

Strong Anion-Exchange HPLC for Size-Range and Topology-Resolved Analysis of Nucleic Acids

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Strong AEX resolves nucleic acids from short ssDNA fragments to plasmid topoisomers. For large DNA, retention is not explained by ion exchange alone. Above the large-fragment regime, flow rate becomes a selectivity variable through post-release slalom retardation.

Summary

- Nucleic acid therapeutics and their manufacturing intermediates cover a wide analytical range, from short oligonucleotides to large plasmid constructs.
- Nucleic-acid analyses therefore must now distinguish fragment length, strand state, and plasmid topology within workflows that can be automated and transferred.
- Electrophoresis remains useful, but it is not an LC method-development platform and is less suited to routine fraction-compatible separations.
- Strong AEX provides salt-gradient control because nucleic acids present a dense phosphate backbone; above the large-DNA threshold, retention can no longer be assigned to charge-density contrast alone.

Question

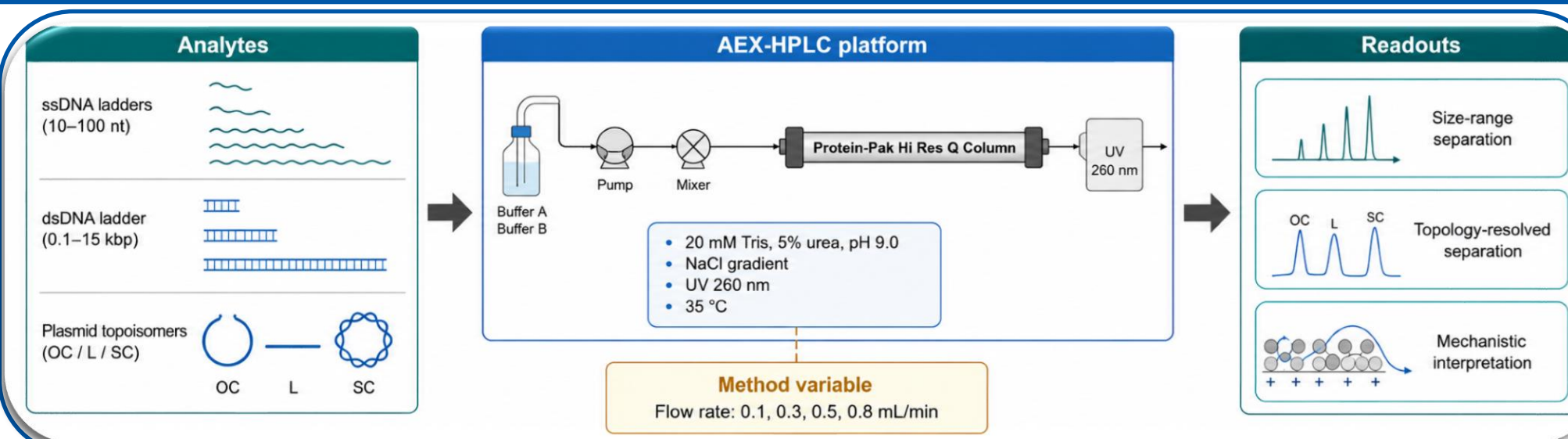
Can one strong AEX method cover fragment sizing and plasmid topology analysis, and at what size does flow rate become part of selectivity?

Experimental

- Column: Protein-Pak™ Hi Res Q, 5 μm, 4.6 × 100 mm Column, nonporous quaternary amine packing.
- Eluent A: 20 mM Tris, 5% urea, pH 9.0. Eluent B: Eluent A + 1.0 M NaCl.
- Ladder separations: 0.5 mL/min, 35 °C, UV 260 nm.
- Mechanism studies: 60 to 95% B over fixed 7.5 mL; flow rates 0.1, 0.3, 0.5, and 0.8 mL/min.
- Pressure controls separated shear-flow effects from static-pressure effects.



Experimental overview

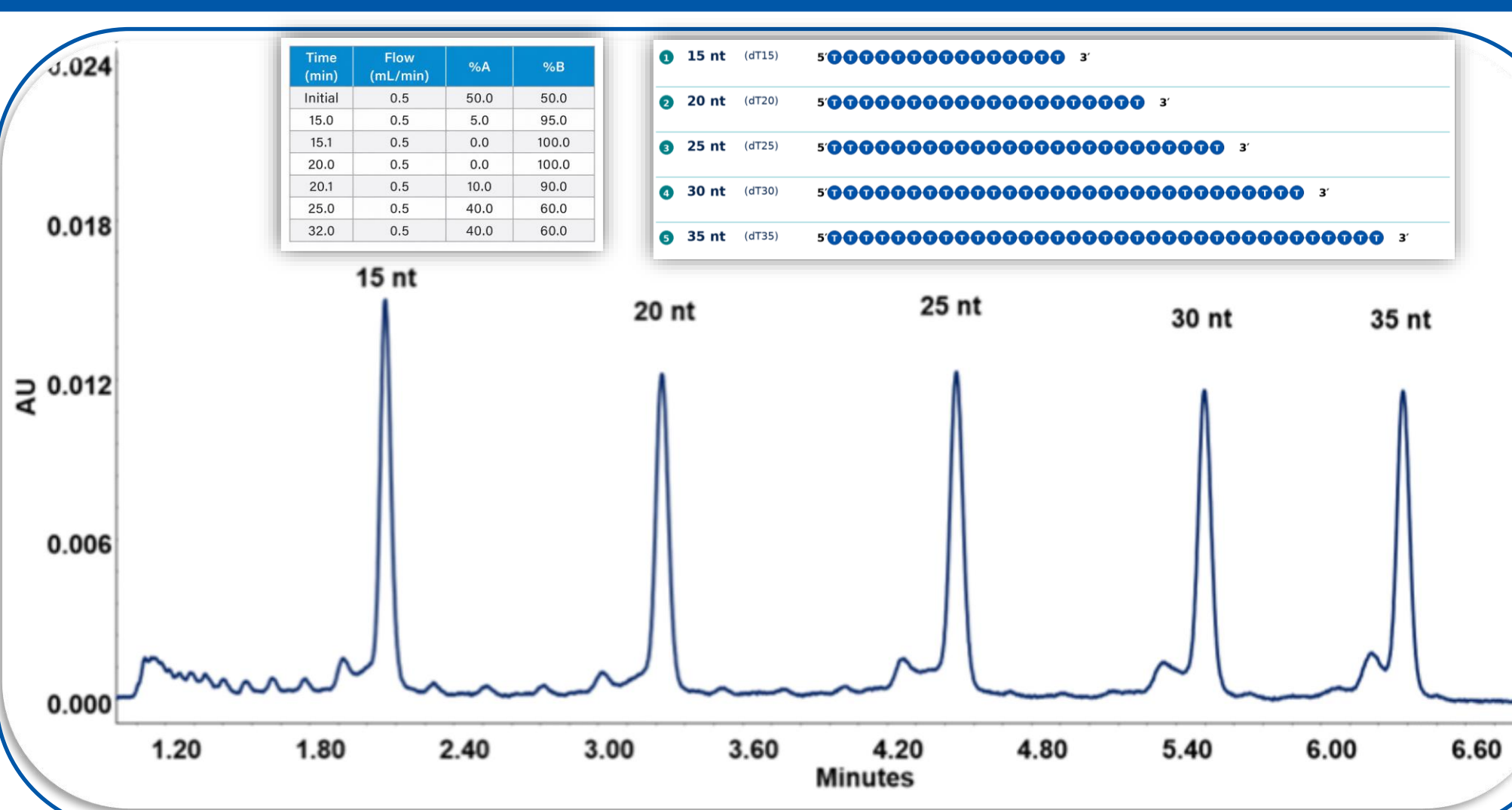


A single strong AEX-HPLC method was used to connect size-range nucleic acid separations with the flow-dependent retention behavior observed for large dsDNA and plasmid topoisomers.

Mechanistic scope

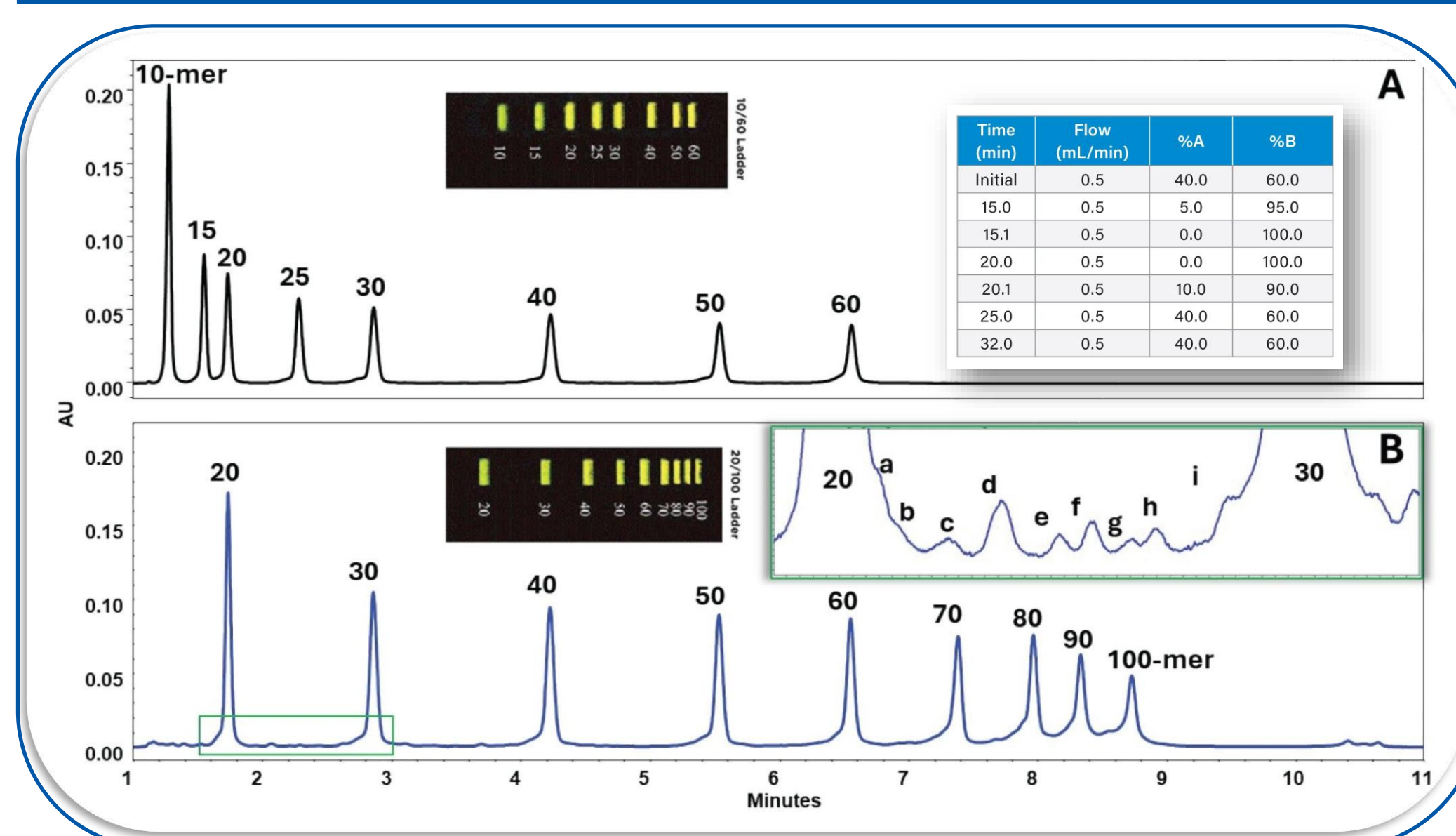
- The slalom contribution is invoked only after AEX release and only where molecular extension can alter migration through the interparticle space.
- Under the tested conditions, this contribution is negligible for short fragments and measurable for large linear dsDNA.
- Thus, flow rate is treated as a selectivity parameter only in the extension-sensitive size and topology regime.

AEX resolves ssDNA length variants from 15 to 35 nt



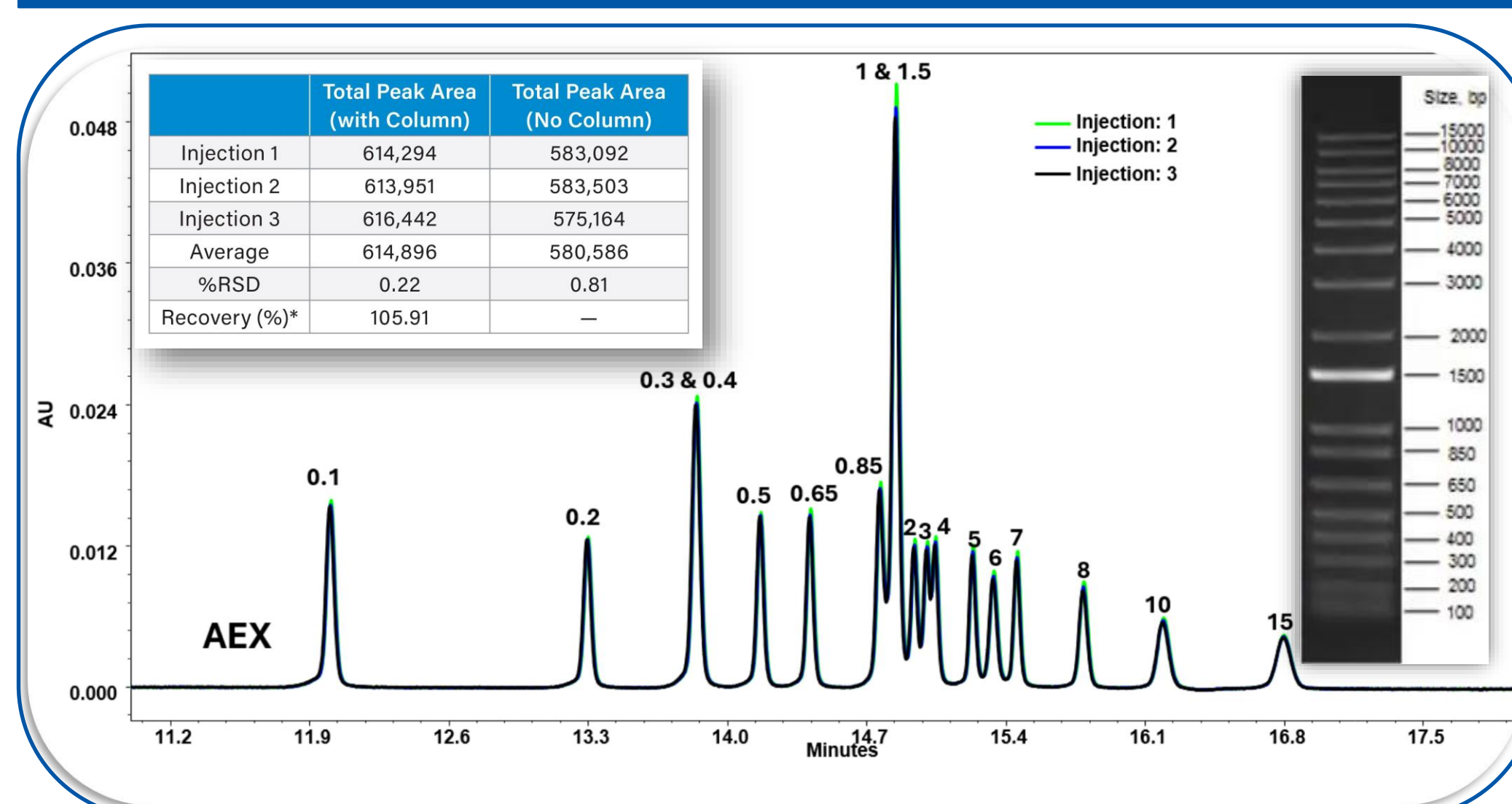
Salt-gradient AEX resolved discrete homopolymer ssDNA length (Poly dT) variants - Excellent separation of failure sequences.

AEX resolves ssDNA length variants from 10 to 100 nt



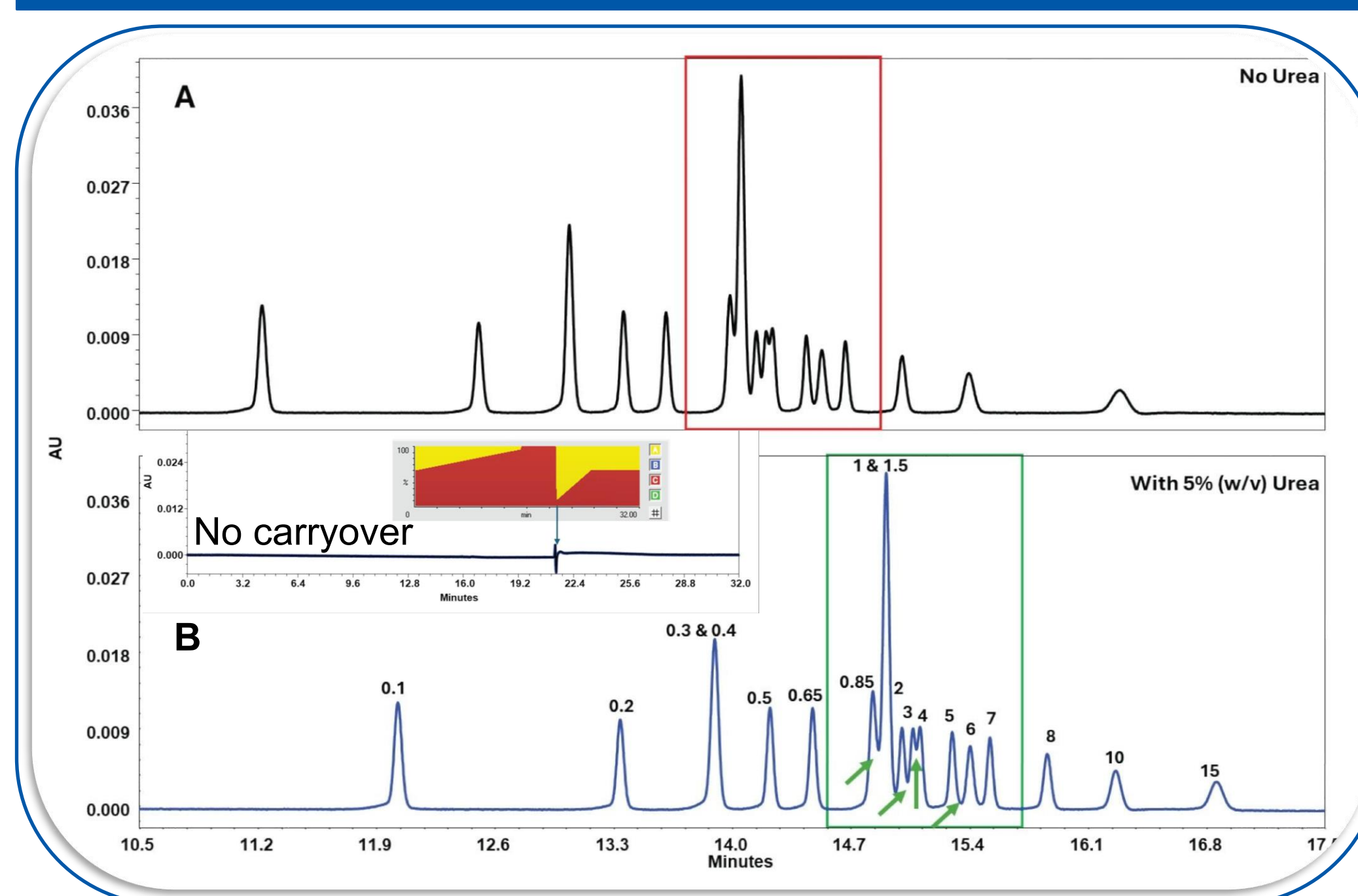
Salt-gradient AEX resolved discrete ssDNA (heteropolymer) length variants across both ladder sets, establishing the low-size charge-dominated regime.

AEX separates dsDNA fragments from 100 bp to 15 kbp



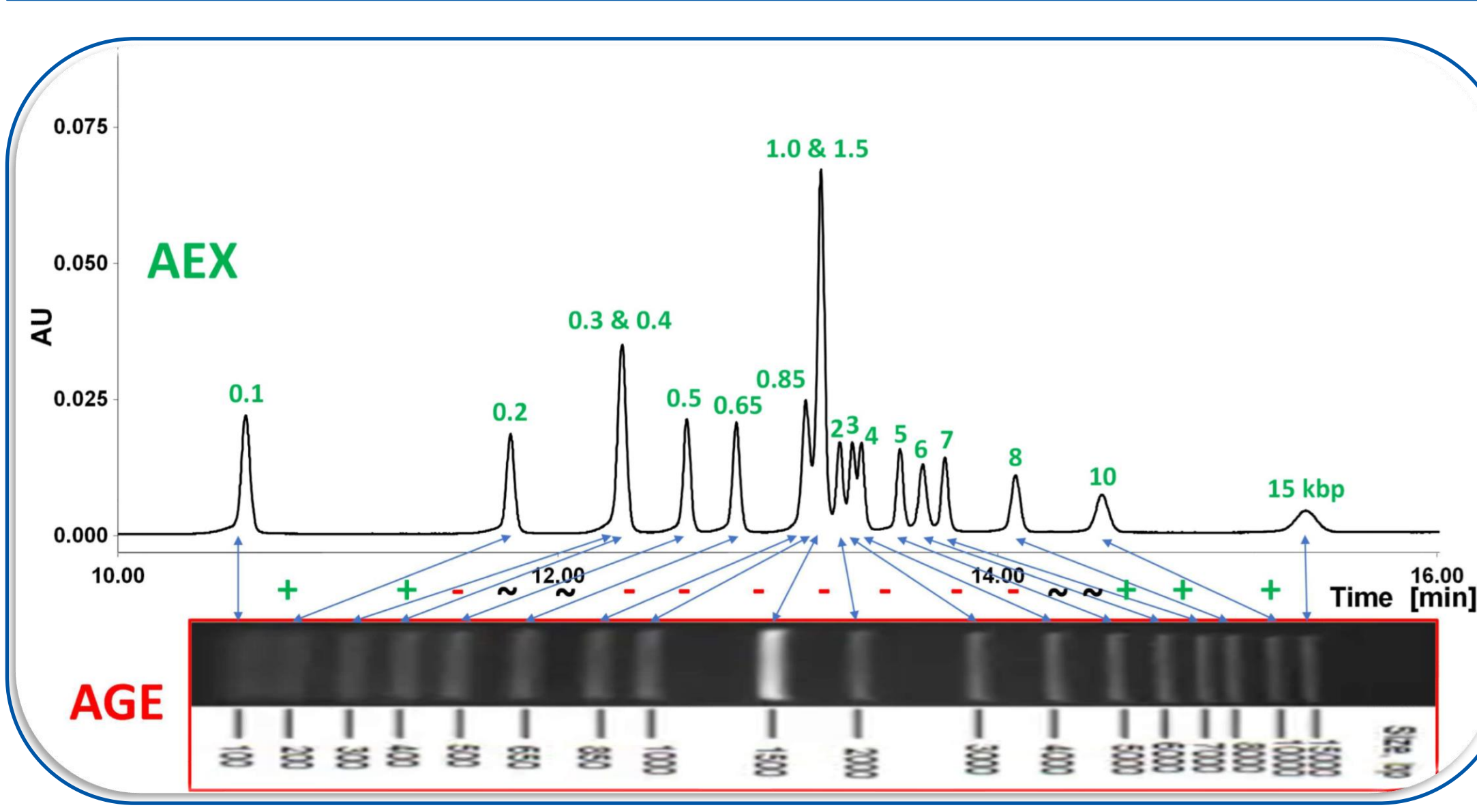
1 Kb Plus DNA Ladder was separated by strong AEX. The chromatographic profile resolved dsDNA fragments across a broad size range, with peak assignments supported by agarose gel electrophoresis.

Urea improves AEX peak quality and recovery behavior



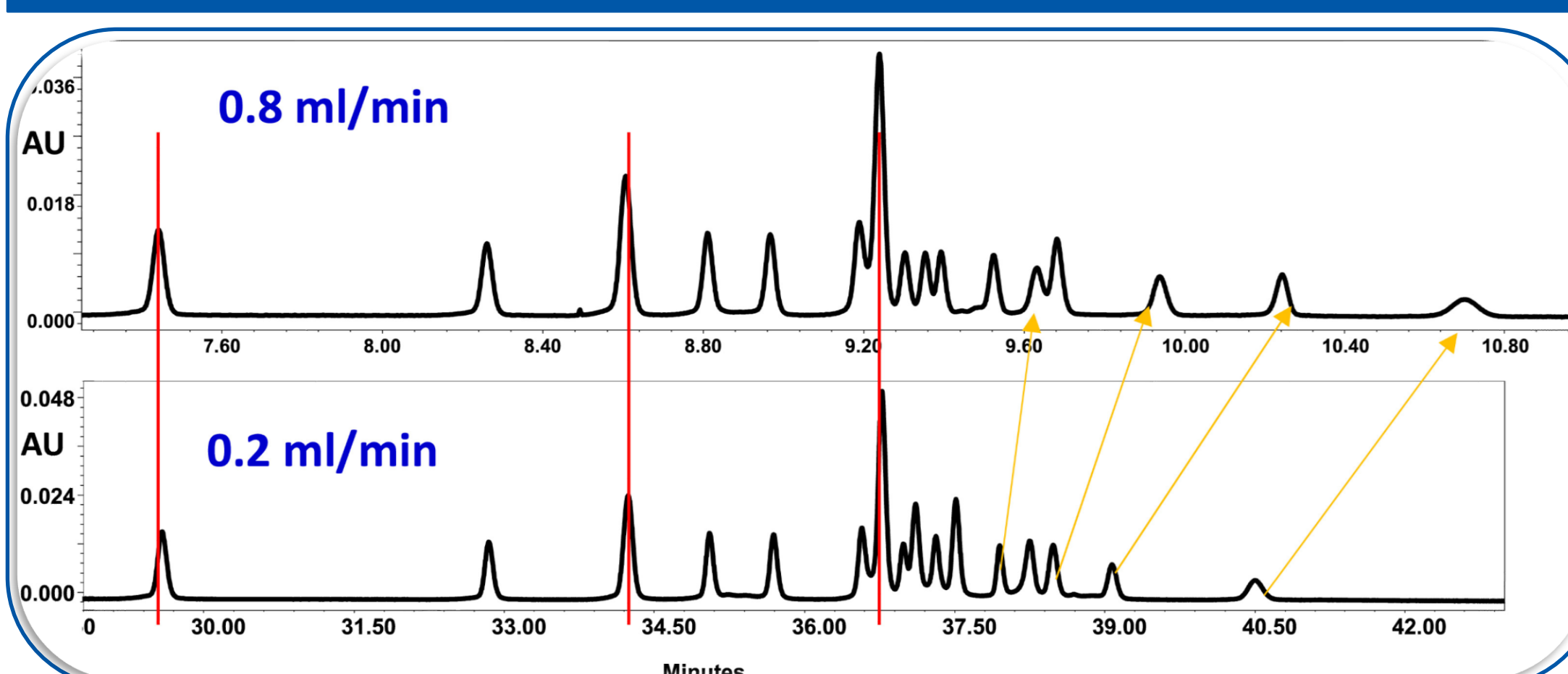
Incorporation of 5% urea improved peak shape and valley definition and reduced structure-dependent chromatographic behavior. Recovery testing with the 1 Kb Plus DNA Ladder showed effectively complete recovery, and blank injections after ladder analysis showed no observable A260 carryover.

Large dsDNA reveals non-classical AEX selectivity



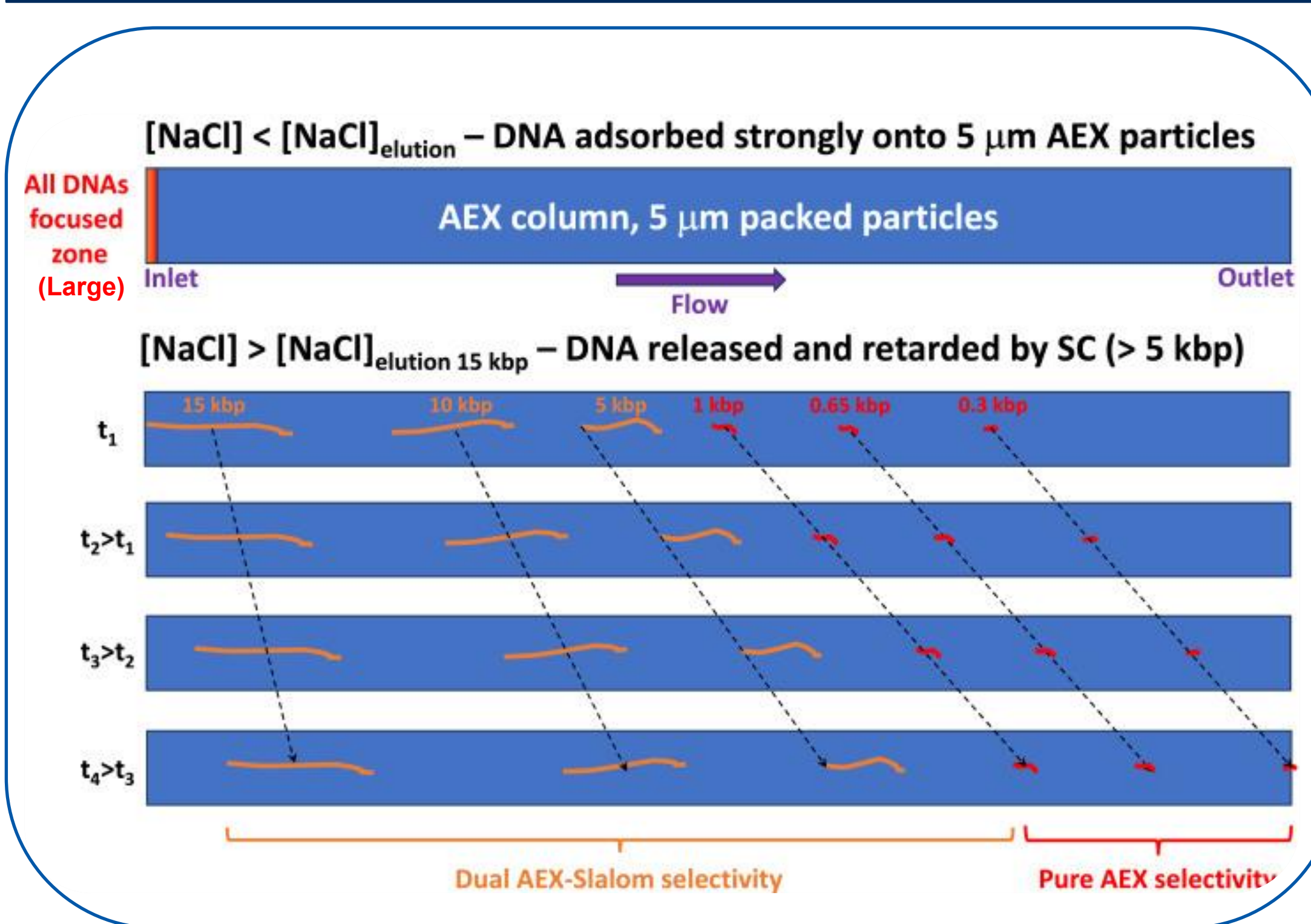
Relative to AGE, AEX selectivity compresses at intermediate size and then increases again for the largest fragments, indicating an additional retention contribution.

Shear flow, not pressure, shifts large-DNA retention



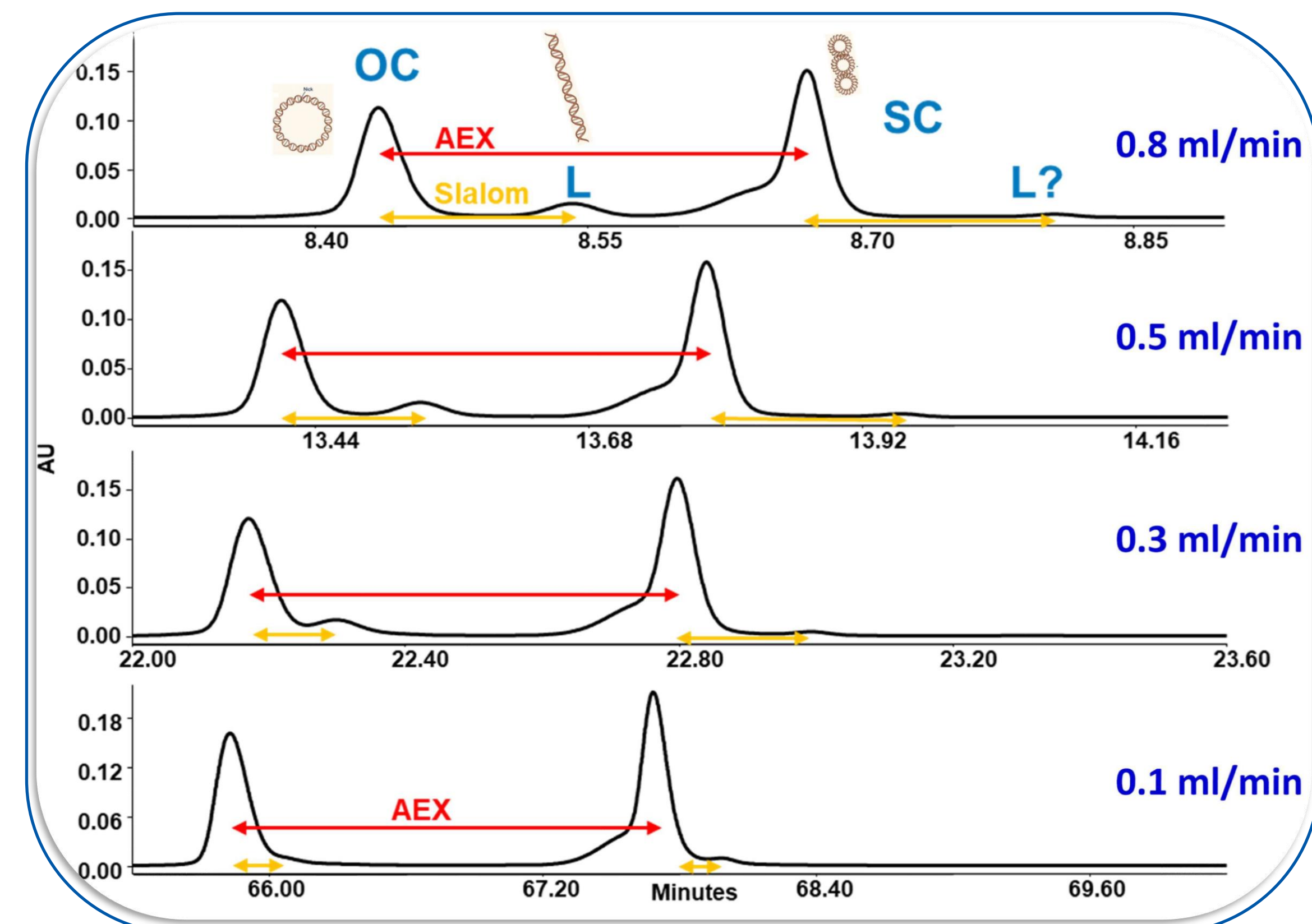
Pressure-control experiments decoupled static pressure from shear rate. Raising pressure at constant flow did not reproduce the large-DNA selectivity shift, while increasing flow at comparable pressure (constant inlet pressure of 450 ± 14 bar) increased retention of the largest dsDNA fragments.

Dual AEX-slalom mechanism explains large-DNA selectivity



AEX controls adsorption and salt-gradient release; after release, large linear dsDNA gains flow-dependent slalom retardation.

Flow rate tunes plasmid topoisomer resolution



Higher flow selectively retards the linear form as per slalom mechanism, improving oc/l and l/sc resolution for plasmid topology analysis.

Mechanistic interpretation

- Short fragments: salt-gradient AEX dominates retention.**
- Large linear dsDNA: AEX release is followed by shear-extension in the interparticle volume (Slalom).
- Plasmids: the linear form is the most impacted as it is the most stretchable topoisomer and hence most responsive to slalom shear effects as flow rate increases.
- Advantages:**
 - oc/l and l/sc selectivity become flow-rate tunable under the tested conditions.
 - Additional experimental parameter that could be used in method development to optimize separation performance.

Conclusions

- Strong AEX resolved ssDNA ladders, dsDNA fragments, and plasmid topological forms within one LC framework.
- 5% urea reduced structure-dependent behavior in ladder separations and supported high recovery without observable carryover.
- Large-dsDNA selectivity required dual retention: AEX adsorption/release followed by post-release slalom retardation.
- For plasmids, increased flow selectively retarded the linear form, improving oc/l/sc resolution.

Method implication: for large DNA, flow rate is a selectivity parameter, not only a runtime setting.

References

Finny, A. S.; Addepalli, B.; Lauber, M. A. **High-Resolution Separations of Single and Double-Stranded Nucleic Acids Using Strong Anion-Exchange Chromatography.** Waters Application Note 720009136, 2025.

Gritti, F.; Fekete, S.; Finny, A. **Dual retention mechanisms in DNA separation: Relevance of slalom chromatography in anion-exchange gradients.** Journal of Chromatography A 2025, 1763, 466457. <https://doi.org/10.1016/j.chroma.2025.466457>

Finny, A. S.; Gritti, F.; Fekete, S.; Addepalli, B.; Lauber, M. A. **Slalom-Aided Anion Exchange Chromatography for Enhanced Analysis of Plasmid DNA Topological Impurities.** Waters Application Note 720008928, 2025.

