Evaluating Affinity Chromatography Media for Capture of Novel Blood-Brain-Barrier Penetrant AAV Capsids

Jacob Guzman, Tom Elich, Roberto Facendola, Kavitha Bodige, Shamik Sharma

Voyager Therapeutics Inc., Lexington, MA, USA

3161

voyager therapeutics

EXECUTIVE SUMMARY

Objective: Evaluate multiple affinity stationary phases for capture of a novel BBB penetrant AAV capsid.

Key Findings:

- Optimal base bead and ligand selection can unlock **up to 10-fold improvement** in affinity resin binding capacity.
- Relationship between binding capacity, residence time, and feed concentration reveal mass transfer dependencies.

Impact: Resin A1 enables shorter residence times, smaller column sizing, and opportunity to eliminate pre-affinity TFF.

MATERIALS & METHODS

Testing was executed on an AKTA Avant using a standard method (Table 1). Four resins with different base bead and ligand properties were evaluated (Table 2). Two separate feed streams were used as load materials for testing:

- **Feed for DBC testing**: Material was pre-purified by affinity and AEX chromatography, concentrated, diafiltered, and adjusted to a known concentration prior to affinity resin experiments. Pre-purified feed material was used to enable online monitoring of capsid breakthrough trends by AKTA UV absorbance.
- **Feed for elution yield testing:** Crude lysate was clarified, concentrated and diafiltered by TFF, and sterile filtered prior to affinity resin experiments.

Table 1. **Affinity Chromatography Method**

Step	Mobile Phase Conditions
Equilibration	Neutral pH with conductivity
AAV Loading	DBC Testing: Pre-purified AAV material
	Yield Assessment: Clarified & TFF concentrated AAV lysate
Wash	Neutral pH with conductivity
Elution	Low pH elution
Strip	Acidic buffer

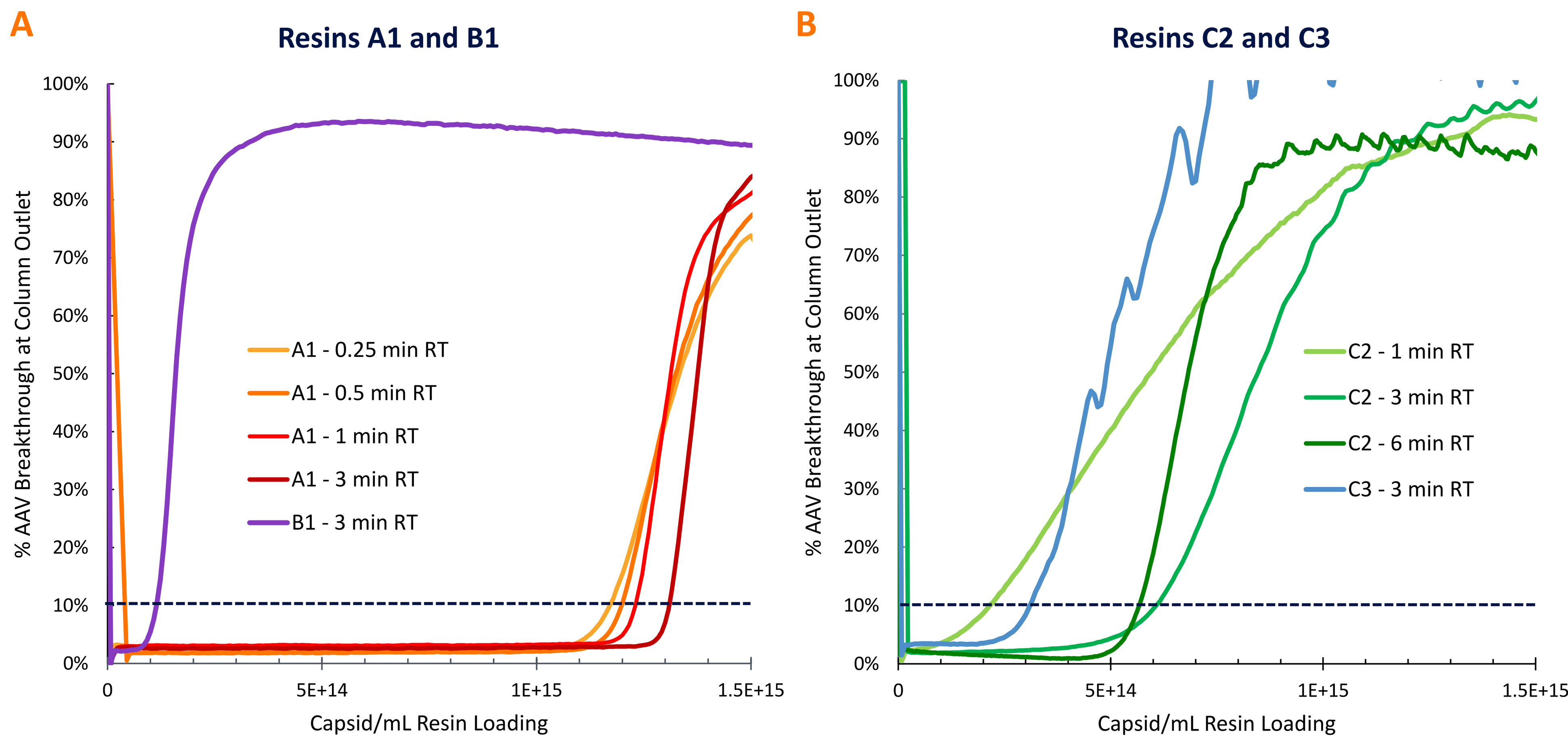
Table 2. **Base Bead and Ligand Conditions for the Experimental Affinity Resins**

Base Bead	Ligand	
A	1	Same ligand, different base bead
B	1	
C	2	Same base bead, different ligand
C	3	

RESULTS & DISCUSSION

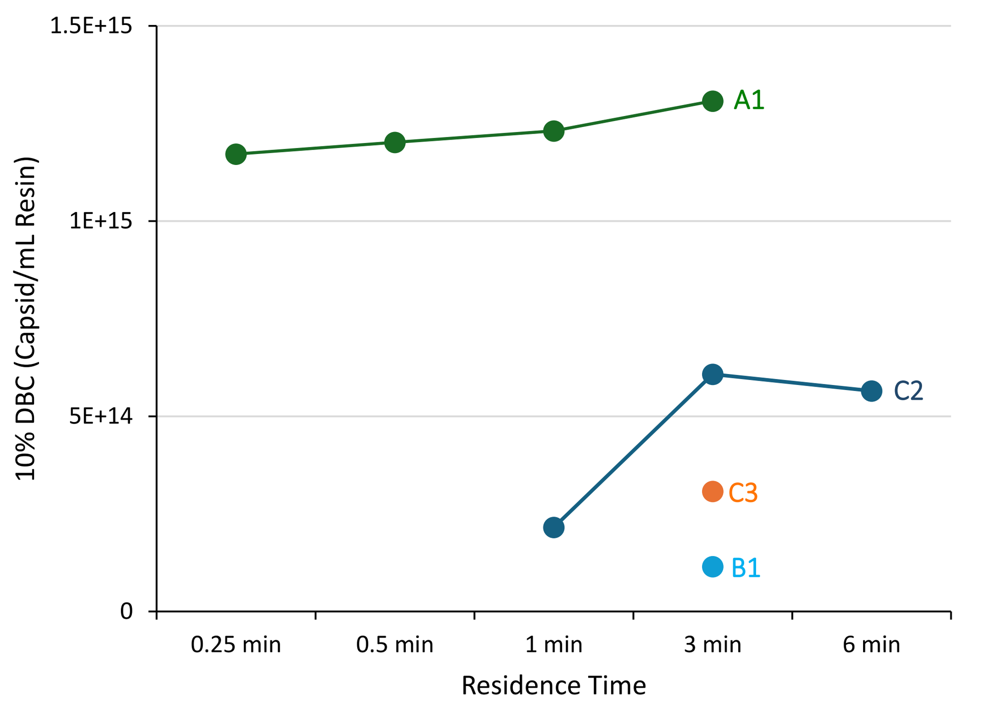
Breakthrough curves vary with residence time and resin selection

Figure 1. **Impact of Load Residence Time on Breakthrough Curve Behavior for the Experimental Resins**



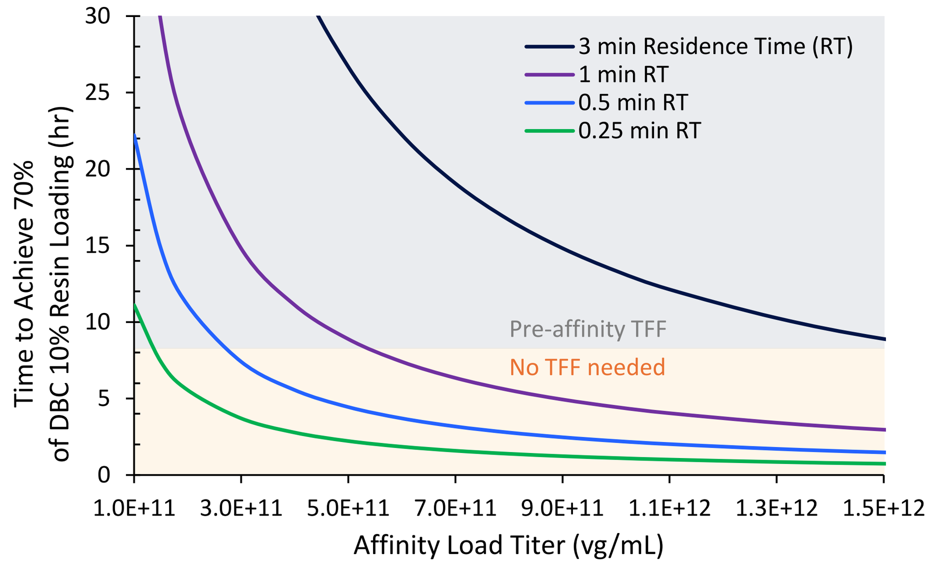
Optimal resin selection improves capacity up to 10-fold; eliminates TFF

Figure 2. **DBC at 10% Breakthrough for Different Affinity Resins and Residence Times**



- **Convection dominates for Resin A1:** High binding capacity with minimal residence time dependence.
- **Diffusion dominates for Resin C2:** Moderate binding capacity with strong dependence on residence time.
- **Resins C3 and B1:** Low binding capacity due to non-optimal base-bead (B1) or ligand (C3) for the tested AAV capsid.

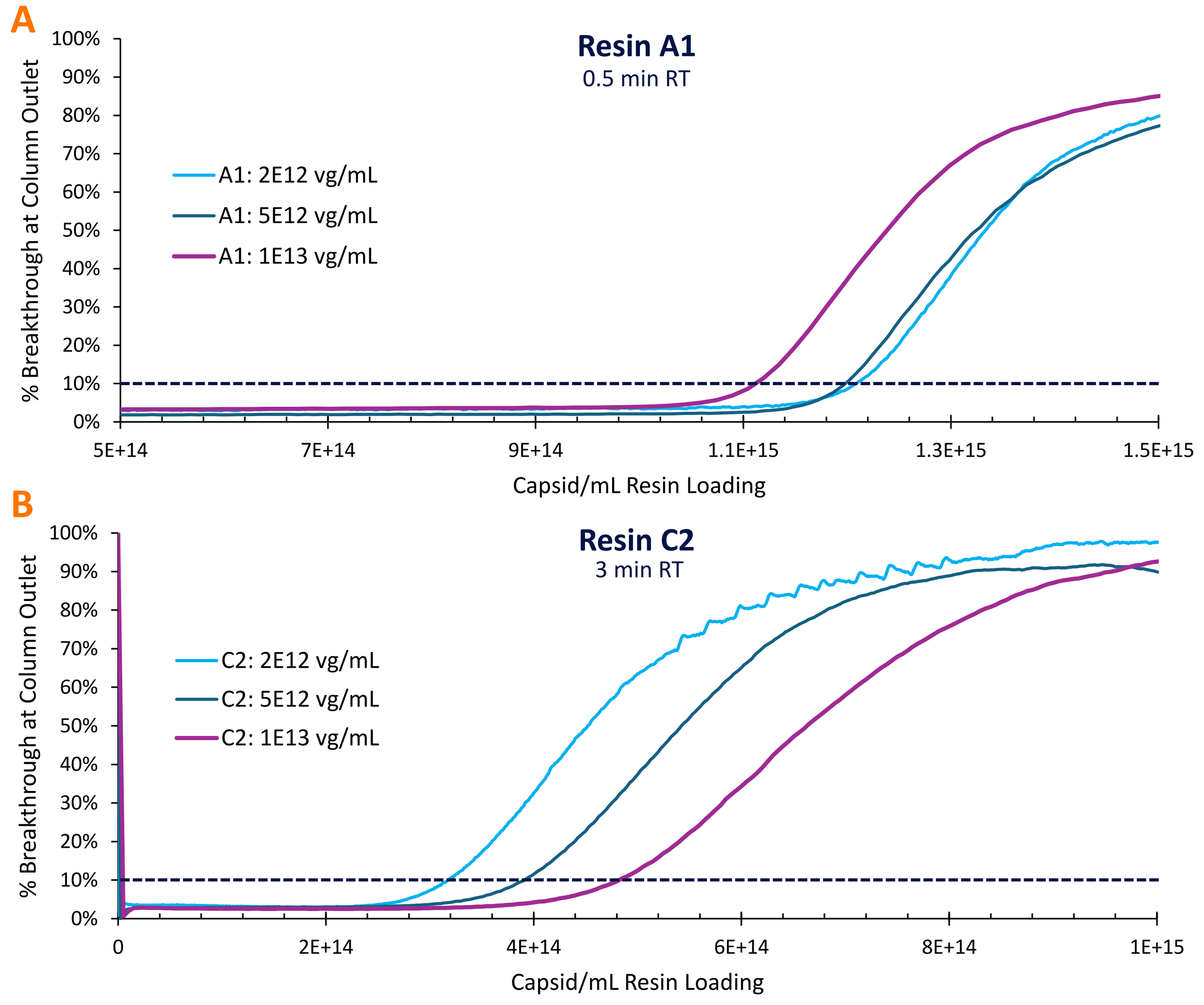
Figure 3. **Short Residence Times Enable Resin A1 to Eliminate Pre-affinity TFF Concentration**



- Pre-affinity TFF concentration may be implemented to reduce column load time, especially in low titer and long residence time scenarios.
- The 0.5-min RT enabled by Resin A1 offers an opportunity to complete affinity load in a standard operating shift without pre-affinity TFF, provided the clarified lysate maintains a titer ≥ 3E11 vg/mL.

Feed concentration effects reveal mass transfer limitations

Figure 4. **Impact of Feed Concentration on Breakthrough Curve Behavior for Resins A1 and C2**



Resin A1: Data at 2e12 and 5e12 vg/mL titer show comparable breakthrough profiles, suggesting minimal transport limitations for feed titers ≤ 5e12 vg/mL.

Earlier breakthrough at 1e13 vg/mL titer indicates mass transfer non-idealities. This may be attributed to **intra-particle transport rate limitations** under high solute flux and short residence time. Data suggests a critical flux threshold was exceeded at 1e13 vg/mL and 0.5 min residence time conditions.

Resin C2: Higher feed concentrations directly correlate to later breakthrough (e.g. higher capacity). This is likely attributed to higher **diffusive driving force**, which increases capacity.

Along with Figure 2 data, these results suggest Resin C2 is controlled by diffusive-mass transfer.

Comparison of relative elution yield using clarified harvest as feed

Figure 5. **Relative Elution Yield Measured After Loading Concentrated Clarified Harvest Feed**

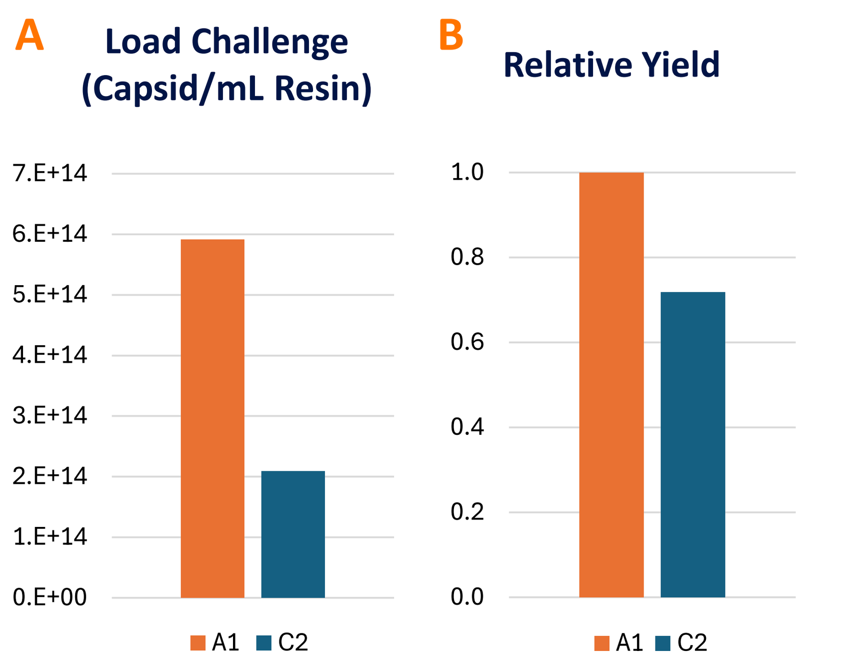
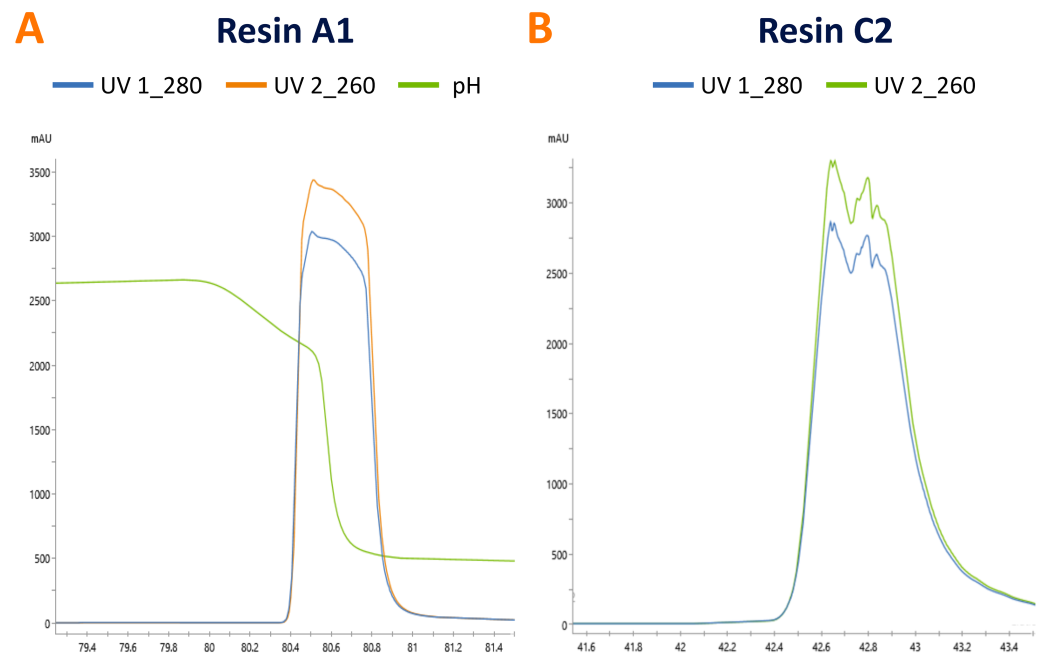


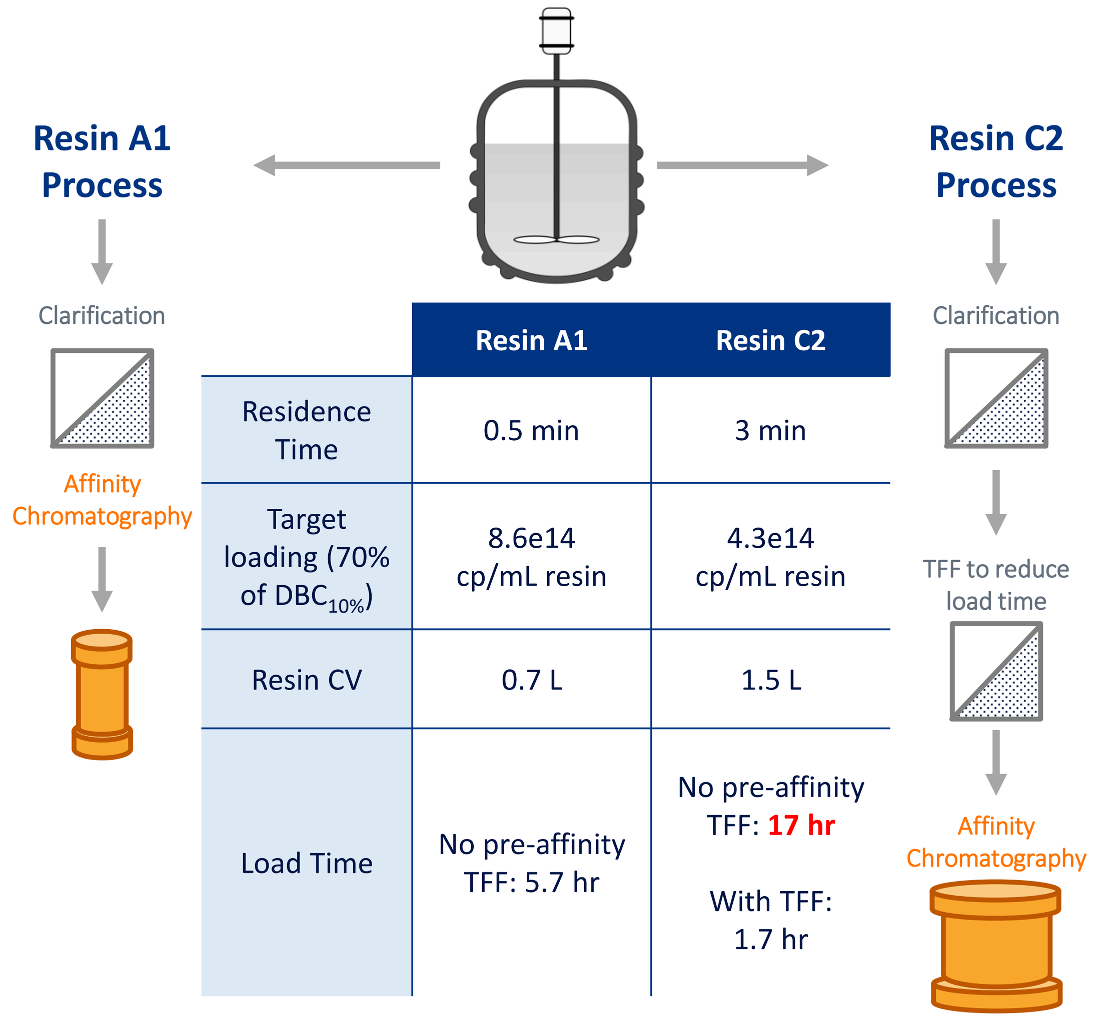
Figure 6. **Elution of Concentrated Clarified Lysate Feed**



Performance using concentrated clarified lysate feed: The best performing resins (A1 and C2) were then evaluated using clarified lysate feed which was pre-concentrated by TFF (Figure 5 and Figure 6). Resin A1 provided higher relative elution yield, in addition to increased load capacity.

Implications on process scale-up

Figure 7. **Case-study: Process Design for Scale-up to 500 L Bioreactor at 5e11 vg/mL Titer**



	Resin A1	Resin C2
Residence Time	0.5 min	3 min
Target loading (70% of $DBC_{10\%}$)	8.6e14 cp/mL resin	4.3e14 cp/mL resin
Resin CV	0.7 L	1.5 L
Load Time	No pre-affinity TFF: 5.7 hr	No pre-affinity TFF: 17 hr With TFF: 1.7 hr

Resin A1 offers Cost of Goods advantages:

- Increased binding capacity and yield
- 50% reduction in column size
- Shorter residence times avoid use of pre-affinity TFF

CONCLUSIONS

Optimal base bead & ligand selection is critical to maximize resin binding capacity:

- Capacity of ligand 1 improved 10-fold when using base bead A.

Residence time dependencies provide insight to mass transfer properties:

- Resin A1 DBC is *independent* of residence time: convective mass transfer.
- Resin C2 DBC is *dependent* on residence time: diffusive mass transfer.

Feed concentration dependencies indicate mass transfer limitations:

- Resin A1 capacity *reduces* at high feed concentration due to intra-particle flux limitations.
- Resin C2 capacity *increases* with feed concentration due to increased diffusive force.

Resin A1 offers compelling Cost of Goods advantages.

ACKNOWLEDGEMENTS

- **Downstream process development:** Preparation of chromatography load materials, execution of affinity testing.
- **Upstream process development:** Generation of AAV capsid.
- **Pilot Production:** Generation of AAV capsid.
- **Process Analytics:** Execution of ddPCR titer and full capsid assays.