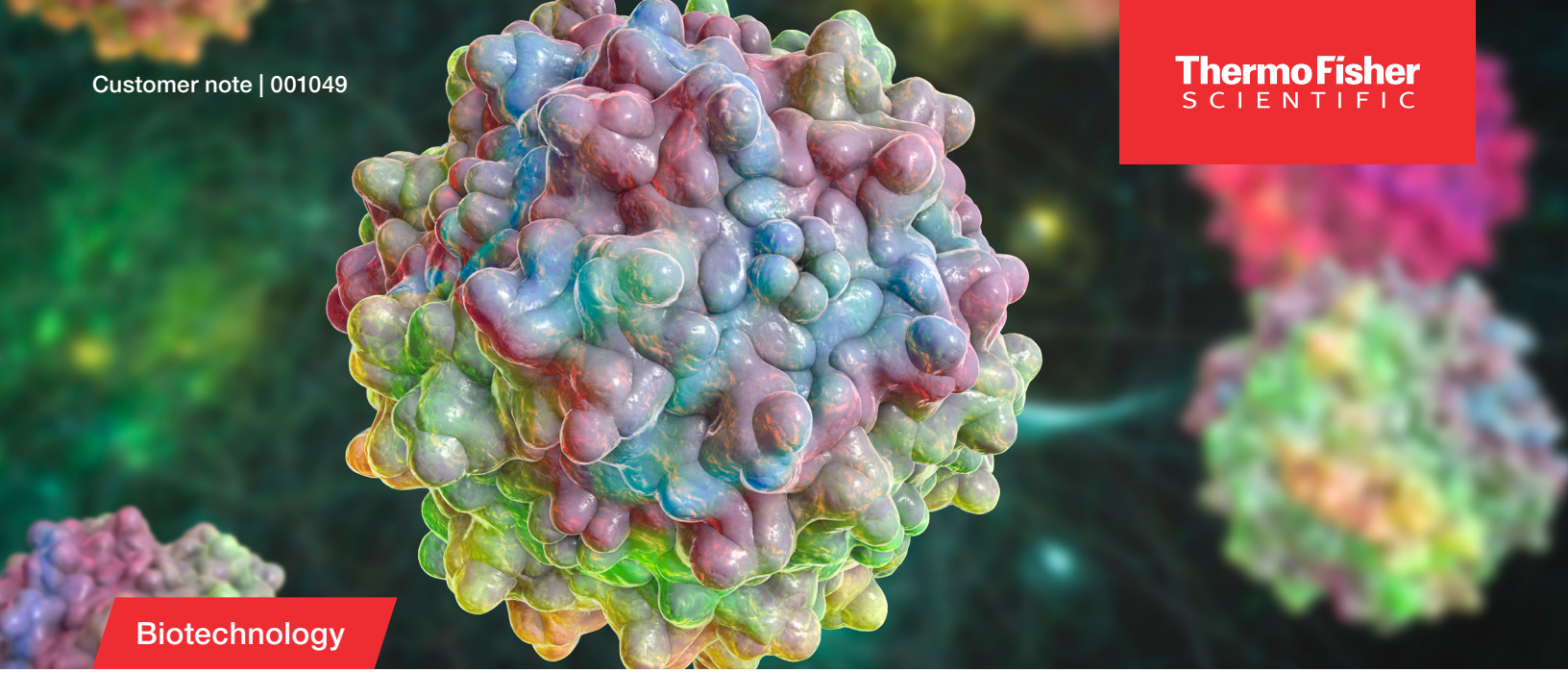

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Biotechnology

Quantitative analysis of genome packaging in adeno-associated viruses using native MS

Authors

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Keywords

Q Exactive UHMR MS, adeno-associated virus (AAV), cell and gene therapy, downstream processing, genome packaging, native mass spectrometry (MS)

Key benefits

- Intact native mass spectrometry (MS) for the analysis of AAV-based viral vectors does not require laborious sample preparation, thus minimizing potential sample loss.
- Native MS analysis of AAV capsids allows for accurate assessment of genome packaging efficiency.
- Short acquisition times enable rapid determination of empty:full capsid ratio.

Goal

To demonstrate the potential of native mass spectrometry for the characterization of next generation biotherapeutics

Introduction

Recombinant adeno-associated viruses (rAAVs) are vectors used for the purpose of gene therapy. They have successfully been modified to treat severe disease caused by a missing or defect gene in humans. AAVs consist of an icosahedral protein capsid built by 60 copies of three proteins (VP1, VP2, VP3), resulting in an average weight of approximately 3.5 MDa. The protein capsid contains a single-stranded DNA genome, adapted for therapeutic purpose.[1,2] Recombinant AAV production has been steadily optimized throughout the last years to yield high genome packaging efficiency, however, empty capsids remain an undesired by-product during manufacturing. Knowing the exact amount of correctly filled AAV capsids is important to determine the correct dosage required for treatment. There is also evidence of immune reactions caused by empty capsids. Therefore, it is of utmost importance to have analytical methods in place, enabling a quantitative evaluation of capsid species present during downstream processing.

There are several techniques available to measure the amount of empty and full capsids. The most common ones are analytical ultracentrifugation (AUC) and, more recently introduced, anion-exchange chromatography (AEX) and capillary electrophoresis (CE).[3] Yet, those methods rely on absorbance-based detection which can be problematic. One limitation is a lack of sensitivity, which is of significance when working with expensive and low concentrated samples. More importantly, absorbance-based methods do not allow for a definite identification of full and empty capsid species, which can be successfully circumvented by employing mass spectrometry (MS).[4]

Still, native MS analysis of intact viral capsids is challenging due to their high molecular weight and complexity. The resulting lack of charge state resolution prevents deconvolution to determine the accurate mass of AAV species present. Nevertheless, previous charge detection mass spectrometry (CDMS) experiments have revealed that full and empty AAV capsids share the same charge state distribution while differing in their mass due to the lack or presence of the cargo genome.[5-7]

Here, we use this knowledge to quantify full and empty AAVs via conventional native MS on the Thermo Scientific™ Q Exactive™ UHMR (Ultra-High Mass Range) Hybrid Quadrupole-Orbitrap™ mass spectrometer.

Experimental

An overview of the experimental workflow is shown in Figure 1. Full and empty AAV8 reference material with a concentration of 2e13 particles/mL (vp/mL) has been sourced commercially. For sample preparation, AAV samples can either be directly diluted 1:20 (v/v) in 100 mM ammonium acetate (pH 6.8, final concentration should not be lower than 1e11 vp/mL) or buffer exchanged into 100 mM ammonium acetate using Thermo Scientific™ Zeba™ spin desalting columns according to the manufacturer's instructions.

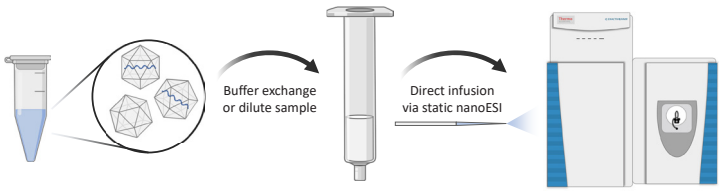

Figure 1. Experimental workflow. AAV reference material was buffer exchanged using SPE followed by native MS analysis using direct infusion via static nanoESI.

Following buffer exchange, the sample concentration was between 5e12 and 1e13 vp/mL. For analysis, 5 µL of the sample were loaded into double-coated borosilicate nESI emitter tips (Thermo Scientific, Hemel Hempstead, UK). For analysis, a Q Exactive UHMR hybrid quadrupole-Orbitrap mass spectrometer equipped with a Thermo Scientific™ Nanospray Flex™ ion source was used. Tune parameters can be found in Table 1. Please note, instrument parameters were optimized using a sample with known concentration of full and empty capsids.

Table 1. Tune parameters used on the Q Exactive UHMR MS for native MS analysis of AAV samples

Parameter	Setting
Acquisition mode	Positive ion mode
Resolution setting	25,000 (at *m/z* 200)
Microscans	10
AGC Target	1e6
Maximum injection time	200 ms
Spray voltage	1.5 kV
Capillary temperature	250 °C
S-lens RF level	200
Ion transfer target *m/z*	High *m/z*
Detector optimization	High *m/z*
In-source trapping	On
Desolvation voltage	-100 V
Source DC offset	-50 V
Extended trapping	150 V
Trapping gas	Sulfur hexafluoride
UHV readout	4.0e-10 mbar
Acquisition time	5 minutes, transient averaging enabled

Results and discussion

To investigate the potential of native mass spectrometry for quantifying genome packaging in recombinant AAV capsids, full and empty AAV8 reference material were analyzed individually. As shown in Figure 2, empty AAV8 capsids were observed as a signal cluster with a *m/z* of approximately 24,000, while signals derived from full capsids were detected at a *m/z* of >28,000. Hence, signals derived from full and empty capsids clearly differ in their *m/z* and thereby seem to follow previously described behavior, indicating similar charge states but differences in mass. CDMS-based experiments reported an average charge of +160 for intact AAV capsids of various species.[5] Taking that into account, the observed *m/z* would correlate with a mass of 3.8 MDa and 4.5 MDa, for empty and full capsids, respectively. The observed mass shift of 800 kDa correctly correlates with a packed genome of approximately 2.5 kb.

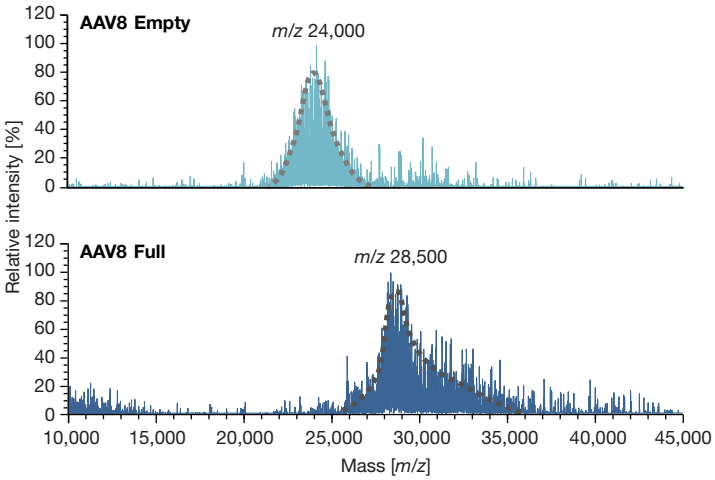

Figure 2. Intact native MS analysis of full vs. empty AAV8 reference material. The dotted line indicates the distribution of corresponding signal clusters.

Next, it was investigated whether capsids could be differentiated in mixtures prepared with different empty:full ratios. As shown in Figure 3, distinct signal clusters are still observed and can therefore be used for quantitation.

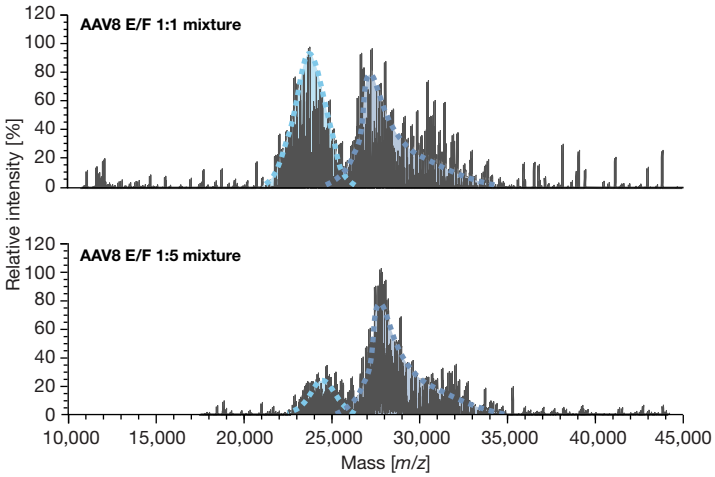

Figure 3. Analysis of full and empty AAV8 mixed at different ratios

To quantify the amount of full capsids present in the analyzed samples, the area below the highlighted signal clusters was integrated using Image J followed by calculation of the ratio and the fractional abundance of full capsids in percent (Table 2).

Table 2. Determining the ratio of full and empty capsids in samples mixed at volumetric ratios of 1:1 and 1:5

Empty:Full (V:V)	Ratio	% Full (MS)
1:1	1.41	58.50%
1:5	3.28	76.62%

The robustness of the established assay was evaluated by analyzing 1:1 mixtures of full and empty AAV8 at different transient lengths. As highlighted in Figure 4, results remained consistent with 61% full capsids detected at various resolution settings ranging from 3,500 to 25,000 @ *m/z* 200.

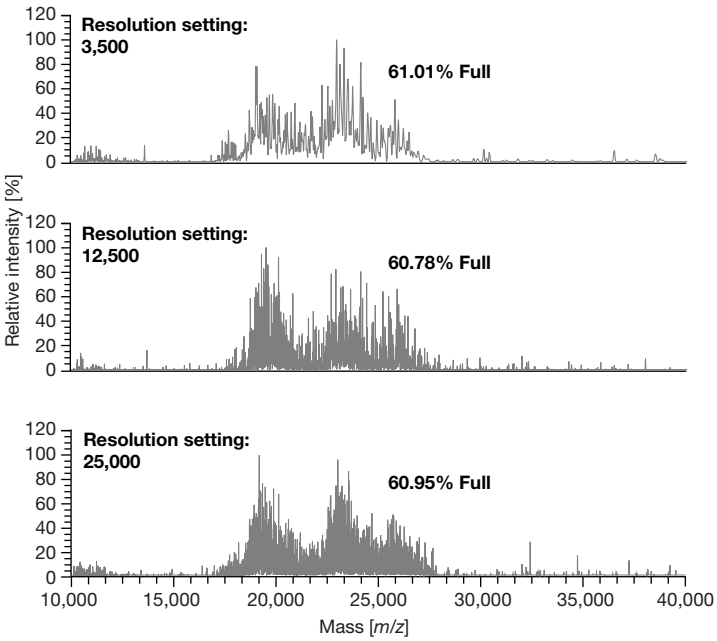

Figure 4. Analysis of full and empty AAV8 at various resolution settings

Conclusion

In this study, the potential of using the Q Exactive UHMR mass spectrometer for the intact native MS analysis of viral capsids was demonstrated. Knowing the packaging efficiency during AAV manufacturing is a crucial step towards successful clinical application. It was shown that native MS can be used to successfully quantify full and empty AAV capsids. The presented method is applicable to various AAV serotypes and has great utility for application during downstream processing. Additionally, the proposed assay has promising potential for further development using either CDMS or charge reduction for detection of partially filled capsids.

References

1. Zhao, Z.; Anselmo, A. C.; Mitragotri, S. Viral vector-based gene therapies in the clinic. *Bioeng. Transl. Med.* **2022**, *7*, e10258, doi:10.1002/btm2.10258.
2. Wang, D.; Tai, P. W. L.; Gao, G. Adeno-associated virus vector as a platform for gene therapy delivery. *Nat. Rev. Drug Discov.* **2019**, *18*, 358–378, doi:10.1038/s41573-019-0012-9.
3. Werle, A. K. et al. Comparison of analytical techniques to quantitate the capsid content of adeno-associated viral vectors. *Mol. Ther. Methods Clin. Dev.* **2021**, *23*, 254–262, doi:10.1016/j.omtm.2021.08.009.
4. Strasser, L. et al. A Native Mass Spectrometry-Based Assay for Rapid Assessment of the Empty:Full Capsid Ratio in Adeno-Associated Virus Gene Therapy Products. *Anal. Chem.* **2021**, *93*, 12817–12821, doi:10.1021/acs.analchem.1c02828.
5. Worner, T. P. et al. Adeno-associated virus capsid assembly is divergent and stochastic. *Nat. Commun.* **2021**, *12*, 1642, doi:10.1038/s41467-021-21935-5.
6. Worner, T. P.; Snijder, J.; Friese, O.; Powers, T.; Heck, A. J. R. Assessment of genome packaging in AAVs using Orbitrap-based charge-detection mass spectrometry. *Mol. Ther. Methods Clin. Dev.* **2022,** *24*, 40–47, doi:10.1016/j.omtm.2021.11.013.
7. Barnes, L. F.; Draper, B. E.; Chen, Y. T.; Powers, T. W.; Jarrold, M. F. Quantitative analysis of genome packaging in recombinant AAV vectors by charge detection mass spectrometry. *Mol. Ther. Methods Clin. Dev.* **2021**, *23*, 87–97, doi:10.1016/j.omtm.2021.08.002.

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