WEBINAR DIGEST

Demystifying AAV affinity capture: mapping AAV affinity ligand footprints with cryo-electron microscopy

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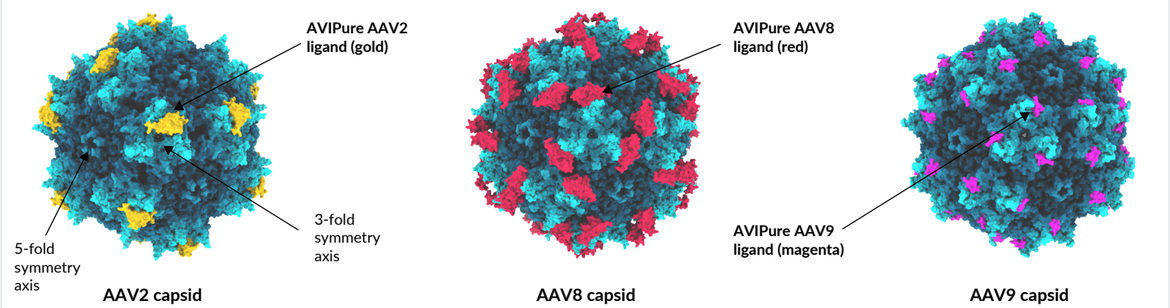
Cryo-electron microscopy (cryo-EM) provides high-resolution structures of AAV capsids and binding partners. Structures of several AAV-affinity ligands with the target capsids elucidate the mechanism of affinity resin capture. This poster delves into how these ligands engage the AAV capsids, and how these results can accelerate process development for these important gene therapy vectors.

CRYO-EM IN AAV AFFINITY RESIN DESIGN

Cryo-EM techniques allow visualization of the three-dimensional structure of AAV capsids. The AAV capsid structure consists of 60 viral protein (VP) monomers, predominantly VP3, which assemble with icosahedral symmetry. To determine where the AVIPure affinity ligands bind on AAV capsids, we determined cryo-EM structures of the ligands:capsid complexes. Identification of the binding site on the capsid helps scientists determine if a given capsid, which often contains proprietary mutations, will bind to a serotype specific AVIPure AAV resin.

Figure 1 illustrates three cryo-EM structures, AAV2, AAV8, and AAV9 capsids bound to their respective AVIPure ligands. These findings reveal that all ligands bind to the capsid's 3-fold symmetry axis. This enables rational pairing of an AAV resin to a specific capsid and enables the selection of capsids that maintain the AVIPure epitopes.

Figure 1. Cryo-EM structures of AVIPure ligands bound to AAV 2, 8, and 9, respectively.

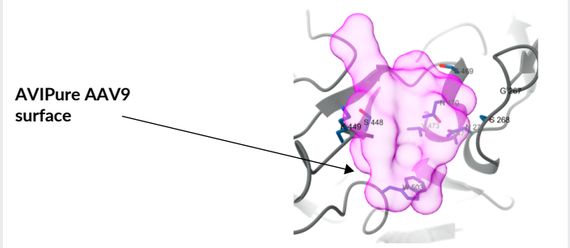


MAPPING LIGAND BINDING SITES

Cryo-EM maps revealed that AVIPure ligands have specific binding "footprints" on each AAV serotype's capsid. The AAV2 ligand binds between lobes of the 3-fold axis, disrupting the axis's natural symmetry. This leads to a highly specific interaction that enhances AAV2 capture and reduces impurities, such as host cell proteins and residual DNA.

The AAV8 ligand also binds at the 3-fold axis but at a different location on the protrusion. Though slightly less extensive than AAV2, this interaction still ensures strong selectivity, which is essential for producing highly pure AAV8 preparations. AAV9's interaction is particularly unique; the ligand is a small 10-amino-acid peptide that binds within the galactose binding pocket on the 3-fold axis, as shown in **Figure 2**. Despite the small size, the surface area of the binding interface is comparable to that of the larger AAV2 and AAV8 ligands. This compact yet highly efficient ligand minimizes the risk of interference from capsid mutations and enables efficient and adaptable purification of AAV9 capsids.

Figure 2. The AAV9 ligand is a small 10-amino-acid peptide that binds with the galactose binding pocket on AAV9 three-fold region.



APPLICATIONS OF CRYO-EM INSIGHTS IN AAV ENGINEERING

One of the key benefits of understanding these binding sites is the ability to predict whether a modified AAV capsid can be effectively purified using AVIPure resins. Compatibility with AVIPure resins can be predicted by comparing capsid sequences with the mapped ligand binding residues. This is valuable for engineered capsids, where specific mutations may affect binding efficiency. Additionally, if this information is integrated into the early capsid engineering and selection stages, the capsids will maintain compatibility with AVIPure capture resins, thus avoiding potential downstream challenges. These cryo-EM insights also support sustainable production. By validating the mechanism of AVIPure affinity capture, producers can be confident in the selection of these resins, which exhibit high stability in sodium hydroxide, and can be reused multiple times, reducing waste and production costs.

SUMMARY

Cryo-EM technology enabled precise mapping of the AVIPure ligand binding sites on AAV capsids. The data on AAV2, AAV8, and AAV9 provide a foundation for developing robust, selective purification methods tailored to gene therapy vectors, supporting advances in gene therapy manufacturing and helping meet growing demands for high-purity viral vectors.

Watch the video and view the poster here

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