The Problem of Partials: Redefining the Analytical Frameworks Surrounding Capsid Characterization

As adeno-associated viral vector (AAV) manufacturing has evolved from research-scale production to commercial operations, the limitations of traditional characterization methods have become impossible to ignore. What was once acceptable heterogeneity is now a major barrier to consistent quality and predictable clinical performance. With modern programs pushing the limits of dose, scale, and regulatory scrutiny, controlling capsid content is no longer a downstream “nice-to-have”; it is a primary determinant of manufacturability, safety, and therapeutic effect.

This shift has re-centered a critical question: how accurately can developers quantify the particles their processes produce? Fifteen to twenty years ago, empty and partially packaged capsids were not viewed as serious impurities because analytical technologies could not reliably measure them, and potency was assumed to correlate primarily with vector genome titer.

Today’s high-resolution analytical methods have overturned that assumption. Many AAV preparations are now known to contain significant proportions of capsids with partially packaged genomes, a byproduct that arises throughout production and is far harder to remove than empties due to overlapping charge and density properties with full capsids. These particles can compete with full genomes during intracellular processing following vector delivery, reducing potency and potentially introducing uncertainty in biodistribution of transgene expression[(1)]. Early data showing that complementary partials can recombine into full genomes delayed their classification as impurities[(2)(3)], but these events are likely inconsistent and cannot be relied upon to restore function in a clinical setting.

Regulators now expect deeper characterization and clearer control strategies for the different subspecies. As a result, the analytical framework used to distinguish empty, partial, and full capsids has become a core requirement for next-generation AAV manufacturing — essential for demonstrating high purity, predictable potency, and reliable safety profiles.

The Functional Cost of Incomplete Packaging

Both empty and partial capsids can enter cells, potentially interfering with full genome delivery and vector potency, but only DNA-containing particles can undergo complete intracellular processing[(4)] and nuclear delivery of partial genomes within target cells may also interfere with the processing of full genomes, reduce expression efficiency and therapeutic efficacy[(1)(5)]. Moreover, while partials do not appear inherently more immunogenic than empties, they still contribute to unwanted antigenic burden and increase dose requirements.

A decade ago, analytical ultracentrifugation (AUC) was one of the only practical approaches for distinguishing capsid density species, but AUC instruments suitable for viral vector characterization were not widely available, leaving many developers to rely on incomplete data. Whether the industry underestimated the importance of

measuring partials or simply lacked tools to quantify them, the result was the same: partial capsids remained a blind spot in AAV release and characterization strategies.

An Overview of Incumbent Analytical Techniques for Capsid Content Characterization

Today, a wave of analytical innovation has shifted the landscape, enabling more precise evaluation of capsid composition. No single method, however, fully resolves the distribution of empty, partial, and full particles in isolation. Instead, developers increasingly rely on orthogonal techniques, cross-confirming results, increasing confidence in data, and building the historical comparability frameworks required for regulatory acceptance[6].

Size-Exclusion Chromatography (SEC-HPLC): SEC-UV-HPLC is often the first method employed to assess capsid populations because it is routinely available in biologics manufacturing environments. By measuring the UV absorbance ratio at 260 nm (nucleic acids) versus 280 nm (proteins), SEC-UV-HPLC provides an approximate estimate of full-to-empty enrichment. However, because it relies on bulk absorbance, this method cannot distinguish partial from full capsids, nor does it resolve heterogeneity within partial species. Its primary value lies not in precision compositional analysis but in rapid quantification of total capsid titer and preliminary packaging assessment[6]. SEC-UV can be coupled to different types of detectors, such as MALS (multi-angle light scattering) which can measure molar mass of particles populations and thus distinguish single-particles from aggregates, but none of them is resolutive enough to quantify partial vs. full particles[7].

Analytical Ultracentrifugation (AUC): AUC remains the historical and regulatory “gold standard” for measuring AAV particle composition. By separating capsids according to their sedimentation coefficients, AUC can resolve empty, partial, and full populations and assign quantitative abundances. Critically, AUC can differentiate partial genomes of distinct sizes, offering not just a binary classification but a profile of intermediate species[8][9].

Despite its strengths, AUC presents operational barriers: it requires highly specialized instrumentation, long run times, and expert interpretation. Even in organizations that deploy AUC, throughput limitations restrict its use to selected development stages and final release testing.

Cryo-Transmission Electron Microscopy (Cryo-TEM): Cryo-TEM provides direct visual evidence of AAV capsid structure by imaging particles preserved in a vitrified state. Unlike spectroscopic or density-based assays, cryo-TEM does not infer composition from a bulk signal — it enables analysts to see individual capsids, assess their morphology, and identify whether particles appear empty, partially filled, or full based on internal contrast [10][11].

Cryo-TEM is particularly valuable for confirming structural integrity or investigating unexpected species that appear in other assays. However, while cryo-TEM can reveal partial genomes, it does not natively provide automated particle-by-particle quantification. Although accurate quantitative data can be obtained using specialized image analysis software, this remains labor-intensive, requires expert interpretation, and is sensitive to sample preparation quality. In most organizations, cryo-TEM is used as a high-resolution investigative tool rather than a routine characterization method.

Mass Photometry: Mass photometry has rapidly gained traction due to its unique combination of simplicity, speed, and sensitivity. By measuring light scattering from individual particles landing on a detection surface, mass photometry assigns a molecular mass to each capsid, enabling classification into empty, partial, and full populations (12)(13). While it lacks the fine partial resolution of AUC, it offers sufficient discriminatory power for most development-stage applications.

Because mass photometry requires only nanogram quantities of sample, it is particularly useful for screening small-scale process development runs or early-stage formulation studies. Analyses can be completed within hours, with minimal method development and the potential for automation.

Charge Detection Mass Spectrometry (CDMS): Charge detection mass spectrometry (CDMS), while not yet widely deployed, represents a promising frontier. Native mass spectrometry techniques can directly measure the mass and charge of intact AAV capsids, enabling precise identification of genomic payloads and capsid stoichiometry (14)(15). CDMS offers single-particle measurement advantages using direct mass resolution rather than image-based classification.

Adoption of CDMS remains limited due to cost and availability, but as equipment becomes more accessible, it is likely to emerge as a valuable orthogonal tool, particularly for resolving subtle genome packaging variants that elude other assays.

The Argument for QuTEM as a Complementary Tool for Capsid Characterization

Quantitative transmission electron microscopy (QuTEM) has emerged as one of the most promising additions to modern AAV analytical frameworks. Unlike traditional cryo-TEM, which has historically only distinguished full from empty capsids, QuTEM applies image-based density analysis to classify individual particles as empty, partial, or full, with the ability to further resolve different partial genome sizes. This capability directly addresses a critical blind spot left by both conventional imaging and bulk analytical assays.

A recent comparative study has demonstrated that QuTEM measurements strongly align with established methods such as AUC and mass photometry, reinforcing its utility as an orthogonal confirmation tool[6]. Today, its greatest value lies in its ability to validate and contextualize results generated by other techniques. Where AUC provides population-level density profiles and mass photometry provides mass estimates, QuTEM offers particle-level evidence, allowing developers to confirm not just how many partials exist, but their type. This single-particle visibility becomes especially powerful when unexpected species appear in upstream development or drug product release testing.

If throughput and accessibility improve, QuTEM could become a reference method, streamlining workflows by collapsing multiple biophysical questions into a single assay readout. As regulatory expectations tighten around partial capsid quantification, developers who invest early in higher-resolution tools will be better positioned to:

- Diagnose upstream assembly and packaging issues
- Establish more predictive purity specifications
- Build comparability datasets that withstand regulatory review

- Reduce development risk tied to potency variability

Currently, wider QuTEM adoption is constrained by implementation realities, including the need for specialized software and expert operators, as well as current availability, as only one Sweden-based commercial group performs it routinely today. Yet as a high-resolution, particle-level method with clear concordance to incumbent tools, QuTEM represents a strong candidate to become a future gold-standard method within broader AAV analytical workflows.

The Case for Orthogonal Methods

Across platforms, no single technique delivers all required attributes: high resolution, low sample volume, fast turnaround, and regulatory acceptance. As a result, leading manufacturers increasingly apply multiple methods in parallel, including combining mass photometry for high-throughput screening with AUC or QuTEM confirmation for pivotal lots.

This strategy is particularly critical for partial capsids, where historical data are sparse and classification thresholds continue to evolve. The result is not redundancy, but risk mitigation. When datasets converge across orthogonal methods, they establish a convincing picture of capsid composition that supports regulatory filings, clinical dosing calculations, and long-term comparability studies.

Improved resolution in capsid composition analysis enables developers to pinpoint the origins of heterogeneity, refining process parameters earlier and establishing purity specifications that meaningfully predict potency. Techniques such as QuTEM, mass photometry, CDMS, and refined separation methods are beginning to close longstanding visibility gaps, but their true value emerges only when integrated into a coherent, multi-modal strategy. In addition to physical methods, orthogonal molecular biology assays should also be implemented, to confirm the results obtained through particles content analysis at the DNA level, such as electrophoresis techniques or multiplex digital PCR, or even long-read next-generation sequencing technologies which can provide sequence identity of both full-length and partial AAV genomes at a single-particle resolution[5][16][17]. By adopting more sensitive, particle-level tools now, manufacturers can reduce development risk, accelerate process optimization, and build products that meet both the scientific and regulatory standards required for next-generation AAV therapies.

As analytical technologies continue to advance, the industry's focus will shift not only toward eliminating partial capsids but understanding their formation mechanisms and quality implications. For now, however, effective characterization still depends on selecting the right toolbox and combining techniques intelligently to ensure that every dose delivered to a patient is as controlled, predictable, and potent as intended.

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