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Assessing the quality of adeno-associated virus gene therapy vectors by sedimentation velocity analysis

INTRODUCTION

Since the first gene therapy study in the late 1980s, there has been an ongoing interest in gene therapy strategies. According to Ginn *et al.* (1) about 2600 gene therapy clinical trials are being completed, ongoing or approved in 2018. The largest number of trials is concerned with cancer, followed by monogenic and infectious diseases (Table 1). In general, gene therapies can be divided into nonviral or viral delivery systems. Nonviral systems are usually characterized by an undirected and inefficient delivery and only transient expression; on the contrary, they show a larger packaging capacity and a better biosafety profile than viral gene therapies (1). However, the great advantage of viral systems lies in their high delivery efficiency and the exploitation of the viral biology (being able to infect host cells and to exploit their replication machinery). Among the known viral delivery systems, adenoviruses are used most often, followed by retroviruses and adeno-associated viruses (AAV). Interestingly, AAV show an increase in interest in recent years in contrast to other virus types (1). One reason might be the fact that AAV is less immunogenic than other viruses (2).

GENE THERAPY CLINICAL TRIALS	
CANCER DISEASES	65%
MONOGENETIC DISEASES	11.1%
INFECTIOUS DISEASES	7%
CARDIOVASCULAR DISEASES	6.9%
OTHERS	10%

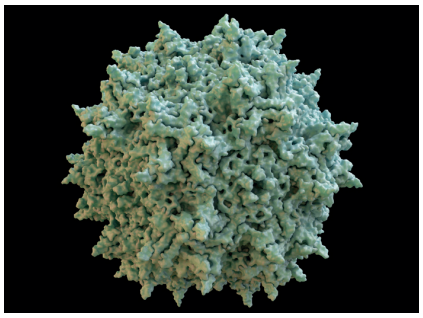
Tab. 1: Distribution of completed, ongoing or approved clinical trails by disease category 2018, in % (1)

Adeno-Associated Viruses

AAV is a nonpathogenic, nonenveloped parvovirus with the size of approx. 22 nm that is not able to replicate without the assistance of a helper virus (adeno- or herpes simplex virus) (2). The virus capsid can carry a transgene of about 5kb (including viral ITR sequences) without significant reduction in viral production yields (2). A promoter, the gene of interest and a terminator are cloned between both ITR sequences. After transduction, the transgene is present as an episome in the nucleus from where the expression of the gene of interest takes place. The episomal DNA limits the risk of integration but it might be diluted out after several cell cycles (3). Several AAV serotypes are used as gene therapy vectors. These serotypes show a specific tissue-tropism thus limit their use to certain organs. Efforts are made to optimize the capsid and therefore modulate the viral tropism to certain cell types (4, 5).

EXAMPLE 1
Quality control of rAAV vectors by AUC

Due to its popularity and increased usage, it is of major interest to assess the quality of clinical-grade rAAV vectors before administration.



3D CG rendered image of Adeno-Associated Virus (AAV) Capsid

Burnham and colleagues (6) showed that sedimentation velocity analytical ultracentrifugation (AUC) experiments are able to characterize the quality of viral vector productions (ratio between empty and filled capsids). A single AUC experiment provides insights into the composition of the encapsulated DNA, the success of the purification, the presence of aggregates and the ratio between empty, partially filled and filled viral particles. The AAV samples are measured at 260 nm at a speed of 20,000 rpm (6). A peak at 63S corresponds to empty vector capsids whereas a peak at 93S shows the proportion of filled capsids. There are also peaks between 63S and 93S visible, which might be partially filled capsids, and finally larger peaks (larger than 93S) appeared that could be aggregated viral particles. Furthermore, the scientists tried to assess if the AUC is able to discriminate between transgenes of different sizes. Firstly, they used a transgene of 3370 nucleotides in length that yields an s-value of about 92 after sedimentation velocity whereas a transgene of 4200 nucleotides results in a peak with an s-value of 101. Taken together, the AUC is a valuable tool to analyze rAAV vectors notwithstanding the composition and length of the transgene or the viral serotype.

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by ingenuity.

Novartis Gene Therapies, Inc. et. al.v. Genzyme Corporation
IPR2023-01044 (U.S. Patent 10,429,288)
Genzyme Exhibit 2005

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EXAMPLE 2

Sedimentation Velocity analysis with exceptional resolution proves to be the gold standard for interpreting orthogonal techniques

In the following example, Wang *et. al.* have investigated the potential of anion exchange chromatography (AEX) for the determination of empty and full AAV capsids and also for other populations in the sample like partially loaded capsids (7). They also mentioned transmission electron microscopy (TEM) and a number of other techniques like ELISA in combination with qPCR as possible methods to verify the quality of AAVs. However, they acknowledge that the AUC is the gold standard in the detection and characterization of AAV particles. TEM is not a quantitative method, giving rather qualitative information on the tested AAV batch. ELISA/qPCR and CDMS are shown to not be accurate enough to provide proper resolution. Finally, they directly compared AEX and AUC: AUC showed a much higher resolution as compared to AEX – as evident in the baseline separation between empty and full capsids in the AUC experiment. The comparable AEX experiment shows the empty capsids as an overlapped shoulder peak of the main filled-capsid peak. The AUC experiment even revealed a small population of what might be fragmented genome capsids. This species was not visible in AEX (Figure 1).

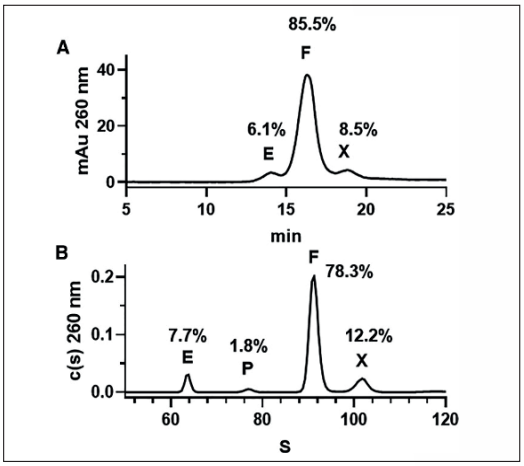

Fig. 1: Characterization of AAV empty and full capsids by AEX and AUC (reproduced with permission from Wang *et al.* (7)) Comparison of the AEX chromatogram (A) and AUC sedimentation coefficient distribution (B) of an affinity-purified AAV6.2 sample. E, empty capsid; F, full capsid; P, capsid with fragmented genome; X, unknown species. The peak percents labeled on the plots are area percents determined at UV 260 nm, without response factor corrections.

In contrast to AUC, every chromatographic technique uses a matrix, which particles have to pass through. Thus, not only can particles interact with the matrix, but also dilution effects might dissolve aggregated AAV particles and finally might falsify the true picture of a virus batch. AUC as a matrix-free in-method allows the characterization in a near-native environment.

OPTIMA AUC

- First-principle technique that does not depend on a matrix and does not require standards
- Samples are analyzed in their native state with almost no buffer restrictions
- One experiment reveals information about heterogeneity (detection and quantification of particle populations), formulation, aggregation, mass, association & shape of a protein or protein complexes. size distribution
- Optical systems:
  - Rayleigh Interference
  - UV/VIS absorption
- Sample volume:
  - 2-sector centerpieces: 450 µl or less
  - 6-channel equilibrium centerpieces: 120 µl or less
- Wavelength range: 190 – 800 nm
- Molecular weight range:
  - 10^2 Da (e.g., Peptides/Oligosaccharides) –
  - 10^8 Da (e.g., Viruses/Organelles)
- Concentration range:
  - UV/VIS absorption:
    0.005 – 1-2 mg/ml Luteinizing Hormone
  - Interference:
    0.025 – 4-5 mg/ml BSA

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