

Literature Reference

AAV on Sepax Analytical SEC

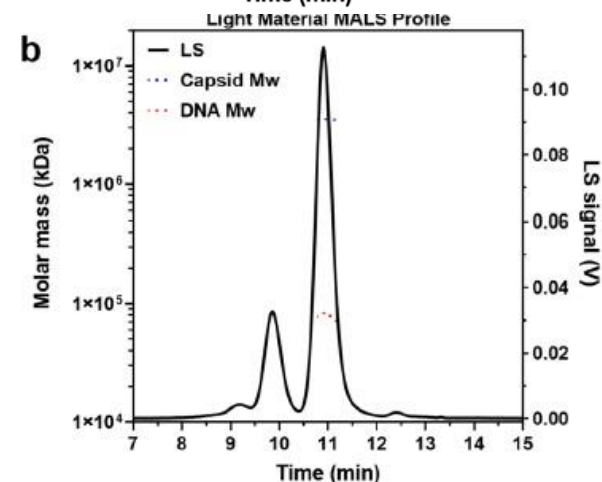
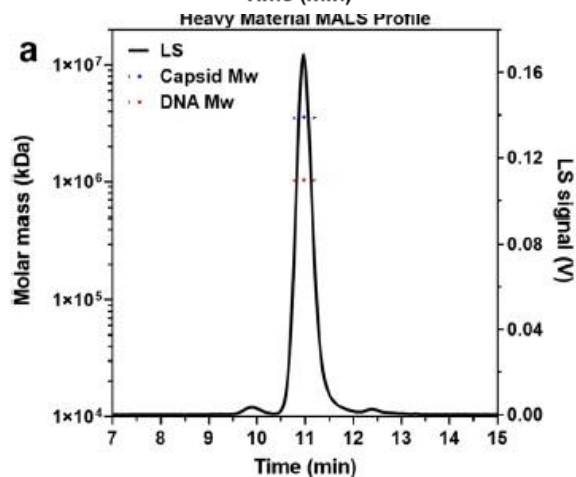
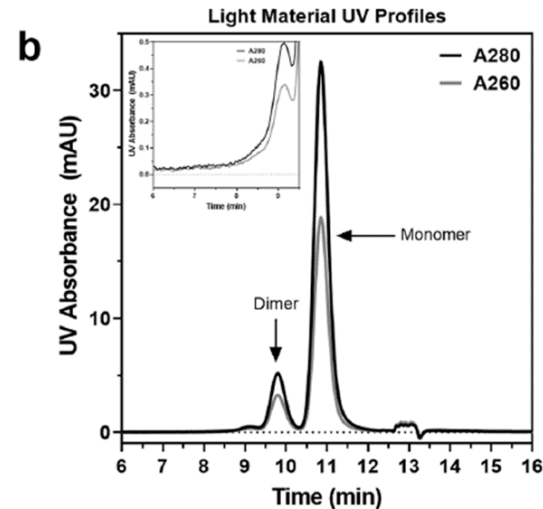
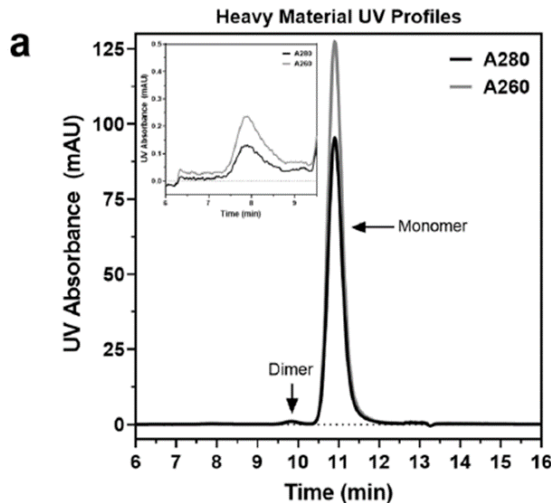
Characterization and Identification of Adeno of Adena Associated Vectors by SEC and MALS

A260/280nm(125/90) = ~1.3

A260/280nm(18/35) = ~0.5

The figure above identifies illustrates heavy vs. light capsids via A260/280 ratio. The figure below verifies the molar mass of the heavy vs. light via MALS.

Column	SRT SEC-1000 7.8 X 300mm and 7.8X50mm Part Numbers: <u>215950-7805</u> and <u>215950-7830</u>
Mobile Phase	2XPBS with 10% ETOH
Flow Rate	1ml/min
Instrument	HPLC; SEC-MALS
Instrument Notes	UHPLC may not be ideal based on: The light capsids, meanwhile, were found to be much more thermally stable at higher temperatures, supporting the idea that internal pressure from the encapsidated DNA causes capsid instability.
Gradient	50uL



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McIntosh, N.L., Berguig, G.Y., Karim, O.A. et al. Comprehensive characterization and quantification of adeno associated vectors by size exclusion chromatography and multi angle light scattering. *Sci Rep* 11, 3012 (2021). <https://doi.org/10.1038/s41598-021-82599-1>

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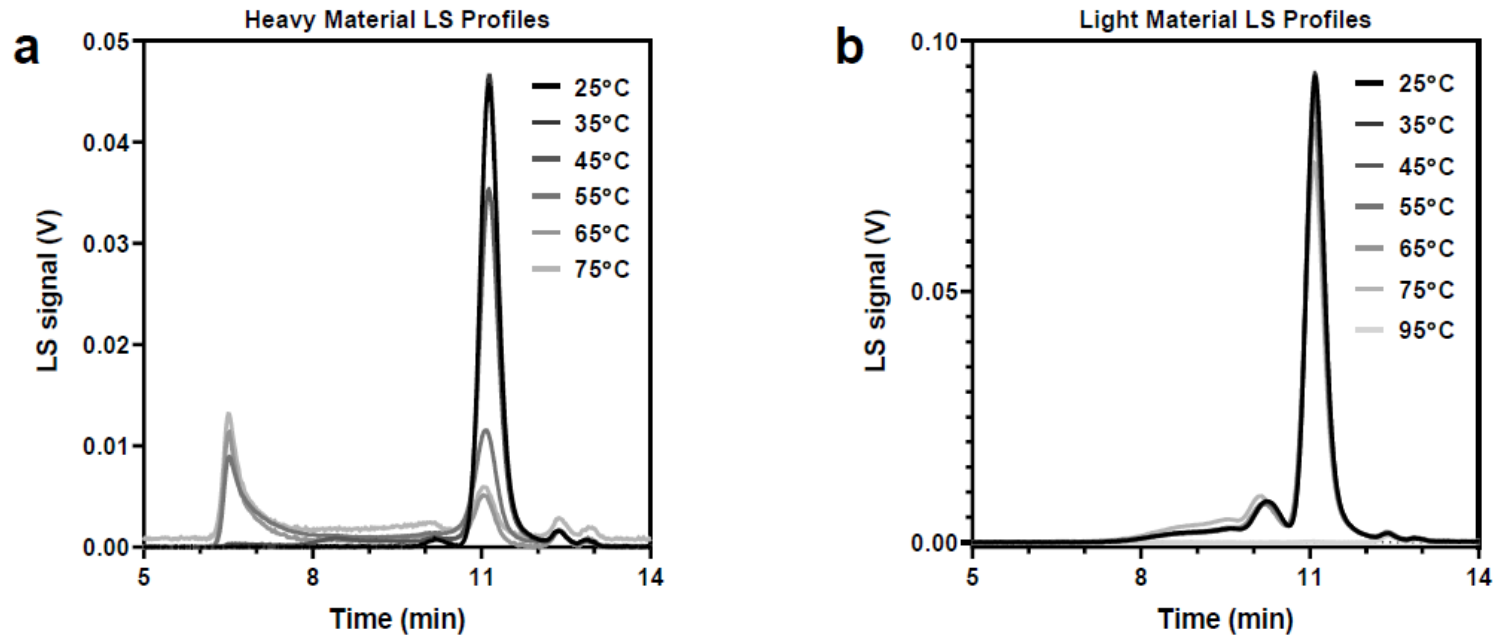


Figure a- Illustrates degradation of the capsid releasing DNA with respect to temperature. Presence of heat may cause internal pressure rupturing the capsid. Internal pressure maybe caused by the DNA in an enclosed space.

Figure b – Demonstrates that the capsid without DNA does not degrade with heat supporting the above hypothesis about internal pressure.

Internal pressure associated with full capsid may cause rupturing with UHPLC



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