Rational design and experimental evaluation of peptide ligands for the purification of adeno-associated viruses via affinity chromatography

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Abstract

Adeno-associated viruses (AAVs) have acquired a central role in modern medicine as delivery agents for gene therapies targeting rare diseases. While new AAVs with improved tissue targeting, potency, and safety are being introduced, their biomanufacturing technology is lagging. The AAV purification pipeline, in particular, hinges on protein ligands for the affinity-based capture step: while featuring excellent AAV binding capacity and selectivity, these ligands require strong acid (pH <3) elution conditions, which can compromise the product's activity and stability; additionally, their high cost and limited lifetime has a significant impact on the price tag of AAV-based therapies. Seeking to introduce a more robust and affordable – yet equally effective – affinity technology, this study introduces a cohort of peptide ligands that (i) mimic the biorecognition activity of the AAV receptor (AAVR) and anti-AAV antibody A20, while (ii) enabling product elution under near-physiological conditions (pH 6.0) and (iii) granting extended reusability by withstanding multiple regenerations. A20-mimetic CYIHFSGYTNYNPSLKSC and AAVR-mimetic CVIDGSQSTDDDKIC demonstrated excellent capture of serotypes belonging to distinct clones/clades – AAV1, AAV2, AAV5, AAV6, AAV8, and AAV9 – corroborating the in silico models documenting their ability to target regions of the viral capsid that are conserved across all serotypes. CVIDGSQSTDDDKIC-Toyopearl resin features binding capacity (˜1014 vp per mL) and product yields (˜60-80%) on par with commercial adsorbents, and purified AAV2 from HEK293 and Sf9 cell lysates affording high recovery (up to 78%) and reduction of host cell proteins (up to 700-fold), and high transduction activity (up to 65%) of the purified vectors.

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