Unraveling the Impact of Manufacturing Process-Related Stresses on AAV Stability, Aggregation, and DNA Release

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Introduction

- The adeno-associated virus (AAV) platform continues to grow as one of the most promising delivery vehicles for gene delivery. Since material availability and cost are two of the main challenges at early-stage development, there is a significant need for high throughput, low volume analytical methods to enable stability screening with limited material.
- Use of Size Exclusion Chromatography coupled with Multi-Angle Light Scattering (SEC-MALS) and Field Flow Fractionation coupled with Multi-Angle Light Scattering (FFF-MALS) have shown promise as multi-attribute assays in assessing the stability of an AAV.
- Coupled with Free DNA detection, and thermal ramping to assess aggregation (Tagg) of a model AAV this study presents an analytical toolkit for formulation and stability screening of AAVs.

Stress types	
Control	
Thermal	
Freeze-Thaw	5x -8 30x -8
Oxidation	קק 10 pp וסס pp
Agitation	Using st 1 Using 100
PН	۶ ۱
Light	

Methods

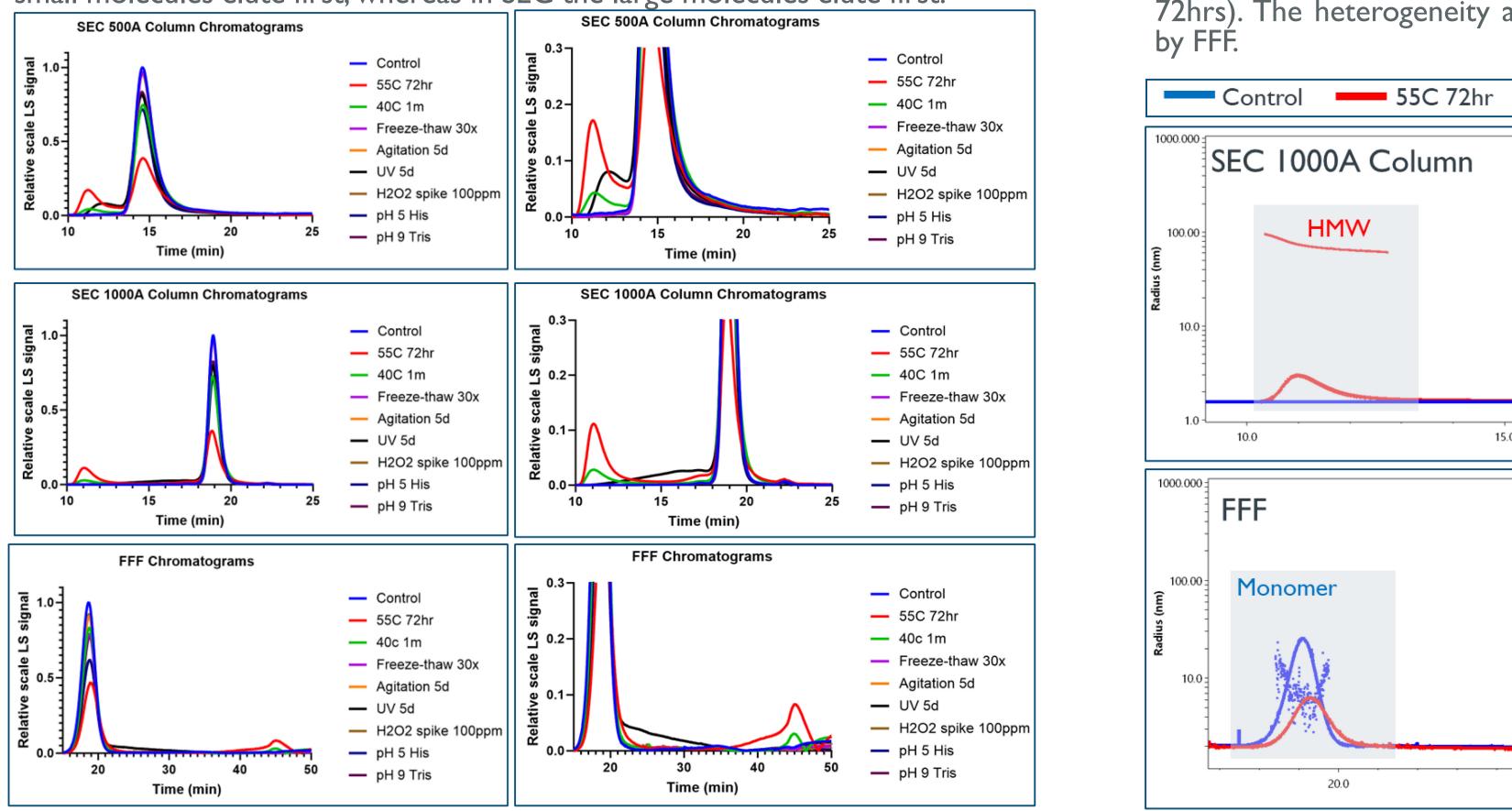
Multi-attribute assays facilitate additional data collection with a small volume of sample

In this study, we explored the advantages of FFF, particularly its capability to measure a wider particle range, extending up to I micron. Additionally, we investigated the use of SEC columns with larger pore sizes, which proved beneficial in capturing high molecular weight (HMW) particles with larger sizes.

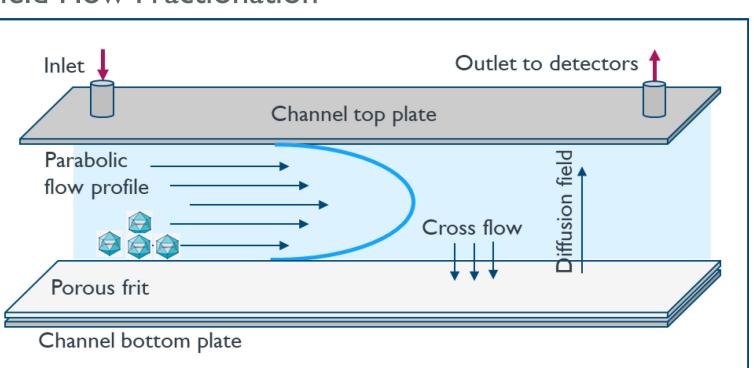
FFF or SEC coupled with MALS enables data collection on:

- Aggregation
- Genome titer
- Capsid titer
- Molecular weight of Capsid and DNA
- Full: Total Capsid Ratio (not able to separate partials)

SEC-MALS chromatograms collected with 500A column and with 1000A column, FFF-MALS chromatograms of all categories of stressed samples along with control presented by 90-angel light scattering signal in relative scale. In FFF separation the small molecules elute first, whereas in SEC the large molecules elute first.

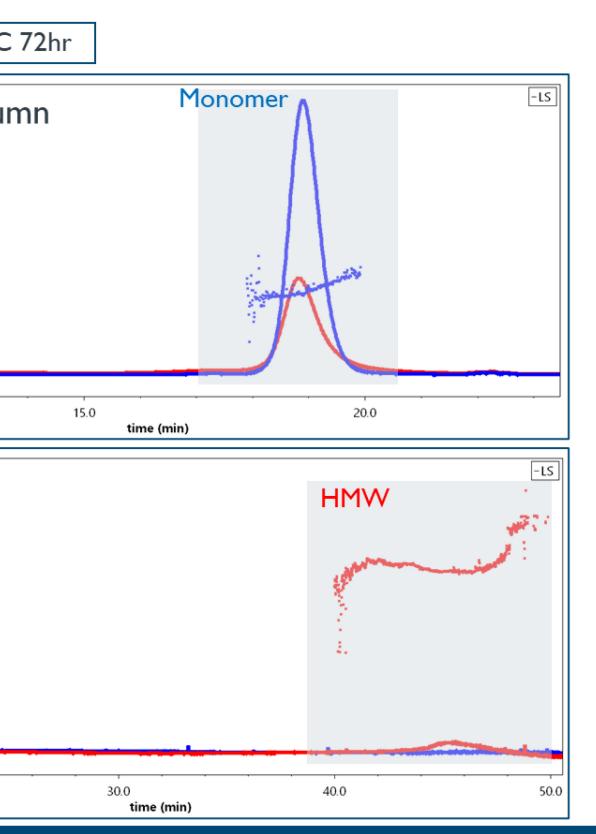


Field Flow Fractionation



MALS allows for measurement of the radius of gyration. The figure below compares MALS signals for SEC using a 1000A column (control and 55C-72hrs) and FFF (control and 55C-72hrs). The heterogeneity and larger size HMW is captured

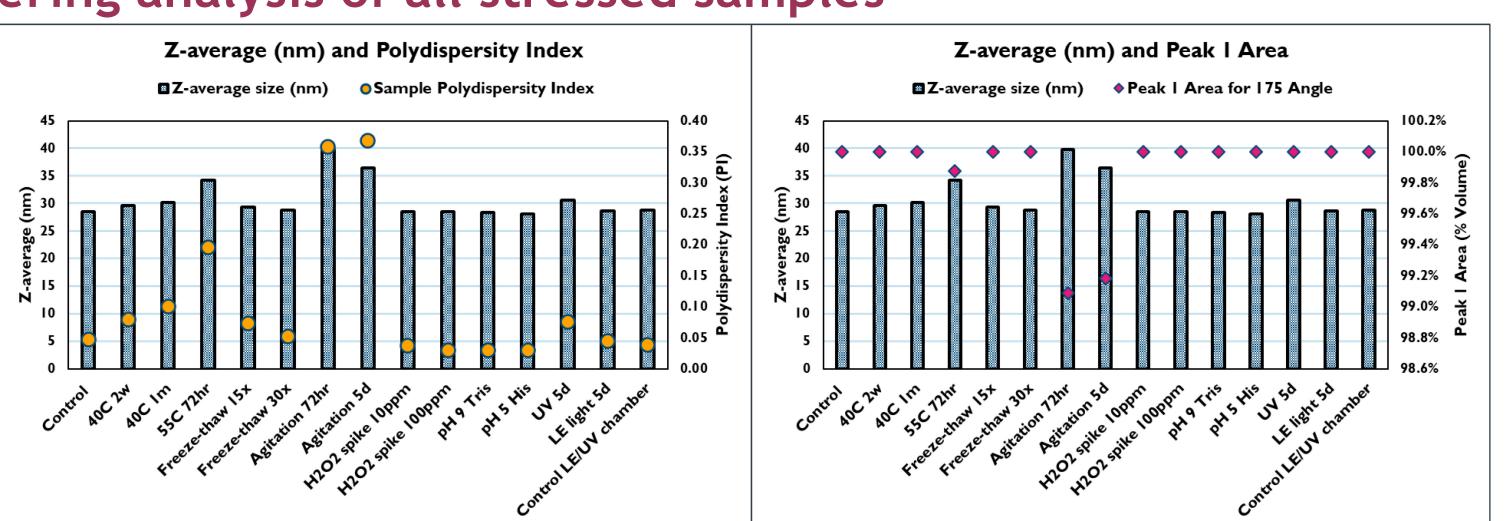
Condition/Duration	
Stored at -80°C	
40°C for 2 weeks	
40°C for I month	
55°C for 72 hours	
80°C to room temp (22°C) uncontrolled	
30° C to room temp (22° C) uncontrolled	
pm H_2O_2 spike, stored at 2-8°C for two	
weeks	
pm H_2O_2 spike, stored at 2-8°C for two	
weeks	
tir bar inside IOR vial after 72hrs at room	
temperature protected from light	
ng stir bar inside IOR vial after 5 days at	
om temperature protected from light	
pH 5, stored at 2-8°C for 1 month	
pH 9 stored at 2-8°C for 1 month	
Exposure to UV light over 5 days	
Exposure to LED light over 5 days	



Results

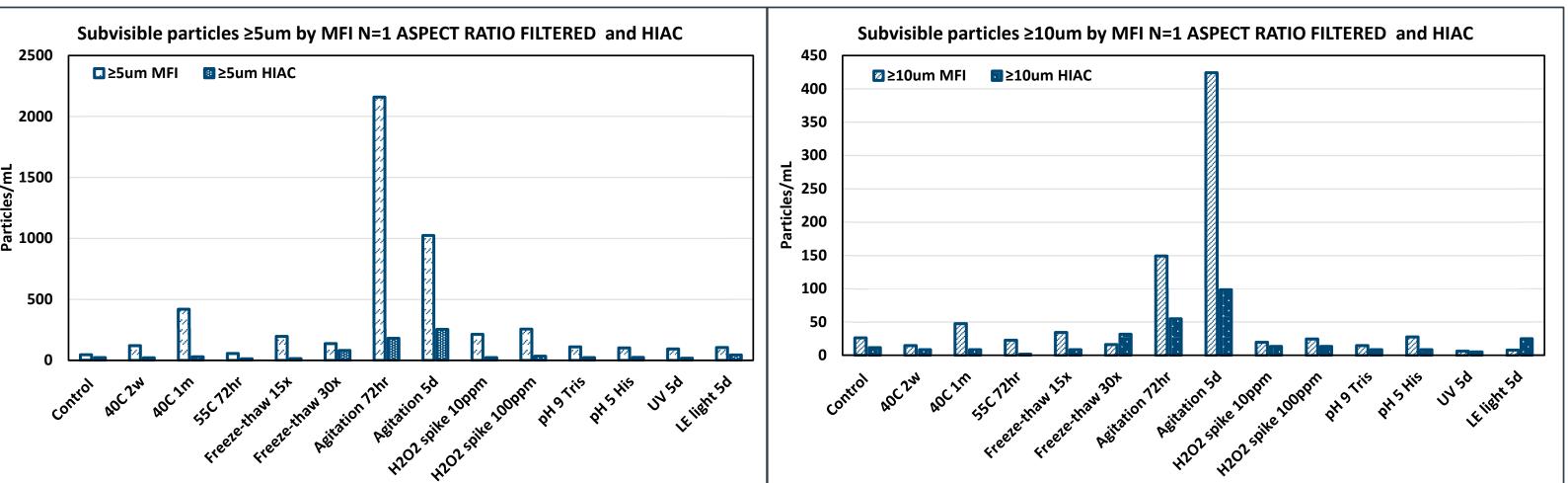
Dynamic light scattering analysis of all stressed samples

The samples subjected to 72 hours and 5 days of agitation exhibited the highest Z-average, indicating the presence large particles An increase in the -average was observed for the sample exposed to 55°C for 7 followed changes in Z-average of sample exposed to UV and 40°C for one month, with no significant impact observed in other assays



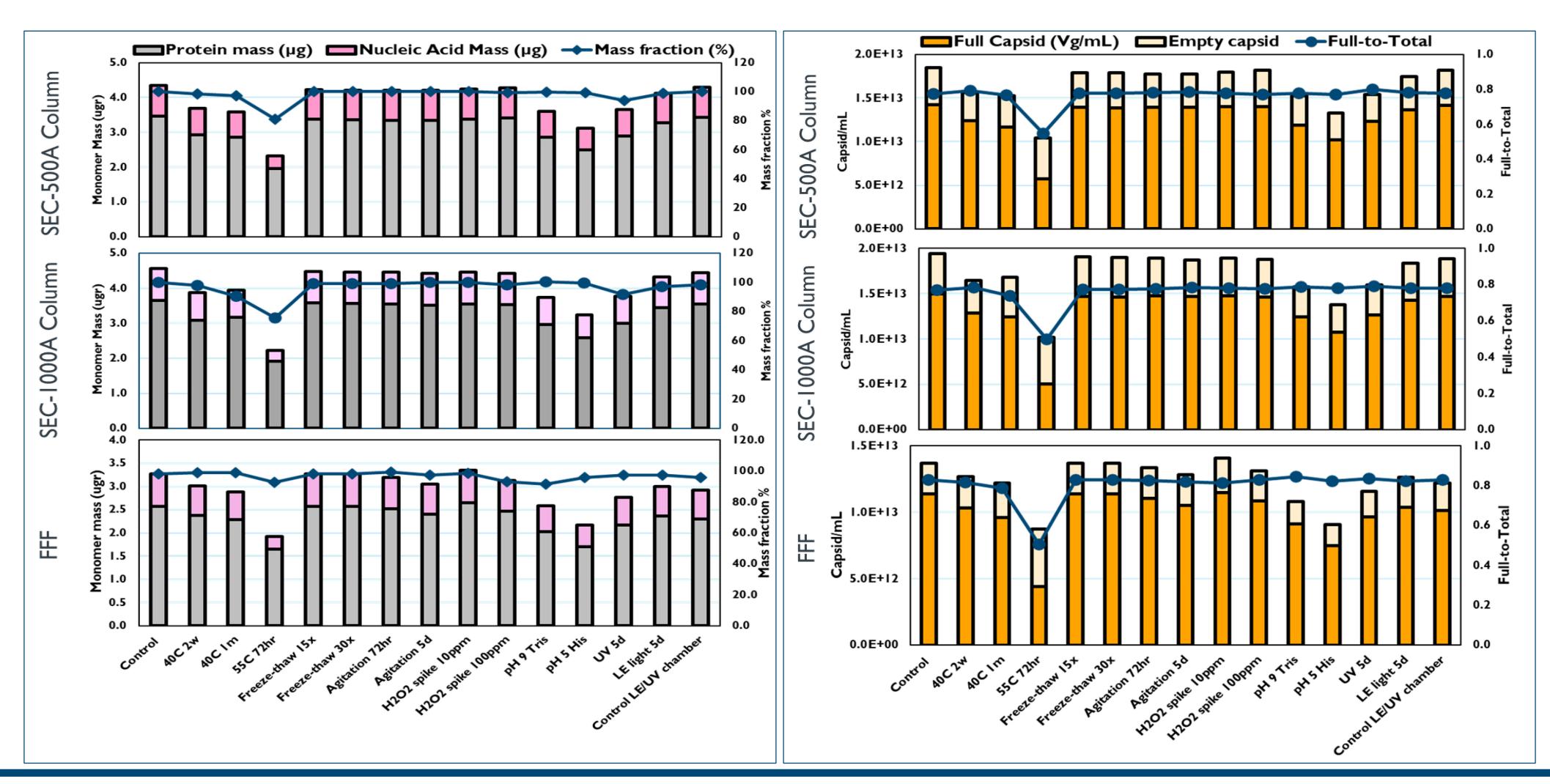
Characterization of subvisible particles by micro-flow imaging (MFI) and Light obscuration (HIAC)

The results indicate that agitation for 72 hours and 5 days resulted in the highest particle इ 1500 count, followed by 40°C I month, H2O2 spiked samples. freeze-thawed samples, and 40°C weeks and 72hours.



Mass fraction or Mass Loss? Aggregate, monomer loss, Capsid titer, and Vg titer by SEC-MALS and FFF-MALS

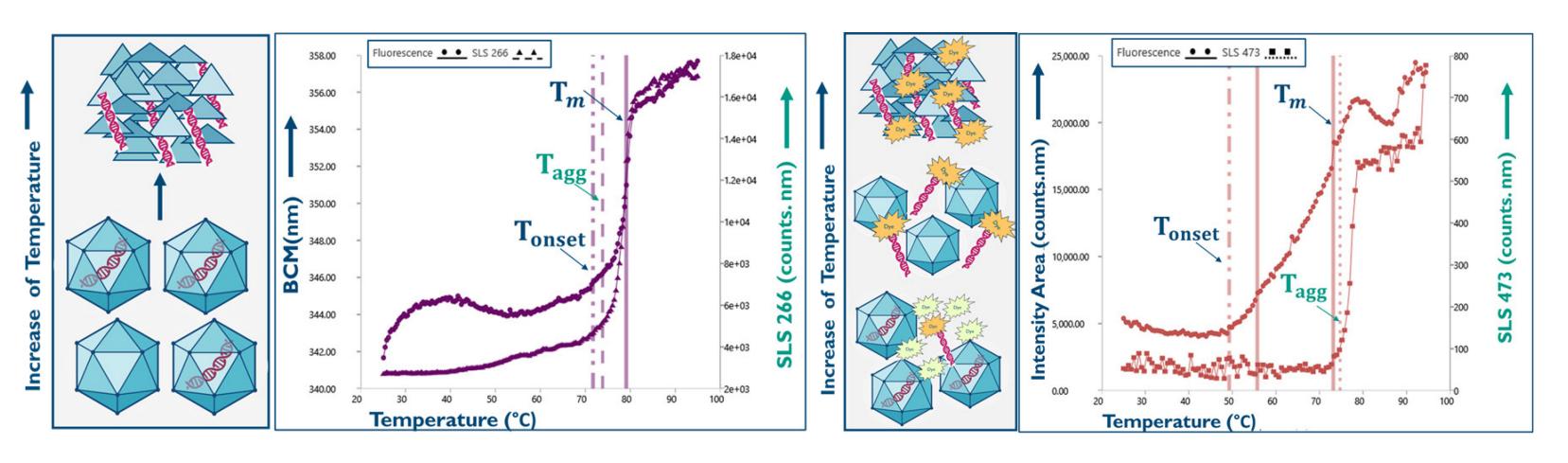
- Across various samples, the 55°C-72hr sample exhibits the highest monomer loss, closely followed by the UV-exposed sample.
- Overall FFF reported mass loss for all stress conditions was slightly higher than SEC, which highlights the ability of FFF to minimize columnrelated effects, allowing for the more accurate measurement of monomer loss, particularly in capturing larger aggregates.



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Thermal stress may lead to the release of DNA from the capsid, while the capsid itself and the DNA can remain intact

These data indicate that for this specific AAV-GOI combination, capsid unfolding and aggregation begin at 70°C and may be complete by 80°C. DNA ejection initiates at approximately 50°C and continues until 75°C. Therefore, storage at temperatures qual to or exceeding 50°C can result in partial DNA ejection. Notably, for the sample stored at 55°C-72 hours, a significant amount of DNA was ejected, aligning with the Tonset and Tagg = of the measurements temperature ramping study.



- The trend in these data indicate that the greatest mass of released DNA was observed at 55°C-72hr, followed by 40°C-Im and 2 weeks, and UV exposure.
- Interestingly, there's a lack of agreement between these data for released Free DNA and the extent of monomer loss determined by SEC or FFF in some stress conditions.
- This suggests that the overlay of data provides insights into the mechanism of aggregation.

Conclusion

- and vector genome (vg) titers suitable for stability screening.
- ranking of different stresses on AAV stability.

References

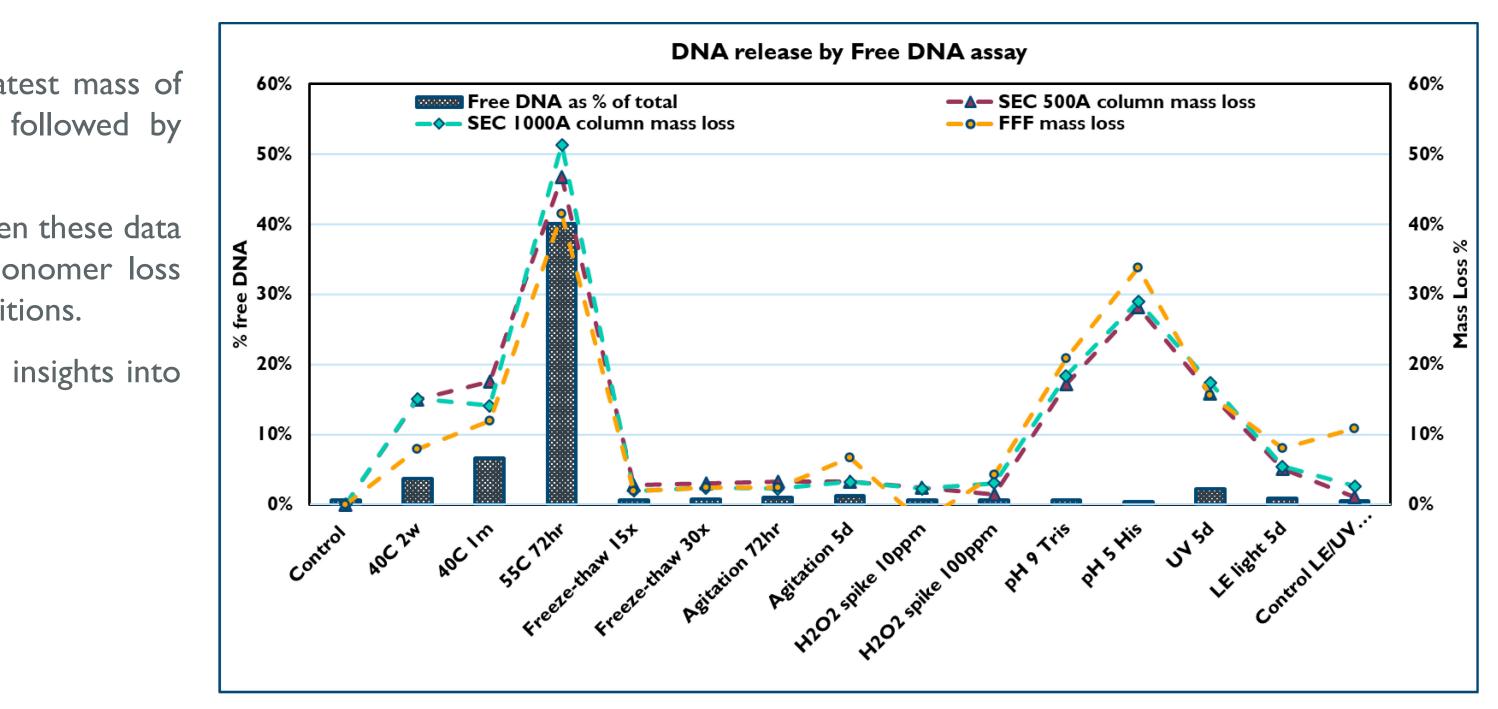
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Poster #1032



• Results showed that thermal stress had the most significant impact on monomer loss, followed by UV light and low and high pH. Agitation created subvisible particles, but not measurable aggregates. Freeze-thaw and exposure to visible light had a minor impact on monomer loss or aggregate formation.

Incorporating separation techniques with MALS furnished additional insights, enabling the quantification of capsid

• Notably, detecting aggregation or subvisible particles by HIAC/MFI or DLS in isolation proved insufficient to resolve the overall stability impact. Lastly, the addition of the free DNA assay and correlation with SEC-MALS/FFF-MALS provides a more comprehensive picture of the release of DNA and aggregation to enable risk

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