Simplifying Progress

Targeted Optimization of AAV8 Upstream Process for Enhanced Downstream Performance

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Introduction

Several recombinant AAV (rAAV)-mediated therapies have been approved so far, and many more are in clinical trials, yet developing an efficient rAAV process platform remains a challenge. First obstacle arises from frequent practice of upstream (USP) and downstream (DSP) process being developed separately. USP optimization activities are usually focused on achieving high viral titer, which does not always translate into maximal purity and recovery in DSP. Second significant challenge is to bridge USP and DSP into a single bioprocess, due to lack of appropriate analytical methodology capable of producing reliable and unbiased results on both sides of the bridge. Furthermore, rAAV-based gene therapy vectors require the removal of process-specific combined with product-specific impurities, as they represent serious safety threats.

Here we showcase the example of AAV8 USP optimization directed towards good performance in DSP (Figure 1). AAV8 is produced in HEK suspension cells via plasmid transfection. Based on the screening results, the best performing set of conditions was successfully scaled up to a 5L stirred-tank bioreactor. Harvested material was then evaluated in DSP, comprised of pre-capture, and two ion-exchange chromatographic steps on CIM monolithic columns. As bridging analytics of choice, we relied on PATfix platform, (d)dPCR and Comassie Bradford protein assay measurements for thorough assessment of relevant parameters such as titer, percentage of full AAV8 capsids and total protein concentration.

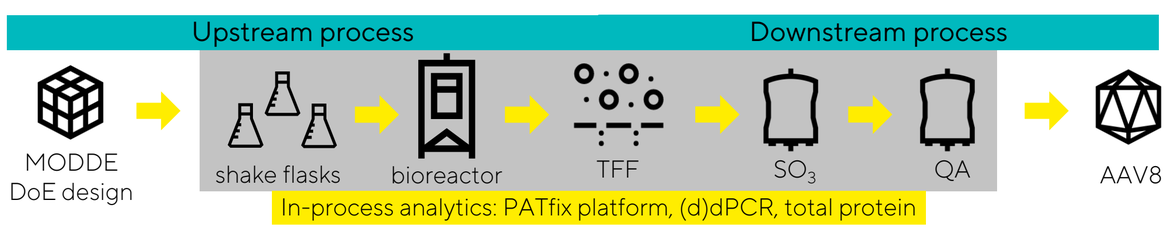

Figure 1. Scheme of AAV8 bioprocess showcased in this publication. Upstream process activities are comprised of Design of Experiments (DoE) conducted in MODDE software, shake flask experiments and scale-up in a stirred-tank bioreactor. At the start of Downstream process, tangential flow filtration (TFF) was used as pre-treatment, followed by two ion-exchange chromatographic steps: capture (SO3) and polishing (QA). All stages in the process were evaluated using PATfix analytics, (d)dPCR and total protein.

1. Upstream process optimization

Experimental design : To enable efficient AAV8 process development in suspension HEK platform, we relied on design of experiment (DoE) approach using MODDE software. D-optimal design was chosen for screening with four center points. Optimized parameters were DNA concentration and the DNA : transfection reagent (TR) mass-to-volume ratio (Figure 2.A), both highly relevant process factors in the triple transfection-based AAV production. Output responses for this study were maximized for AAV titer and % of full capsids, and minimized for total protein concentration.

Small-scale screening: For DoE screening experiment, suspension HEK cells were seeded in 125-mL shake flasks at density 1 million viable cells per mL one day before transfection, in a final working volume 20 mL. DNA : TR complex formation was performed in 10% of the final working volume, for 30 min at room temperature in production media. Cells were harvested 72 hours post-transfection, and samples were collected, lysed and frozen.

Analytics and results: Total protein concentration was determined by Comassie Bradford protein assay (Thermo Fisher). Titer of full capsids was measured by ddPCR. Percent of full capsids was assessed using PATfix system and CIMac AAV full/empty monolith column, and analysed in PATfix software. For complex samples like harvests, multi-angle light scattering (MALS) surface area is used to determine respective peaks of samples, with a correction factor of 1,27 that accounts for the differences in molar masses between empty and full AAV8 particles (Zoratto *et al.* 2021). Joined data is presented in Figure 2.B.

Scale-up: The most optimal set of conditions (1,5 µg pDNA; 1:3 total DNA : TR ratio) were chosen for scale-up in 5L stirred-tank Biostat B-DCU bioreactor (Sartorius). Cells were seeded at density of 500.000 viable cells per mL, and diluted to 1 million viable cells per mL 24 hours before transfection. Transfection conditions were kept the same, and due to the high cell density throughout the process, feed was added 24 hours post-transfection. Cells were harvested and lysed in bioreactor 72 hours post-transfection. Figures 2.D and 2.E show comparison between shake flask and a 5 L scale-up process, where increase in titer and improvement of full capsid percentage are visible, while total protein concentration was kept within similar value range. This difference could be explained by additional control over parameters such as dissolved oxygen and pH, and modification from batch to fed-batch. The switch from ddPCR to dPCR platform likely contributes only to the minor extent, based on thorough assessment of both approaches in a separate study (data not shown in this poster).

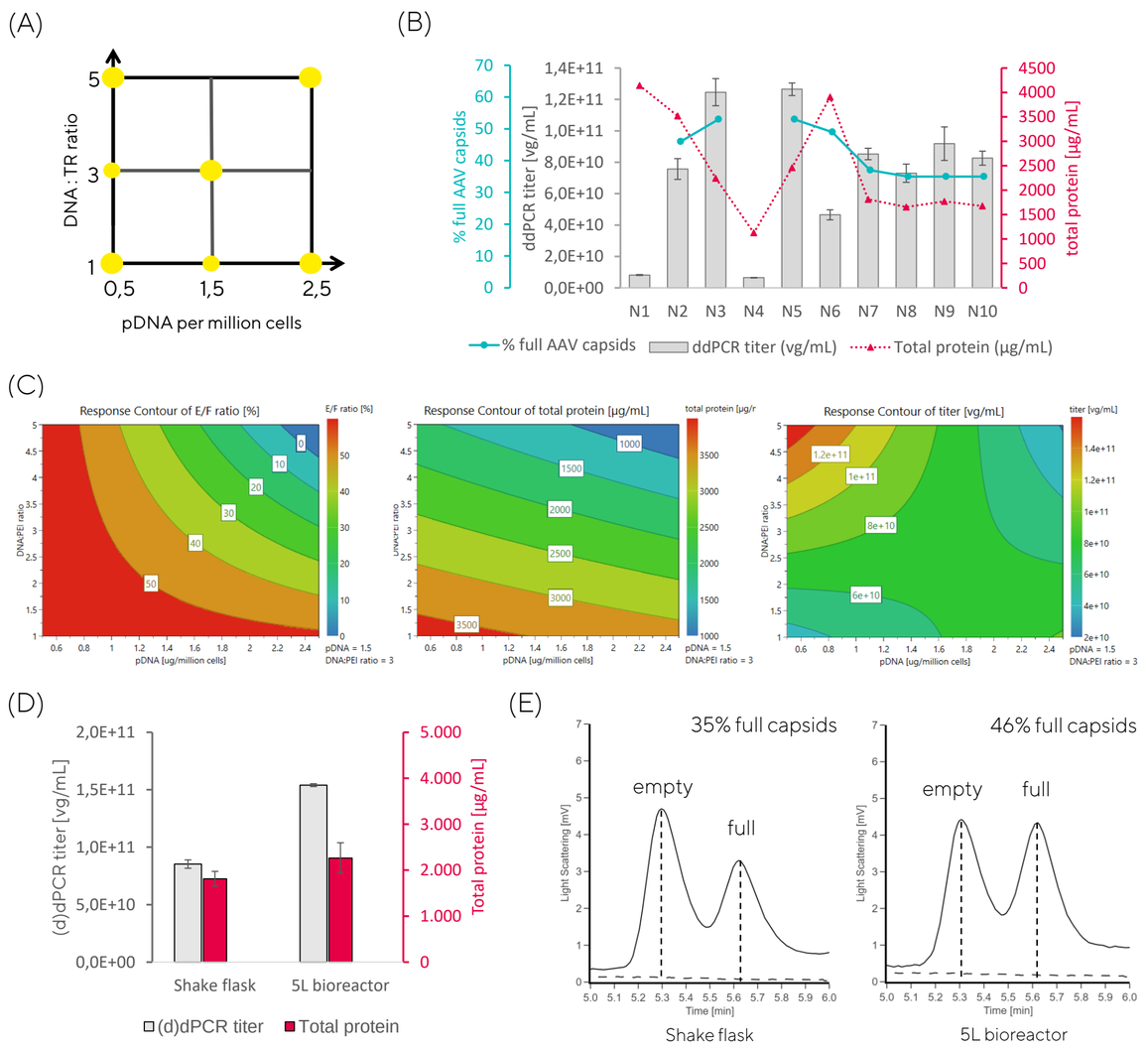

Figure 2. Upstream process optimization and scale-up of AAV8 production in HEK suspension cells. (A) Scheme of transfection parameters from Design of Experiment software MODDE. vg = vector genome. (B) Joined results of small-scale screening experiment. AAV8 titer was determined by ddPCR (grey columns), percent of full AAV8 capsids was analyzed with PATfix AAV platform (blue circles connected with full line) and total proteins measured with Bradford protein assay (red triangles connected with dotted line). For N1 and N4 samples percent of full AAV8 capsids was below the limit of detection of the method. (C) Heatmaps from MODDE for the three output responses. (D) Comparison of vector genome titer and total proteins produced in shake flask and 5L stirred-tank bioreactor with the same conditions. (E) Zoom-in chromatograms of elution on CIMac AAV column, where empty and full capsids (peaks marked with vertical dashed line) are visualised using MALS, % full was calculated in PATfix software based on MALS area with the use of a correction factor (Zoratto et al 2021).

2. Evaluation of optimized harvest in downstream process

Performance challenge: To test the outcome of USP optimization, we assessed the performance of harvest in sequence of purification steps (Žigon *et al.* 2022). As parameters for evaluation, we used DSP recovery and purity. Cumulative recovery was calculated as a percent of total vector genome of the input material. For purity evaluation we followed two parameters: percent of full capsids obtained after polishing step, and removal of total protein and total DNA impurities.

Downstream process: Lysed harvest was clarified and pre-treated by tangential flow filtration (TFF) coupled with a salt-tolerant nuclease. TFF was performed using Hydrosart membranes (Sartorius), loaded at 15,8 L/m2 of clarified lysate. In the next step, AAV8 was captured, concentrated and partially purified using cation exchange chromatography. In that step, 1,5E+13 vg was loaded per 1 mL CIMmultus SO3 column. For the polishing step, SO3 eluate was further purified and enriched using an anion exchange CIMmultus QA column, loaded with 1,12E+14 vg of AAV8 per 1 mL of column.

Analytics and results: Entire process recovery in downstream was 48%, calculated based on dPCR measurements (Figure 3.A). In terms of purity, PATfix analytics on CIMac AAV full/empty column showed enrichment of full capsids. Readouts from FLD and MALS detectors were both evaluated, MALS showing the enrichment from 46% in harvest, to 91% after polishing, and FLD showing 86% in QA eluate (Figure 3.B). Unlike MALS, FLD signal does not require a correction factor in empty/full ratio calculations (Goričar et al 2019), but its application for harvest samples is limited to relatively pure samples. Finally, DSP has successfully removed 5,2 log of DNA and more than 3,7 log of total proteins (Figure 3.C and 3.D).

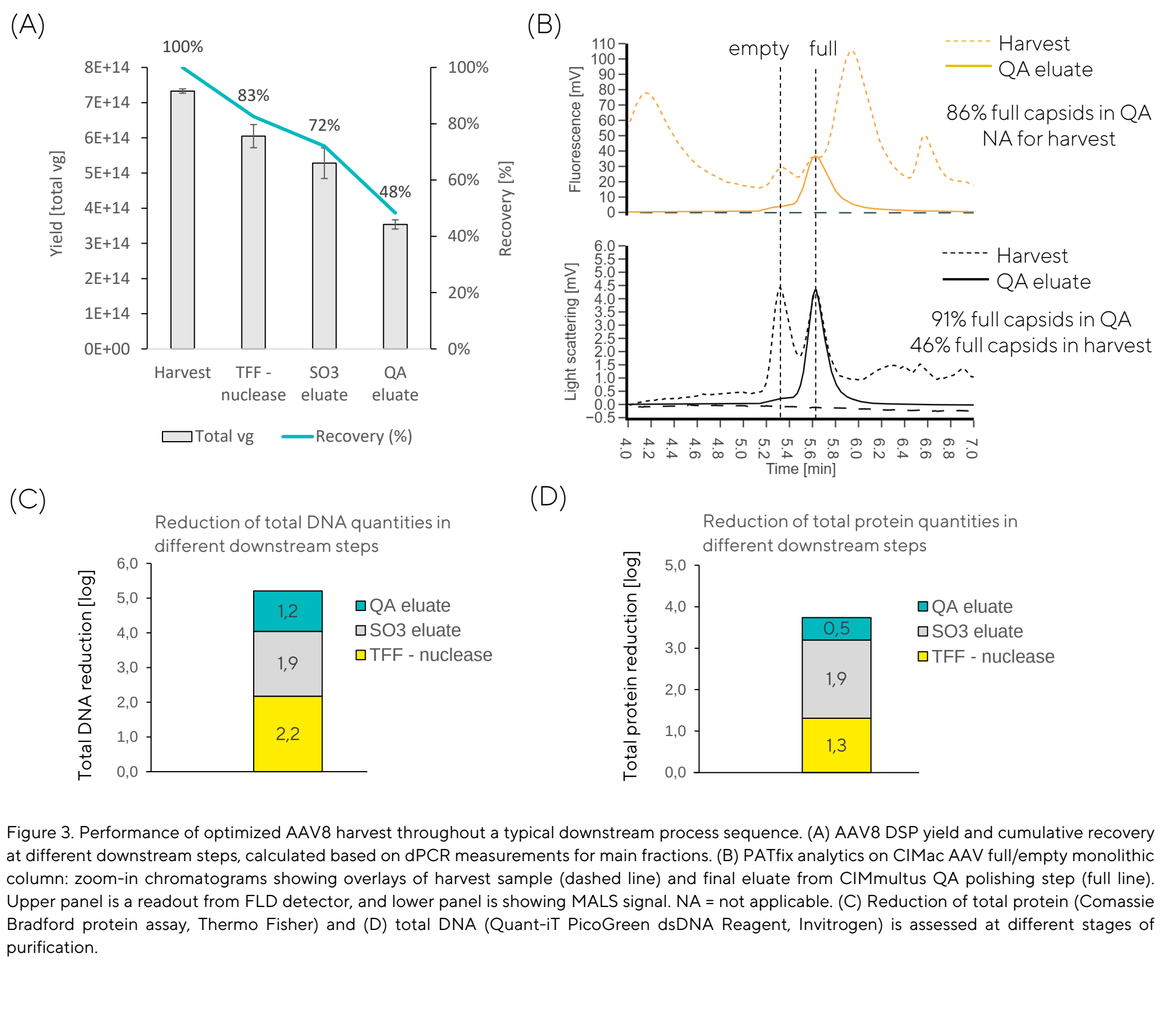

Figure 3. Performance of optimized AAV8 harvest throughout a typical downstream process sequence. (A) AAV8 DSP yield and cumulative recovery at different downstream steps, calculated based on dPCR measurements for main fractions. (B) PATfix analytics on CIMac AAV full/empty monolithic column: zoom-in chromatograms showing overlays of harvest sample (dashed line) and final eluate from CIMmultus QA polishing step (full line). Upper panel is a readout from FLD detector, and lower panel is showing MALS signal. NA = not applicable. (C) Reduction of total protein (Comassie Bradford protein assay, Thermo Fisher) and (D) total DNA (Quant-iT PicoGreen dsDNA Reagent, Invitrogen) is assessed at different stages of purification.

3. Conclusions

In this work we showcase the example of a gene therapy bioprocess, where upstream process was successfully optimized for high yield, purity and recovery of AAV8 full capsids in the downstream part of the process. Starting from Design of Experiments in MODDE software, through screening transfection experiments, the optimal set of conditions was defined and successfully scaled up. As a result, downstream process has achieved 48% of full AAV8 capsid recovery, with close to 90% of full particles and reduction of impurities of 5,2 logs and over 3,7 logs of total DNA and total proteins, respectively.

References

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