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

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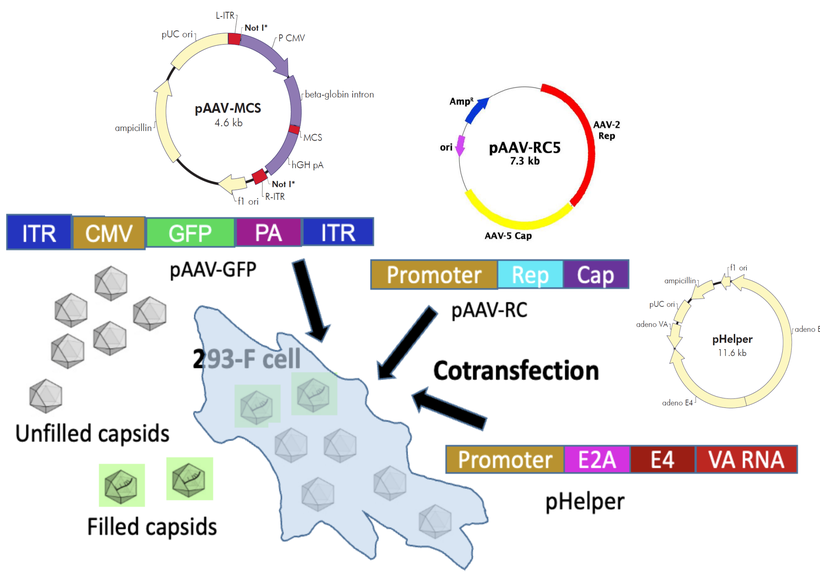
Overview

rAAV viral vector (VV) used for in vivo gene therapy are often produced using a triple transfection of HEK 293 cells. Although the triple transfection method is fairly well established, challenges remain in achieving high VV titer and high ratio of filled vs. empty capsids. The goal of the project presented here is to build mechanistic understanding and knowledge of amino acid consumption to effectively guide the development of advanced manufacturing processes. The resulting model-driven approach is used to design, test, and predict capsid assembly to drive high production of filled capsid – scaling up and transferring to a continuous process at bioreactor scale.

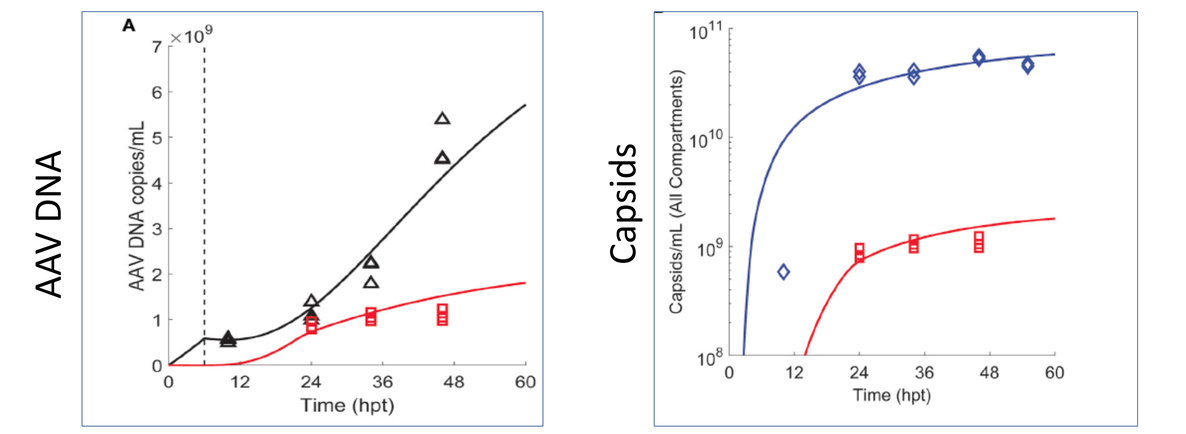
Media optimization can be a lengthy and complex process involving multiple DoEs. Analytical tools at the point-of-need are required to assess key-nutrient consumption and provide feedback to enable feed strategy optimization. Presented here are spent media analysis leveraging automated at-line microfluidic capillary electrophoresis mass spectrometry (CE-MS) analyzer, the REBEL, requiring only 10 µL per sample and 10 minutes analysis time.

Engineering plasmids and developing new transfection approaches



Shown here is a typical rAAV viral vector production using a triple transfection approach with HEK 293 cells. Plasmids coding for the gene of interest (here *GFP*), Rep and Cap AAV proteins and helper genes (to assist viral genome replication) were co-transfected using cationic polyethylenimine polymer (PEI).

Modeling the dynamics of capsid and plasmid DNA production



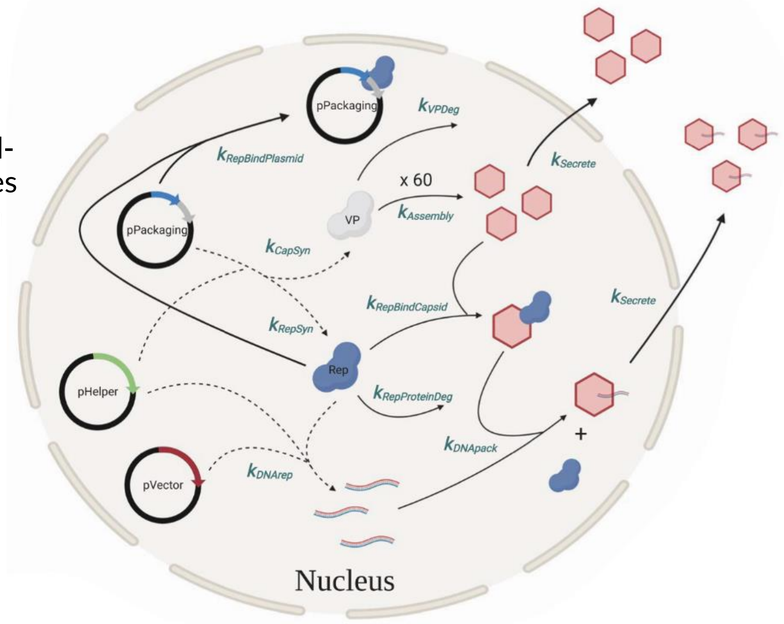
Model vs. data for the dynamics of viral production

Data (MIT experiment) for parameter estimation and fit to model developed (*Ref. 1*):
Quantity of capsids over time; Quantity of viral genome copies over time; Quantity of replicated AAV DNA and capsids over time.
Majority of empty capsids in harvest can be explained by discoordination of capsid formation and DNA synthesis.

The team at MIT developed a mechanistic model (*Ref 1.*) for viral production.
The model is adapted from wild-type AAV synthesis and includes

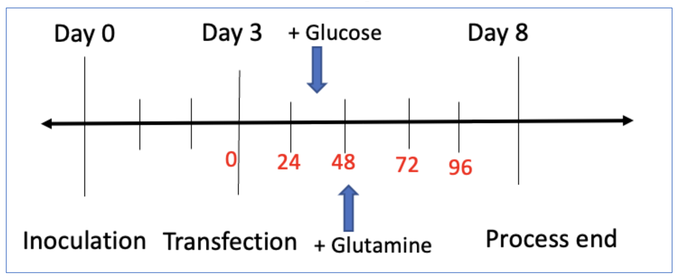
- Rep protein and viral protein (VP) synthesis
- Rep protein regulatory functions
- Capsid assembly
- Viral DNA replication
- DNA packaging into capsid
- Capsid secretion from the nucleus to the cytosol

We assume empty and filled capsid has the same secretion kinetics

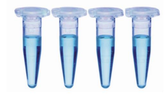


Shake-flask scale experiment on capsid production

A fed-batch timeline to optimize transfection reaction



rAAV Samples collected at different time points after triple plasmid transfection



High-throughput Amino acid screening

AA concentration (mM) at different time points

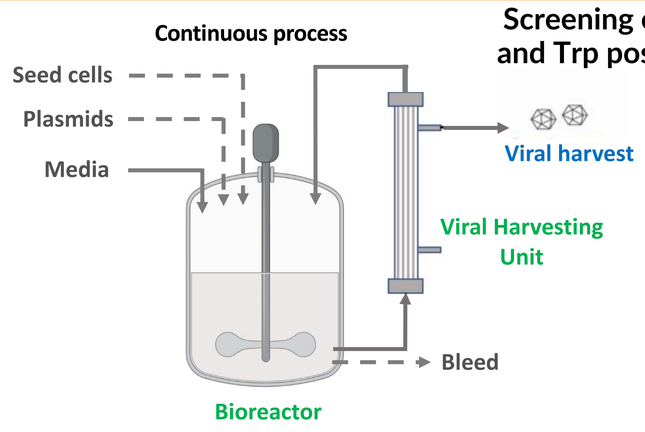
Time (hpt)	Arg	Ala	AQ	Asn	Asp	Cys	Glu	Gln	Glu+Gln	His
0	2.58	-	4.62	0.26	1.1	0.51	0.08	ND	0.08	0.33
12	1.84	3.66	ND	0.2	0.74	0.35	0.75	1.05	1.57	0.15
24	1.71	3.65	ND	0.17	0.67	0.3	0.69	0.84	1.48	0.11
36	1.5	3.26	ND	0.1	0.63	0.28	0.57	0.6	1.18	0.09
48	1.12	2.6	ND	0.08	0.45	0.27	ND	ND	12.23	0.05
72	1	2.48	ND	0.12	0.44	0.27	ND	10.56	11.14	0.04
96	0.72	1.9	ND	0.13	0.35	0.2	ND	13.67	13.71	0.03

Time (hpt)	ILE	Leu	Lys	Met	Phe	Pro	Thr	Trp	Tyr	Val	βAla	Gly
0	0.6	0.88	1.25	0.79	0.73	ND	1.08	0.22	0.37	>LOQ	0.05	ND
12	0.81	1.23	0.73	0.53	0.29	0.22	0.6	0.13	0.17	>LOQ	0.06	0.31
24	0.78	1.17	0.72	0.51	0.24	0.27	0.52	0.11	0.11	1.77	0.06	0.31
36	0.65	1.05	0.45	0.5	0.19	0.35	0.46	0.09	0.08	2.34	0.06	0.35
48	0.44	0.71	0.41	0.36	0.09	0.34	0.32	0.07	0.05	0.77	0.04	0.3
72	0.4	0.63	0.34	0.38	0.06	0.94	0.34	0.06	0.03	0.53	0.06	0.33
96	0.29	0.46	0.38	0.26	0.05	0.84	0.27	0.06	0.03	0.39	0.05	0.29

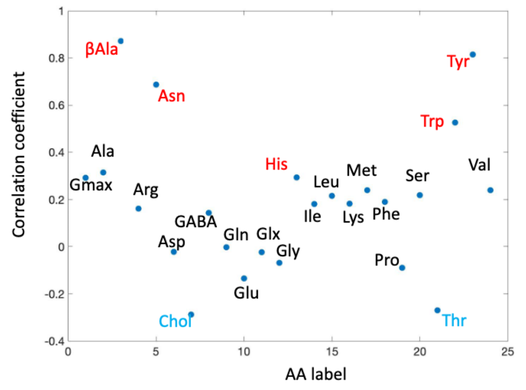
High-throughput amino acid (AA) screening showed that Asn and other amino acids, including several essential amino acids, were depleted during AAV production in a fed-batch process. Asn and Asp are known to exhibit high flux for AA consumption during AAV production (*Ref 2., 3.)*. Results show a decrease in Asn concentration as Gln is consumed by the cells and an increase as Gln is fed into the cell culture (starting at 48h). Proliferation and protein synthesis in the absence of glutamine requires asparagine (*Ref. 4.*)

REBEL data is used to design a feed strategy, and support the development of continuous process, and provide the model input on the key nutrients for the cell growth and production of filled capsids.

Continuous Manufacturing of rAAV using a Benchtop Bioreactor



Screening experiments revealed Ala, Asn, His, Tyr, and Trp positively correlate with capsid production



- High throughput screening of small molecules and amino acids using REBEL can help identify potential candidates that influence AAV production –
- Screening experiments revealed Ala, Asn, His, Tyr, and Trp positively correlate with capsid production.
- Asn is a non-essential AA and constitute about 10% of AAV capsid protein. It is indispensable for AAV production.
- Earlier studies (*Ref 2., 3.*) report that Asn is known to affect *Vaccinia* virus production.

Conclusions and Future work

The REBEL device enables data-driven bioprocess development for rAAV manufacturing in HEK293 cells by accurately and precisely quantitating amino acids, and some biogenic amines. Analysis of cell culture media from shake flask experiments with the REBEL shows that Asn and other amino acids, including several essential amino acids, deplete during AAV production in a fed-batch process. Preliminary data from a perfusion benchtop bioreactor scale show that Ala, Asn, His, Tyr, and Trp positively correlate with capsid production. More experiments are required to confirm how different amino acid directly influence in AAV production.

References

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