# Quantifying heterogeneous AAV capsid loading using mass photometry

*Adeno-associated viruses (AAVs) are used in approved gene therapies to deliver genetic cargo to target cells. Distinguishing heterogeneously filled AAV capsids is an important aspect of sample characterization when developing these therapeutics. Here, data collected by Pharmaron Gene Therapy show that mass photometry can distinguish populations of AAV capsids with heterogeneous loading of genomic content and quantify the proportion of each population in a sample. Mass photometry measurements take only minutes, require minimal training and have very low titer requirements.*

AAV-based therapeutics have been approved to treat some genetic disorders, while AAV-based therapies for diseases such as Parkinson's disease, Duchenne muscular dystrophy and even heart disease are being pursued.[1] As the development and use of AAVs accelerate, there is a growing need for quick and reliable methods to assess the quality of AAV preparations using small amounts of sample from each batch.

One important component of AAV characterization is to assess sample purity and the heterogeneity of capsid loading. Empty, partially filled and overfilled capsids are undesirable in gene therapy treatments because improperly filled capsids compete with full capsids for cell entry receptors and stimulate unintended immune responses, diminishing therapeutic efficacy.[2]

Mass photometry is well-suited to quantifying AAV capsid loading because it measures the mass of individual particles independently of their size or shape.[3] The Samux$^{MP}$ (Fig. 1), a mass photometer optimized for AAV characterization, was used here to distinguish and quantify heterogeneously filled AAVs. Each measurement is fast and requires low sample volumes and concentrations (20 µL of sample at 1x10$^{11}$ cp/mL), which is ideal for AAV sample evaluation, especially when batch sizes are small.


**Fig. 1 The Samux$^{MP}$ mass photometer, a benchtop instrument.** The Samux$^{MP}$ requires very little sample for each measurement (20 µL of sample diluted to 1x10$^{11}$ capsid particles/mL is optimal). Measurements are simple and can be performed in a wide range of buffers.

## The Samux$^{MP}$ quantifies heterogeneously filled capsids

Quantifying the proportions of heterogeneously filled AAV capsids is a critical step in AAV therapy characterization. To determine if mass photometry can fulfill this need, our collaborators at the CDMO Pharmaron Gene Therapy analyzed a sample from a commercially available AAV standard using the Samux$^{MP}$. The instrument was calibrated with a protein of known mass.

In each technical replicate, three distinct peaks appeared in the mass range for AAVs (Fig. 2). The first peak corresponds to the known mass of empty AAV capsids, and the two other peaks are filled with distinct quantities of genetic cargo.

The measured proportions of these populations of capsids in the sample were consistent across the three technical replicates (Table 1), highlighting the precision of the mass photometry measurement.

These results demonstrate that the Samux$^{MP}$ can consistently differentiate and quantify populations of heterogeneously filled capsids in an AAV sample.

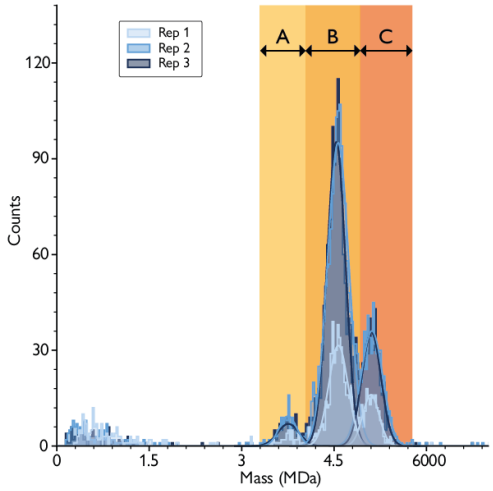


**Fig. 2 Mass photometry measurements of heterogeneously filled AAV capsids.** The mass histograms obtained clearly showed three distinct peaks, likely corresponding to empty capsids (labeled 'A'), and two additional peaks of heterogeneously filled capsids (peaks 'B' and 'C'). The three repeats were drawn from the same AAV sample (technical replicates). The measurements were performed on the Samux$^{MP}$ by the CDMO Pharmaron Gene Therapy.

## Mass photometry agrees with AUC

To assess the accuracy of mass photometry measurements, Pharmaron analyzed the same sample with analytical ultracentrifugation (AUC), a gold-standard (but time-consuming and sample-intensive) technique in AAV analysis.[4] AUC confirmed the presence of the three distinct capsid populations that mass photometry detected. Comparison of the percentages of these three populations measured by mass photometry (averaged for each of the three technical replicates) showed that the mass photometry data were in good agreement with those from AUC (Table 1).

Although both methods produce similar results, analyzing AAVs by AUC is slow and requires considerable expertise as well as much more sample than mass photometry. Mass photometry measurements, in contrast, take only minutes and are straightforward to perform. These results show that with the Samux$^{MP}$, it is possible to achieve results comparable to those from AUC with much less sample and in a fraction of the time.

**Table 1 Comparison between mass photometry and AUC.** Averaged mass photometry measurements from three technical replicates of the percentages of three distinct capsid populations in a sample (data from Fig. 2) are in good agreement with measurements of the same sample from AUC. The mass photometry measurements were performed on the Samux$^{MP}$; all data were provided by the CDMO Pharmaron Gene Therapy.

| | Peak A (%) | Peak B (%) | Peak C (%) | Peak B + Peak C (%) |
|---|---|---|---|---|
| Samux$^{MP}$ Rep1 | 5.8 | 72.1 | 22.1 | 94.2 |
| Samux$^{MP}$ Rep2 | 5.4 | 72.8 | 21.8 | 94.6 |
| Samux$^{MP}$ Rep3 | 5.7 | 68.0 | 26.3 | 94.3 |
| Samux$^{MP}$ Avg | **5.6** | **71.0** | **23.4** | **94.4** |
| AUC (230nm) | **5.9** | **66.6** | **26.3** | **92.9** |

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## Summary

Mass photometry provides essential data for AAV sample assessment. The data presented here demonstrate that the Samux$^{MP}$ mass photometer can quantify the proportion of heterogeneously filled capsids in AAV samples – determining relative abundances of each population with results on par with the gold standard approach, AUC.

The Samux$^{MP}$ has been optimized for AAV analysis, meaning it has excellent resolution in the mass range of AAV capsids. The Samux$^{MP}$ is an easy-to-use benchtop instrument that provides results in just minutes and uses very little sample (20 µL of a sample diluted to 1x10$^{11}$ cp/mL) – offering distinct advantages over AUC. Mass photometry measurements from the Samux$^{MP}$ can be used to streamline downstream process development and characterization of AAV product quality.[5]

### Experimental details

- All experiments were performed by the CDMO Pharmaron Gene Therapy
- The Samux$^{MP}$ was calibrated with a protein of known mass
- AAVs used were a commercially available AAV standard
- 10 µL of AAV sample at a concentration of 1.4x10$^{12}$ cp/mL were added to 10 µL of PBS to give a final AAV concentration of ~7.0x10$^{11}$ cp/mL at the time of measurement
- Mass photometry measurements were made on a Refeyn Samux$^{MP}$ with data recorded for 60 seconds


**References**

[1] *Li & Samulski, J Nat Rev Genet. 2020*

[2] *Samulski & Muzyczka, Annu Rev Virol. 2014*

[3] *Refeyn, How does mass photometry work? [Blog]. 2021*

[4] *Penaud-Budloo et al., Mol Ther Methods Clin. 2018*

[5] *Refeyn, Optimizing and monitoring AAV capsid purification using mass photometry [App Note]. 2022*





Unit 9, Trade City, Sandy Lane West, Oxford OX4 6FF, United Kingdom

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