

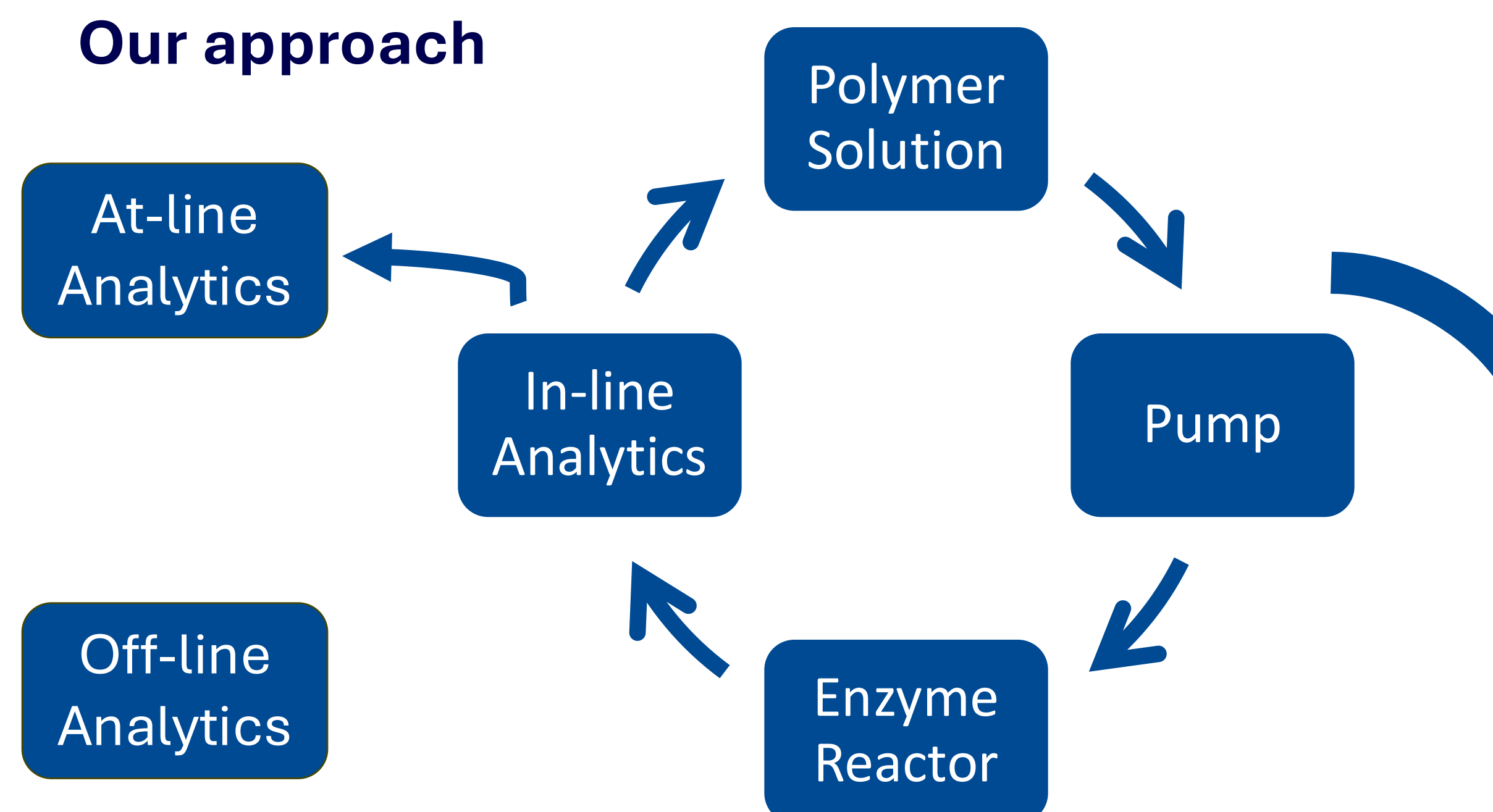
Real-time monitoring of enzymatic polymer degradation in continuous flow

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Why we need new tools

Water-soluble polymers may be released into certain environmental compartments through agrochemicals, personal care and cleaning products, making biodegradability a highly desirable characteristic and a key enabler of circularity. Standard biodegradation tests (OECD 301/310, ISO 17556) tell us *whether* a polymer degrades, but not mechanistically *how*. Understanding the molecular-level progression of degradation requires the development and application of new analytical capabilities.

Our approach



A recirculating flow-chemistry platform that watches polymer degradation as it happens, with an immobilised enzyme and automated at-line SEC.

- Immobilised enzyme on resin beads
- Programmable 6-port valve: 20 μL aliquot every 45 min
- ~32 SEC chromatograms per 24 h run

Current platform

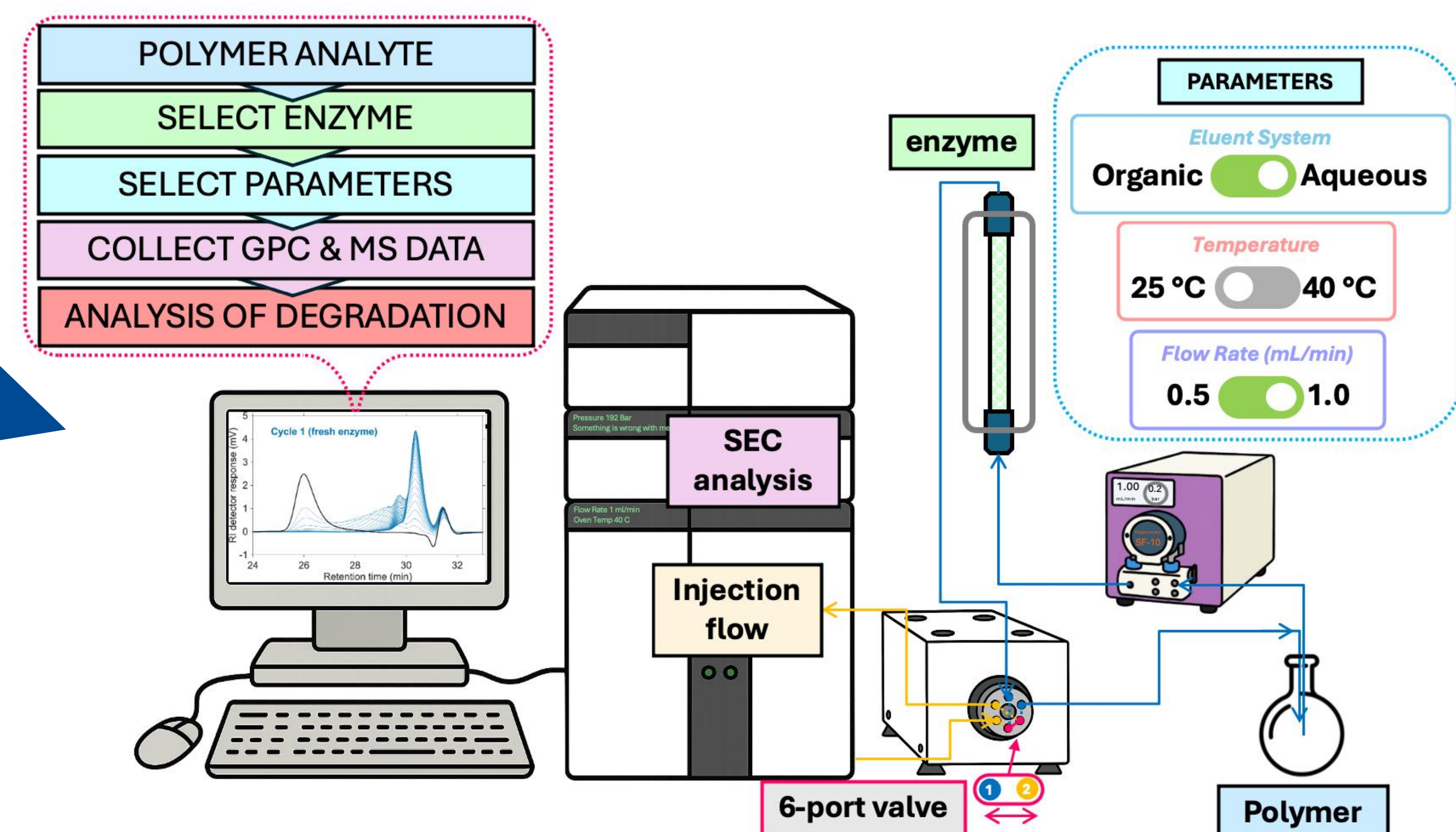


Figure 1. Continuous-flow platform with automated at-line SEC. polymer solution (1 mg/mL, phosphate buffer pH 7) circulates over an immobilised-enzyme fluidised bed at 37 °C and 1 mL/min. A 6-port valve injects 20 μL into the SEC every 45 min.

Reproducibility + reuse

Three experiments: (Fig. 2) batch-to-batch reproducibility of a fresh enzyme charge, and enzyme reusability at two operating conditions.

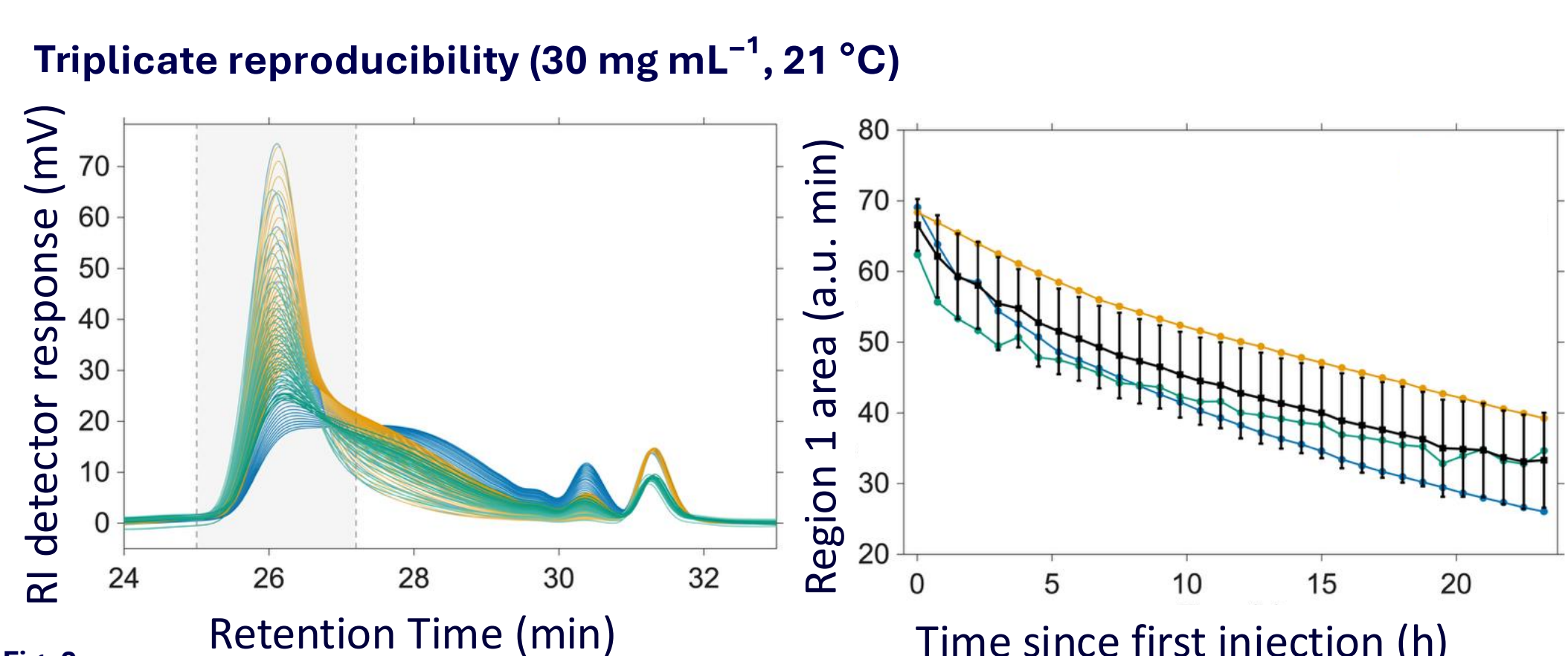


Fig. 2. Tripartite reproducibility at 30 mg mL⁻¹, 21 °C. Left: overlaid chromatograms; three fresh enzyme batches, 32 injections each. Right: Region 1 area vs time, mean \pm SD (black).

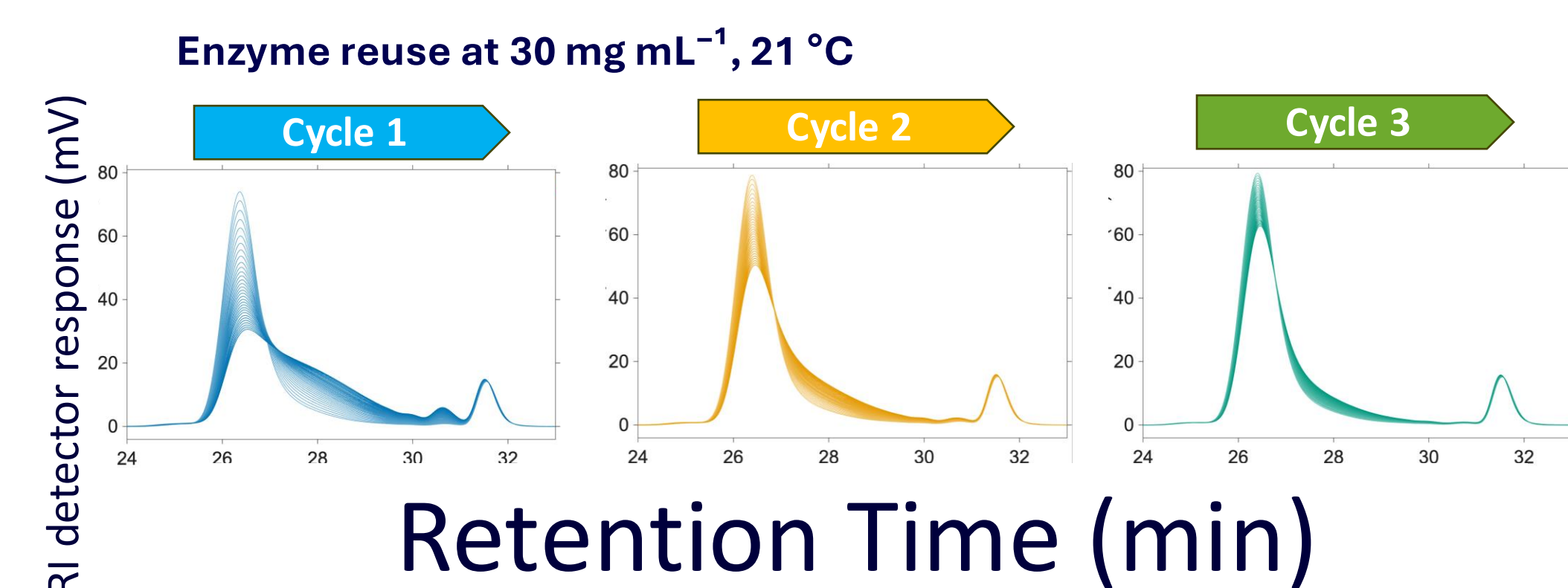


Fig. 3. Enzyme reuse at 30 mg mL⁻¹, 21 °C. Three successive 24 h runs on the same enzyme with fresh polymer each cycle.

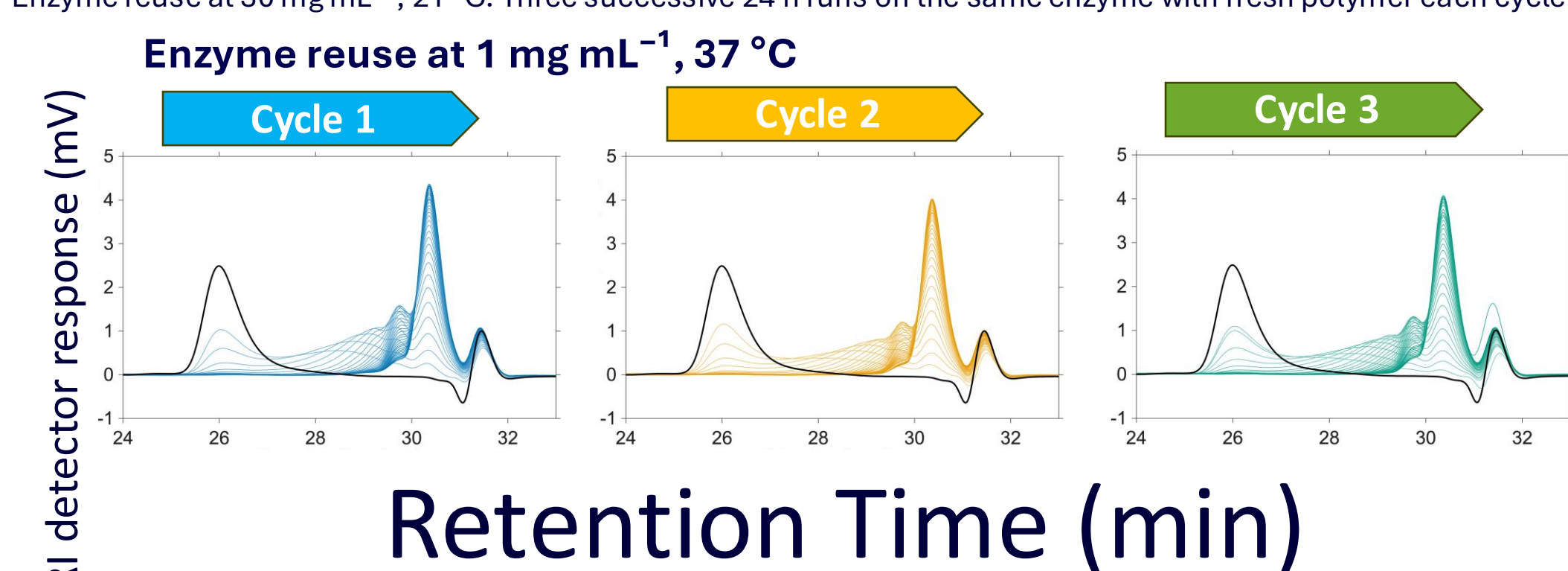


Fig. 4. Same protocol at BASF-relevant conditions (1 mg mL⁻¹, 37 °C). Activity is retained far better here: 89% / 64% of Cycle 1 performance after Cycles 2 / 3.

Kinetic Fits

Two complementary analyses tell the same story from different angles. (1) Region-1 decay (Figs. 5, 6) tracks how fast the polymer disappears in this enzymatic assay. (2) Mn / Mw decay (Figs. 7, 8) tracks how fast the remaining chains shorten. Together: one says *how much* is being consumed; the other says *what's left is getting smaller*.

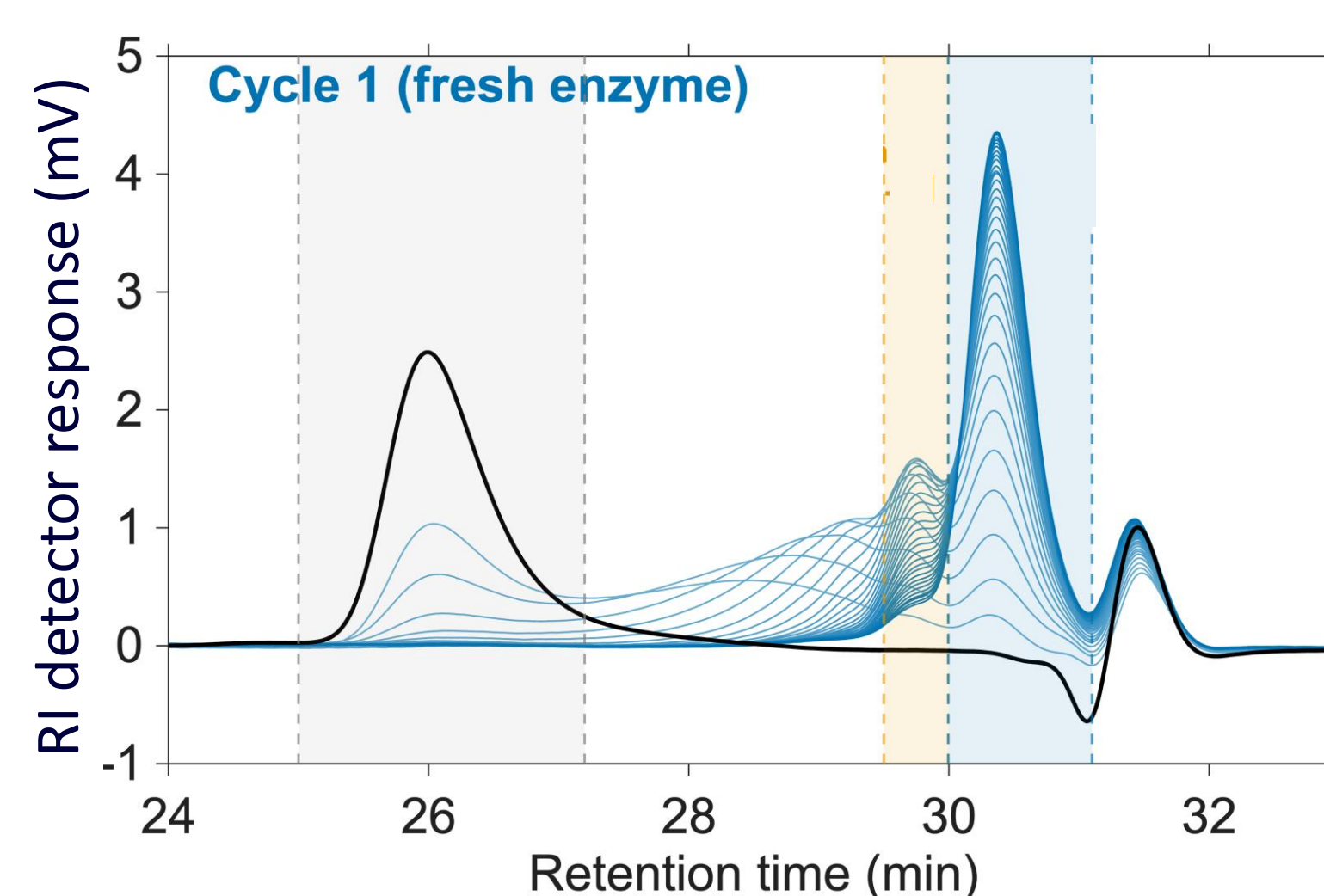


Fig. 5. SEC chromatogram with three diagnostic regions. Region 1 (Gray) = intact polymer (decreases). Region 2 (Yellow) = transient oligomers (rise then fall). Region 3 (Blue) = degradation product (grows to plateau).

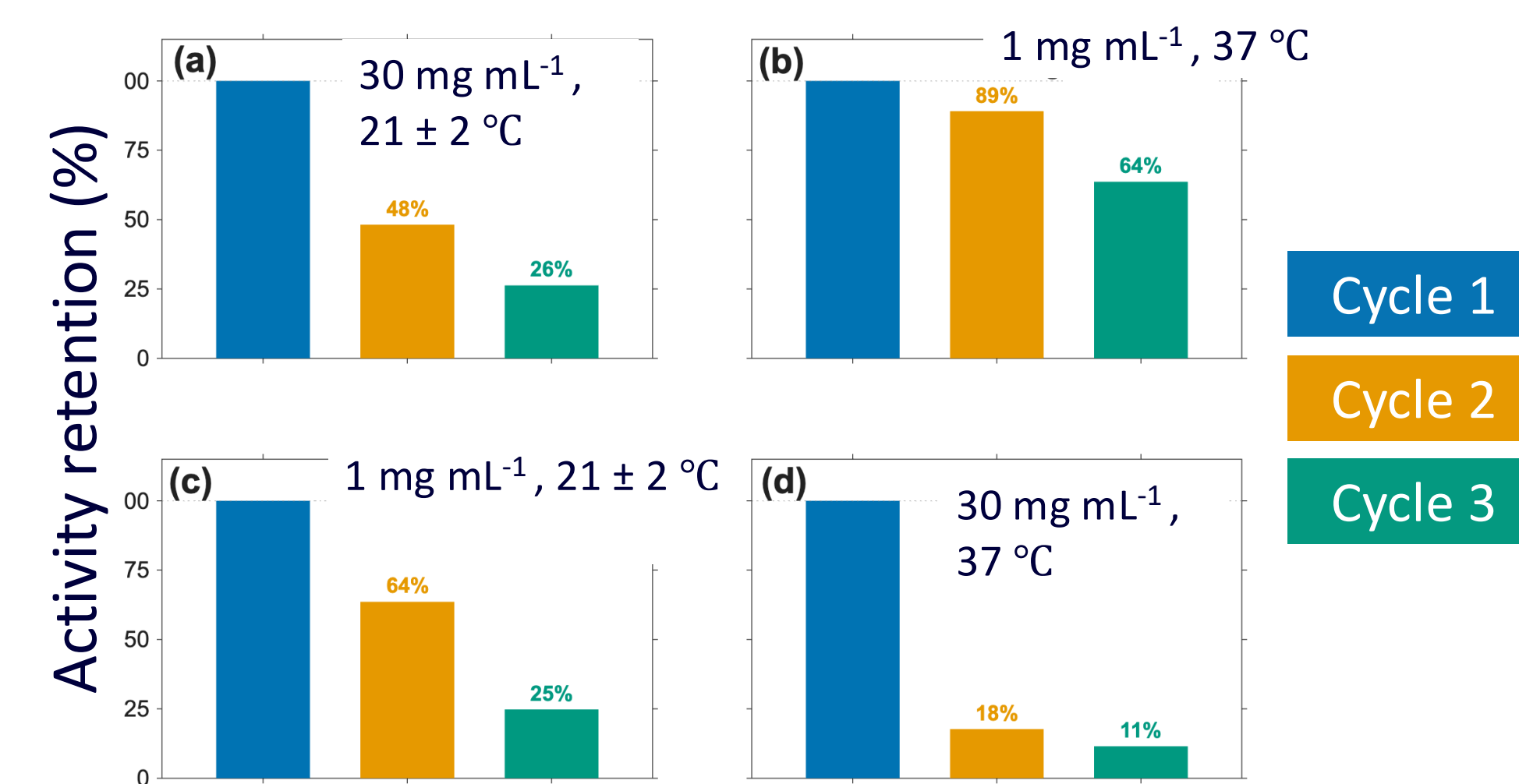


Fig. 6. Region-1 activity retention across four operating conditions. 1 mg mL⁻¹ / 37 °C (b) gives the best reuse; 30 mg mL⁻¹ / 37 °C (d) the worst.

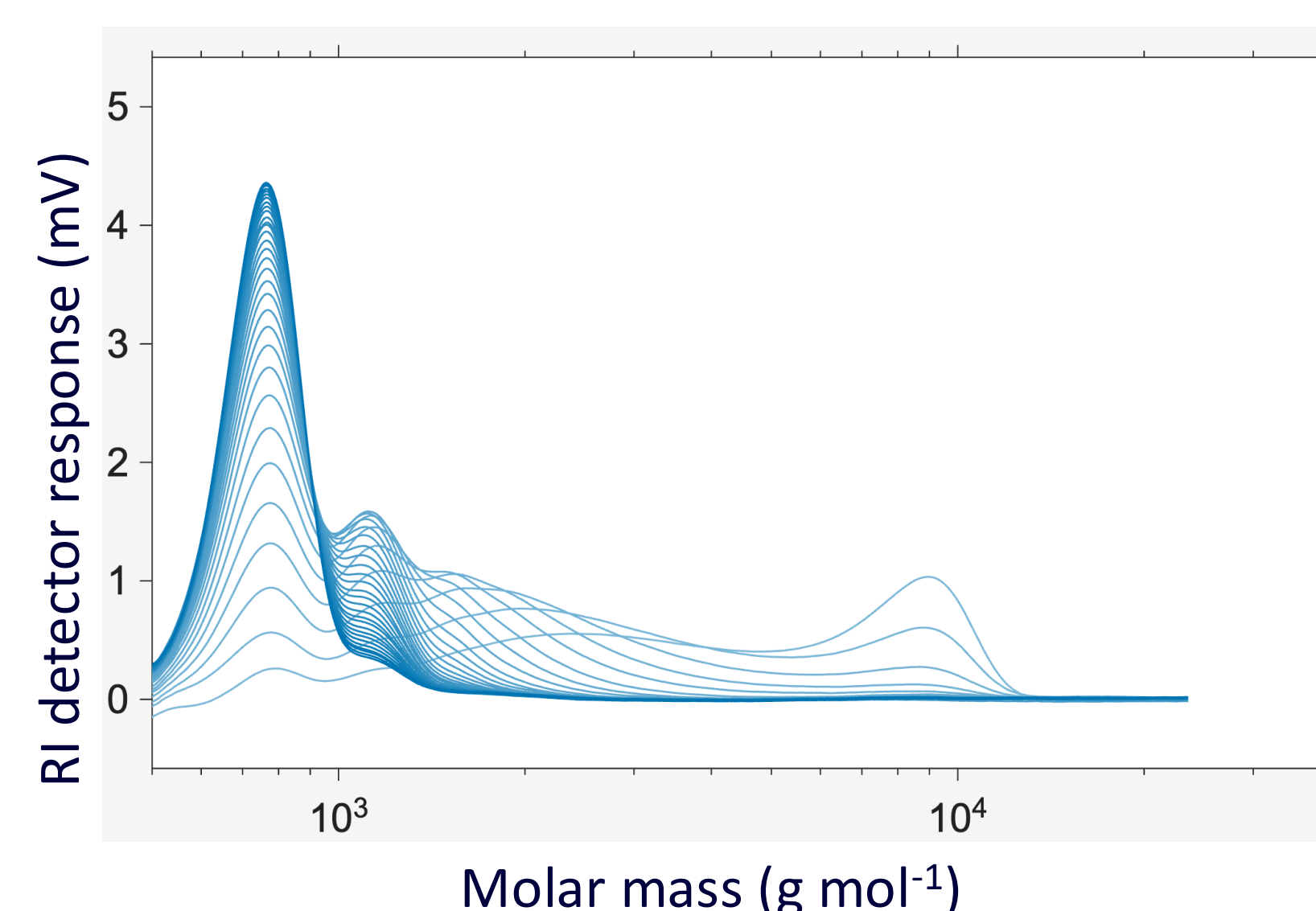


Fig. 7. P2VP-equivalent molar-mass distribution over 24 h (1 mg mL⁻¹, 37 °C). Values are relative to P2VP standards, not absolute.

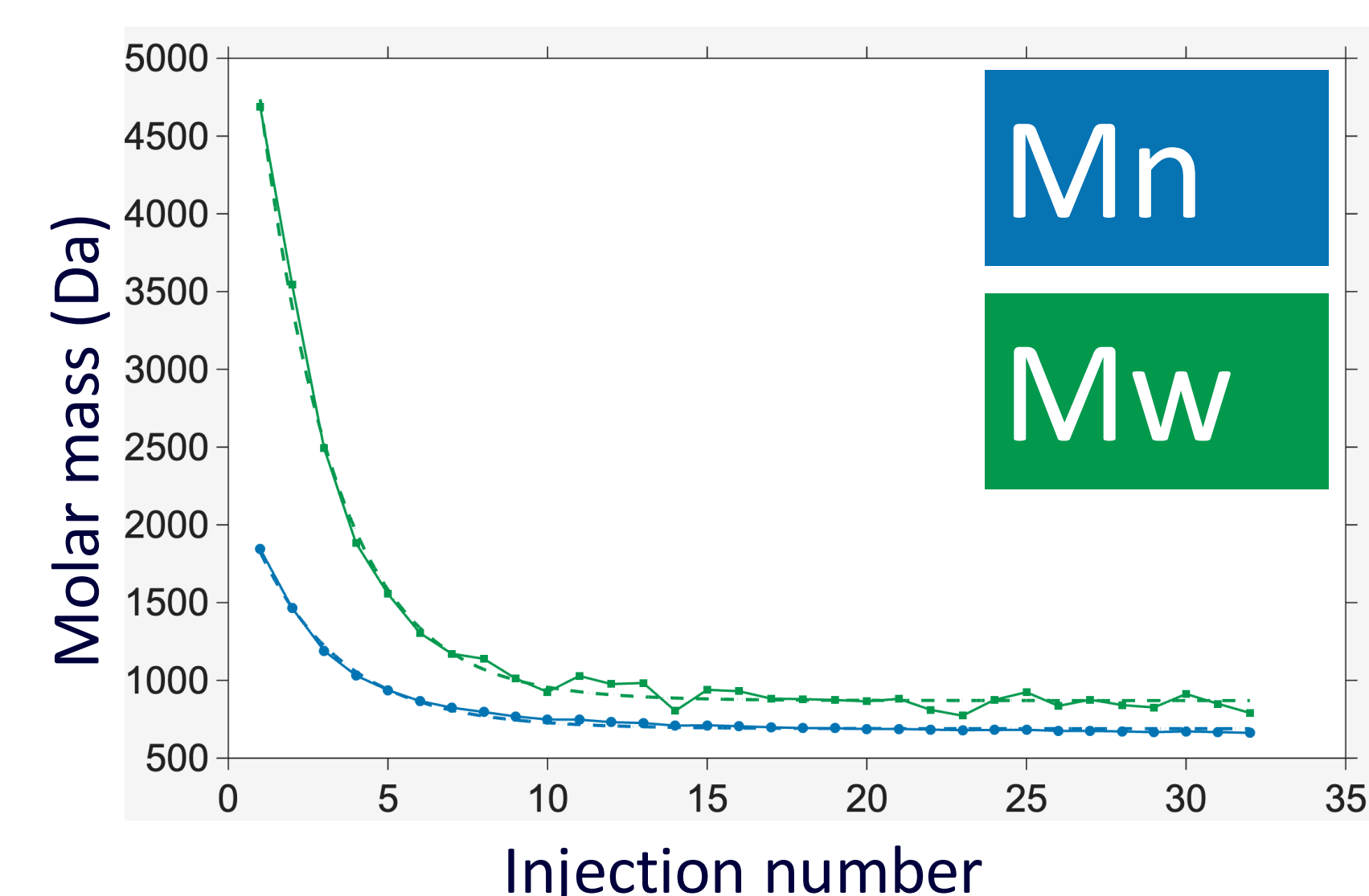


Fig. 8. P2VP-equivalent Mn and Mw decay, 1 mg mL⁻¹ / 37 °C. Moments of the molar-mass distribution (Fig. 6) plotted against injection number, fitted to $y = C + A \cdot \exp(-kt)$. Mn $k = 0.38 \text{ h}^{-1}$; Mw $k = 0.42 \text{ h}^{-1}$.

What next: integrate online ¹H NMR to track monomer release in real time • couple to at-line MS for product identification • scope other water-soluble polymers • move toward a live-cell bioreactor for closer environmental relevance.

Acknowledgements

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Key references

1. Zumstein et al., Acc. Chem. Res. 2022, 55, 2163.
2. Patterson et al., J. Flow Chem. 2023, 13, 103.
3. Osanai et al., Macromol. Biosci. 2004, 4, 936.