Simplifying Progress

Efficient rAAV8 Capsid Quantification in Upstream Process Development with Two-Dimensional Liquid Chromatography

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Introduction

Obtaining a high concentration and a significant proportion of full recombinant adeno-associated viruses (rAAV) capsids during production is important for the success of the entire AAV bioprocess. Analyzing complex upstream samples is challenging and typically involves a combination of analytical methodologies, such as PCR and ELISA. However, this approach is known for being time-consuming and frequently prone to higher standard deviations. To overcome these limitations, the PATfix® AAV Switcher, an automated two-column analytical device, was developed.
The PATfix AAV Switcher system (Figure 1A) enables rapid and accurate detection of rAAV empty (E) and full (F) capsids in harvest samples. The method showcased in this poster efficiently binds and polishes rAAV8 on the CIM® Trap column, before directing it to the CIMac QA HR column where different capsid species are separated, utilizing a novel, patented baseline separation technique. [1] (Figure 1B).

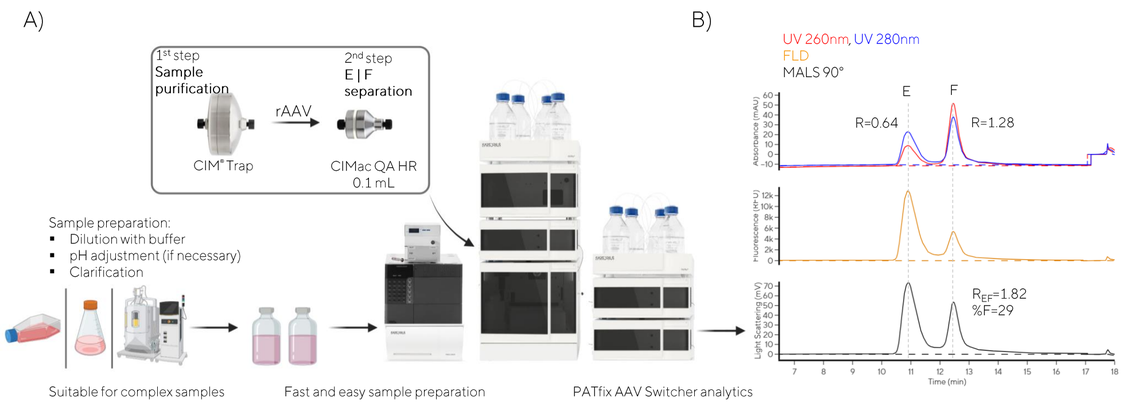


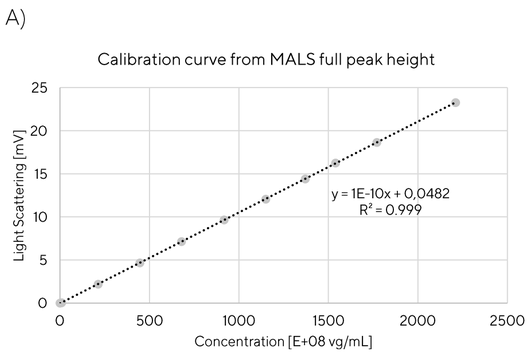
Figure 1: A) Schematic representation of PATfix AAV Switcher system setup and B) sample characterization using PATfix Software. Chromatogram represents innovative patented baseline empty (E) and full (F) capsid separation of rAAV8 reference material [1]. On chromatogram are shown UV 260nm and UV 280nm signals (in red and blue, respectively), as well as Tryptophan fluorescence (FLD, in orange) and readout of multi angle light scattering detector under a 90° angle (MALS, in black). R represents the ratio of UV260 to UV280 signals, and R_{EF} indicates the resolution between empty and full peak, which is automatically calculated by PATfix software. Percent of full capsids (%F) for the reference material shown is 29%, which was additionally confirmed by other available orthogonal methods for downstream samples, such as mass photometry and ultracentrifugation density gradient profiling (data not shown).

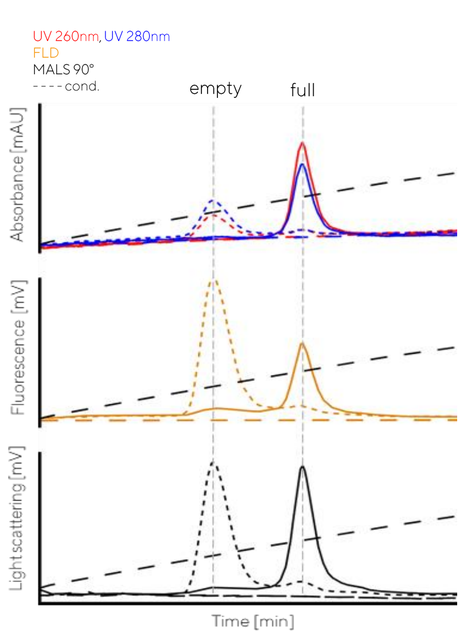
Experimental approach

Before the Switcher analysis, the whole harvest sample is subjected to lysing and diluted with a low pH buffer to allow binding to the first column, followed by clarification. The sample is then introduced to the CIM® Trap column, where the virus is captured and partially purified. Subsequently, the virus is eluted and automatically transferred to the CIMac QA HR column for precise separation and quantification of various capsid species. The complete process, from harvest lysate to obtaining an E/F chromatogram, takes 24 minutes.
The percentage of full capsids (%F) can be assessed using PATfix software, utilising either Tryptophan fluorescence (FLD) or multi-angle light scattering (MALS) signals. Fluorescence detection (FLD) offers advantages in quantifying the percentage of full AAV particles (%F) owing to its superior sensitivity. However, it can be affected by background noise from the matrix with rich protein content. In contrast, multiangle light scattering (MALS) demonstrates greater reliability in complex environments due to its ability in detecting larger particles. The downside of MALS is that it requires a correction factor to account for the molecular weight discrepancies between empty (E) and full (F) capsids [2]. Based on our comprehensive experimental data, we typically select MALS detection for complex upstream samples.

Quantification of the percentages of full capsids

A successful analytical tool for upstream applications is required to effectively accommodate samples with low AAV concentrations and minimal sample volumes, particularly during the early stages of process development.
To determine the limit of detection (LOD), the rAAV8 standard was serially diluted, and a calibration curve was constructed based on the MALS height response of the full peak (Figure 2A). The limit of detection for the MALS signal corresponds to 1.1E+09 vg/mL in a 3.5 mL injection volume. To evaluate the method's flexibility in detecting samples rich in empty and full capsids, two enriched rAAV8 fractions obtained from a CIMmultus® QA HR polishing step were quantified using the PATfix AAV Switcher, and compared with a single-column AAV E/F analysis. The results showed a 3% difference in quantification between the single-column and two-column analyses for both samples. (Figure 2C).





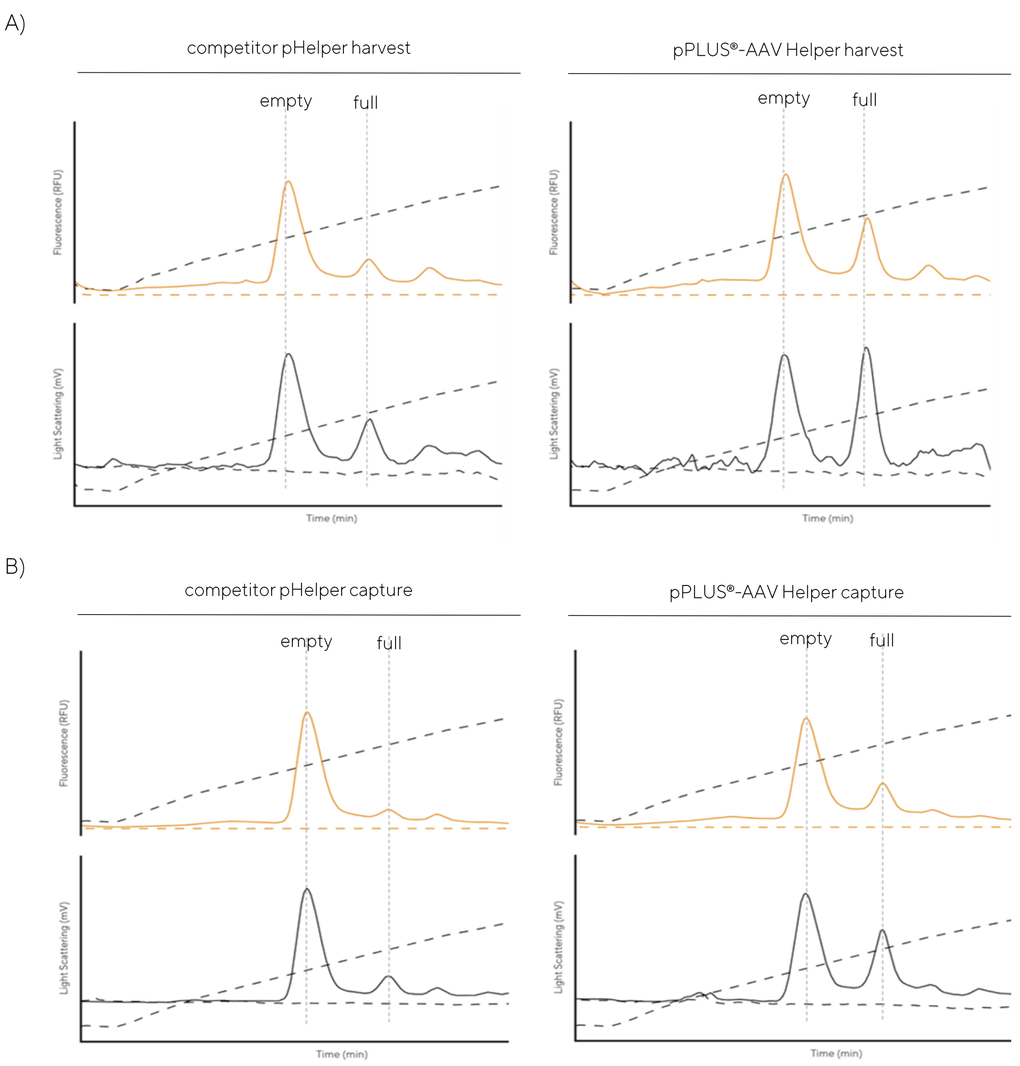
C)

	MALS* % full	
Sample	PATfix AAV Switcher (CIM Trap + CIMac QA HR column)	PATfix AAV E/F method (CIMac QA HR column)
E-enriched	8	9
F-enriched	93	90

Figure 2: Determination of limit of detection for PATfix AAV Switcher analytical method for rAAV8 samples. A) Calibration curve generated with ten serial dilutions of rAAV8 standard, where MALS full peak height is plotted against dPCR titer of full capsids. B) PATfix AAV Switcher chromatogram showing two purified samples, empty capsid enriched (dashed line) and full capsid enriched (full line). UV and FLD signals are shown for comparison purposes, while the quantification was performed using only MALS output. C) Table showing quantification of the %F for the two enriched samples using PATfix AAV Switcher (two-column) and the PATfix AAV E/F (single-column) method employing CIMac QA HR only.
*MALS correction factor of 1,29 was implemented.

The effect of sample matrix on sample characterization

For early upstream process development, the analytical method must not only exhibit low limits of detection and volume but also facilitate reliable capsid detection and quantification in the presence various impurities. To confirm that the sample matrix has no effect on the determination of the E/F ratio, we evaluated two rAAV8 harvests and their respective capture fractions obtained using CIMmultus SO3 chromatography. The samples were produced through FectoVIR®-AAV triple-transfection of HEK suspension cells, assessing the influence of the helper plasmid on the E/F ratio by utilising either the novel pPLUS®-AAV Helper plasmid or the competitor pHelper plasmid. The acquired data was compared to ultracentrifugation density gradient profiling (UCDG) as an orthogonal method for pure samples (Figure 3B). Both methods provided well-aligned results, except for pPLUS-AAV Helper harvest, which showed a 7% difference between the two approaches when FLD quantification was used (Figure 3B).



C)

	FLD % full		MALS* % full	
Sample	PATfix AAV Switcher	UCDG	PATfix AAV Switcher	UCDG
pPLUS®-AAV Helper harvest	23	/	30	/
Competitor pHelper harvest	10	/	11	/
pPLUS®-AAV Helper rAAV8 capture	22	22	22	22
Competitor pHelper rAAV8 capture	10	8	10	8

Figure 3: PATfix AAV Switcher analysis of A) harvest lysates and B) their respective partially purified rAAV8 capsids. C) FLD and MALS signals were quantified, and compared with UCDG. Both analytical methods showed that pPLUS®-AAV Helper led to at least a 2-fold increase in % of full rAAV8 capsids. *MALS correction factor was implemented.

Contributions to the upstream analytical toolbox

PATfix AAV Switcher system:

- is fast (24 minutes per sample) and reliable analytical tool suitable for early process development samples;
- has a limit of detection of ~1.1E+09 vg/mL (for MALS signal) present in 3.5 mL injection volume;
- when compared to orthogonal approaches, it gives comparable values for the percentages of full rAAV8 particles, is fully automated, and equipped with software that controls the method and analyses samples;
- can be used with both complex and purified samples, making it suitable for connecting upstream and downstream portions of the AAV bioprocess.

References

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