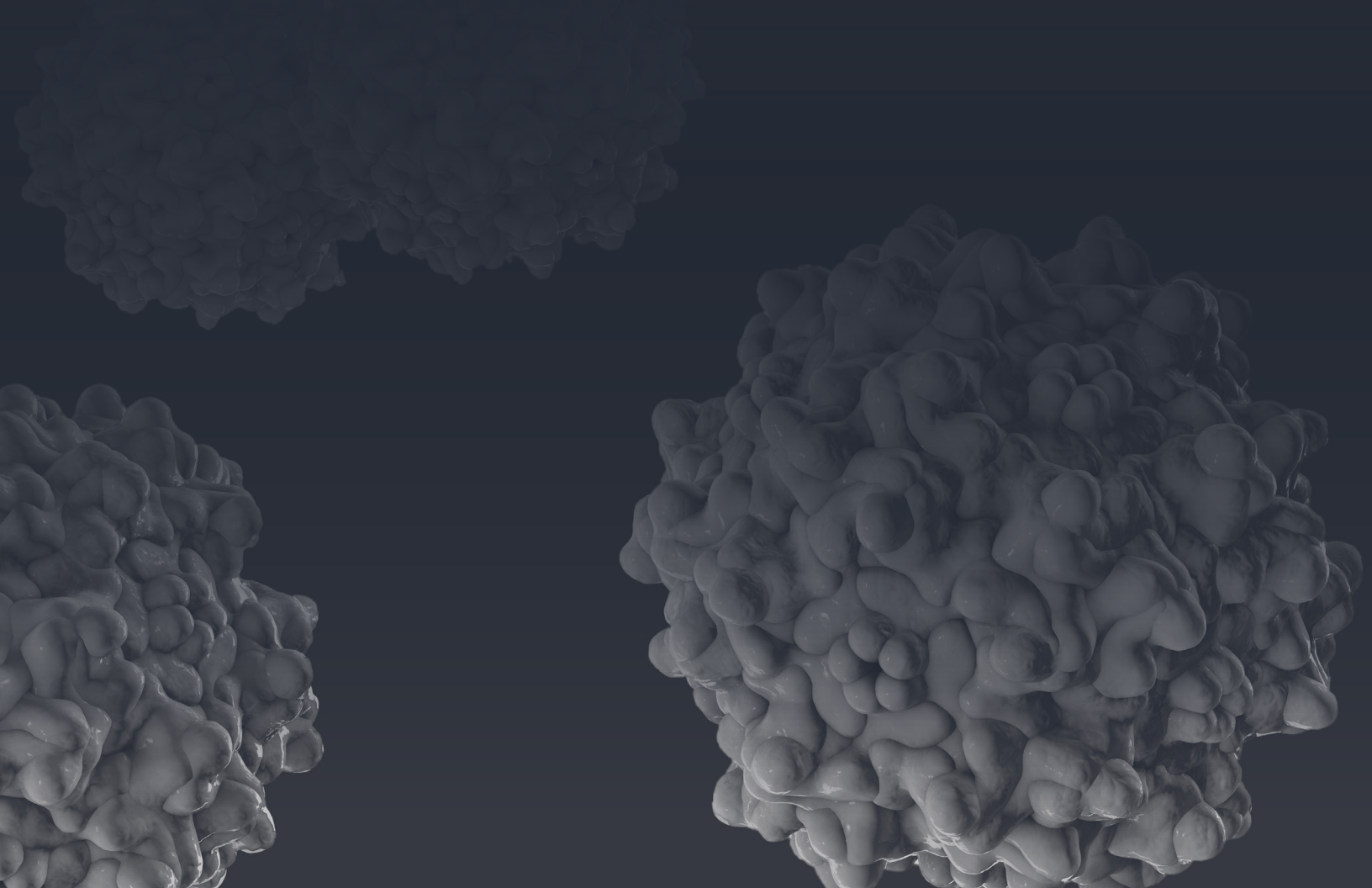ProtaGene
Analytical Excellence

INNOVATION SPOTLIGHT

Advanced AAV Capsid Analysis

Leveraging Serotype-Independent LC-MS Methods with Intact Mass and Peptide Mapping for Comprehensive Characterization

Innovation Spotlight

The analysis of capsid viral proteins is essential to developing an AAV-based gene therapy product. The primary sequence of the viral proteins (VPs) and the relative ratio of the major VP species (VP1, VP2, VP3) are critical quality attributes (CQAs) and are assessed as part of the analytical control strategy.

In addition, assessment of certain post-translational modifications that can impact potency are often monitored to inform process development decisions. Because significant differences among AAV serotypes exist, product-specific methods have been utilized to support development of individual products; however, platform approaches are desirable to reduce cost and move more quickly.

To support early-stage characterization and beyond, we have leveraged our extensive understanding of diverse AAV systems to develop two platform LC-MS methods to analyze AAV capsid viral proteins that can be applied to a diverse set of serotypes for efficient evaluation of early-stage samples.

VP Ratio - Intact Mass Method

◆ Our Intact Mass LC-MS method was developed to separate VP1, VP2, and VP3 and other commonly observed variants of multiple serotypes (Figure 1).

◆ The platform LC method results in separation of the major species, as confirmed by mass spectrometry analysis (Bottom/Lower Panel), enabling VP Ratio determination (Table 1).

◆ The method utilizes mild conditions to avoid inducing degradation artifacts during analysis, as oxidation and deamidation can alter the LC profile (UV detection) and complicate VP Ratio determination*.

*VP Ratio may be calculated from the LC chromatogram data once MS confirms the position of variant species. In some cases, coelution of modified species with major species may occur, and MS data is used to determine VP Ratio more accurately.

Table 1

Product	VP Ratio
AAV2	1:1:10
AAV5	0.5:1.5:10
AAV8	1:2:10
AAV9	1:1:10
AAVX	1:1:10

Figure 1
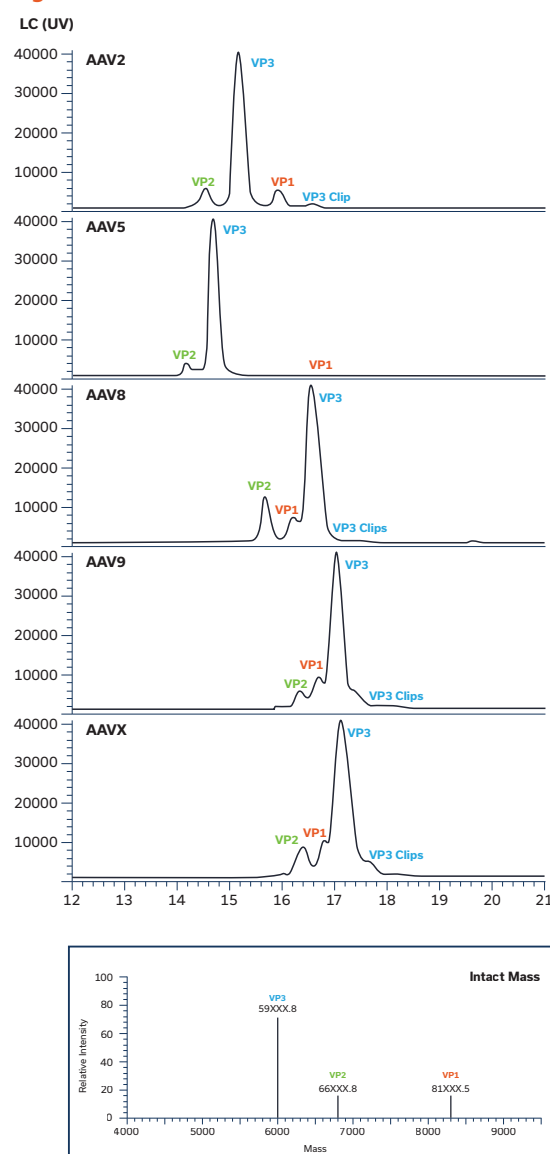
Identification of VP Primary Sequence by LC–MS Peptide Mapping

Fast, efficient confirmation of VP identity is enabled by our platform method for diverse AAV products (Figure 2), including confirmation of the three N-termini and determination of relative levels of Met excision and acetylation to VP1 and VP3 (Table 2). The differences among serotypes lead to unique LC-MS profiles for individual products, as can be seen in Figure 3, yet the platform method achieves greater than 93% sequence coverage across these diverse serotypes.
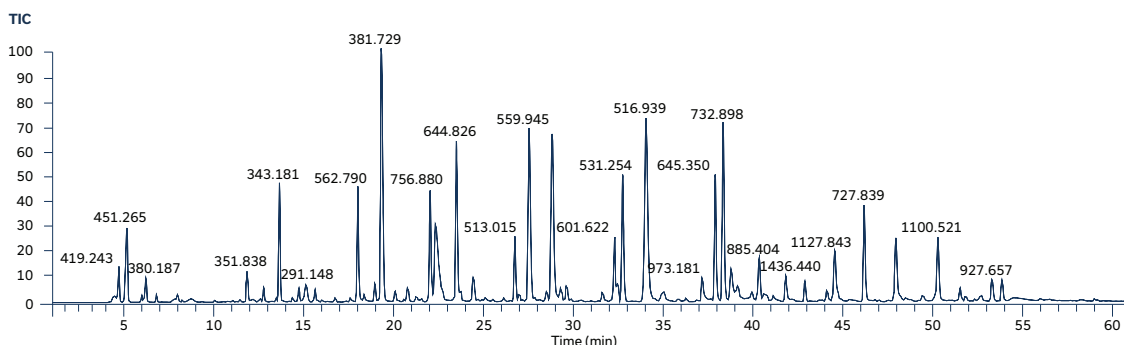
Figure 2

Table 2

Product	Ac-VP1	Ac-VP2
AAV5	97%	95%

LC–MS Analysis of Five AAV Serotype Samples

Using the platform Peptide Mapping method, complete digestion is achieved for all AAV serotypes, and the total ion chromatograms (TIC) show well-resolved LC-MS profiles. Very high primary sequence coverage is obtained, and the platform method further enables the monitoring of specific attributes.

Figure 3
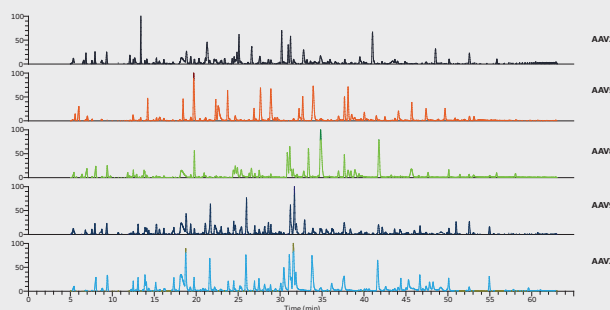
Meet the Innovator

"ProtaGene offers unique methods for deep characterization. These approaches can be applied to a variety of AAV serotypes, enabling us to streamline the development process and reduce costs in the long run."

Annie He
Principal Scientist