Impact of Full vs Empty AAV Capsids on Vector Potency

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INTRODUCTION

"Full" capsids in an adeno-associated virus (AAV) product are viral particles that contain a complete (ITR-ITR) recombinant AAV vector packaged inside, enabling gene delivery, protein production and biological activity.

"Empty" capsids lack DNA and therefore do not contribute to potency, though they can affect immune responses, biodistribution and potential efficacy.

Empty, partial, or otherwise nonfunctional capsids are product-related impurities resulting from the recombinant AAV manufacturing process. There are several concerns around how these impurities may impact downstream product safety and efficacy, particularly at the high vector doses required to achieve therapeutic effect. One such concern is that impurities may trigger both innate and adaptive immune responses, potentially leading to an increased risk of (serious) adverse events.

Preparations containing partial or non-functional particles require higher total capsid exposure to deliver the necessary vector genomes, thereby increasing the potential for dose-related safety concerns. Additionally, product-related impurities may have a potential impact on overall therapeutic efficacy by potentially competing with fully loaded capsids and preventing efficient entry into cells, through various mechanisms.

Therefore, the full-to-empty (F/E) ratio is considered a critical quality attribute in AAV manufacturing, and achieving higher F/E ratios may be essential to mitigating each of these key concerns.

MATERIALS AND METHODS

- In this study, we assessed POLARIS-101™ (formerly known as AAV-SLB101, part of a new generation of capsids engineered to enhance AAV transgene delivery, transduction, and expression in skeletal and cardiac muscle tissues). To test our hypothesis, a set of samples with varying F/E ratios were prepared using one of Solid's preclinical targets (TNNT2 transgene) and tested in the expression assay developed for this program. 84% full sample was the source from the remain dilutions and, empty capsid were added to the same amount of lot of full capsids.
- The studies were conducted using a myoblast cell line derived from mouse skeletal muscle (C2C12), which can be very efficiently transduced by the POLARIS-101 capsid. Experiments were performed in a 96-well plate format, where the 100% nominal drug concentration (NDC) was prepared from a well-defined reference standard and used as Condition 1. Following transduction from the cells by viral genome content and incubation for four days at 37°C, with the six F/E conditions from Table 1, expression readouts were generated on a mesoscale discovery device and quantified using a four-parameter logistic (4PL) model. The 4PL curve features distinct regions corresponding to critical analytical parameters, each contributing to interpretation of the sample performance (Table 2).

Table 1: Percentage of full transgene in POLARIS-101™-TNNT2 capsid used in this study

Condition	Full %
1	84
2	60
3	41
4	30
5	27
6	13
7	9
8	0

VECTOR POTENCY IN FULL VS EMPTY AAV CAPSID RATIOS

PROTEIN EXPRESSION ASSAY IN 4 PL CURVE READOUT

The dataset relates **percentage of full capsids (%)** to **relative potency (%)**, showing how biological activity changes as the fraction of full particles decreases: Potency declines moderately as full capsids decrease from 84% to 41% (100% to 43% relative potency) but falls disproportionately once full content drops to ~30% and below (21% at 30% full; 17% at 27% full), approaching near-zero at 13% and 9% full (0.06% and 0.02%, respectively). These results indicate that full capsid content is a dominant driver of potency, with an apparent threshold behavior in which low full ratios lead to functional collapse, consistent with non-linear effects such as competition from empty/nonfunctional particles and multiplicative inefficiencies in productive transduction. **(Figure 1)** Data was analyzed using 84% full AAV capsid as Reference Standard. 4PL curve analysis can be seen in table 2 and interpretation from each cohort in table 3.

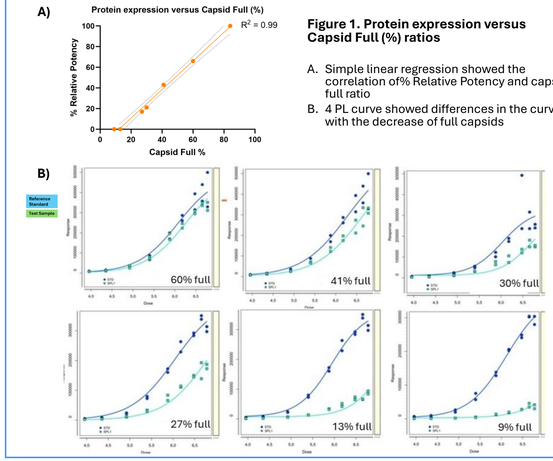


Figure 1. Protein expression versus Capsid Full (%) ratios

A. Simple linear regression showed the correlation of% Relative Potency and capsid full ratio
B. 4 PL curve showed differences in the curve with the decrease of full capsids

Table 2: 4-parameter Logistic (4PL) Curve Parameters from Vector Potency of Full and Empty AAV Ratio

4PL curve is a nonlinear regression model used to describe sigmoidal dose-response relationship. It is appropriate when: a) the response plateaus at low and high concentrations; b) the transition between plateaus is symmetric; c) Potency or relative activity (EC50, IC50) is required.
4PL model is the most accepted model for pharmacopoeia bioassays (USP/EP)
In this study, the dose response is correlated with high % full capsid. 4PL curve parameters are lost below 50% (data not shown)

Parameters	Analytical meaning	60%	41%	30%
A (Lower Asymptote) D (Upper Asymptote) **A-D**	Indicates the maximum achievable response at very high concentrations Represents the baseline or background signal when the dose is near zero	0.784	1.210	0.796
C (Inflection Point/EC50)	The concentration where the response is halfway between A and D	916896	446498	201511
B (Hill Slope)	Describes the steepness of the curve at midpoint	1.007	0.634	0.352*
% R2 SPL		0.99	0.97	0.97

GENE EXPRESSION PROFILE IN FULL VS EMPTY RATIOS

GENE EXPRESSION OF FULL:EMPTY AAV CAPSIDS RATIO

Gene expression decreases as % full capsids decrease, consistent across both MOIs. High MOI can partially rescue gene expression at intermediate full levels (30-60%). Below ~25-30%, increased MOI no longer meaningfully restores expression. Full capsid content is a dominant driver of transcriptional outputs, with MOI acting only as a partial compensatory factor.

Figure 2: Gene expression from full: empty AAV capsid ratio

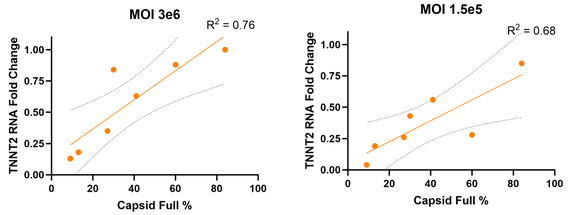


Table 3: Relative Potency from Full and empty AAV capsids ratio

Full (%)	Relative Potency (%)	Interpretation
84	100%	Reference condition – maximal biological activity
60	66%	Moderate potency reduction; system largely functional
41	43%	Continued decline, still proportional to full content
30	21%	Start to sharp, non-linear potency loss (infection point)
27	17%	Disproportionate drop; functional efficiency compromised
13	0.06%	Near-complete loss of activity
9	0.02%	Functional inactivity

If potency were purely proportional to %Full, then:

$$\text{Relative Potency} \approx \frac{\%Full}{84}$$

But we observed potency drops *more* than that at low %Full. A quick "efficiency" check is:

$$\text{Efficiency} = \frac{\text{Relative potency}}{\%Full/84}$$

60% full: expected 71% (60/84), observed 66% → efficiency ~0.92
41% full: expected 49%, observed 43% → efficiency ~0.88
30% full: expected 36%, observed 21% → efficiency ~0.58
27% full: expected 32%, observed 17% → efficiency ~0.53
13% full: expected 15%, observed 0.06% → efficiency ~0.004
9% full: expected 11%, observed 0.02% → efficiency ~0.002

IMPACT OF EMPTY CAPSIDS ON POTENCY ASSAYS

Proliferation assay was conducted simultaneously during the expression assay. 9 multiplicity of infection (MOI) were evaluated and readouts were performed at 4h, 24h and 72h.

The extreme conditions were evaluated 84% Full and 10% full. At **4 h:** both conditions sit in a similar range; At **24 h:** both conditions are essentially at the same high plateau (~3.0), implying **no meaningful separation** under these conditions (consistent with saturation) At **72 h:** both conditions remain broadly similar; if anything, the **10%** series sometimes appear **slightly higher** at some MOIs, but the difference is not large visually. Data suggested the spike of empties in the sample 10% full did not show impact in cell death in time and in any of MOI's used.

Figure 3: Cell Proliferation during expression assay

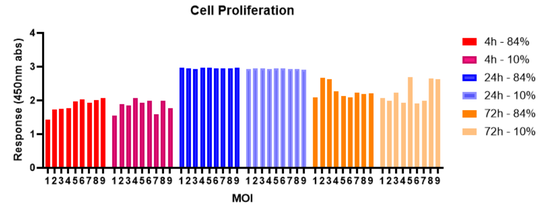
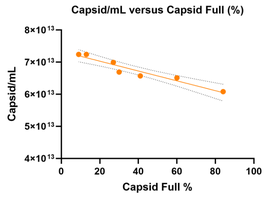


Figure 4: Capsid total amount per mL and capsid full %.



All F/E ratio samples presented in this study showed similar capsid per mL. Readout was performed by capsid titer by SE-HPLC. Average from capsid/mL was 6.84e13, with 6.5% of coefficient of variation.

CONCLUSIONS

- **Full capsid content is a dominant driver of potency**, but the relationship is strongly **nonlinear**. The data suggest a **minimum threshold of full capsids** (~50–60%) is required to maintain meaningful biological activity.
- At low full percentages, empty or non-functional particles likely dominate, resulting in **functional collapse** despite the presence of some full capsids.
- This pattern is consistent with **cooperative or multiplicative effects** in vector transduction rather than a simple dilution model.
- In CMC or assay context: Small reductions in full capsid percentage can have **outsized impacts on potency**. Specifications based solely on titer or particle number may be misleading without considering full/empty composition.
- Potency assays are expected to be **highly sensitive** to changes in full capsid ratio, particularly below mid-range values.
- Each readout may be dependent on the both the cell line used and the incubation period selected, warranting further research and analysis.

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