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Elucidating the effect of polymer flexibility, molecular geometry, and charge neutralization on siRNA-polycation complexes free energy landscape: a computational study

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Introduction

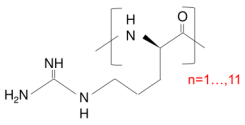
The success of medical threatment with DNA and silencing interference RNA is strongly related to the design of efficient delivery technologies [1]. Cationic polymers represent an attractive strategy to serve as nucleic-acid carriers with the envisioned advantages of efficient complexation, low cost, ease of production, well-defined size, and low polydispersity index. At present, polyethylenemine (PEI), polylysine (polyLYS) and polyarginine (polyARG), among others, have been investigated for gene delivery. However, the balance between efficacy and toxicity (safety) of these polymers is a challenge and in need of improvement [2-3]. With the aim of designing more effective polycationic-based gene carriers, computer simulations and in particular enhanced sampling techniques may be applied to support the design of potent and selective nucleic acids carriers characterized by the best compromise between drug complexation stability and release ability of the delivery system.

Computational Methods

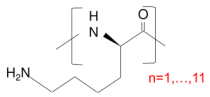
System Coordinates and Topologies

- The **siRNA sequence** dGdG(AGCAGCACCUUCAGGAU)dUdU [33] was selected for the present work.
- Three different **polycationic polymers** of 10 repeating units were considered: polyARG, polyLYS, and PEI with different protonation ratios (27% and 45 %, respectively)

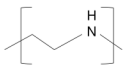
polyARG



polyLYS



LINEAR PEI



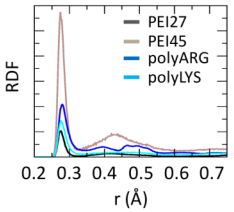
Enhanced Sampling Techniques

- **Repilca Exchange Molecular Dynamics.**
  - 128 replicas from 300K to 530K (CSCS CRAY XC50)
  - Average exchange probability of 0.4
  - A cumulative simulated time of 6.5 s for each system.
  - Force Field: AMBER99-ILDN force-field and TIP3P water.
  - GROMACS-5
- **Metadynamics Simulations.**
  - CV1: distance between the polymer and the major inertia axis of the target siRNA (DFA)
  - CV2: projection of the polymer on the major inertia axis of the target siRNA (POA)
  - Time-dependent gaussian deposition rate of 2 kJ/mol·ps
  - Gaussian widths of 0.5Å (DFA) and 1 Å (POA)

Results

SiRNA-polycations complex formation at the atomistic level

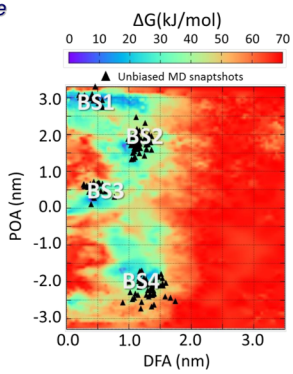
REMD trajectory at 300K was analyzed to compute the Radial Distribution Funcion of the polycationic amine nitrogen atoms around electronegative atoms of siRNA backbone.The first peak results from the hydrogen bond between the amine hydrogen atoms and the phosphate oxygen, while the second peak indicates the water-mediated hydrogen bonding.



SiRNA-polycations free energy landscape

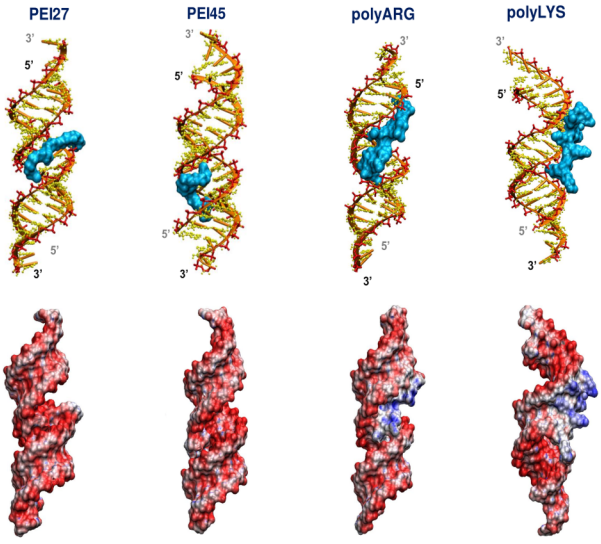
For each polymer, the free energy surface was represented as a function of two collective variables.

Molecular system	Binding Affinity (kJ/mol)
PEI27	70 ± 3
PEI45	120 ± 5
polyARG	250 ± 9
polyLYS	120 ± 4



Electrostatic potential and charge neutralization mechanism

While PEI27 and PEI45 are able to neutralize the siRNA electrostatic potential in the binding region, polyARG and in particular polyLYS were surrounded by a positive potential, which may allow the formation of multivalent interactions with other siRNA molecules.



Conclusions

Our work provides novel insight into the siRNA-polycation complexation mechanism, elucidating how polycation-siRNA binding modes and free energy landscapes are influenced by the physico-chemical properties of the interacting polymers. We demonstrated that polyARG has the best binding affinity compared to polyLYS, PEI45, and PEI27. Moreover, significant differences between polyLYS and polyARG binding modes have been identified. The free energy estimation provided in the present work may be used within the design process of potent nucleic acids binders able to finally reach the best compromise between complexation stability and release ability.

References

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[2] Wiseman et al. Gene Ther. (2003);10: 1654–1662.
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