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

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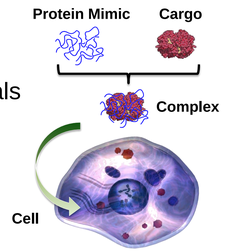
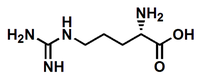
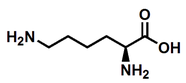
Protein Transduction Domain Mimics: Overview[1,2,3]

- **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



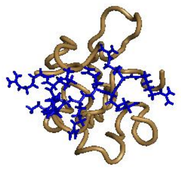


Arginine

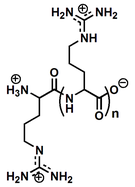


Lysine

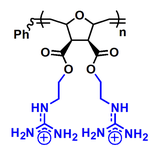
- These residues are used as inspiration for the design of **tunable, synthetic mimics of proteins and peptides**
  - Function: delivery agents of various essential biomolecules



HIV-1 TAT protein
PDB Code: 1tiv
(Blue Residues 49-57 = RKKRRQRRR)



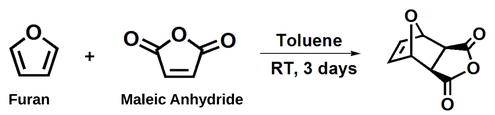
Polyarginine



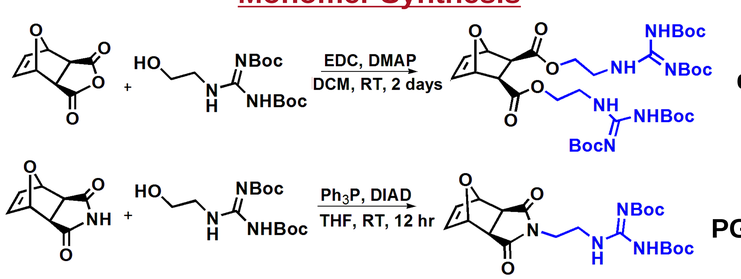
Synthetic guanidinium-rich polymer

- **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

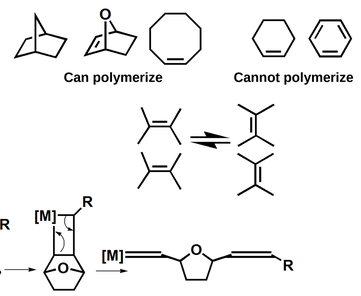


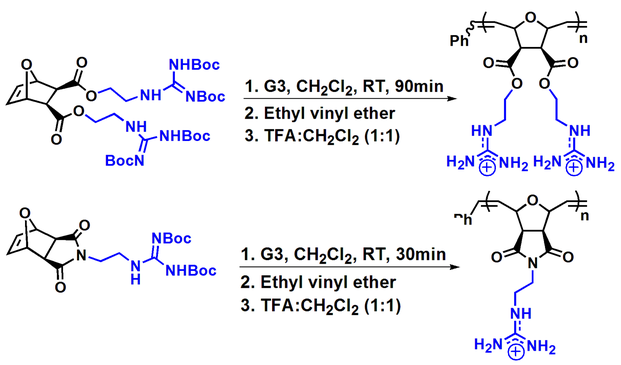
Monomer Synthesis

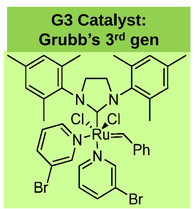


Ring-opening Metathesis Polymerization (ROMP)

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





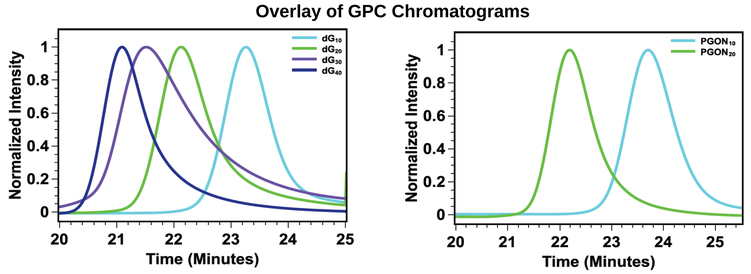


Degree of Polymerization (DP)	dG: n= 10, 20, 30, 40, 60, 80 PGON: n= 10, 20, 30, 40, 60, 80, 100, 120, 160

*DP shown in black have been synthesized, in red are to be made in the future

Molecular Weight Data

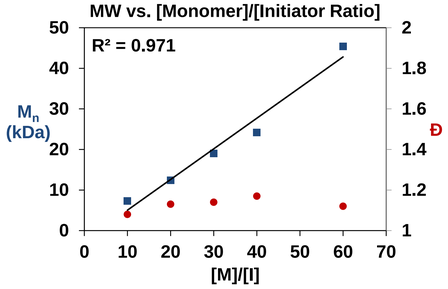
Overlay of GPC Chromatograms



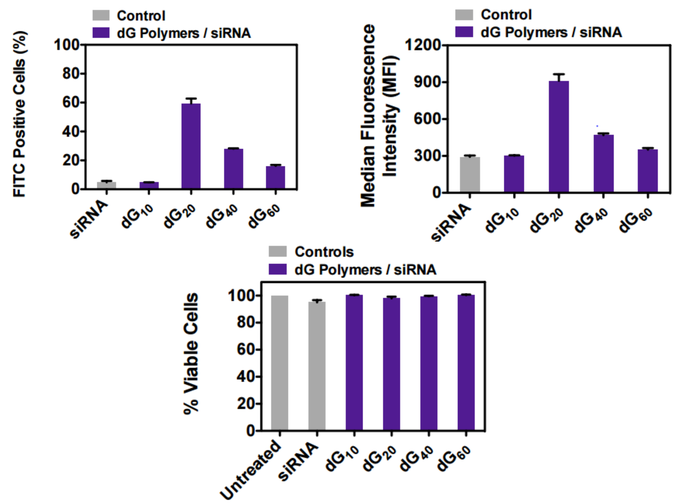
Molecular Weight Summary

Polymer	M_p (Da)	M_n (Da)	M_w (Da)	Đ
dG_{10}	8000	7300	7900	1.08
dG_{20}	15300	12400	14000	1.13
dG_{30}	25000	19000	21800	1.14
dG_{40}	30700	22800	27700	1.17
dG_{60}	40500	45400	48700	1.12
$PGON_{10}$	5600	5100	5500	1.08
$PGON_{20}$	15000	12900	14100	1.10

MW vs. [Monomer]/[Initiator Ratio]



Preliminary siRNA Delivery Experiments in Jurkat T Cells



Critical guanidinium charge content required for efficient delivery

Jurkat T Cells: N/P = 8/1; Media = RPMI with 10% FBS; Cell Density = $4x10^5$ cells/mL; 4 h incubation.

Summary

- dG and PGON polymer precursors **successfully synthesized and characterized**
- PGON 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_{20}** delivers 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_{30}** 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 assaying using model vesicle systems experiments

Acknowledgements

Tew Research Group

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NSF

References

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[3] deRonde, B. M.; Tew, G. N.; Development of protein mimics for intracellular delivery. *Biopolymers. Epub* 2015 Apr. 9.
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