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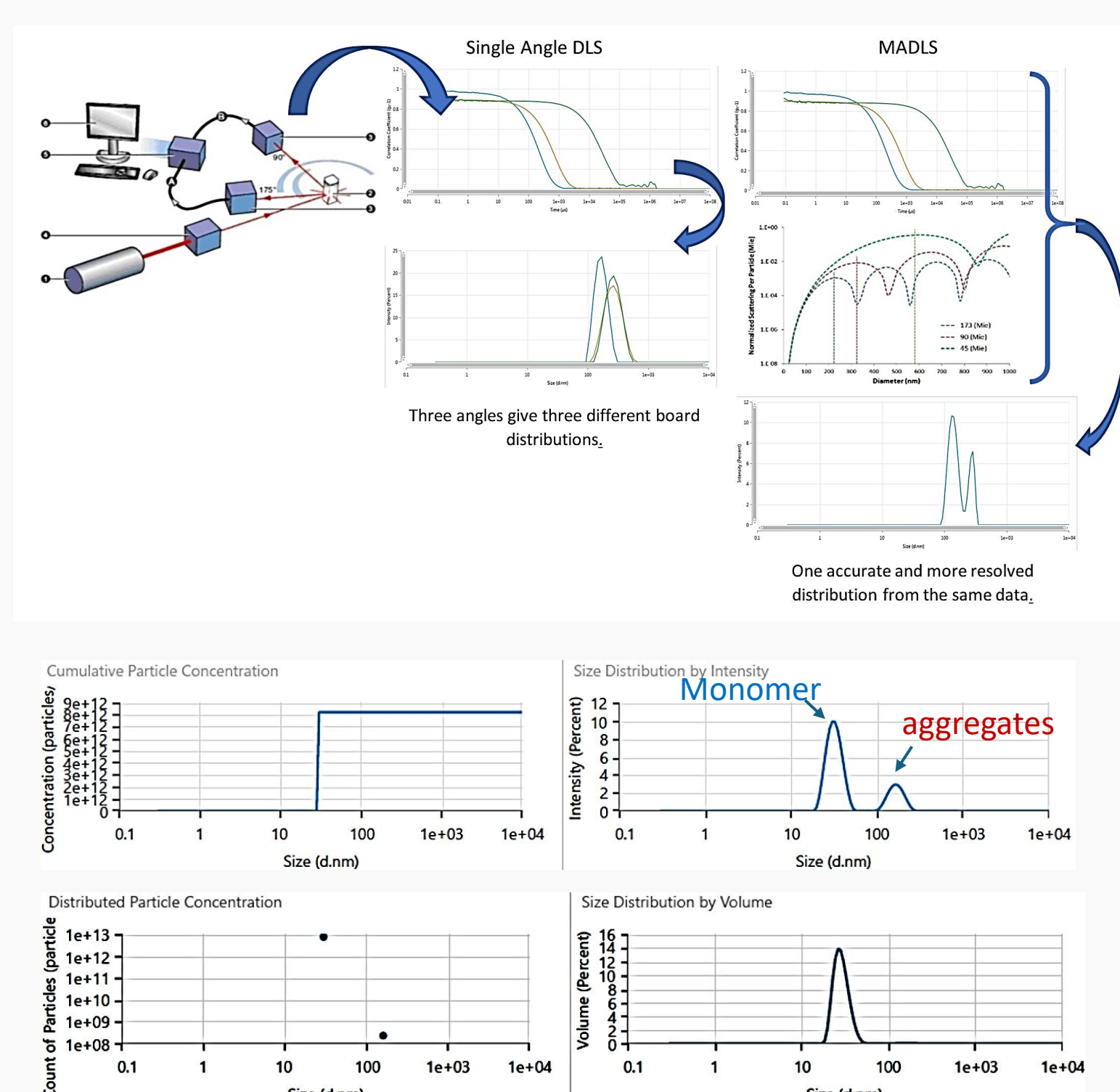
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Introduction

Adeno-associated viruses (AAVs) are widely used as vectors for gene delivery in therapeutic applications. Accurate quantification of AAV capsid titers is essential to ensure product quality and safety during manufacturing. While ELISA is commonly used, it is time-consuming and involves multiple steps, making it less ideal for fast-paced in-process testing. We evaluated Multi-Angle Dynamic Light Scattering (MADLS) and Mass Photometry (MP) as rapid, reliable alternatives for measuring AAV capsid titer and aggregation. Both are one-step assays requiring minimal sample handling and deliver results in under 10 minutes—ideal for real-time, in-process monitoring. By streamlining key analytics, these methods can enhance manufacturing efficiency, improve process control, and accelerate timelines, ultimately supporting the timely delivery of high-quality gene therapy products to patients.

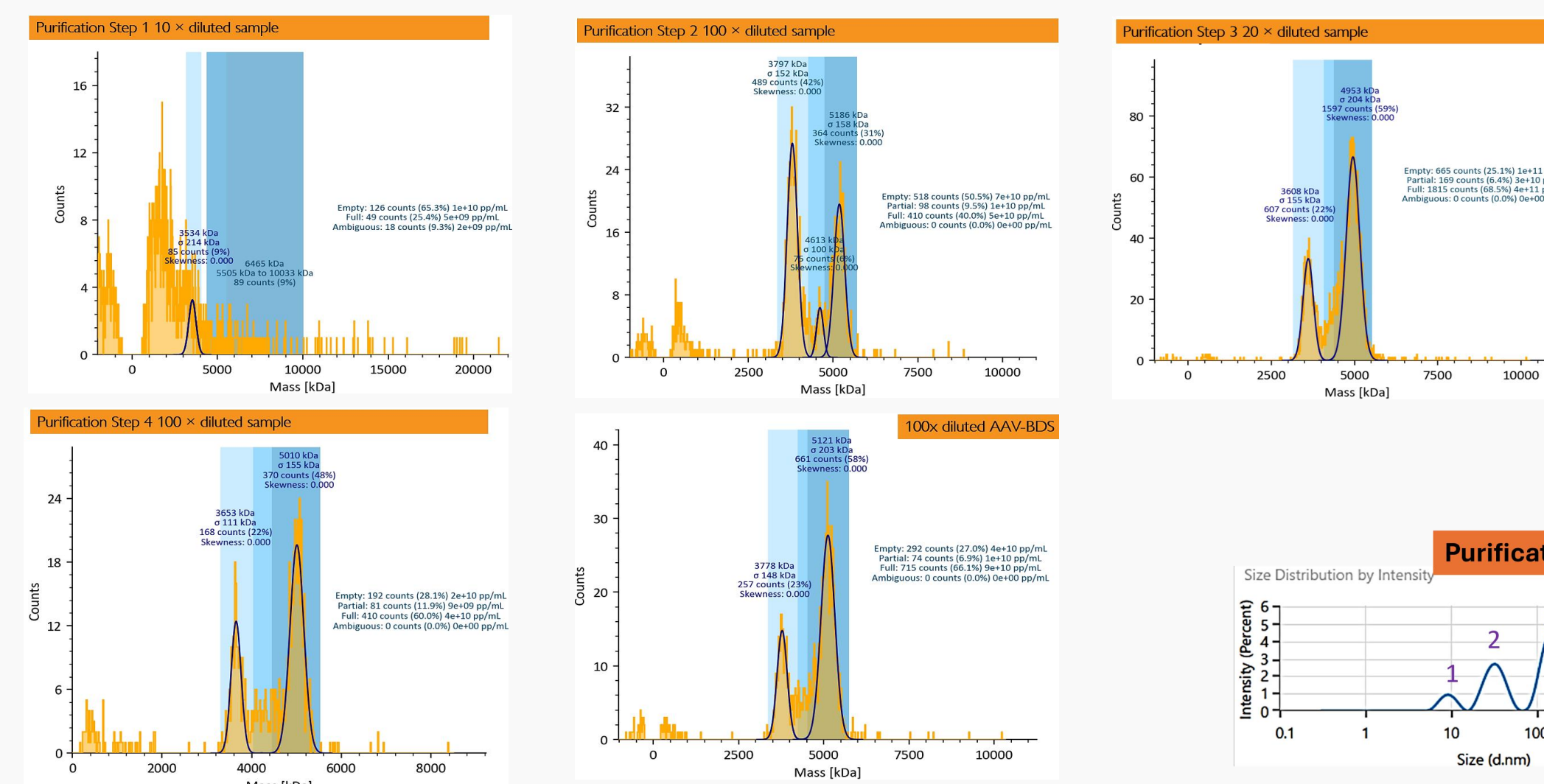
Multi-Angle Dynamic Light Scattering (MADLS)

MADLS measures particle concentration using an ensemble approach, providing size-resolved data without the need for a calibration curve. In purified AAV samples, particle size distribution is determined through light scattering, where signal intensity increases with particle size and aggregation. This allows quantification of both monomeric AAV capsids and aggregates, reflecting overall capsid concentration. While MADLS is effective at detecting AAV aggregation, it cannot differentiate between empty and full capsids.

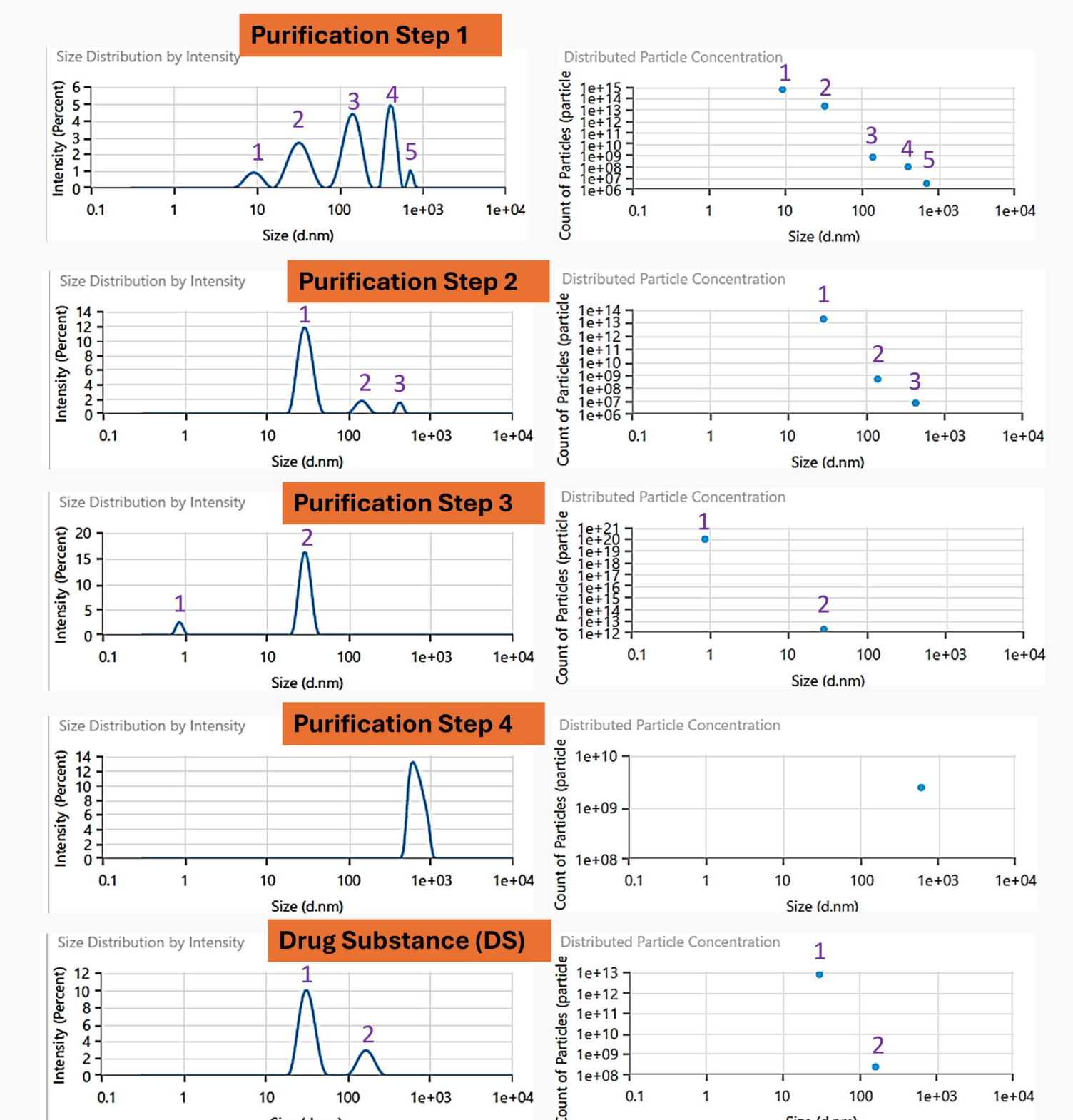


AAV in-process sample and drug substance test results from MADLS and Mass Photometer

Mass Photometer Results

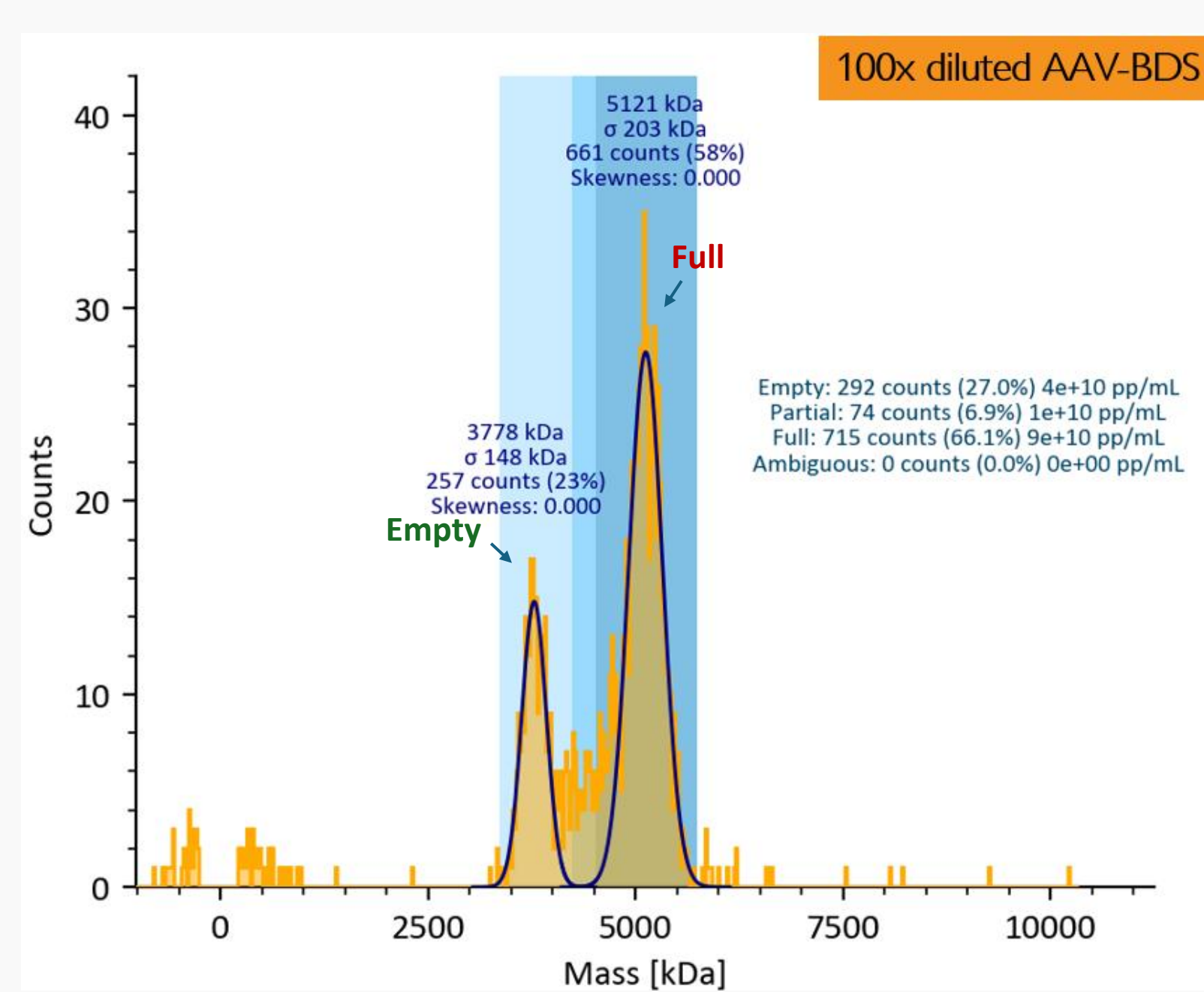
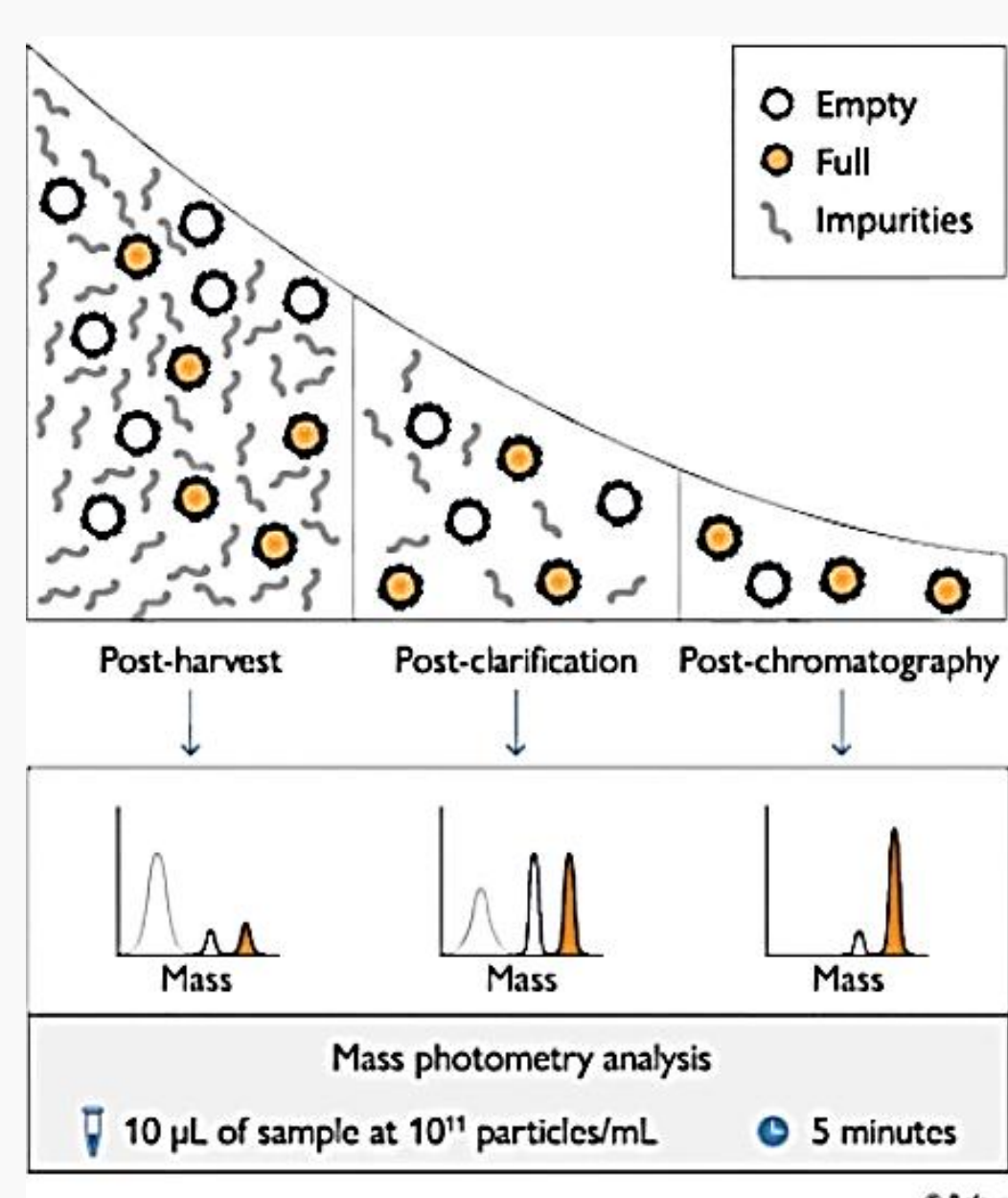


MADLS Results



1. MADLS method identify different particle populations based on size.
2. MP method identify different particle populations based on mass (molecular weight)
3. MADLS method is very sensitive in detecting aggregation
4. MP method may require sample dilution, which can reverse the aggregation status
5. Both MADLS and MP methods are impacted by impurity.

Enhancing Tissue Infectivity through Random Peptide Insertion in Capsid Protein



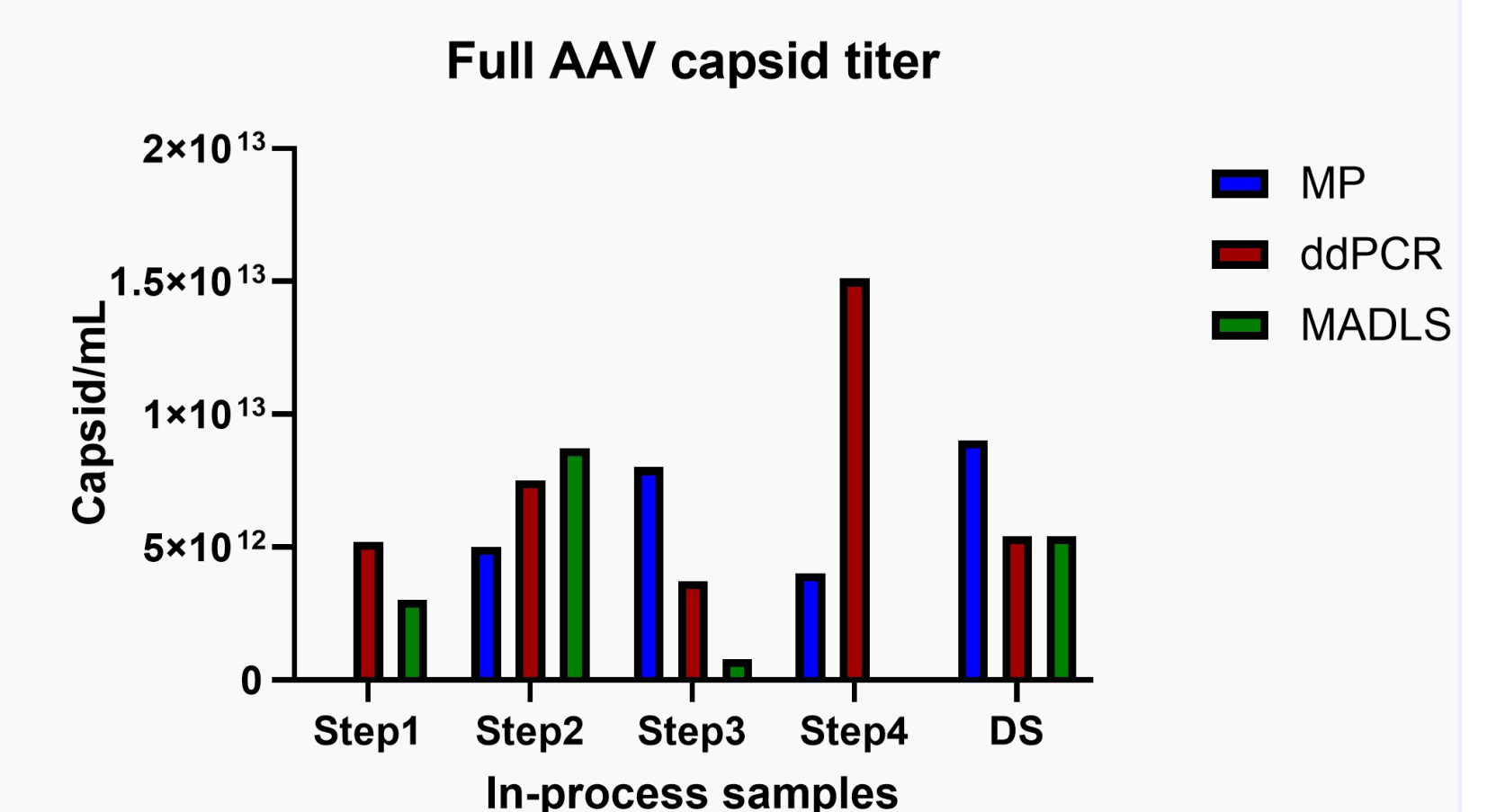
Mass photometry (MP) measures light scattered by individual AAV particles as they interact with a glass surface, enabling precise determination of each particle's mass. This allows accurate assessment of empty/full capsid ratios across any AAV serotype, along with detection of partially filled, overfilled capsids, impurities, and aggregates. A rough capsid titer estimate is also included in standard data analysis.

MP requires samples to be within an optimal concentration range (~1E11/mL), so dilution is necessary for more concentrated samples. The method captures cumulative particle events, and peak annotation for empty, partially filled, and full capsids is required to calculate capsid titer and empty/full ratios.

3-way comparison of full AAV capsid titer test results from MADLS, MP and ddPCR

For measuring AAV empty/full capsid ratios, Mass Photometry (MP) produces results comparable to Analytical Ultracentrifugation (AUC), providing both total and full capsid titers. In contrast, MADLS measures only total capsid titer; full capsid titer is estimated by applying the MP-derived empty/full ratio to the MADLS total titer.

We compared full capsid titers from MP and MADLS to ddPCR-based genomic titers. After purification (Step 2), AAV purity significantly improved, and both MP and MADLS results closely matched ddPCR values. However, Step 3 samples contained many small particles, reducing accuracy for both methods. Step 4 samples showed reversible aggregation, which particularly affected MADLS measurements.



Purification	MP Results					ddPCR Results		MADLS Results		AUC Results
	Empty	Partial	Full	Full%	Total Particle	Genomic titer	Total Particle	Full	Full %	
Step1	1.00E+12		5.00E+10	25.4%	1.05E+12	5.21E+12	1.18E+13	3.00E+12		
Step2	7.00E+12	1.00E+12	5.00E+12	40.0%	1.30E+13	7.50E+12	2.18E+13	8.72E+12		
Step3	2.00E+12	6.00E+11	8.00E+12	68.5%	1.06E+13	3.70E+12	1.13E+12	7.74E+11		
Step4	2.00E+12	9.00E+11	4.00E+12	60.0%	6.90E+12	1.51E+13	1.19E+09	7.14E+08		
Drug Substance	4.00E+12	1.00E+12	9.00E+12	66.1%	1.40E+13	5.41E+12	8.35E+12	5.41E+12	64.8%	

1. For purified AAV samples with majority monomer population and clean sample matrix, both MADLS and MP methods can provide close test results for AAV capsid titer.
2. MP method might perform better for samples with aggregation and some background.
3. No need for dilution, MADLS method might perform better for assay accuracy and monitoring aggregation status.

Reference

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