



Nano-Reverse Phase Liquid Chromatography-Mass Spectrometry Methods for In-Depth AAV Capsid Characterization

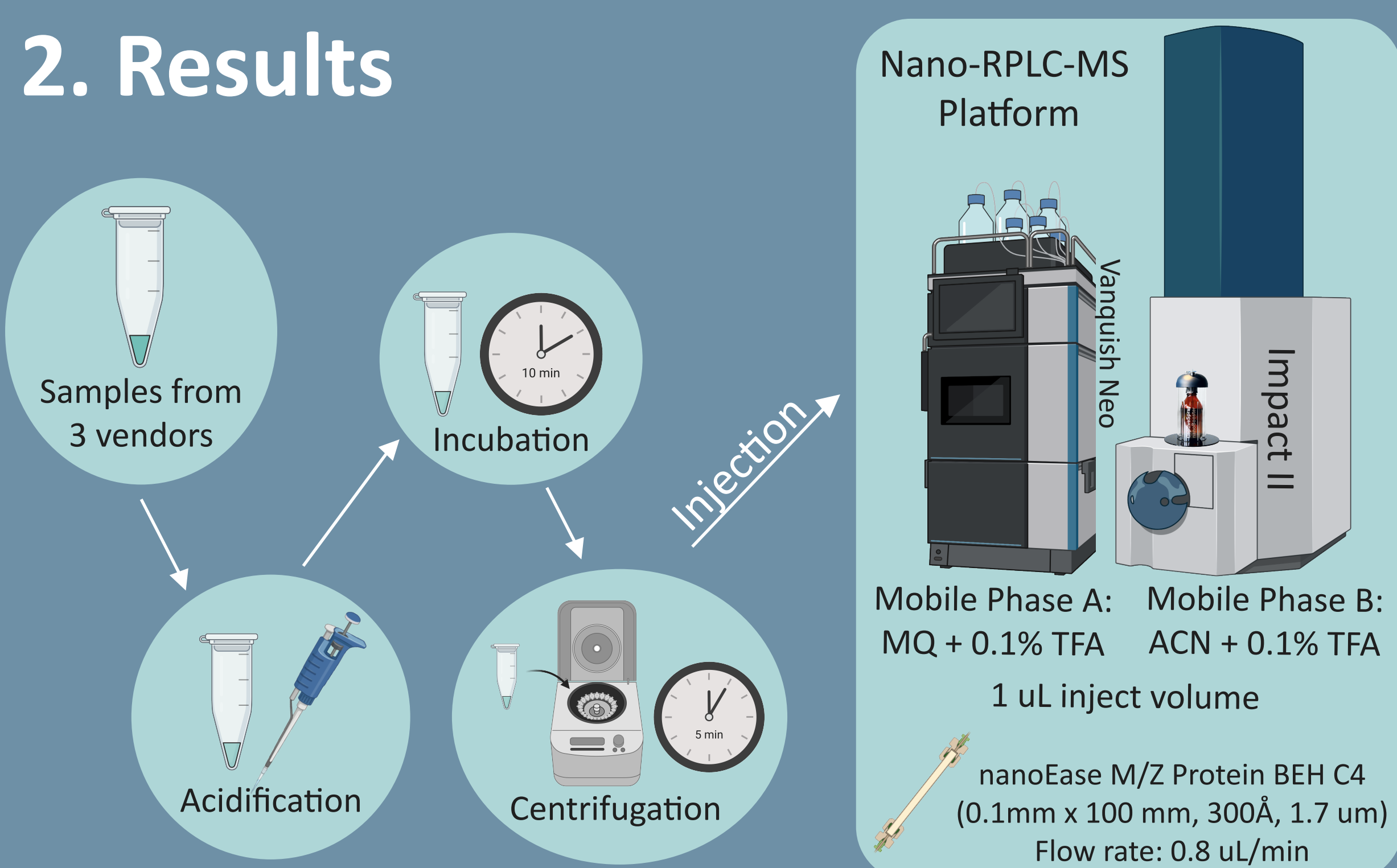


1. Introduction

Rising use of AAVs in gene therapy demands low-volume, low-concentration characterization methods because current approaches require high sample volumes and limit throughput. As capsid composition and post-translational modifications can influence potency, stability, and therapeutic efficacy, robust analysis is needed to assess each batch and support product comparability. We present a nano-RPLC-MS workflow that combines nano-LC separation with high-resolution MS1 to enable intact viral protein (VP) separation, serotype identification, PTM detection, and lot-to-lot comparisons during downstream processing (DSP) using minimal material.

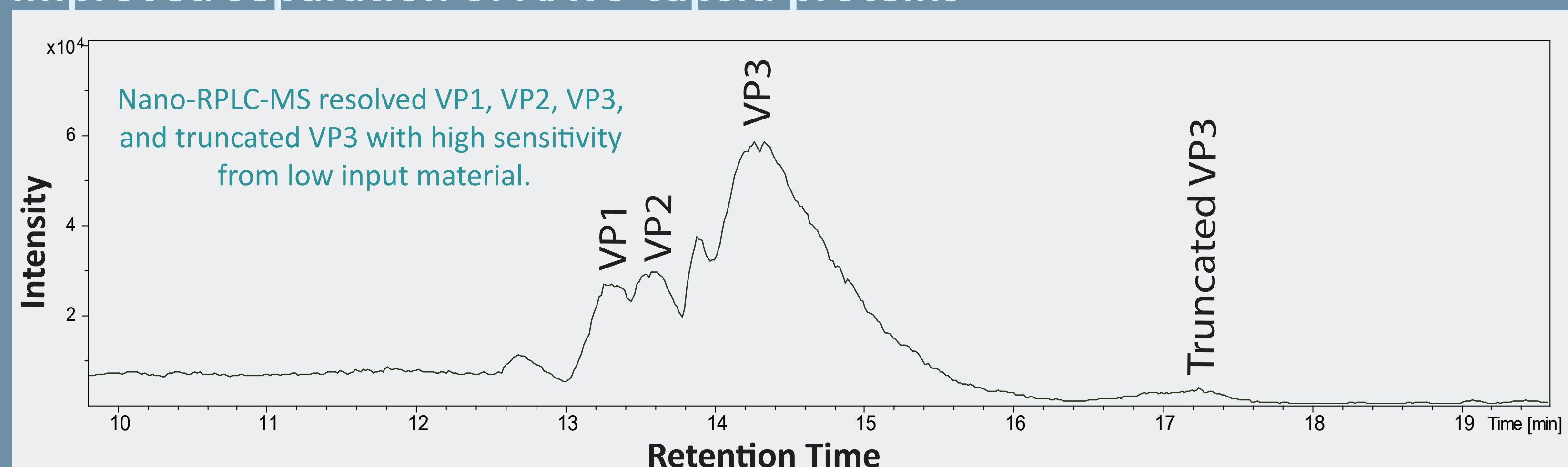
Aim: Develop an ultrasensitive nano-RPLC-MS platform for intact AAV capsid profiling from $\leq 1 \mu\text{L}$ ($\sim 0.3 \text{ ng}$) enabling detailed assessment of capsid heterogeneity relevant to gene therapy performance and batch comparability.

2. Results

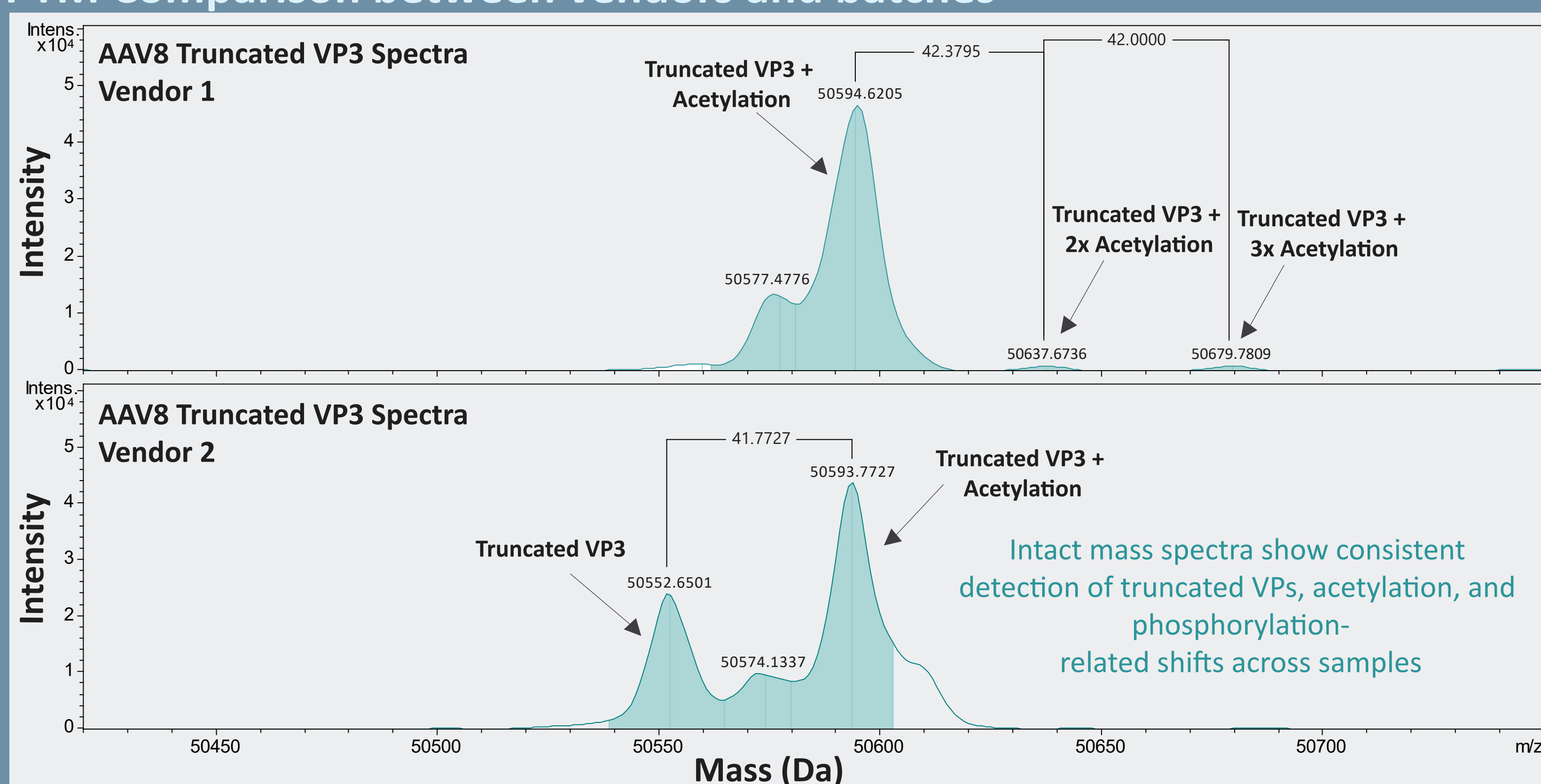


The nano platform achieves intact profiling from $\leq 1 \mu\text{L}$ ($\sim 0.3 \text{ ng}$) with improved VP separation, reproducible VP1/2/3 ratios, and PTM detection

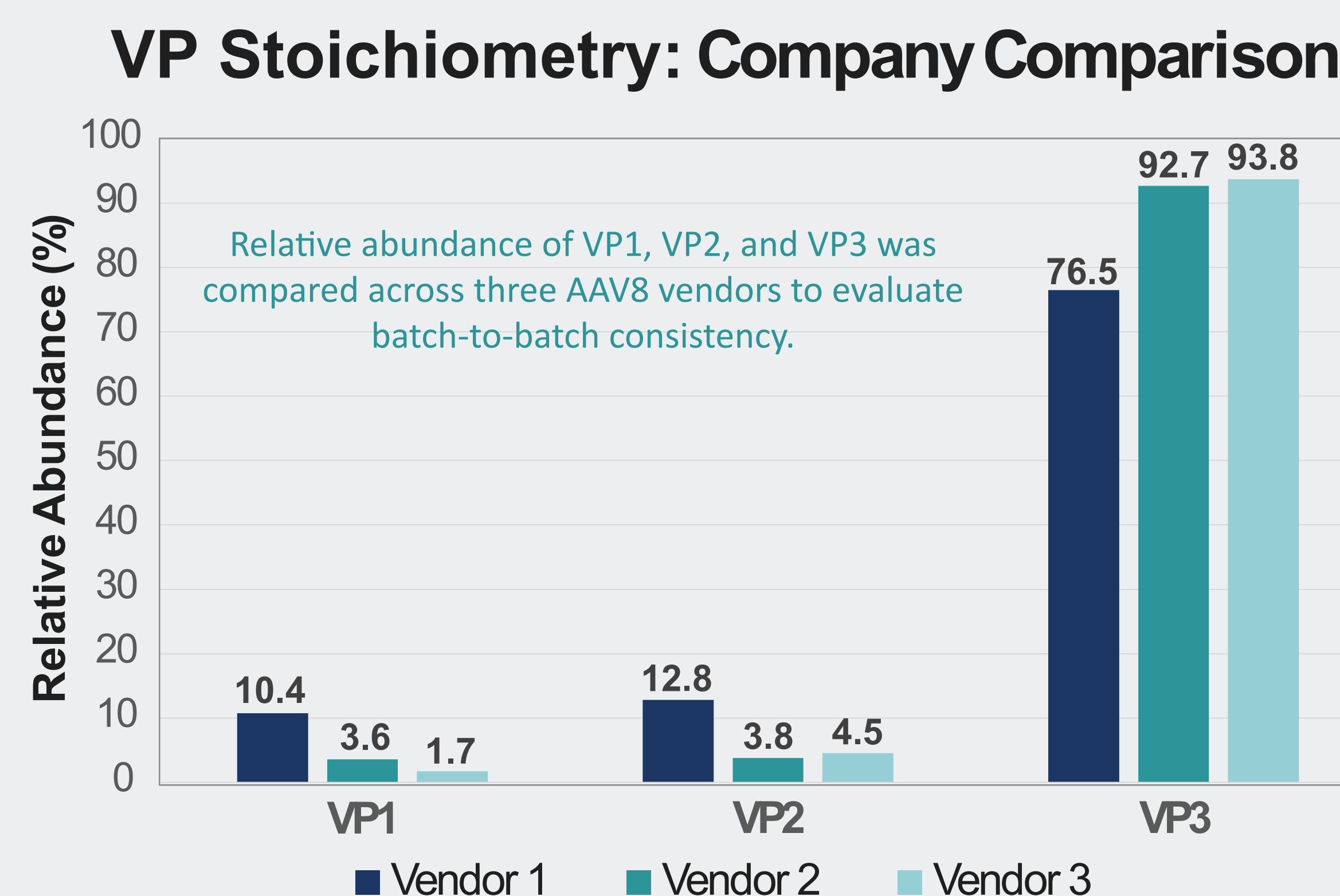
Improved separation of AAV9 capsid proteins



PTM Comparison between vendors and batches



VP Stoichiometry assessment between vendors (AAV8)



3. Conclusions

- Enables intact AAV capsid profiling from minimal material.
- Resolves VP species, detects PTMs, and compares stoichiometry across samples.
- Supports evaluation of potency, stability, and product consistency.
- Promising for consistent testing during DSP and earlier batch assessment.

4. Future plans

- Expand to additional AAV serotypes and batches.
- Apply the workflow to in-process DSP monitoring.
- Integrate complementary techniques for broader characterization.

5. Contact

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6. Acknowledgements



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