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Supplemental information

Evaluation of a rapid multi-attribute combinatorial high-throughput UV-Vis/DLS/SLS analytical platform for rAAV quantification and characterization

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SUPPLEMENTAL METHOD

Stunner Data Analysis

Stunner uses Lunatic & Stunner Analysis software (version 8.1.0.244) for data analysis. The UV-Vis absorbance of a sample is used to simultaneously determine the total amount of protein and DNA present in each aliquot. The measured ensemble absorbance is deconvoluted into the underlying absorbance curves for protein (A280), DNA (A260), and for other common impurities, using proprietary algorithms as previously described.[1] The concentrations of protein and single-stranded DNA (ssDNA) are determined based on calculated molar extinction coefficients.[2] Molar extinction coefficients for each naturally occurring AAV serotype were calculated based on the published amino acid sequences and ratios of VP1, VP2, and VP3.[3] Engineered novel AAV capsid amino acid sequences and ratios of VP1, VP2, and VP3 were added as appropriate. Molar extinction coefficients for each viral genome were calculated using expected genome size. From the protein and DNA concentrations, the calculated molecular weight information of capsid amino acid and packaged genome size, and the ratio of viral genome titer and capsid titer are used to calculate the percentage of full capsids.

Stunner also collects DLS and SLS data using a 660 nm LED laser light source with the former characterizing the size, polydispersity index (PDI), and size distributions of the sample. DLS data for a typical AAV sample show a major peak at approximately 25 nm diameter corresponding to the main AAV population. Additional peaks may represent aggregates or smaller particulates/impurities that may not have been removed during purification. Intensity distributions are used to determine the percentage of particles in each size population. The software highlights the main AAV population in the green bracket using two vertical lines at 15 and 45nm, respectively.

SLS measures the intensity of scattered light as a Rayleigh ratio and is proportional to the molecular weight of the sample. Consequently, an increase in the particle titer and/or an increase in %Full causes an increase Rayleigh ratio. As a result, a given Rayleigh ratio corresponds to a range of particle

titers depending on the %Full of the sample. The aggregation state also interferes with determining AAV titer by SLS alone; the Rayleigh ratio used in calculations is adjusted by the percentage of the SLS signal coming from the main AAV population, which is defined according to the size of AAV monomers. For example, if DLS information shows that 70% of SLS signal intensity is coming from the main AAV population, then only 70% of the measured Rayleigh Ratio is used in calculating titer. Alternatively, if no SLS signal intensity is detected at the appropriate size, then a capsid titer of zero is calculated, even if a non-zero A280 is measured. Finally, the %Full value from UV-Vis analysis is combined with the adjusted Rayleigh Ratio to compute the optimal capsid titer.

Stunner uses information from SLS, DLS, and UV-Vis in concert for accurate and precision calculations of titer. The theoretical basis for this comes first from one definition of the Rayleigh Ratio in equation (S1):

$$R_\theta = KCM \tag{S1}$$

Where K is the optical contrast constant, C is mass concentration, and M is molecular weight. The Rayleigh Ratio for an AAV sample is adjusted based on the percentage of the SLS signal coming from the main AAV population described in equation (S2):

$$R_{\theta,capsid} = [Capsid\ Intensity\ (\%)] \times R_\theta \tag{S2}$$

K is determined from the refractive index of the buffer (n_0) that is either calculated or user input and a calculated refractive index increment of the AAV (dn/dC), described in equation (S3):

$$K = \frac{4\pi^2 {n_0}^2}{N_A \lambda^4}\left(\frac{dn}{dC}\right)^2 \tag{S3}$$

From those equations we first express mass concentration, described in equation (S4) in terms of titer (T), molecular weight, and Avogadro's Number (N_A), then convert into Rayleigh Ratio expressed as an AAV titer with N_A and molecular weight (equation S5):

$$C = \frac{TM}{N_A} \tag{S4}$$

$$R_{\theta,capsid} = K\frac{T}{N_A}M^2 \tag{S5}$$

Rearranging the equation, we arrive at an expression of titer as a function of measured Rayleigh Ratio, K, and molecular weight, described in equation (S6). AAV molecular weight is calculated based on the capsid and genome information input by the user.

$$T = \frac{R_{\theta,capsid} N_A}{K M^2} \quad \text{(S6)}$$

A mass balance analysis is also performed after the calculation of full and total capsid titers. The difference between the total capsid titer determined by AAV Quant and the total amount of protein determined by UV-Vis alone (both expressed in cp/mL) is excess protein (i.e., protein not included in the AAV capsid) and labeled as "Free and Aggregated Protein" in the software. Similarly, the difference between the full capsid titer and the total DNA computed by UV-Vis alone (both expressed in vg/mL) is excess DNA not encapsidated in AAV and is labeled as "Free and Aggregated DNA" (Figure 1).

SUPPLEMENTAL DATA

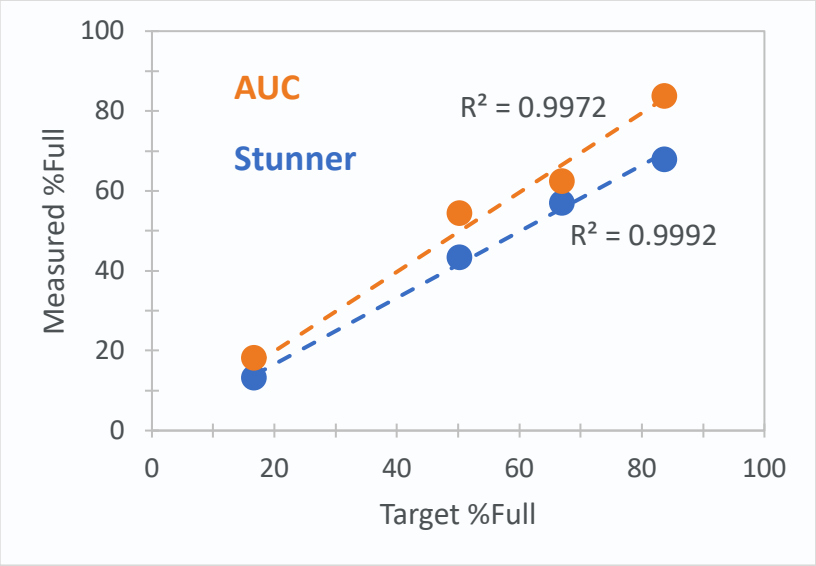

Figure S1. Stunner versus AUC for the % Full determination

AUC was read for the highest enriched %Full sample and dilutions with empty capsids used to ID the target %Full. Sample was seen by AUC to have some over-packaging, which could account for some of the variability shown.

SUPPLEMENTAL REFERENCE

1. Boonefaes, T. (2015). Optical characterisation of dna and/or rna. 2015-08-13.

2. Sommer, J.M., Smith, P.H., Parthasarathy, S., Isaacs, J., Vijay, S., Kieran, J., Powell, S.K., McClelland, A., and Wright, J.F. (2003). Quantification of adeno-associated virus particles and empty capsids by optical density measurement. Mol Ther *7*, 122-128. 10.1016/s1525-0016(02)00019-9.

3. Gill, S.C., and von Hippel, P.H. (1989). Calculation of protein extinction coefficients from amino acid sequence data. Anal Biochem *182*, 319-326. 10.1016/0003-2697(89)90602-7.