# Characterizing the Formation of Polyethyleneimine-based Polymer-DNA Nanoparticle Complexes for Improved Gene and Drug Delivery Applications

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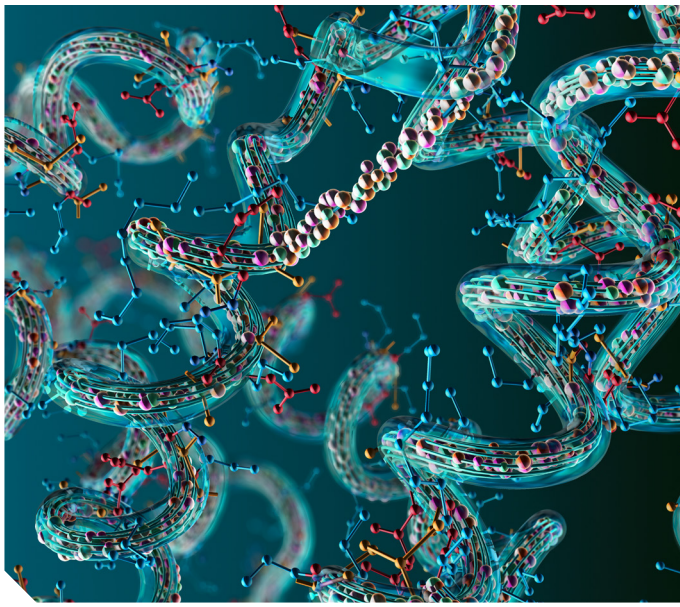
**In this note:** Characterization of polyplex formation at millimolar concentrations of PEI.

Polyethyleneimines (PEIs) are widely studied as non-viral vectors for gene and drug delivery due to their ability to compact nucleic acids into nanoparticles known as polyplexes [1]. Compared to viral vectors, PEIs offer reduced immunogenicity, higher loading capacity, and lower production costs [2].

However, their clinical translation has been limited by relatively low transfection efficiencies [3]. One key factor influencing transfection is the nature of polyplex formation, which governs cellular uptake and intracellular release. Effective delivery systems require polyplexes that are stable enough to prevent pre-mature release of nucleic acids, yet weak enough to release the cargo at the correct time and location inside the cell [4].



## Introduction

Understanding the intermolecular interactions and conformational changes involved in polyplex formation and stability is essential for optimizing PEI-based delivery platforms. As recently reported by Mustafa *et al.* [5], attenuated total reflectance infrared (ATR-FTIR) spectroscopy is well suited for this purpose. It provides molecular-level insights into DNA structure and binding through vibrational modes of the phosphate backbone and nucleobases. This application note demonstrates how ATR-FTIR can be used to characterize polyplex formation with both linear and branched PEI architectures.

## Experimental

A full description of the methods are described by Mustafa *et al* [5]. ATR-FTIR measurements were performed on a Bruker INVENIO FTIR spectrometer equipped with a Harrick ConcentratIR2™ multiple reflection silicon ATR accessory. Two PEI architectures were studied: linear PEI (LPEI, 15 kDa) and branched PEI (BPEI, 10 kDa). Polyplexes were formed at various nitrogen-to-phosphate (N/P) ratios from 0 to 3 using 0.01 M phosphate buffer (pH 7.4). Final concentrations were 3 mM for LPEI, 5 mM for BPEI, and 1 mM for DNA. Samples were incubated for one hour at room temperature prior to measurement.

To confirm spectral interpretation, additional samples were prepared with increasing sodium chloride concentrations (50–250 mM) to screen electrostatic interactions. All spectra were background subtracted, baseline-corrected and normalized.

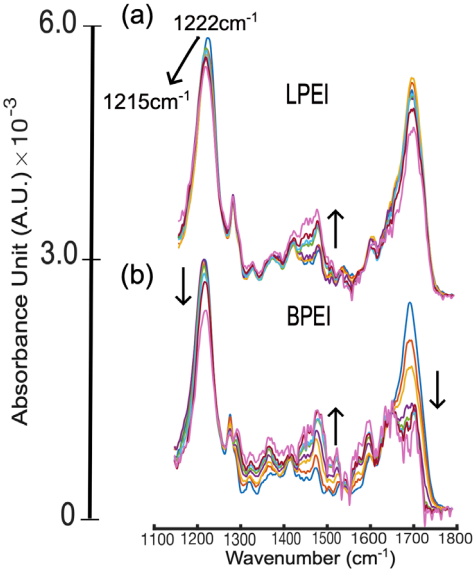



**Figure 1: ATR-FTIR spectra of DNA in the presence of LPEI (a) and BPEI (b) in phosphate buffer (pH = 5.0). The black arrow indicates increasing N/P ratio from 0-3.**

## Results and Discussion

Figure 1 shows ATR-FTIR spectra of DNA in the presence of increasing N/P ratios for LPEI and BPEI. With LPEI, the asymmetric phosphate stretching band at 1222 $cm^{-1}$ exhibits a clear frequency shift as N/P increases, indicating strong electrostatic binding to the DNA backbone. This frequency shift also indicates a conformational change in the DNA structure from the B-form to the Z-form. Moderate changes in the nucleobase ring stretching region (1400–1500 $cm^{-1}$) and carbonyl band at 1680 $cm^{-1}$ were also observed. These changes indicate that LPEI form weak binding interactions to the nucleobases of DNA.

In contrast, spectra of BPEI-based polyplexes showed no shift at 1222 $cm^{-1}$, but a significant intensity decrease and splitting of the 1680 $cm^{-1}$ band, indicating interactions with nucleobases via intercalation, without significant disruption of the phosphate backbone.

Salt screening experiments confirmed these interpretations. As shown Figure 2, increasing NaCl reduced LPEI binding by ~70%, consistent with electrostatic interactions. BPEI binding, however, decreased by only 20–30% at the highest salt concentrations, confirming binding is predominately facilitated by interactions with DNA nucleobases.

## Conclusion

This study demonstrates that ATR-FTIR with the Harrick ConcentratIR2 accessory can effectively differentiate binding modes in PEI-DNA polyplexes. The 1222 $cm^{-1}$ phosphate band serves as a sensitive marker for LPEI-DNA electrostatic interactions, while the 1680 $cm^{-1}$ band reports on BPEI intercalation with DNA nucleobases. These insights provide a foundation for designing more effective non-viral gene delivery systems.

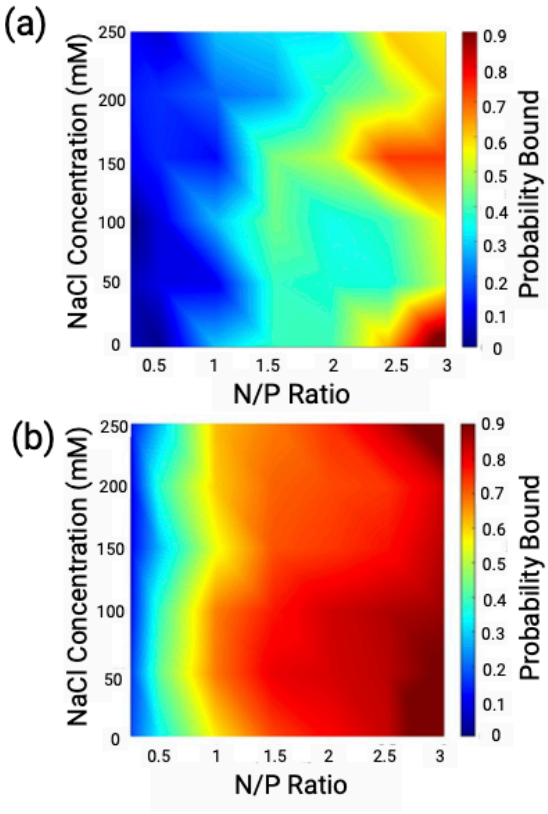



**Figure 2: Diagrams showing the probability of bound DNA by polymers obtained from the ATR-FTIR spectra of LPEI and BPEI polyplexes in the presence of varying NaCl concentrations (50-250 mM).**

## References


1. I., Degors; C., Wang; Z., Rehman; I., Zuhorn. "Carriers Break Barriers in Drug Delivery: Endocytosis and Endosomal Escape of Delivery Vectors". Acc. Chem. Res., 52 , 1750 —1760 (2019).
2. D. Puhl, A. D'Amato and R. Gilbert. "Challenges of gene delivery to the central nervous system and the growing use of biomaterial vectors". Brain Res. Bull., 150 , 216 —230 (2019).
3. J. Casper, S. Schenk, E. Parhizkar, P. Detampel, A. Dehshahri and J. Huwyler, J. "Polyethylenimine (PEI) in gene therapy: Current status and clinical applications". Control. Release, 362, 667 —691 (2023).
4. R. Kumar, C. Santa , M. Chalarca Bockman, C. B. Van, C. Grimme, R. Dalal, M. Hanson, J. Hexum and T. Reineke. "Polymeric Delivery of Therapeutic Nucleic Acids". Chem. Rev.,121, 11527 —116520 (2021).
5. R., Mustafa, D., Danielle, M., Harper, D., Punihaole, "Revealing two distinct molecular binding modes in polyethyleneimine-DNA polyplexes using infrared spectroscopy". Soft Matter, 21, 4192-4200 (2025).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