Optimizing Mass Spectrometer Conditions to Accurately Determine Full/Empty AAV Ratios: A Sample Half-Full Approach

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Abstract

Purpose: To determine which instrument parameters impact full/empty AAV ratio measurements and suggest what settings provide accurate ratios.

Methods: Commercially-available empty and full AAV2 were mixed to known ratios and measured with a variety of instrument parameters on a Thermo Scientific™ Q Exactive™ UHMR Hybrid Quadrupole-Orbitrap™ Mass Spectrometer with Direct Mass Technology mode. Empty/full ratios were determined using the STORIboard analysis package (Proteinaceous).

Results: A select few instrument parameters are shown to influence the measured full/empty ratios. Typically, these parameters bias against full capsid transmission.

Introduction

Adeno-associated viruses (AAVs) have been shown to be safe, valuable vectors for gene therapy. AAVs are small, non-pathogenic viruses that can be engineered to deliver genes to specific tissues and cells. The successful application of AAVs as a therapy begins with accurately determining the percentage of engineered capsids containing the desired gene. To date, analytical ultracentrifugation (AUC) has been used to provide full-to-empty AAV capsid ratios. However, ratio determinations using AUC typically require large amounts of sample (>100 µL at titers of ~10^{12} vg/mL). Orbitrap™-based charge-detection mass spectrometry (CDMS) can provide full-to-empty AAV ratios on the order of minutes using < 1 µL of sample at comparable titer. The role of instrument settings on ratio accuracy and reproducibility are presented.

Materials and methods

Sample Preparation

Empty (2E+13 vp/mL) and full (CMV-GFP, 2E+13 vg/mL) AAV2 capsids were purchased separately from Virovek (Hayward, CA). The empty and full were mixed and buffer exchanged with 200 mM ammonium acetate using a 100 kDa molecular weight cutoff filter to produce samples containing 50% and 75% empty capsids.

Test Method(s)

CDMS experiments were performed using Direct Mass Technology mode on a Thermo Scientific™ Q Exactive™ UHMR Hybrid Quadrupole-Orbitrap™ Mass Spectrometer. All ions were produced by electrospray ionization using borosilicate emitters and a spray voltage ~1.2 kV in a Thermo Scientific™ Nanospray Flex™ ion source.

Data Analysis

RAW files were processed using the latest version of the data analysis software STORIboard (Proteinaceous) resulting in masses (MDa) and percentages for empty, partial-filled, full, and over-filled capsids. The data here will be presented as fraction of empty capsids, as empty capsids are simpler to distinguish from filled, partially-filled, and over-filled capsids.

Results

After an initial round of optimization, baseline values for the parameters discussed in this study were set as recorded in Table 1.

Table 1. Default values, baseline values and ranges for instrument parameters in this study

Parameter	Default Value	Baseline Value	Range
Ion Transfer Tube Temp (C)	275	**350**	[250, 350]
Source DC Offset (V)	21	**0**	[-21, 30]
In-Source Trapping (Desolvation) (V)	Off	**-10**	[-100, -1]
Injection Flatapole (V)	4	**4**	[1, 10]
Injection Flatapole RF (V)	700	**700**	[250, 700]
InterFlatapole Lens (V)	3	**3**	[1, 8]
Bent Flatapole (V)	2	**2**	[1, 7]
Extended Trapping (eV)	Off	**5**	[5, 300]
Trapping Gas (N2)	1.0	**10.0**	[2.0, 10.0]
HCD Purge Time (ms)	5	**15**	[5, 50]
HCD Field Gradient	65	**65**	[20, 100]
HCD RF (V)	900	**900**	[250, 900]

Figure 1. Screenshot from the latest STORIboard analysis program for a 50% empty AAV2 sample (*empty, filled, over-filled labels added*). Separate measurements on a nominally 100% full sample contained ~5% empty capsids. For more information on new, AAV-specific capabilities within STORIboard, visit MP 816 by Ryan Fellers *et al.*.

Figure 2. Box-and-whisker plot demonstrating the accuracy and reproducibility for AAV ratio determination for samples containing 50% and 75% empty capsids from 10 replicate measurements.

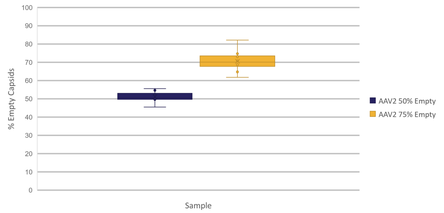
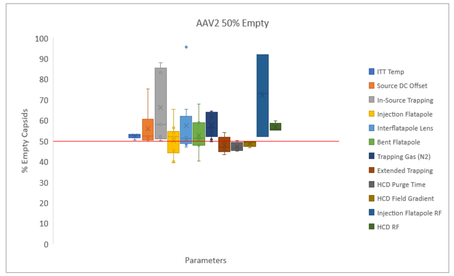
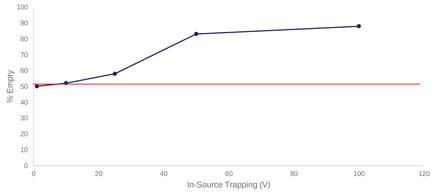


Figure 3. The impact of individual instrument parameters on measured AAV ratios for 50% empty AAV2. The larger the box, the more sensitive the measured ratio to the parameter setting. Ranges for each instrument parameter studied are given in Table 1.



As can be seen in Figure 3, many of the parameters have minimal impact on the measured AAV empty/full ratios. For example, it has been widely believed that heavier gases, such as sulfur hexafluoride (SF_6) or xenon were necessary to properly trap AAV capsids in the HCD cell. However, the data presented indicate that accurate AAV empty/full ratios can be measured using relatively low nitrogen pressures in the HCD cell. In contrast, two parameters, in-source trapping (desolvation) voltage and injection flatapole RF voltage, stand out as significantly biasing against full capsids. Figure 4 highlights how drastically the measured full/empty ratio changes with a modest increase in desolvation voltage.

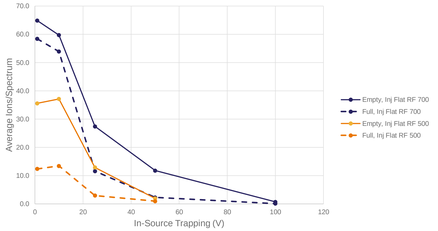
Figure 4. Percent empty capsids versus in-source trapping voltage for 50% empty AAV2.



A possible explanation is that at higher injection energies, it is more difficult to retain ions in the radial direction. For higher *m/z* ions, this effect would be larger. In general, RF voltages on the injection flatapole radially confine the ions, allowing for better desolvation during in-source trapping. Higher voltages allow for better confinement of higher *m/z* ions[1]. However, the default injection flatapole RF voltage at high-*m/z* transmission is the maximum that can be applied. So, as a test, the injection flatapole RF voltage was lowered and the individual rates of empty and full AAV ions (average ions/spectrum) were measured as a function of in-source trapping (desolvation) voltage. The results are presented in Figure 5. The rate decreases for all ions as in-source trapping is increased. At the maximum injection flatapole voltage, the rate decreases more rapidly for full AAV ions. Lowering the injection flatapole RF voltage results in a significant difference in average ions per spectrum between the empty and full ions, even at low in-source trapping voltages. These findings are consistent with the original assumption that full ions are being lost radially with more in-source trapping.

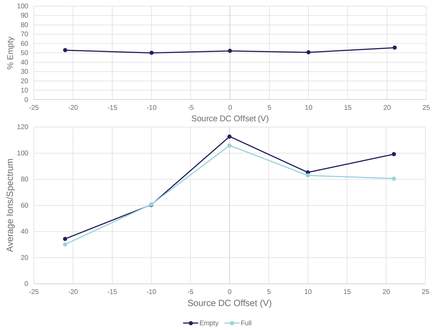
As a result, desolvation should be performed more in the HCD cell. Extended trapping, trapping gas setting, HCD purge time, and HCD field gradient have all shown minimal impact to the measured AAV full/empty ratios. Furthermore, recent improvements in signal processing have demonstrated the ability to account for incomplete desolvation when measuring heavier species (see WP 451, Goodwin *et al.*)

Figure 5. Rate (average ions/spectrum) of empty and full-plus-overfilled capsids versus in-source trapping voltage for 50% empty AAV2 at different injection flatapole RF voltages.



It should also be mentioned that certain parameters may not impact the measured full/empty ratio but may result in lower sensitivity. Examples include trapping gas setting and source dc offset, as shown in Figure 6.

Figure 6. Percent empty capsids versus a select range of source dc offset voltages and the associated average empty and full-plus-overfilled capsid ions per spectrum for 50% empty AAV2.



Conclusions

Of the many adjustable parameters on the Thermo Scientific™ Q Exactive™ UHMR Hybrid Quadrupole-Orbitrap™ Mass Spectrometer, a select few, namely in-source trapping, demonstrate an impact on the measured AAV full/empty ratios. However, these deviations are largely predictable for high-*m/z* ions and can be avoided by optimizing starting from the baseline set of parameter values found in Table 1. From the optimized settings, which differ slightly from the default values, a range of full/empty ratios for any AAV serotype can be measured accurately and reproducibly.

References

Fort, K.L., van de Waterbeemd, M., Boll, D., Reinhardt-Szyba, M., Belov, M.E., Sasaki, E., Zschoche, R., Hilvert, D., Makarov, A.A., Heck, A.J.R.: Expanding the structural analysis capabilities on an Orbitrap-based mass spectrometer for large macromolecular complexes. *Analyst* **143** 100-105 (2018)

Acknowledgements

Our thanks to Z. Szabo, W. Liu, and the Kelleher Lab for their generous gifts of experiment time and various AAV samples. Determining AAV ratios were greatly simplified by the work of R. Fellers and Proteinaceous.

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