Rewriting Bioprocessing with Synthetic Affinity Ligands

A. Guerreiro, D. Broomhead, F. Canfarotta, J. Czulak, E. Daniels, A. Thomson, A. Shephard, A. Sufan
Tozaro, Bedford, United Kingdom

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 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

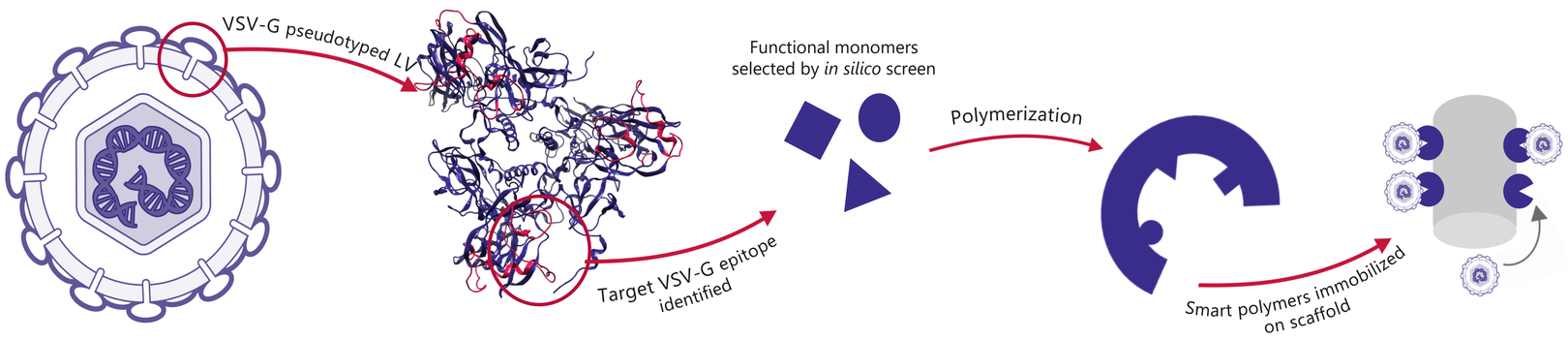


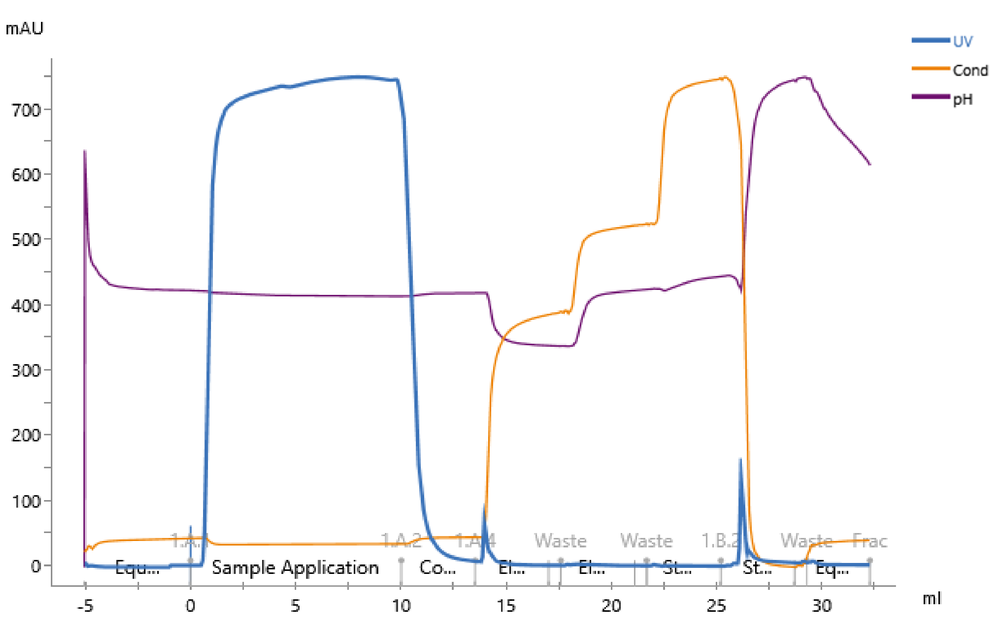
Figure 1 Smart Polymer™ design process. After *in silico* modeling and screening, polymers are synthesized and immobilized on a variety of scaffolds for testing.

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).

High Recovery, High Purity Lentivirus Purification

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 on an AKTA FPLC.

A



B

Total LV Recovery	**50%**
Functional LV Recovery	**43%**
HCP Reduction	**99.8%**
DNA Reduction	**99.9%**

Figure 2 (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 and functional assays. HCP and DNA reduction in the LV containing fractions was measured via UV absorbance.

Lentiviral vector recovery was quantified via p24 ELISA and functional analysis. **50%** of bound lentiviral particles were recovered during elution, with functional recovery of **43%** (Fig 2A & B). Elution buffers consisted of 500 mM NaCl pH 6.

Smart Polymers™ Demonstrate Pan-AAV Binding

Smart Polymers™, designed to target conserved regions across wildtype serotypes, were immobilized on agarose beads for evaluation against AAV1, 2, 5 & 9. AAV samples consisted of crude harvest material which had not been benzonase or nuclease treated.

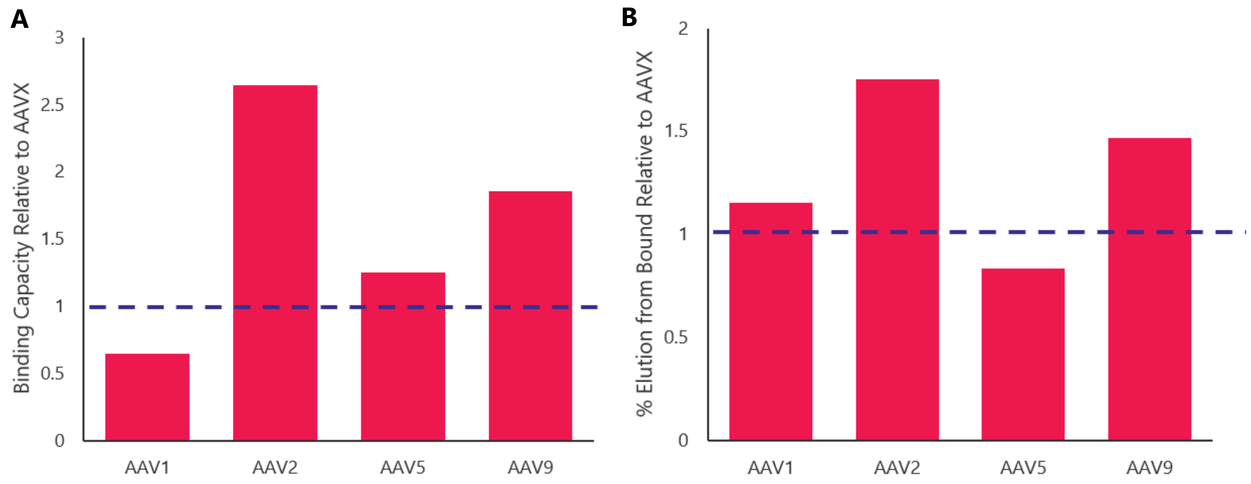


Figure 3 Lead Smart Polymer™ candidate, designed against multiple AAV serotypes, exhibited pan-serotype binding when immobilized on agarose beads. Binding capacity (A) and elution performance (B) were benchmarked against AAVX (dashed line) immobilized on the same bead type, run in parallel.

Pan-serotype binding was observed for several Smart Polymers™ with SP-003 exhibiting the highest level of specific binding. Binding capacity and elution performance were benchmarked to beads functionalized with AAVX (Fig 3). SP-003 matched, or bettered, the performance of AAVX for 3 of the 4 serotypes tested, including AAV9. Total recoveries of up to 92% of bound AAV were achieved, using neutral pH elution conditions and 1M NaCl (Fig 4).

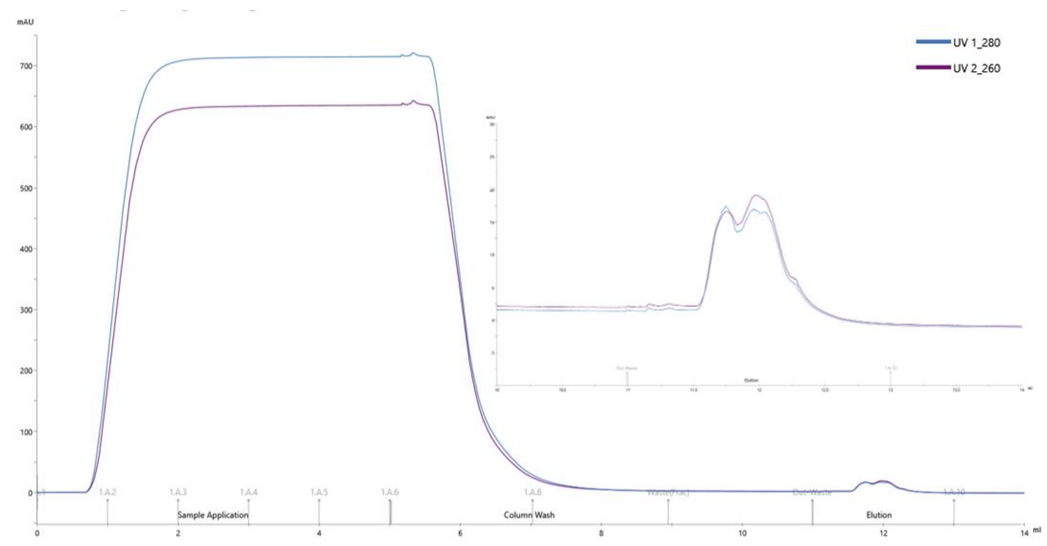


Figure 4 Chromatogram showing elution of AAV2 from Smart Polymer™ functionalized agarose beads on the AKTA FPLC. AAV samples were crude cell harvest no pre-treatment.

Transforming viral purification: Smart Polymers™ boost
LVV & AAV recovery while delivering **high impurity removal**