# Rationally Designed Smart Polymers for Affinity Purification of AAV and Lentiviral Vectors

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## Introduction

Manufacturing bottlenecks continue to limit patient access to cell and gene therapies, with low recovery and high costs of viral vector bioprocessing hindering commercial viability. Improved purification is critical for maximizing viral vector yields and streamlining manufacturing. Yet, a key constraint is the lack of high-performance, economical affinity ligands tailored to viral vectors. Here, we introduce a platform of synthetic Smart Polymers, rationally designed as pseudo-affinity ligands for the selective capture, release, and purification of AAV and lentiviral vectors. These reagents are engineered to enable serotype-specific or pan-serotype binding, with tunable affinity for both purification and analytical use.

## Smart Polymer Design and Synthesis

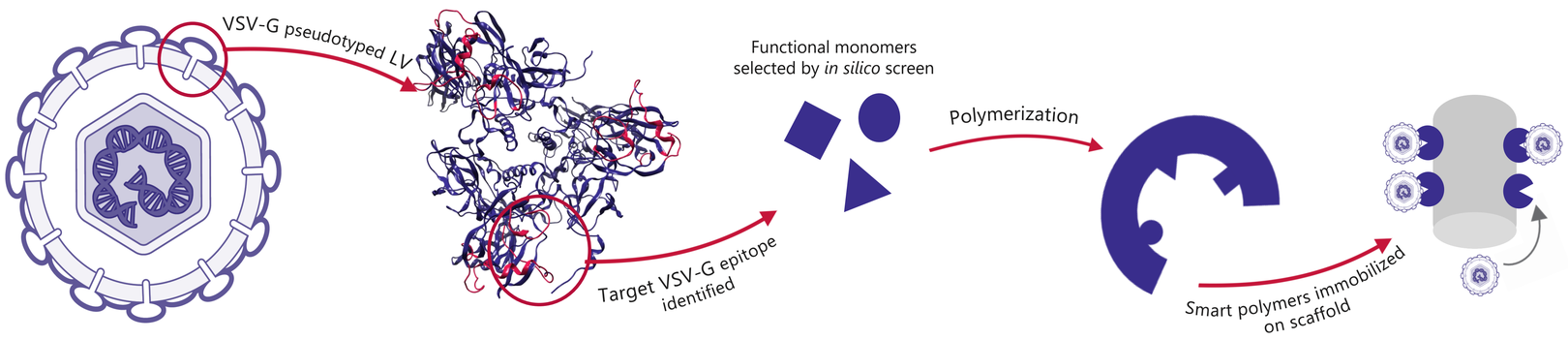


**Figure 1** Smart polymer design process. After *in silico* modeling and screening, polymers are synthesized and immobilized on a variety of scaffolds for testing.

*In silico* molecular modeling of VSV-G, and a range of AAV serotypes, was used to select target epitopes. Each epitope was then screened against a proprietary *in silico* monomer library to identify the key monomers for epitope binding. After validation of these monomers via an *in vitro* HPLC screen, the optimal polymer composition was determined to achieve the desired binding kinetics against the selected viral epitopes. Cross-linkers were added and synthetic affinity ligands targeting VSV-G and multiple AAV serotypes polymerized (Fig 1).

## Synthetic Affinity Ligands Enable Lentiviral Capture

Post polymerization, Smart Polymers targeting VSV-G were immobilized on AR2G biosensor tips, exposed to lentivirus samples with titers between 1 x $10^7$ – 1.8 x $10^8$ TU/mL and analyzed on the Octet BLI. Biosensors without polymer immobilized were used as a control.

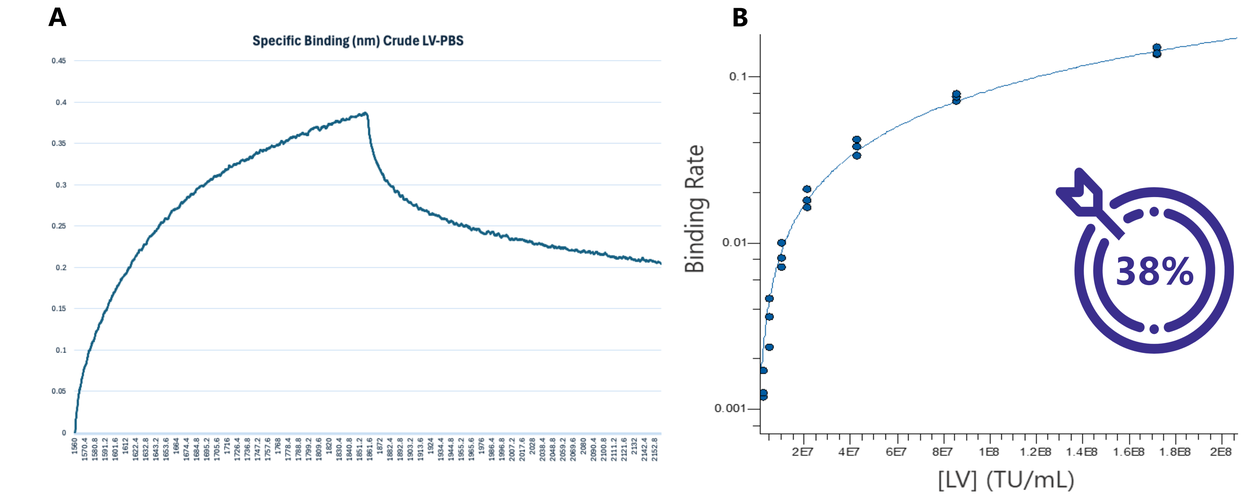


**Figure 2** (A) LV-specific binding was observed for Smart Polymer-functionalized biosensors, including in crude harvest samples. (B) Binding was shown to be titer-dependent.

Polymers demonstrated lentivirus-specific binding (Fig 2A), in a titer-dependent manner (Fig 2B), confirming their ability to recognize and bind VSV-G pseudotype lentivirus. From the 50 polymers synthesized post *in silico* screening, 19 were confirmed to bind lentivirus *in vitro,* resulting in a hit rate of 38%.

## Smart Polymers Demonstrate Pan-Serotype AAV Binding

BLI proof-of-binding experiments were replicated for AAV Smart Polymers. Based on *in silico* data the most promising polymers for pan-serotype recognition were tested against AAV1, 2, 5, 6 & 9 (testing against remaining serotypes ongoing).

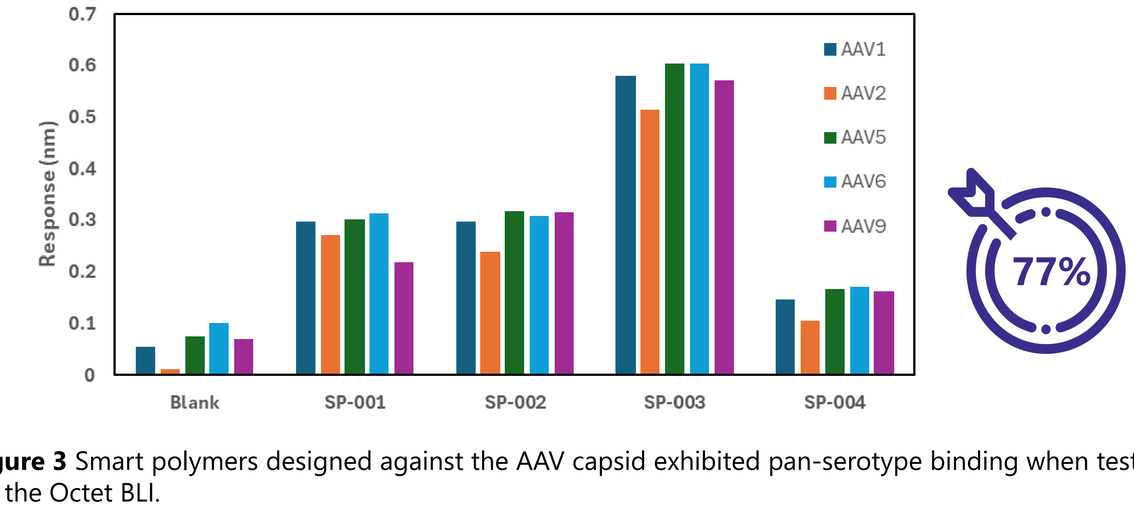


**Figure 3** Smart polymers designed against the AAV capsid exhibited pan-serotype binding when tested on the Octet BLI.

Pan-serotype binding was observed for several Smart Polymers with SP-003 exhibiting the highest level of specific binding (Fig 3). AAV-targeting Smart Polymers have exciting potential as a cheaper, more versatile alternative to pan-serotype antibodies for AAV analytics and purification. For AAV 17 of the 27 polymers synthesized were shown to bind all AAV serotypes tested, equaling a hit rate of 77%.

## High Recovery, High Purity Lentivirus Affinity Purification

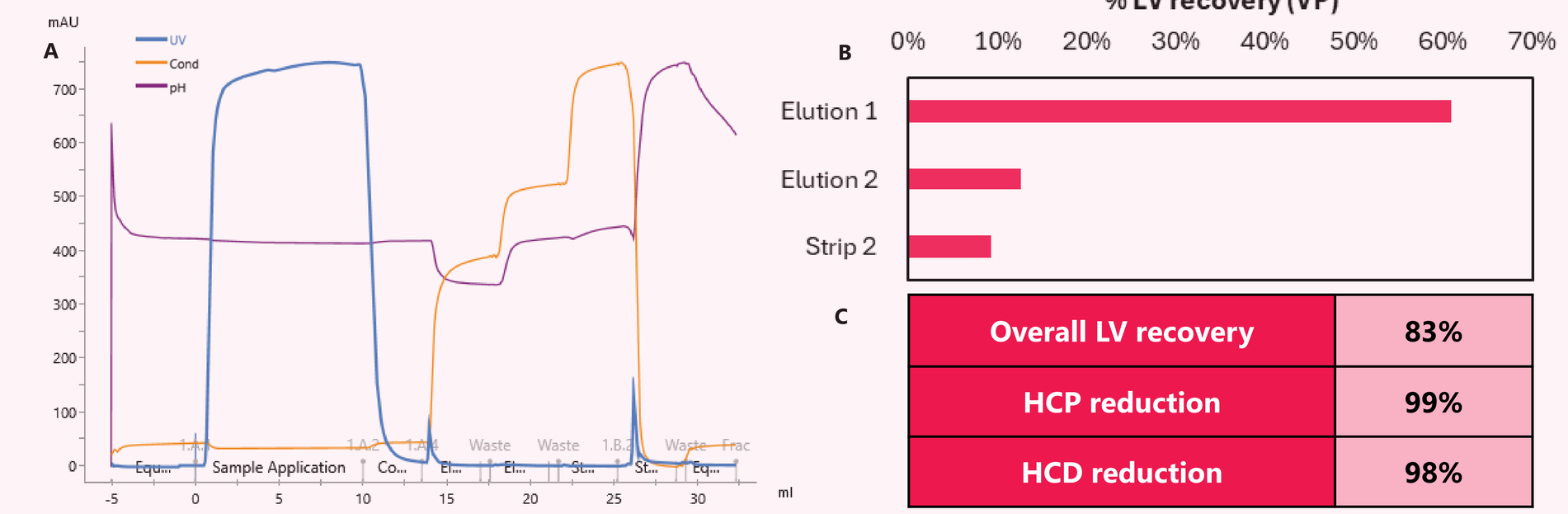


**Figure 4** (A) Chromatogram showing elution of LV from Smart Polymer functionalized membranes on the AKTA FPLC. (B) Recovery of bound LV particles in various fractions as quantified by p24 ELISA. (C) HCP and HCDNA reduction in the LV containing fractions was measured via UV absorbance.

To evaluate their suitability for viral purification, VSV-G targeting Smart Polymers were immobilized on a 13 mm membrane and used to purify lentiviral samples via the AKTA FPLC.

Lentiviral recovery was quantified using p24 ELISA. **>60%** of bound lentivirus was recovered during elution 1 (Fig 4A & B) using 500 mM NaCl at pH 6. A further 21% was eluted in subsequent steps resulting in an **overall recovery of bound lentiviral particles of 83%.** Equivalent work is ongoing to evaluate the potential of Smart Polymers for AAV affinity purification.

Transforming lentiviral purification: Smart Polymers boost **lentiviral recovery to >80%** while maintaining **high impurity removal**