# Optimization of Scalable rAAV Production for Gene Therapy – Leveraging At-Line Amino Acid Measurements for Bioprocess Modeling-Driven Approaches

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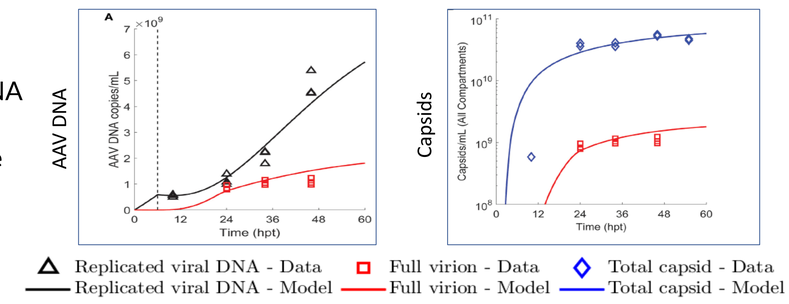
## Overview

- **Background:** the triple-plasmid transfection method used to produce rAAV in HEK 293 cells is fairly well-established, challenges remain in achieving high viral vector (VV) titer and high ratio of filled vs. empty capsids.
- **Project Goals:**
  - Build mechanistic understanding and knowledge of amino acid (AA) consumption to effectively guide the development of advanced manufacturing processes.
  - Design, test, and predict capsid assembly to drive high production of filled capsid – scaling up and transferring to a continuous process at bioreactor scale.
- **Summary of results:** Spent media analysis from an at-line analyzer, the REBEL, was used to further understanding of AAV production process and identify correlation between key nutrient consumptions and capsid titers in fed-batch and continuous processes.

## Continuous Manufacturing of rAAV using a Benchtop Bioreactor

### Previous Results and Background

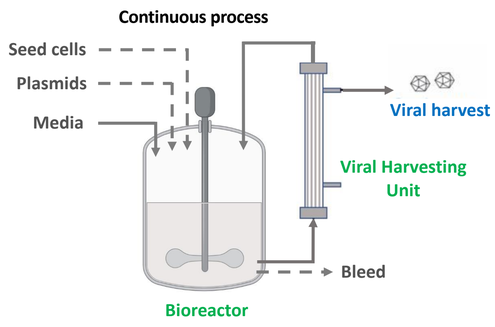
- A mechanistic model for viral production was developed describing capsid and plasmid DNA production (1).
- Majority of **empty capsids can be explained by discoordination** of capsid formation and DNA synthesis.



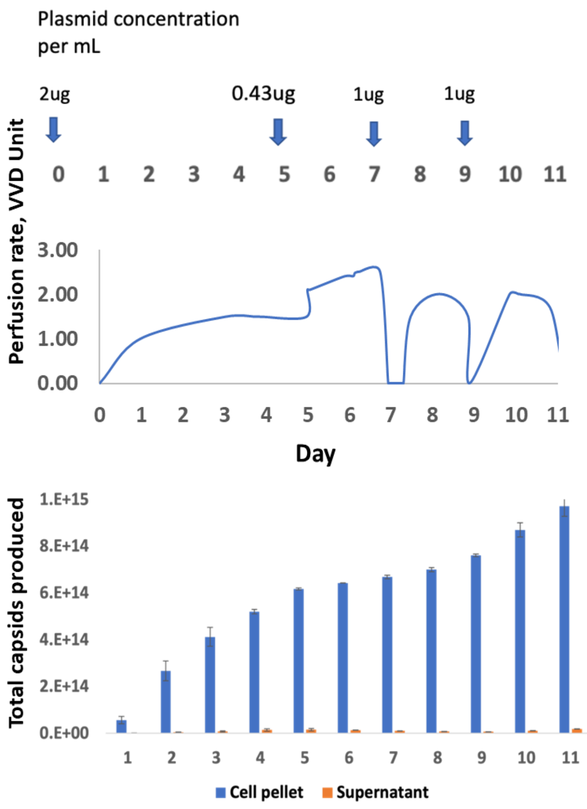

- Asn constitutes 10% of VP3 capsid protein which is a major component of an AAV particle and is therefore indispensable for AAV production.
- Earlier published studies (2) show that Asn is known to affect *Vaccinia* virus production.
- In previous spent media analysis of AAV production performed in shake flasks, using the **REBEL,** we observed **a decrease in Asn concentration as Gln is consumed** by the cells and **an increase as Gln is fed** into the cell culture. These experiments also showed essential AA severely depleted throughout the culture (3).
- The REBEL is an automated, at-line microfluidic capillary electrophoresis mass spectrometry (CE-MS) analyzer that provide amino acid, vitamin and other bio-amine data in 10 minutes using a 10 µL sample volume.

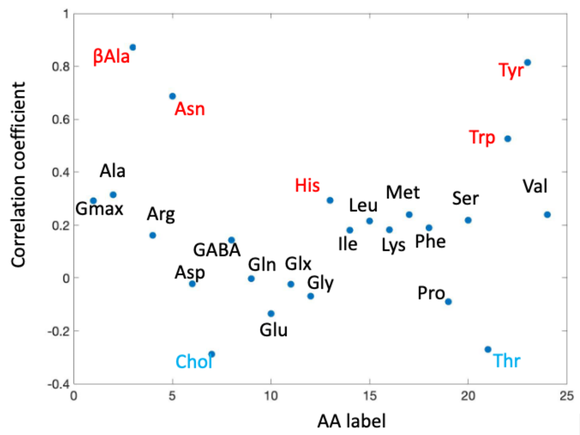
### Experimental Design

- **Goal:** Assess the feasibility of continuous culture for the production of rAAV from the triple-plasmid transfection in HEK293 cells. Evaluate the correlation of AA in spent media and capsid titer during AAV production.
- **Materials & Methods:**
  - AAV5 capsids produced by triple-plasmid transfection of HEK 293 cells in FreeStyle 293 Expression Medium (Thermo Fisher Scientific).
  - 300 mL Applikon bioreactor with Artemis Biosystems VHU filter for perfusion.
  - Cells were transfected starting on day 0 with plasmid addition strategy and perfusion rate based on model prediction. Daily samples were analyzed on the REBEL.




### Results



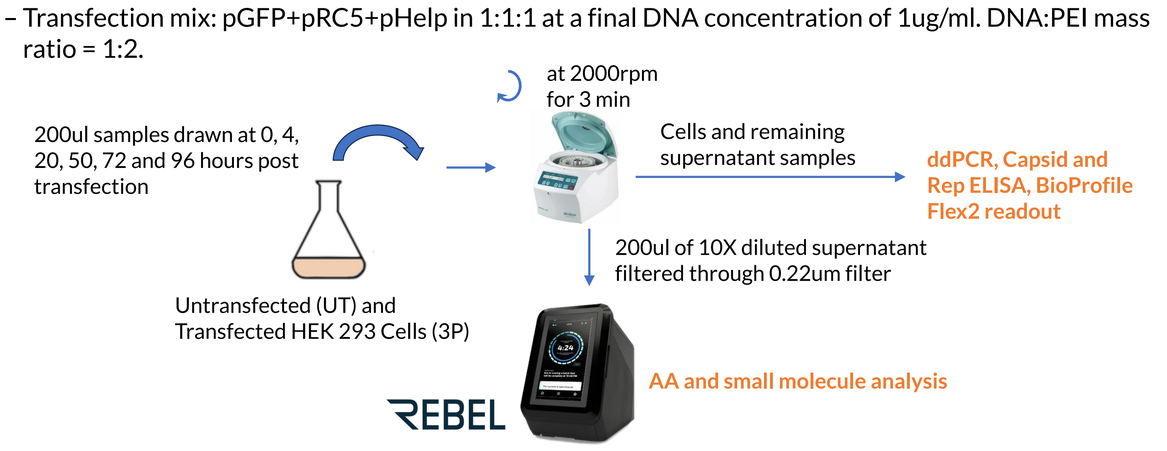

*Correlation coefficient plot was obtained by performing (Pearson) cross-correlation between concentration and capsid titer values measured at different time points for each amino acid.*

**REBEL measurements indicate anti-correlation signatures of Gln-Asn levels with dynamic capsid production during continuous AAV manufacturing**
- Ala, Asn, His, Tyr, and Trp positively correlate with capsid production
- Gln and Glu correlates negatively with the capsid production
- Positive correlation of Asn is compensated by negative correlation of Gln with capsid production

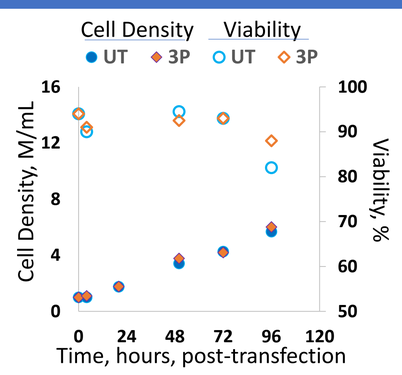
## Development of AA supplementation strategy for AAV production

### Experimental design

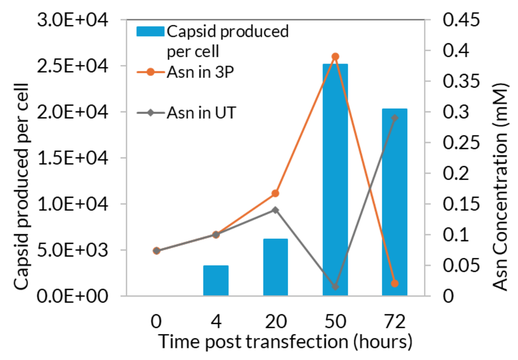
- **Goal:** Assess depletion of AAs during production of rAAV from triple-plasmid transfection of HEK 293 cells. Identify AAs that influence the capsid titer and are good candidates for supplementation.
- **Materials & Methods:**
  - AAV5 capsids produced by triple-plasmid transfection of HEK 293 cells in shake flasks in FreeStyle 293 Expression Medium (Thermo Fisher Scientific) or EX-CELL 293 Medium (Millipore Sigma).
  - Transfection mix: pGFP+pRC5+pHelp in 1:1:1 at a final DNA concentration of 1ug/ml. DNA:PEI mass ratio = 1:2.



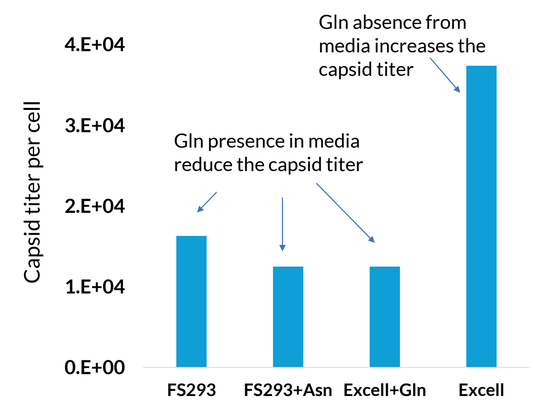

### Results




**No appreciable differences observed in cell growth under different transfection conditions in FreeStyle 293 medium (UT vs 3P)**

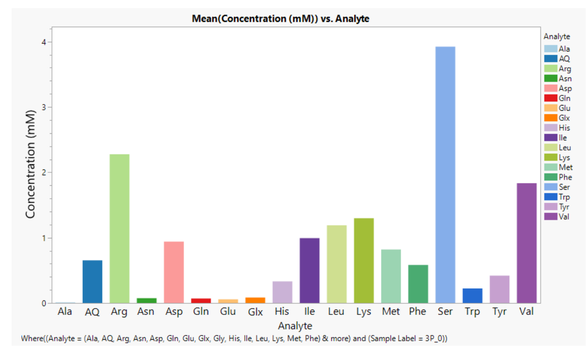



**Capsids produced per cell correlates with the endogenous Asn production (FreeStyle 293 medium)**

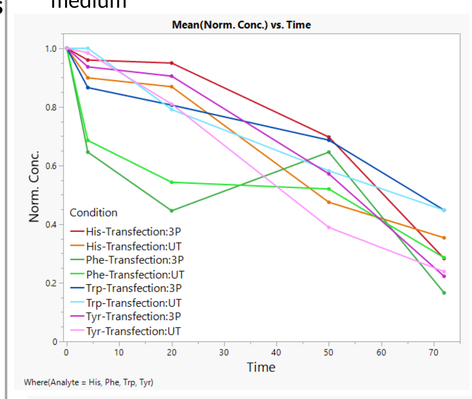



**Capsid titer per cell increase when Gln is deprived from the culture media**

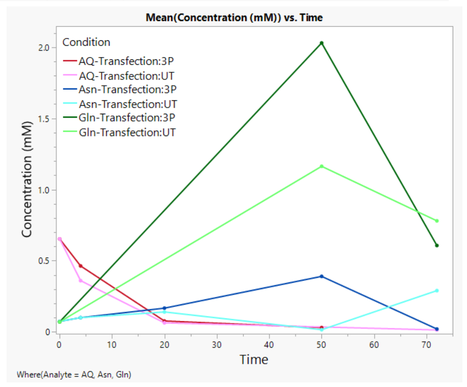
***REBEL time course analysis showing selected amino acids in fresh media, UT and 3P samples. Graphs generated with JMP add-on tool.***




Amino acid and AQ profile in fresh FreeStyle 293 Expression medium




In FreeStyle 293 medium, His, Phe, Trp and Tyr showed progressive depletion during time course post transfection similarly between 3P and UT samples




**Asn profile in time course post-transfection (PT):**
- **20 h. pt:** slight rise in 3P and UT, correlating with increase in Gln and decrease in AQ (Ala-Gln dimer)
- **50 h. pt**: significant rise in 3P vs UT is most likely enhanced by Gln release from AQ
- **72 h. pt: sharp depletion in 3P vs UT can be attributed to the utilization of Asn for capsid production which is known to maximize after 48hpt**

## Conclusions and Future work

- Data from a perfusion benchtop bioreactor scale show that Ala, Asn, His, Tyr, and Trp positively correlate with capsid production, while Gln correlates negatively
- Analysis of cell culture media from shake flask experiments shows that Asn and other amino acids, including several essential amino acids, deplete during AAV production
- Capsid titer per cell increases when Gln is absent from media
- The REBEL device enabled data-driven bioprocess development for rAAV manufacturing in HEK293 cells by accurately and precisely quantitating amino acids, leading to deeper process understanding
- More experiments are ongoing to optimize the perfusion process to achieve high titer of full capsids

## References and disclaimer

1. Molecular Therapy: Methods & Clinical Development Vol. 21, 2021, pp. 642-655
2. Pant A, Yang Z. Asparagine: An Achilles Heel of Virus Replication? ACS Infect Dis. 2020 Sep 11;6(9):2301-2303.
3. Poster ASCGT 2022 . 908 Devices Web site. https://908devices.com/resource/rebel-ps-13/

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