Novel Ligand Outperforms State-of-the-art Ligand in Lentivirus Purification by Anion Exchange Chromatography

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Introduction

Lentiviral vectors (LVV) have emerged as versatile tools for gene delivery in various biomedical applications, including cell and gene therapy. LVVs are involved in the manufacturing of increasing numbers of products such as Chimeric Antigen Receptor (CAR) T-cells. However, increasing market needs cannot be fulfilled by current virus production pipelines, and downstream processing has been identified as a critical bottleneck in LVV manufacturing[1].

Successful application of LVVs relies heavily on the purity and yield of lentiviral preparations. Among the various purification techniques, anion exchange chromatography (AEX) stands out as a robust method capable of meeting growing demand for LVV in the pharma-industry.

The pK_a and hydrophobic properties of a ligand are crucial for optimal binding and elution of LVVs. Diethylaminoethyl (DEAE) has historically been the industry-leading ligand of choice for LVV AEX. Unfortunately, DEAE requires high salt concentrations (up to 650 mM) for optimal LVV elution. The need to use harsh salt conditions often contributes to low LVV recovery due to high sensitivity to pH and salt concentration.

We developed a novel ligand for LVV AEX compatible with the sensitive nature of the viral vectors, that overcomes current yield limiting challenges in LVV downstream processing.

Novel Ligand Screening

- We searched for new ligands focusing on enhanced pK_a values and hydrophobic properties.
- pK_a titration screening of novel ligands was used to select candidates with lower pK_a than DEAE's 11.5[2].
- ANJ-ligands with enhanced pK_a levels and hydrophobic properties are available in-house.
- These pK_a values allow for on/off mode of positive charge on the support matrix by pH change.
- The ANJ-Ligand and resin matrix are coupled by a covalent bound.
- Resins carrying ligands were screened for binding and elution on enveloped biological particles and the best performing "ANJ-Resin" was selected.

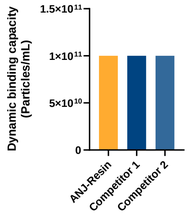


Figure 1: ANJ-Resin capacity binding LLV is equal to competitors. Particles/mL: Viral particle measured by p24 ELISA per mL of resin.

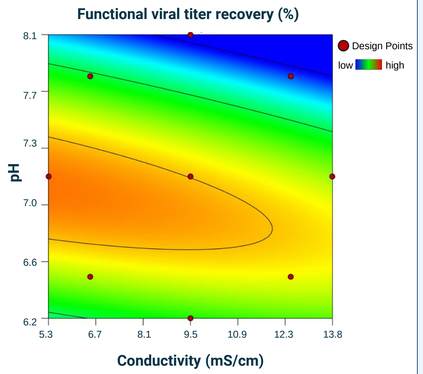


Figure 2: Design of Experiments with ANJ-Resin reveals pH 6.8-7.2 and conductivity >10 mS/cm as optimal for LVV binding. Conductivity is a proxy for salt content and negative charged ions. LVV in conditioned chemically defined medium used.

[1] Pezzulo et al. 2023 The Woes and Wins of CAR-T Manufacturing: Lessons from Abecma and Carvykti. *Health Advances Analysis*
[2] Aljaf et al. 2020 Diethylaminoethyl cellulose (DEAE-C): applications in chromatography and organic synthesis. Arkivoc part I, 153-179

ANJ-Resin Outperforms DEAE-Resin in LVV Capture

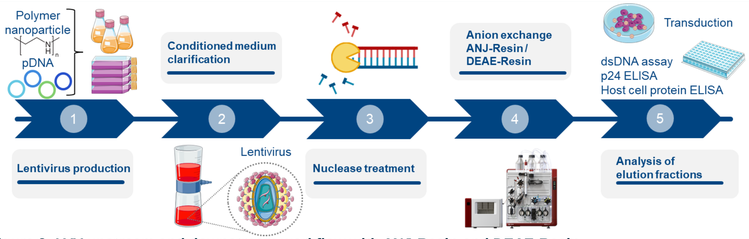
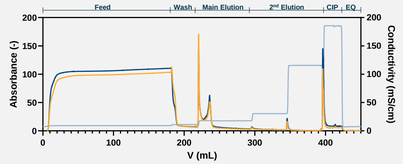


Figure 3: LVV upstream and downstream workflow with ANJ-Resin and DEAE-Resin.



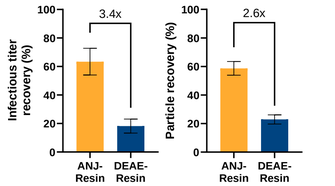
ANJ-Resin Chromatography

Category	NaCl [mM]	pH
Feed	≤75	7
Wash	75	7
Main Elution	150	8
2nd Elution	275-1300	8

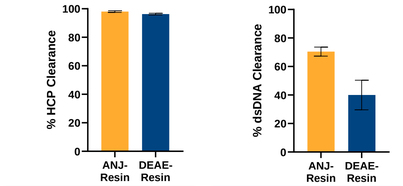
CIP: Cleaning in place 1 M NaOH, EQ: Equilibration

Figure 4: LVV capturing chromatogram by ANJ-Resin. Majority of LVV eluting in the main elution fraction at 150 mM NaCl pH8. The double peak results from a delay in pH adjustment compared to the conductivity change, although this shift can be overcome by increasing buffer strength.

LVV Recovery



Contaminants Clearance



Host cell protein [µg]	9.6 ± 2.4	18.2 ± 1.7		dsDNA [µg]	1.8 ± 0.6	4.1 ± 0.3
Clearance [%]	98.0 ± 0.6	96.3 ± 0.6		Clearance [%]	70.5 ± 3.1	40.0 ± 10.4

Figure 5: ANJ-Resin outperforms DEAE-Resin in infectious virus and physical virus recovery. ANJ-Resin recovers 3.4x more infectious virus than DEAE and 2.6x higher physical particles, whereby 150 mM NaCl for ANJ-Resin and 650 mM NaCl for DEAE was considered. Infectious titer was determined by evaluating the transducing units by HEK293T transduction. Particle recovery was evaluated by p24 ELISA.

Figure 6: ANJ-Resin clears more HCP and DNA from clarified media than DEAE-Resin. ANJ-Resin removes 1.9x more HCPs and 2.3x more dsDNA than DEAE-Resin.

ANJ-Resin Superior to DEAE-Resin for LVV Polishing Applications

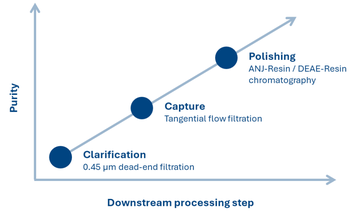


Figure 8: LVV downstream workflow of ANJ-Resin and DEAE-Resin comparison. Tangential flow filtration (TFF) was done by a 300 kDa hollow fiber.

LVV Recovery

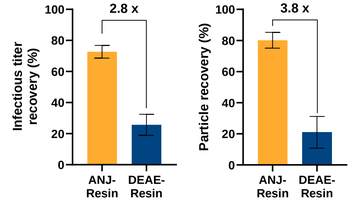
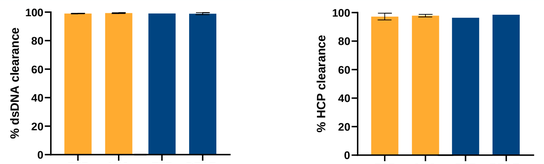


Figure 9: ANJ-Resin surpasses DEAE-Resin in infectious virus and physical virus recovery. ANJ-Resin recovers 2.8x more infectious virus than DEAE and 3.8x higher physical particles. Infectious titer was determined by evaluating the transducing units by HEK293T transduction. Particle recovery was evaluated by p24 ELISA.

Contaminants Clearance



dsDNA Clearance [%]	99.07 ± 0.15	99.34 ± 0.27	99.00	98.90 ± 0.71	HCP Clearance [%]	97.23 ± 2.39	99.81 ± 0.04	96.48	98.56

Figure 10: ANJ-Resin clears more HCP and DNA from clarified media than DEAE-Resin. ANJ-Resin removes 1.9x more HCPs and 2.3x more dsDNA than DEAE-Resin.

Conclusions

- On/Off mode of novel ANJ-Resin enables functional LVV release at nearly physiological pH (7 to 8) and salt concentration (150 mM)
- ANJ-Resin can be used for capturing or polishing in downstream processing
- **ANJ-Resin outperforms commercially available best-in-class DEAE-Resin in both capturing and polishing**

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