Effect of Polymer Backbone Architecture on Delivery Efficiencies of ROMP-Based Protein Mimics

Leah M. Caffrey¹; Brittany M. deRonde²; Gregory N. Tew²
¹Department of Chemistry, University of Massachusetts Amherst
²Department of Polymer Science and Engineering, University of Massachusetts Amherst

Protein Transduction Domain Mimics: Overview[1,2,3]

- Protein transduction domains (PTDs), also known as cell penetrating peptides (CPPs), expedite cellular uptake of small molecules
- siRNA, plasmid DNA, proteins, antibodies
- Rich in cationic amino acid residues that enable peptide to cross cell membrane and deliver materials

- These residues are used as inspiration for the design of tunable, synthetic mimics of proteins and peptides
- Function: delivery agents of various essential biomolecules

Goal[1-4]: To explore the role of polymer backbones in siRNA delivery using two different ROMP scaffolds
- Vary both degree of polymerization (numbers of monomer repeat units) and cationic charge count per monomer
- Diguanidine (dG) series: 2 charges/monomer
- Polyguanidinium oxanorbornene (PGON): series: 1 charge/monomer

Starting Materials Synthesis

Monomer Synthesis

Monomer Synthesis

Monomer Synthesis

Monomer Synthesis

Monomer Synthesis

Monomer Synthesis

Monomer Synthesis

Ring-opening Metathesis Polymerization (ROMP)

- Ring-opening:
  - Reaction driven by relief of ring strain
- Metathesis:
  - Breaking and reforming of bonds
- Polymerization:
  - Connecting monomers together to make chain structures

Preliminary siRNA Delivery Experiments in Jurkat T Cells

Critical guanidinium charge content required for efficient delivery

Jukat T Cells: N/P = 8:1; Media = RPMI with 10% FBS; Cell Density = 4x10⁶ cells/mL; 4 h incubation.

Summary

- dG and PGON polymer precursors successfully synthesized and characterized
- dG 10, 20 polymers and dG 10, 20, 30, 40 polymers were successfully synthesized and characterized
- Plot of Mn vs. [M]/[I] is linear: controlled polymerization of dG monomer
- dG 20, 40, 60 polymers deliver siRNA
- Optimal charge content for efficient FITC-siRNA delivery
- dG polymers deliver most amount of siRNA; delivery decrease with higher DP
- dG polymers have extremely high cell viability: minimal cell death

Future Work

- Synthesize full series of dG and PGON polymers
- Test siRNA delivery and cytotoxicity with entire series of polymers
- dG polymers included to determine point at which DP increase leads to delivery decrease
- Size and zeta potential measurements
- Perform experiments to assess complex formation and stability
- Dye release assay using model vesicle systems experiments

Acknowledgements

Tew Research Group

References