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A Study on Gene Therapy: Using Poly (β -aminoesters)

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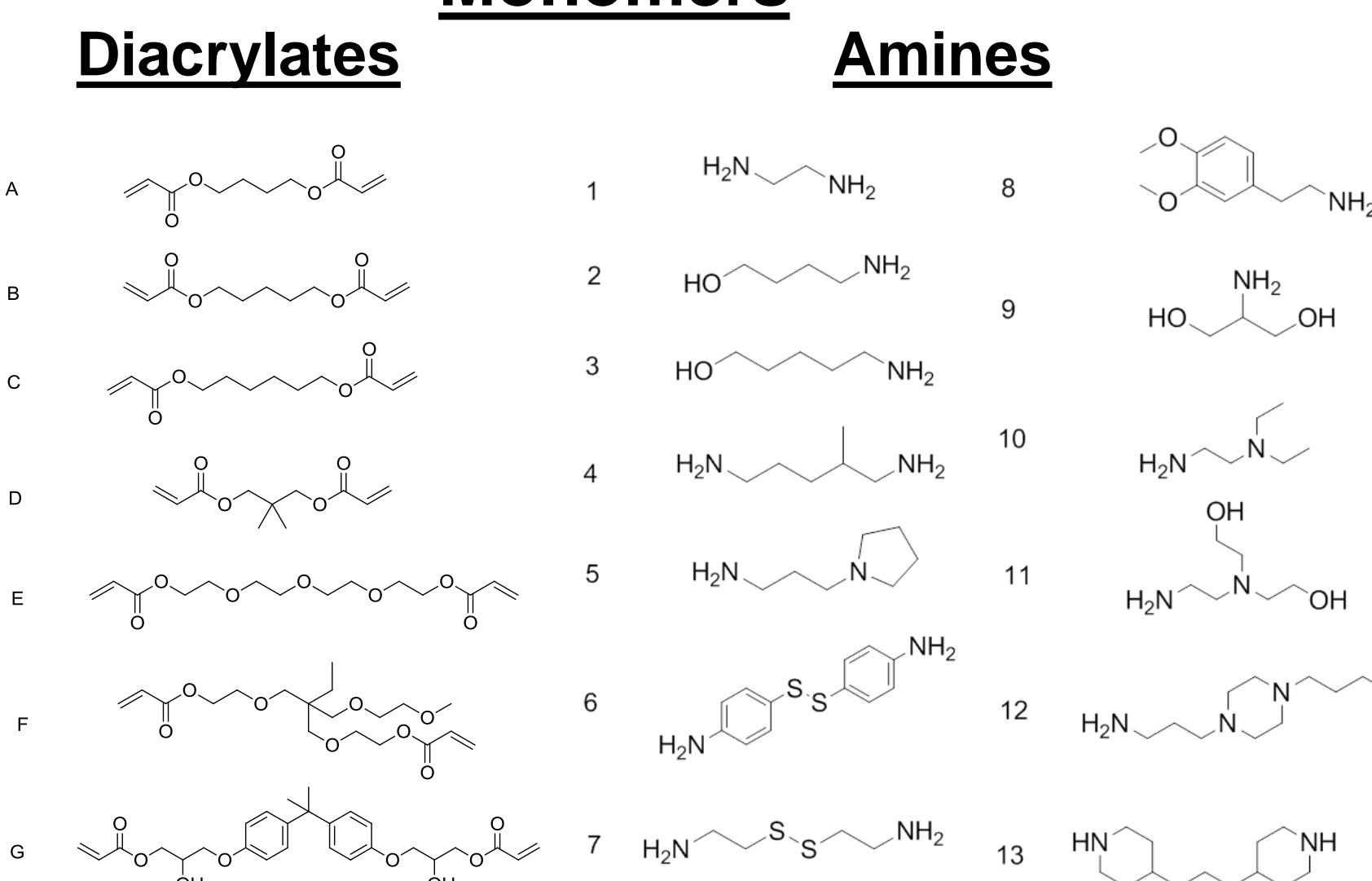
ABSTRACT

Gene delivery is the process of administering therapeutic genes to a patient's cells, where upon uptake, their modulation of the protein expression patterns in the host can result in a desired phenotype. This is especially beneficial for the treatment of a large number of diseases at the genetic level, including cancer. Cationic polymers, which are known for their ease of synthesis that can allow for a large library of polymers to be created for studies, have garnered much attention as potential gene delivery vectors. One type of cationic polymer, poly (β -aminoesters) (PBAEs), is being utilized as a potential type of vector for gene delivery. Unlike other vectors that have been observed in the past, which include viral vectors, poly-L-lysine [PLL], and poly(ethylene imine) [PEI], PBAEs are structurally diverse and non-cytotoxic. In addition, PBAEs are easily synthesized (via Michael addition), biodegradable, and capable of controlled DNA release into the cell.

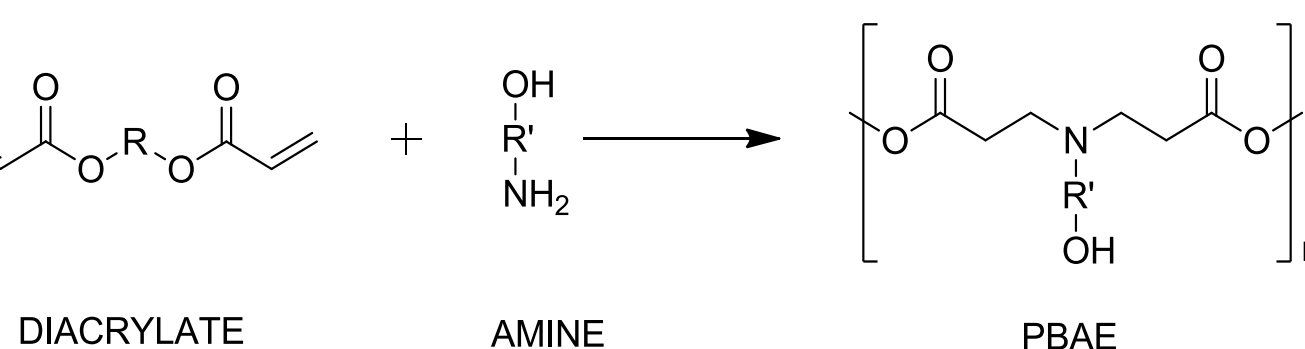
INTRODUCTION

PBAE Synthesis

Monomers



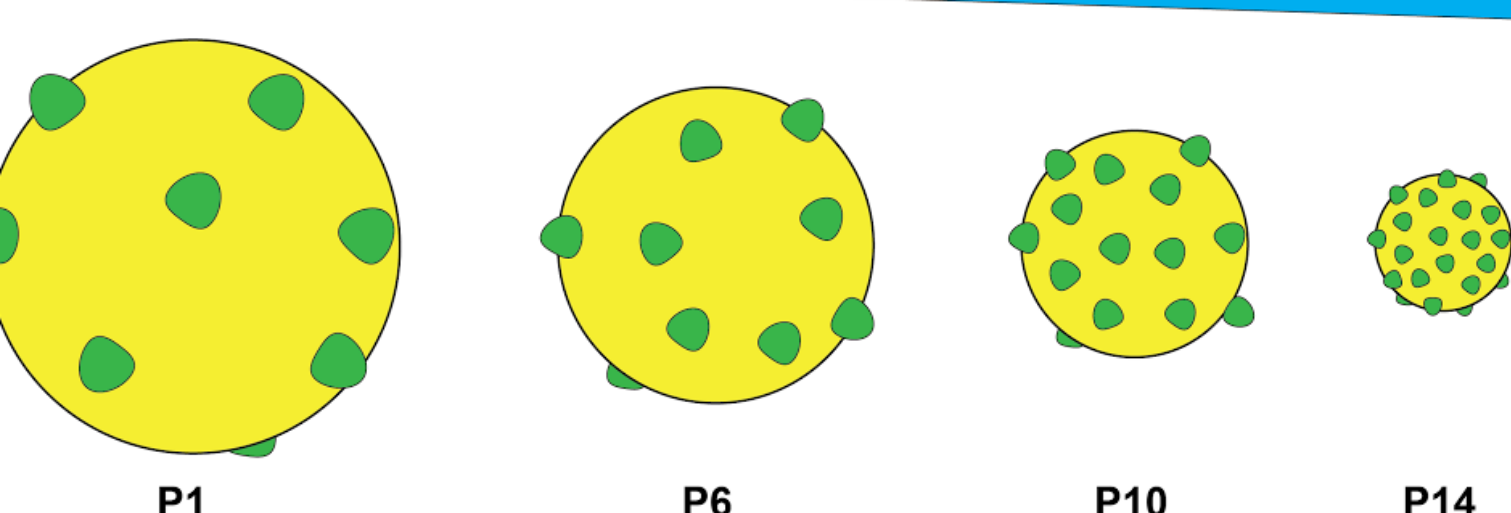
Reaction Scheme



A diacrylate and an amine combine, via the one-step Michael Addition, to create a PBAE. The simplicity of Michael Addition allows for a large library of PBAEs..

Polymer Molecular Weight

Mannose Surface Density

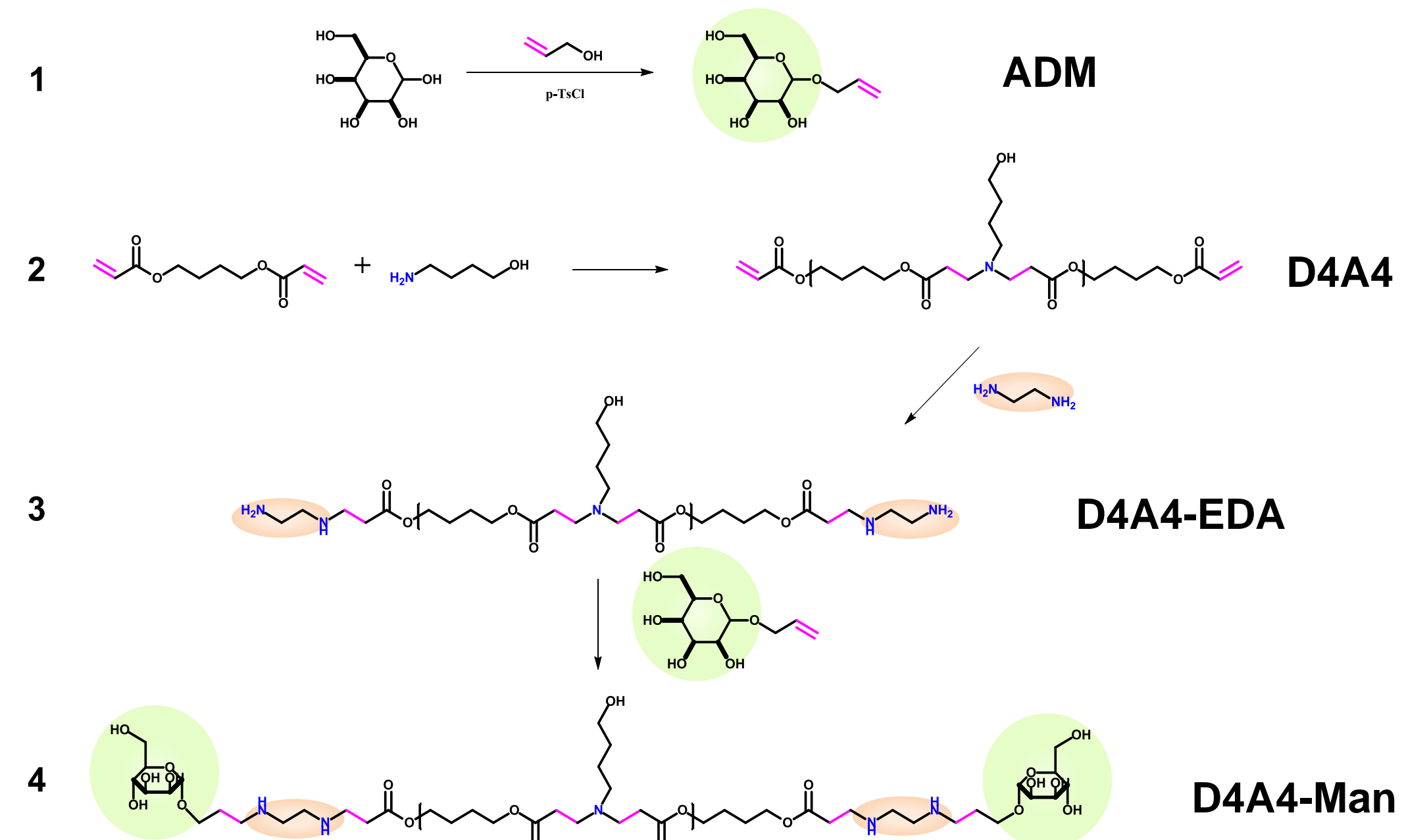


We analyzed the effects of mannosylation on PBAEs of a wide range of molecular weights. As polymer molecular weight decreased, the surface density increased.

RESULTS & DISCUSSION

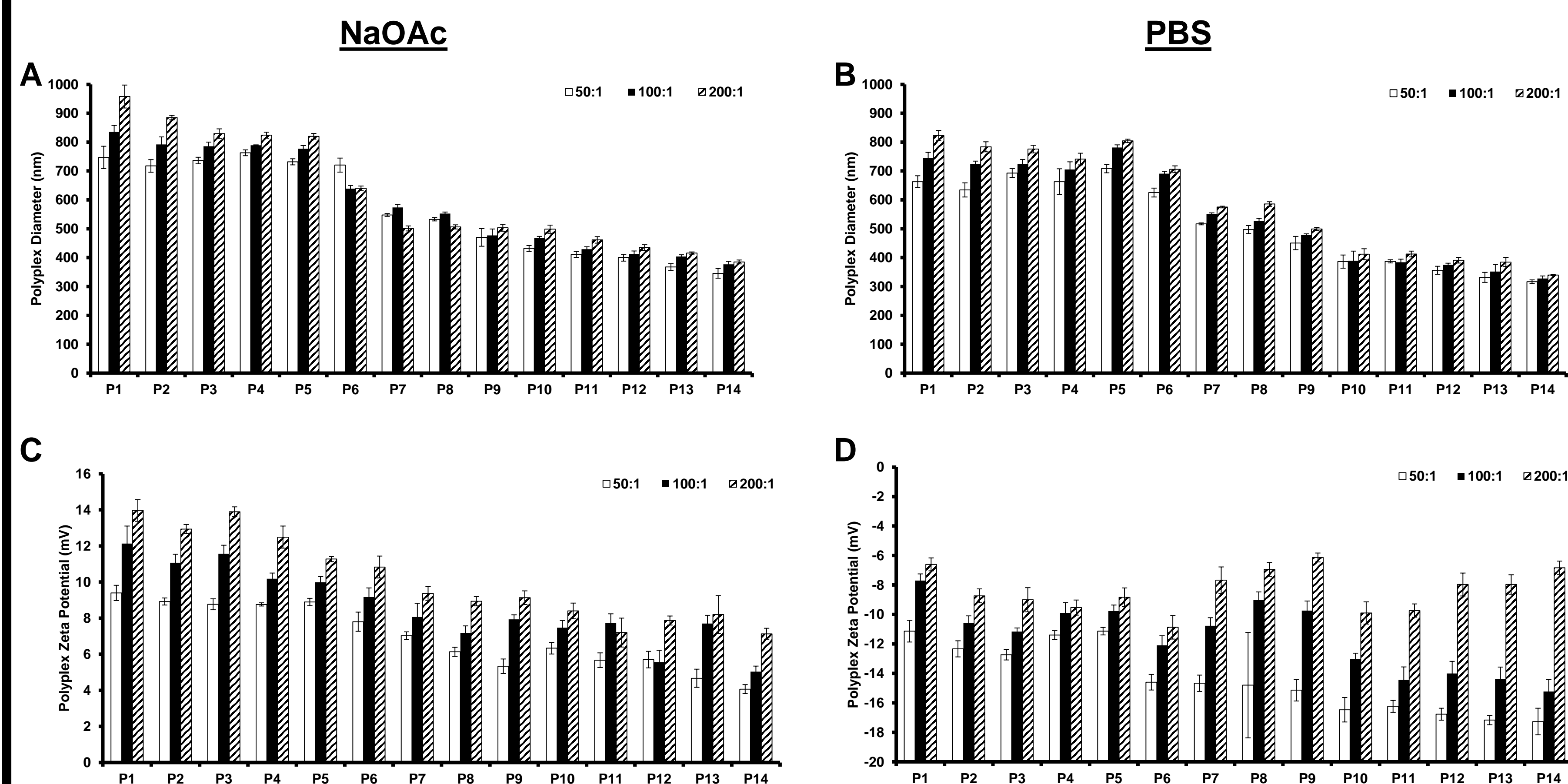
Mannosylation of PBAE

- Mannose is used as a grafting ligand.
- It has been previously shown that mannosylating PBAEs improved gene delivery efficiency.¹
- In these studies, we examine the effects of both mannosylation and molecular weight on the gene delivery efficiency of one specific PBAE – D4A4.



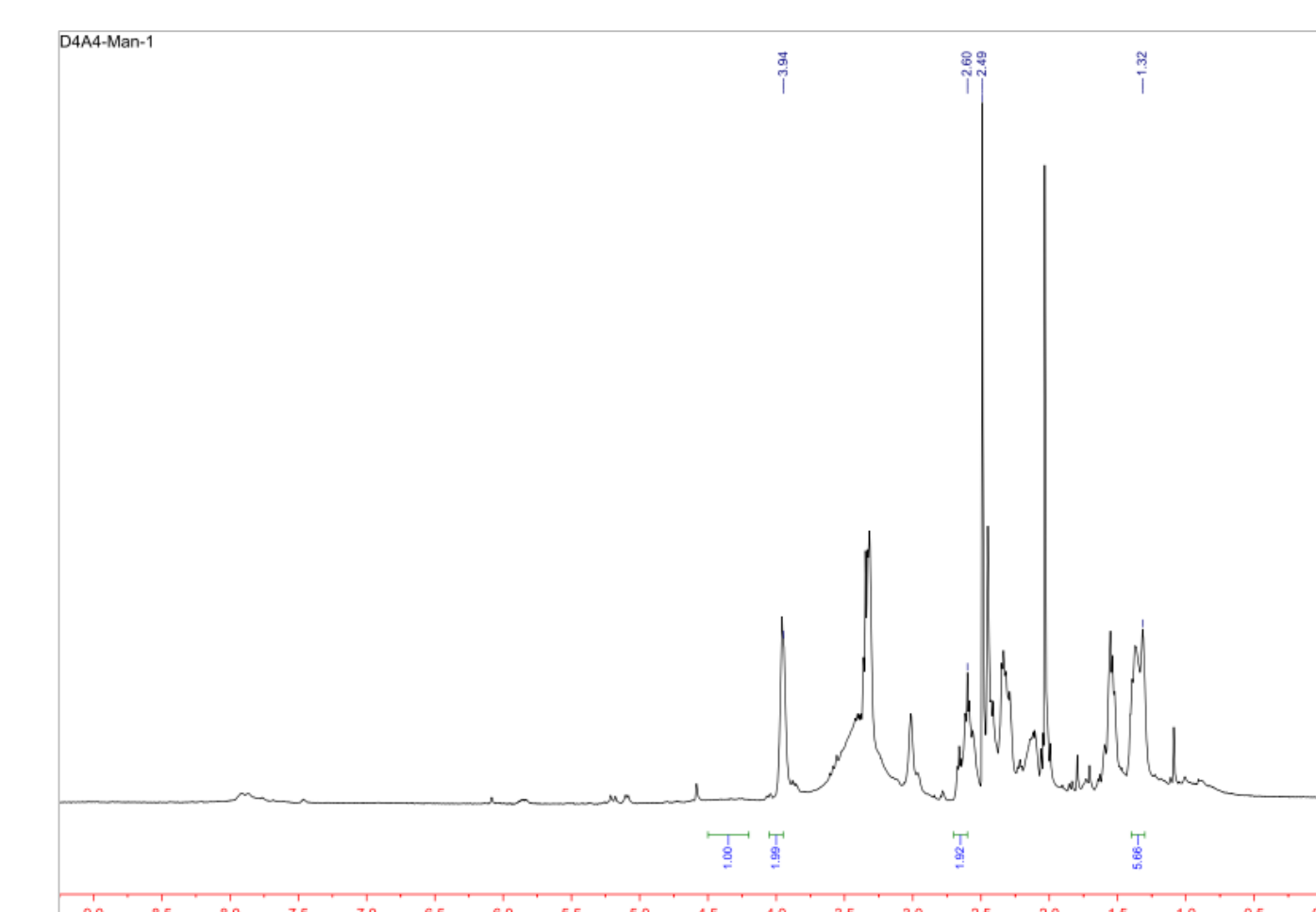
Polyplex Characterization

- To form polyplexes, three ratios of polymer to pDNA were used: 50:1, 100:1, and 200:1. Polyplexes are ordered on these graphs in descending molecular weight, with P1 having the highest molecular weight, and P14 having the lowest.
- Using dynamic light scattering (DLS), both the polyplex diameter (nm) and the polyplex zeta potential (mV) were measured. Sodium acetate (NaOAc) and PBS were used as solvents.
- As molecular weight decreases, both the polyplex diameter and zeta potential decrease.



NMR Spectroscopy

- Nuclear magnetic resonance spectroscopy (NMR) allows us to determine (and confirm) the structure and purity of each polymer
- NMR was also used to evaluate polymer degradation profiles. This significantly reduced instrumentation time, as well as experimental costs.



The NMR Spectroscopy of D4A4-Man-1

- In order to determine the degradation of a polymer, the resonance intensities of the OH protons of degradants relative to the 12 CH₂ protons at 3.95-4.05, 2.6-2.7, and 1.3-1.4 ppm (indicated above).
- Relative Hydroxyl Group Generation (RHGI) was then calculated:

$$RHGI = \frac{OH}{Index} - H$$

In which:

$$OH = I_{(4.5-4.2)}$$

$$H = (I_{(4.05-3.95)} + I_{(2.7-2.6)} + I_{(1.4-1.3)})/12$$

CONCLUSIONS & FUTURE DIRECTIONS

- As molecular weight increases, both zeta potential (mV) and diameter of polyplex (nm) decrease.
- NMR Spectroscopy also allowed us to determine the polymer degradation profiles at a significantly lower cost and time.
- Future experiments that can be considered include:
 - Imaging using SEM, TEM, Confocal Microscope
 - Antigen Presenting Cell (APC) Gene Delivery studies
 - Infectious disease and cancer models

ACKNOWLEDGEMENTS

- Mingfu Chen, Charles Jones, Anitha Ravikrishnan, and Dr. Blaine Pfeifer of the Pfeifer Group in the Chemical and Biological Engineering Department at the University at Buffalo
- University Honors College

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- Jones, C.H., Chen, M., Gollakota, A., Ravikrishnan, A., Zhang, G., Lin, S., Tan, M., Cheng, C., Lin, H., Pfeifer, B. A. Structure-function assessment of mannosylated poly(β -amino esters) upon targeted antigen presenting cell gene delivery. *Under revision at Biomacromolecules*