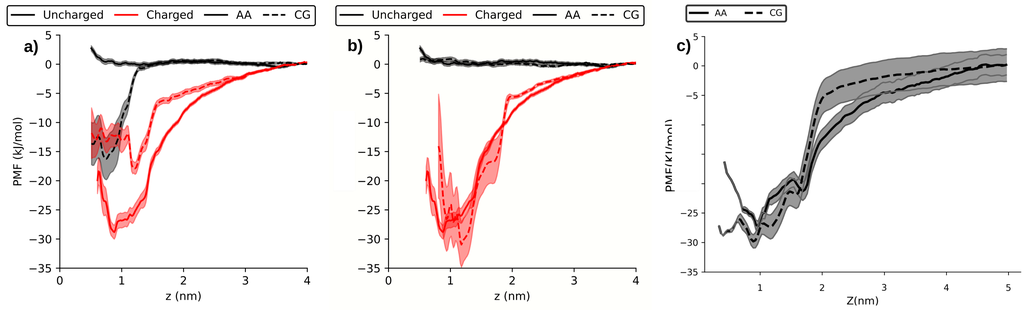

Figure 5: Comparison of AA and CG PMF between siRNA and a 500 Da PEI with a) default and b) modified nonbonded parameters, as well as c) 500 Da PEI-CA. The PEI is either uncharged or charged at 40% protonation ratio, while PEI-CA had a protonation ratio of 30%. The nonbonded interaction parameters from b) are used without additional modification in c).

Conclusions

- Achieved compatibility between CG PEI forcefield and siRNA.
- Extended the CG PEI forcefield to model CG PEI substituted with caprylic acid.
- Compatibility of CG PEI-CA and siRNA was demonstrated, which did not need additional tuning of nonbonded interactions.

Acknowledgements

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