AAV Aggregation: Ultrafiltration/Diafiltration as a Stress Step in AAV Manufacturing

Mechanistic insights and mitigation strategies for sensitive capsids

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Introduction

Ultrafiltration and diafiltration (UFDF) are standard terminal operations for concentration and buffer exchange in adeno-associated virus (AAV) manufacturing; however, their impact on capsid stability and recovery remains poorly characterized. In this study, UFDF process optimization was conducted using sensitive AAV capsid serotypes, including an engineered variant, selected for their heightened propensity toward aggregation.

The research demonstrates that process conditions impacts product stability. Specifically, the combination of elevated bulk concentration and increased shear rates can trigger capsid aggregation and unstable permeate flux behavior during UFDF. Furthermore, the buffer transition phase during the early stages of diafiltration represents a critical window where capsids are prone to loss from localized membrane polarization and surface adsorption. This manifests as an immediate yield loss at the onset of diafiltration, resulting from localized membrane polarization and surface adsorption. The incorporation of modulated flow during diafiltration improved permeate flux and reduced formation of the membrane boundary layer, consistent with disruption of localized concentration polarization.

Methods

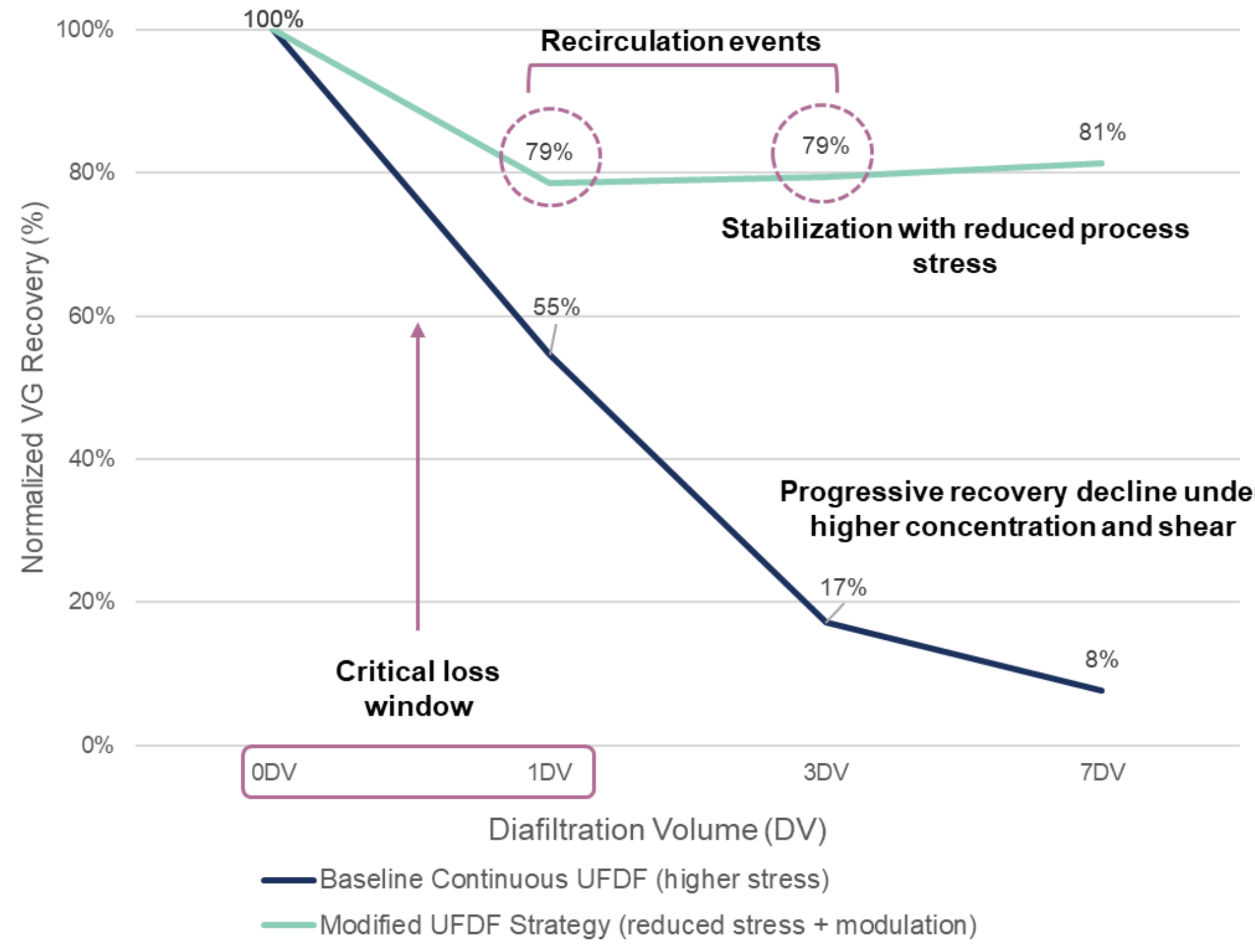
Overarching Experimental Approach

Sensitive AAV1 and engineered AAV9 capsids were evaluated in hollow fiber UFDF under varying process stress conditions.

- Multiple UFDF conditions were assessed, and two representative conditions were selected for presentation to contrast baseline continuous operation (Condition 1: ▬) with the lead modified strategy (Condition 2: ▬).
- These two cases represented a **higher-stress continuous UFDF operating mode** and a **lower-stress modified setup incorporating reduced effective loading and modulation/circulation,** including forward/reverse flow in the absence of TMP.
- In-process samples were collected across diafiltration to monitor retentate behavior and recovery trends.
- Final formulation mitigation strategies included ionic strength adjustment, a 2-8°C hold, and freeze-thaw across varying sample volumes.
- Unchained Labs Stunner™ was used for rapid in-process protein characterization (titering and aggregation propensity) and ddPCR corroborated endpoint trends.

Results

Figure 1: Normalized Recovery Across Diafiltration



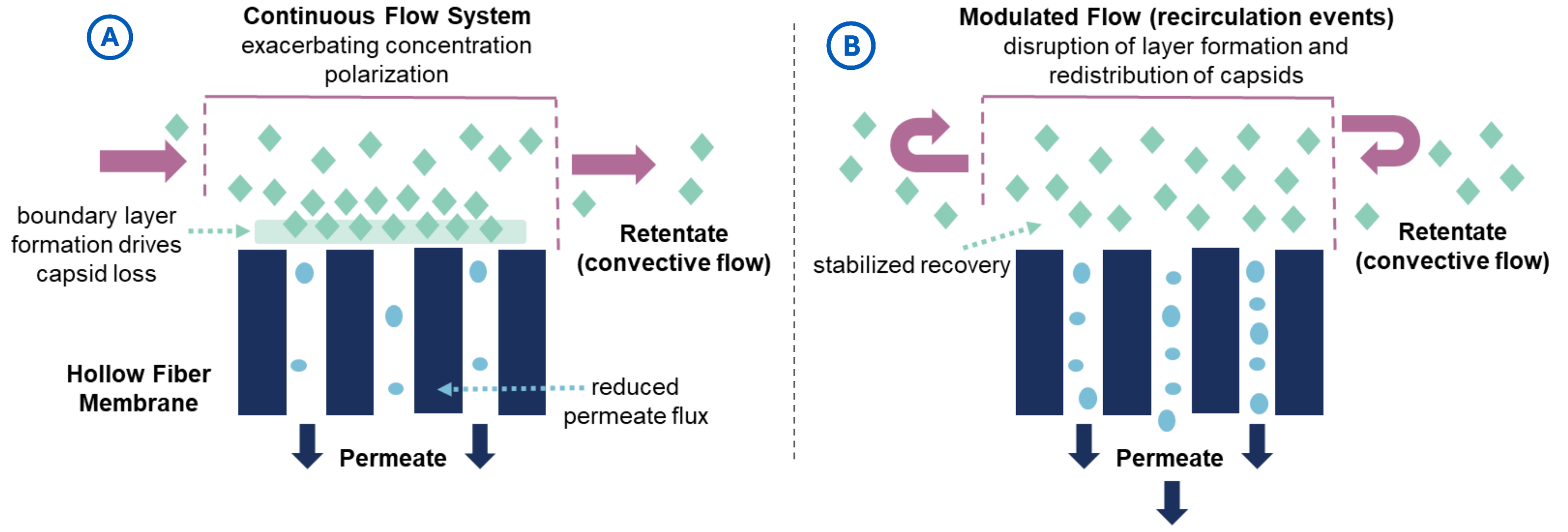
① UFDF Recovery Diverges After an Inherent Early Loss Window

Figure 1: Representative UFDF recovery profiles for a sensitive engineered capsid under combined higher-stress continuous operation and combined lower-stress/modulated conditions.

- Both conditions exhibit an immediate loss near the onset of diafiltration, consistent with an inherent early loss window at the membrane interface during the buffer exchange.
- Recovery continues to decline under compounding higher-stress conditions, whereas the combined lower-stress/modulated strategy stabilizes recovery, revealing that continued loss is process-dependent.
- *These conditions are tolerated by robust/wild-type capsids, and exhibit behavior to that of the latter lower-stress/modulated parameters.*

Ⓐ **Figure 2A:** Schematic of concentration polarization during hollow fiber UFDF for a sensitive engineered AAV capsid. Under continuous baseline operation and elevated process stress, capsids accumulate at the membrane interface and gradually form a localized boundary layer that further promotes adsorption and continued loss, resulting in a decrease in bulk concentration from the retentate and eventually a reduction in permeate flux.

② Figure 2: Concentration Polarization Governs Capsid Stability during UFDF



Ⓑ **Figure 2B:** Under the modified UFDF strategy, reduced process stress together with modulation/recirculation disrupts the boundary layer, redistributes capsids into the retentate, and stabilizes recovery (avoiding loss in permeate flux). Monitoring product condition alongside process parameters provides a more complete assessment of UFDF performance

③ Figure 3: Hydraulic and product-focused measurements together help explain UFDF behavior

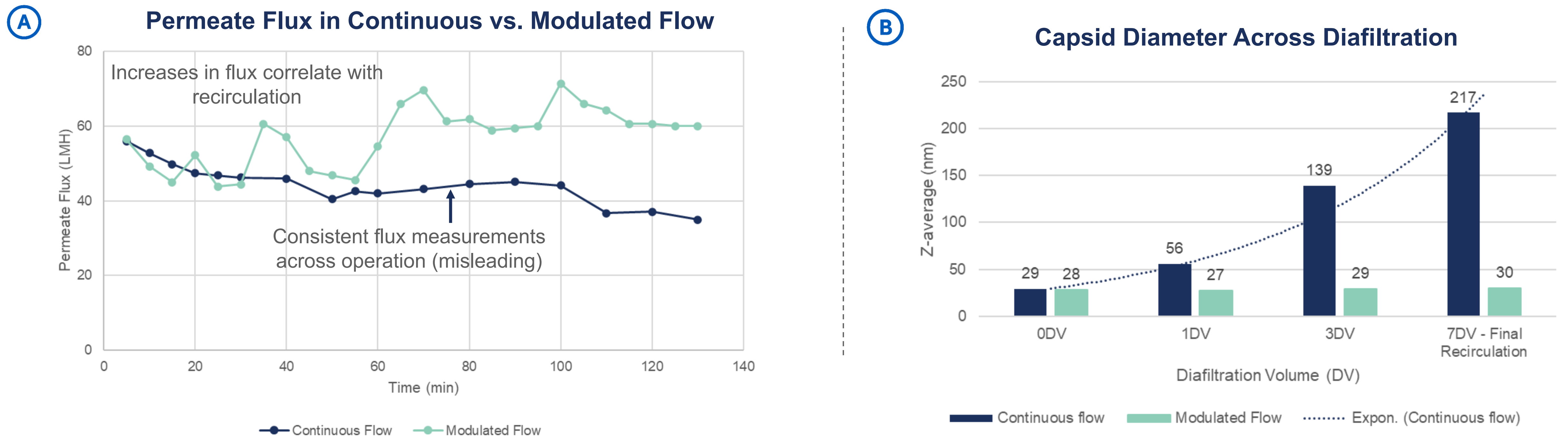


Figure 3: Schematic Flux, retentate concentration, and DLS-based particle size measurements under baseline continuous and modified UFDF conditions. While hydraulic trends (**3A**) provide useful process context, product-focused measurements (**3B**) more clearly distinguish capsid behavior under each condition. Under baseline continuous operation, retentate concentration declines and z-average increases across diafiltration, consistent with progressive loss from the retentate and increasing instability. In contrast, the modified strategy better preserves retentate concentration and prevents particle/capsid size growth beyond its monomeric range (~27 - 30nm). Ultimately, this reinforces the added value of evaluating hydraulic and product measurements together.

④ Formulation-Conserving Mitigation can Partially Reverse Final Formulation Aggregation

If after the terminal UFDF step aggregation is present, can we re-solubilize capsids without changing the formulation framework?

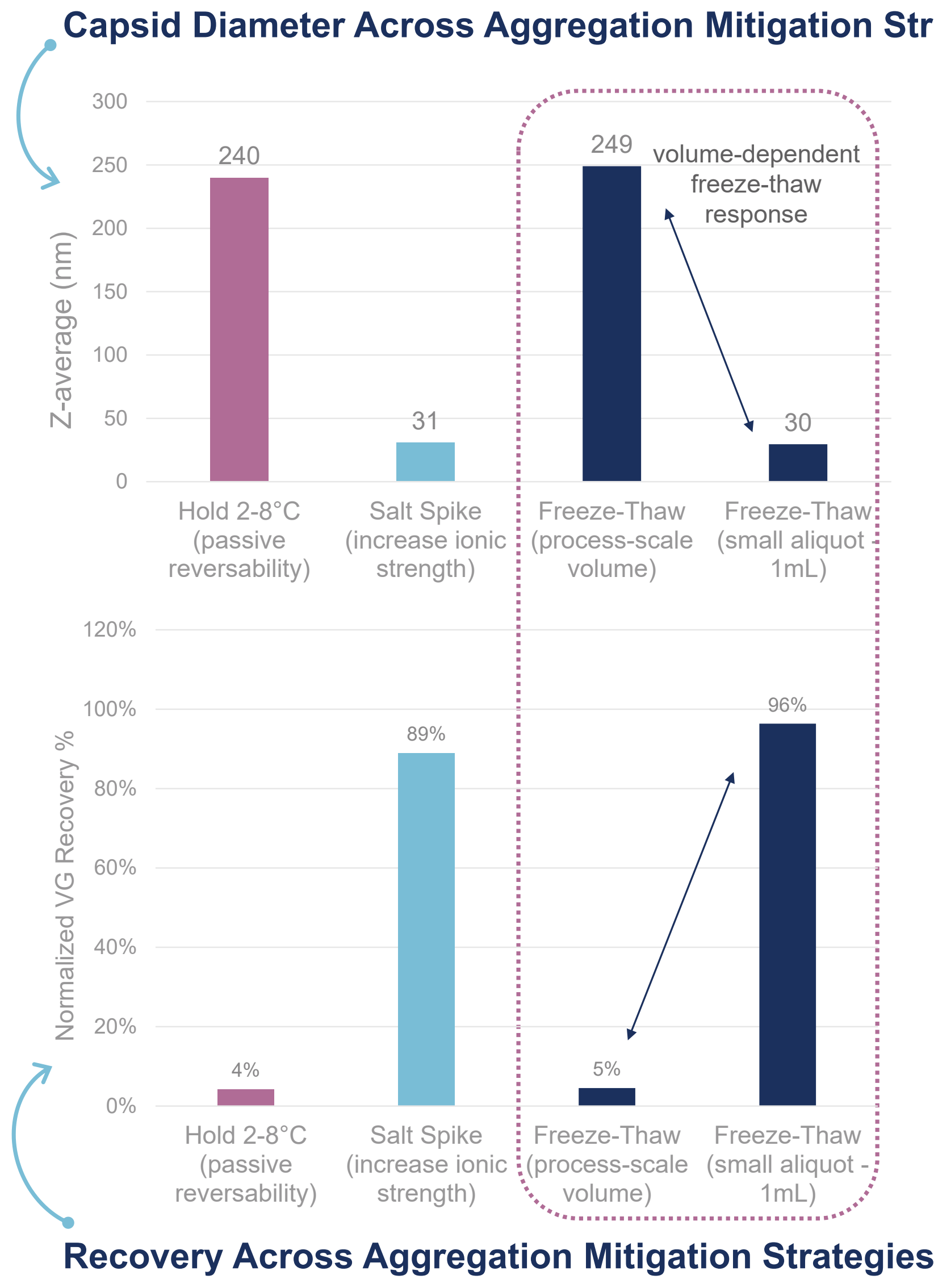


Figure 4: Aggregation mitigation strategies were evaluated in multimeric post-terminal UFDF material.

- Increasing ionic strength reduced z-average and improved normalized recovery consistent with re-solubilization of aggregated vector without altering the core formulation matrix.
- Freeze-thaw response was largely scale-dependent, with improvement observed at low aliquot scale but not at process-scale volume, suggesting that freeze-thaw kinetics influence potential reversibility of aggregation.
- 2-8°C passive hold alone was ineffective at resolving aggregation.

Overall, final formulation aggregation was most effectively reversed by ionic strength adjustment, as a hold alone was ineffective and the freeze-thaw response was scale-dependent; this data supports an aggregation mechanism that is electrostatically driven and **favors ionic strength adjustment as a more reliable aggregation mitigation strategy.**

Conclusion

Ultrafiltration/diafiltration (UFDF) can act as a condition-dependent stress step for sensitive engineered AAV capsids as opposed to simply being a buffer exchange and concentration step. Across the datasets presented here, an inherent early loss window was observed during early diafiltration, while continued loss depended on the degree of process stress and behavior in the hollow fiber. The data supports a model in which concentration polarization, localized capsid accumulation, and aggregation drive capsid loss under higher-stress conditions. However, this behavior could be mitigated through process intervention and potential reversal once in final formulation.

Key Takeaways:

- Initial diafiltration introduces an **inherent early loss window**
- **Continued loss is process-dependent** and linked to membrane interface instability
- Product-focused measurements should be evaluated alongside process parameters
- Ionic strength adjustment was the most promising formulation mitigation strategy

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