Novel Plasmid DNA Bottlebrush Polymers

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Introduction

Advances have been made toward generating bottlebrush polymers with unique topologies and assemblies, but synthetic methods and dispersity limitations remain. **In this work, biologically derived pUC19 plasmid DNA (pDNA) serves as a backbone for synthetically made poly(ethylene glycol) methyl ether mustargen (mPEG-CEA) to alkylate under biologically relevant conditions (Figures 1-2).** By varying the molecular weight of mPEG-CEA and the concentration relative to the pDNA, **we aim to generate variety of linear and cyclic bottlebrush polymers with this method (Figures 3-4)**. Visualization of the formed bottlebrushes will be attempted through the combination of atomic force microscopy (AFM) and agarose gel electrophoresis. This method serves as a facile route to achieve PEGylated DNA materials which can significantly improve DNA's ability to be used as a material in the future.

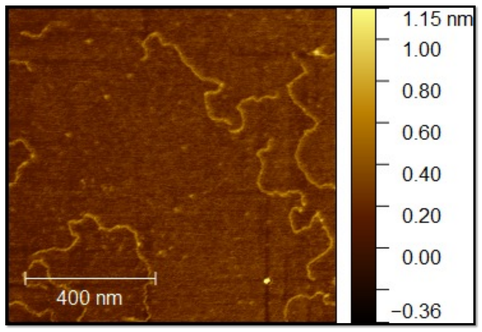


Figure 1. Biologically derived linear pUC19 plasmid DNA (double-stranded) from DH5α competent cells and grown via a fed-batch fermentation procedure; captured via Cypher VRS1250 Video-Rate High Speed AFM (Oxford Instruments, Asylum Research).

Graft-to Mechanism

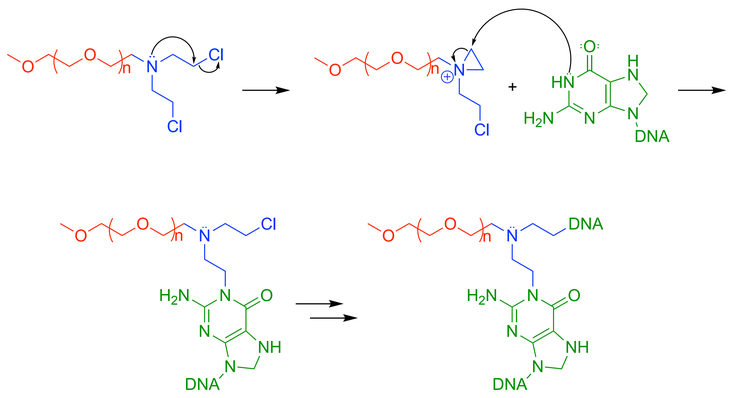


Figure 2. Proposed mechanism for alkylation of DNA with via intramolecular cyclization (aziridinium formation) of the mustargen followed by nucleophilic attack by the N7 position of guanidine

Methods & Results

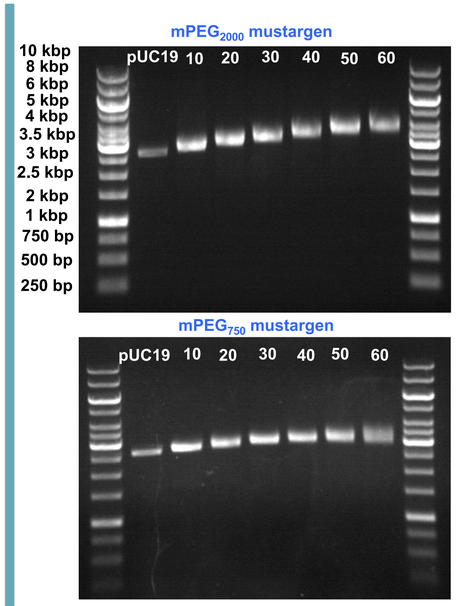


Figure 3. 0.5% (w/v) agarose gel run at 75V for 1 hour. This gel is showing samples of increasing molar equivalence of mPEG-mustargen relative to pDNA (10-60 equivalence). Lane 1 & 9: BioMarker 10kb DNA ladder. Lane 2: **linearized** pUC19. Lanes (3-8) are increasing molar equivalence of mPEG-mustargen 10, 20, 30, 40, 50, 60.

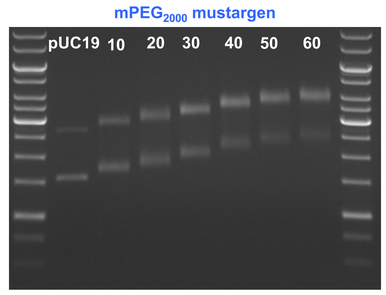
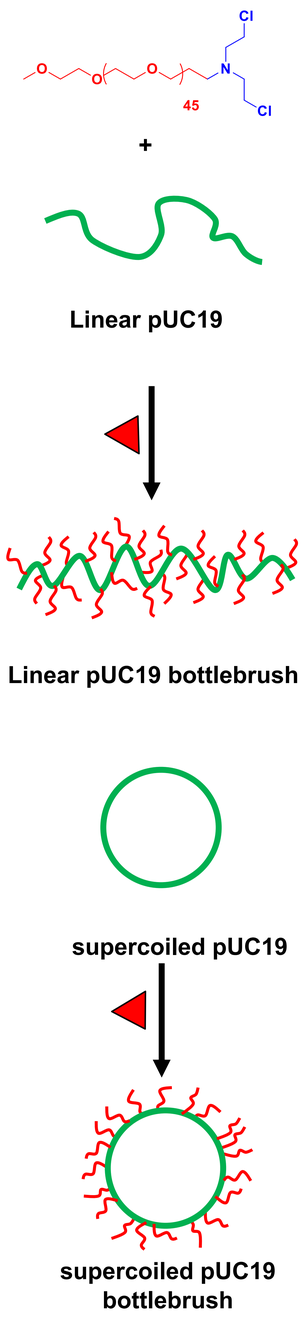
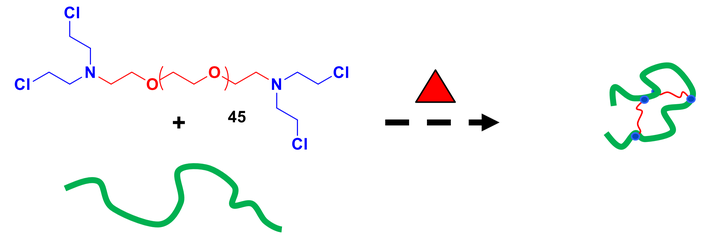


Figure 4. 0.5% (w/v) agarose gel run at 75V for 1 hour. This gel is showing samples of increasing molar equivalence of mPEG-mustargen relative to pDNA (10-60 equivalence). Lane 1 & 9: BioMarker 10kb DNA ladder. Lane 2: **supercoiled** pUC19. Lanes (3-8) are increasing molar equivalence of mPEG-mustargen 10, 20, 30, 40, 50, 60.



Single-Chain Nanoparticles

Preliminary results. Attempts are currently underway to find experimental conditions to form single-chain nanoparticles (SCNPs). **Under dilute conditions and using a PEG bis-mustargen, intramolecular crosslinks may form between two different sites on pDNA.** The resulting crosslinks would cause a characteristic collapse of the resulting conjugate and lowering of the hydrodynamic radius. Early results have been showing possible formation of dimers or trimers of pUC19. Conditions must be optimized under dilute conditions to yield an intramolecular crosslink.



Conclusions & Future Work

Conclusion

We have generated encouraging results for the possible formation of pDNA bottlebrush via a novel mPEG mustargen. Gel electrophoresis allows for simple characterization of the possible pDNA conjugates. **These results aim to provide a facile route for a grafting-to PEGylation approach of pUC19 plasmid DNA.**

Future Work

Future work includes getting AFM images of the plasmid-PEG conjugates and a restriction digest study to confirm chemical modification and overall structure. Additionally, optimizing conditions for pUC19 SCNP formation.

References

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2. Alconcel, S. N. S.; Baas, A. S.; Maynard, H. D. FDA-Approved Poly(Ethylene Glycol)-Protein Conjugate Drugs. *Polymer Chemistry* **2011**, *2* (7), 1442–1448. https://doi.org/10.1039/c1py00034a.

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