

Continuous biomanufacturing using virus-based expression systems enabled by multi-stage bioreactors

From Knowledge
to Competitiveness

Portugal-US Partnerships 2026 Summit

Portugal
Program



Ricardo Correia

rccorreia@ibet.pt

Supervisor(s): António Roldão^{1,2}, Manuel JT Carrondo^{1,2}

1. iBET, Instituto de Biologia Experimental e Tecnológica, Portugal
2. ITQB NOVA, Instituto de Tecnologia Química e Biológica António Xavier, Portugal

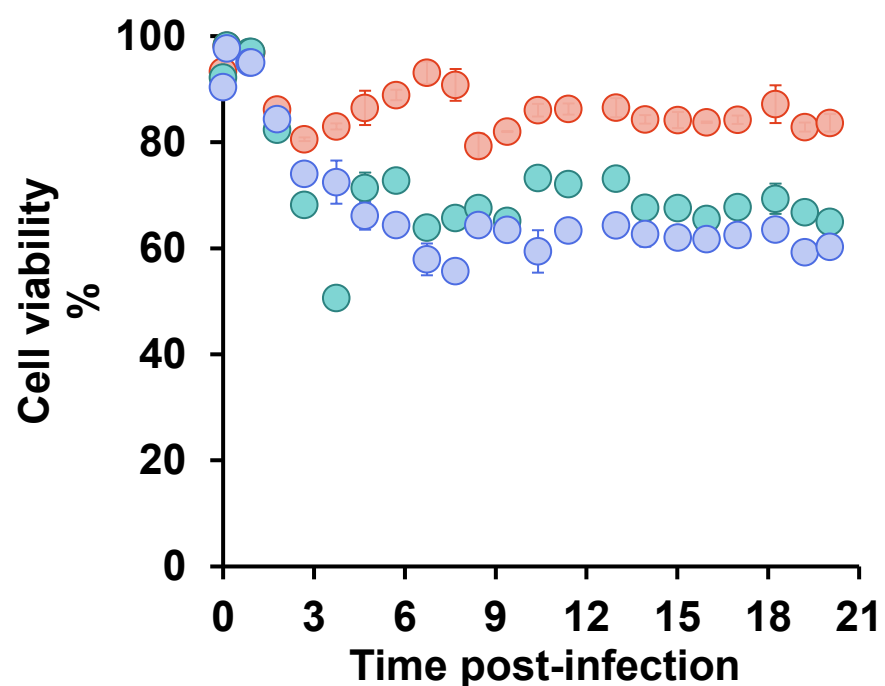
INTRODUCTION

As the biotechnology field advances towards intensified bioprocesses, continuous operations arise as leading modalities for efficient biomanufacturing. The lytic nature of virus-based expression systems used for production of many vaccines and viral vectors limits their transition from traditional batch-based to intensified continuous bioprocessing. Here, we implemented process intensification strategies and continuous multi-stage bioreactor setups to mitigate this bottleneck. This endeavor aims at improving production across different types of biopharmaceuticals, namely influenza virus-like particles (VLPs) as vaccine candidate, adenovirus-like particles (ADDOmer) as nanoparticle-based snake-bite therapy, and recombinant adeno-associated viruses (rAAV) as gene therapy vector; all of which via virus-based expression systems. In addition, these approaches allow seamless integration with continuous downstream processing, paving the way for fully integrated, end-to-end biomanufacturing.

Influenza VLPs as vaccine: continuous production using multi-stage (cascaded) bioreactors

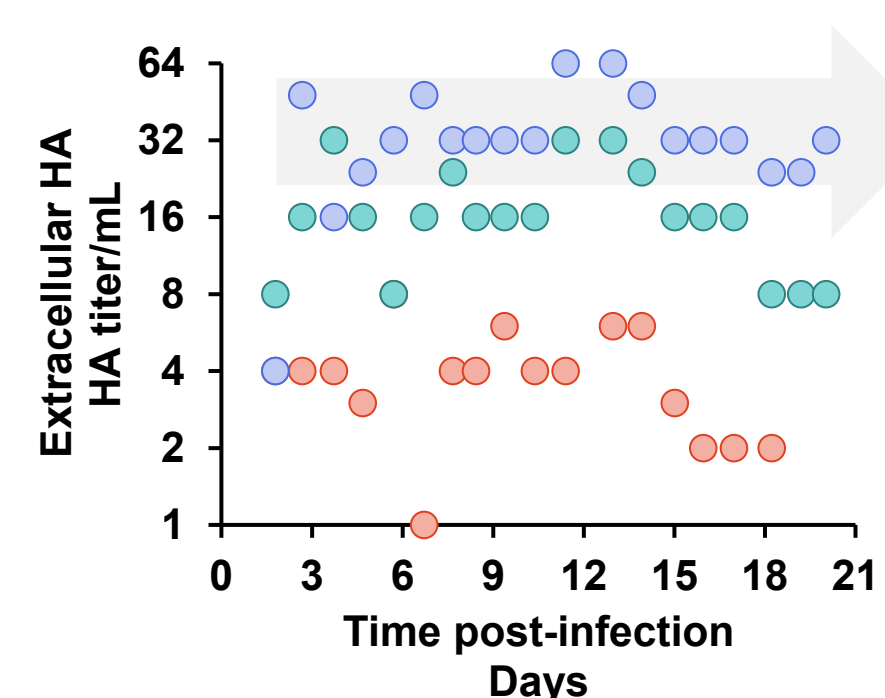
Strategy

Cell growth kinetics



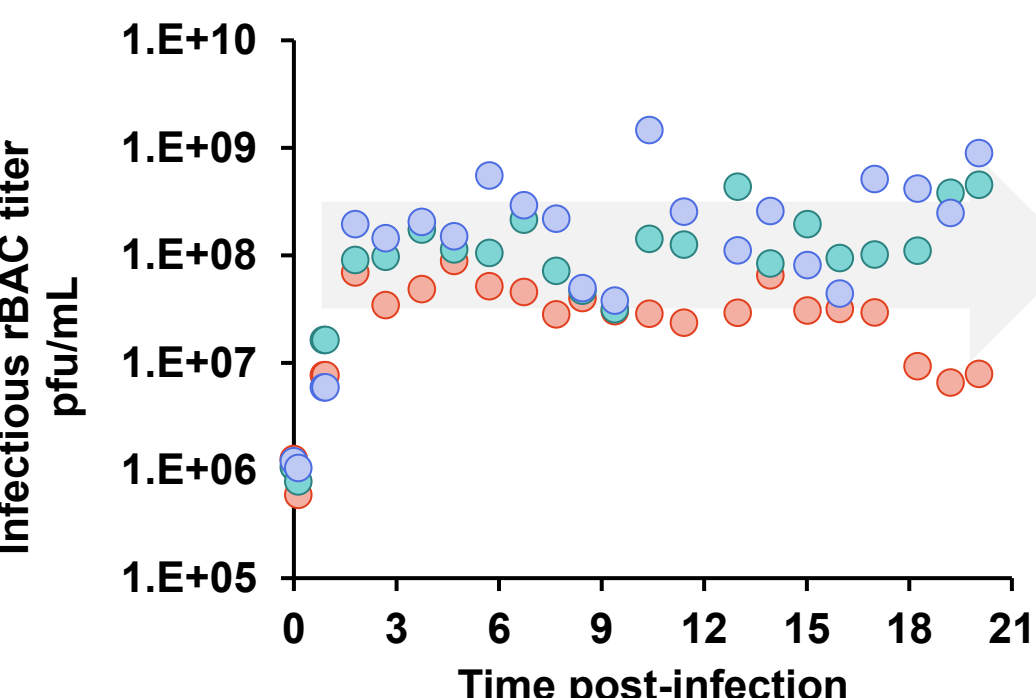
Kinetics change with RT

HA-VLPs production

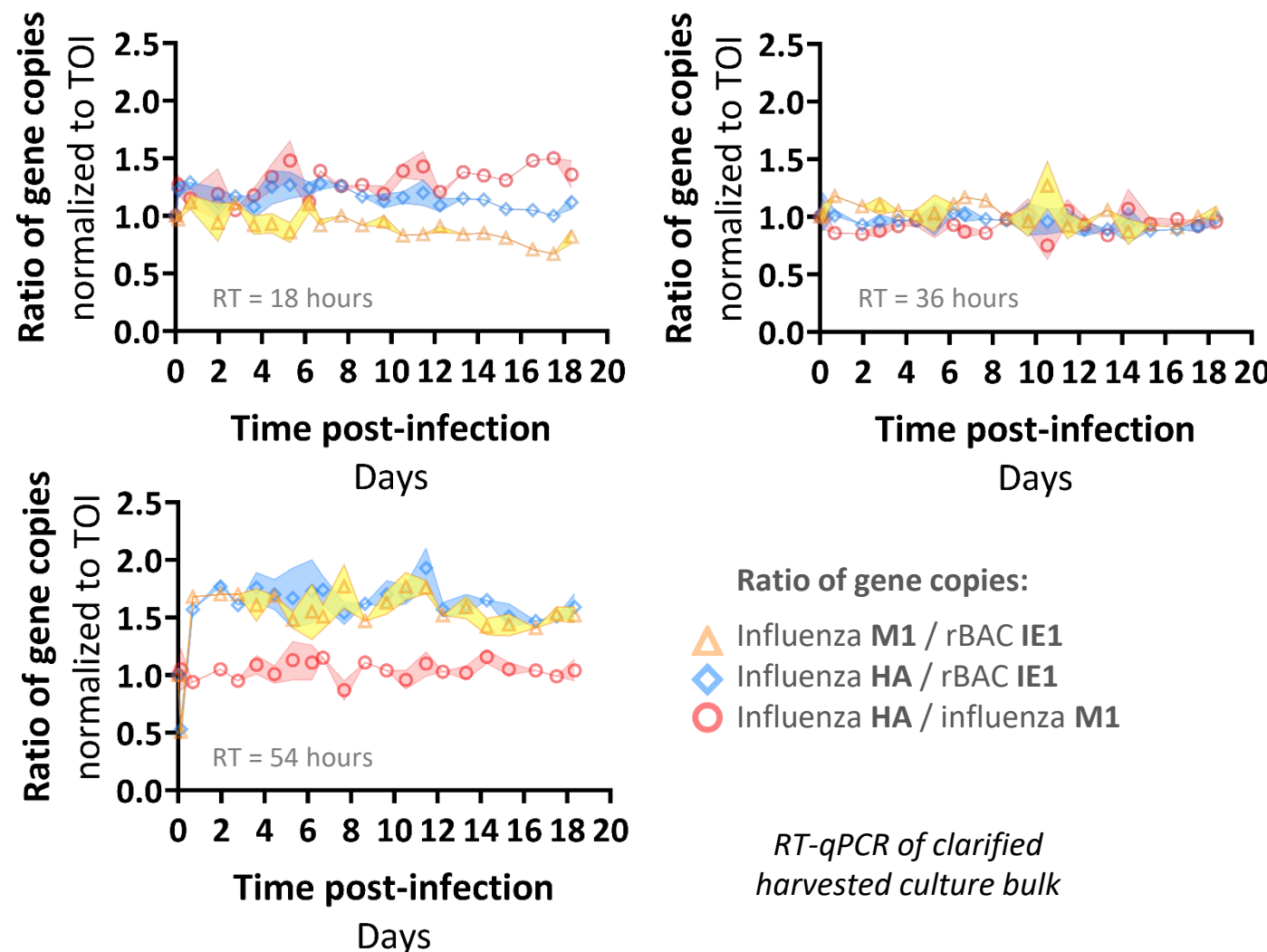


HA-VLPs titers remained constant

Virus passage effect



Infectious rBAC titers are maintained throughout continuous operation, and are higher than in Scheme 1



Genes of interest are maintained in rBAC
Percentage of rBAC particles comprising the genes of interest (HA and M1) is maintained throughout continuous operation

ADDOmer as snakebite therapy: intensified production via perfusion at High Cell Density (HCD)

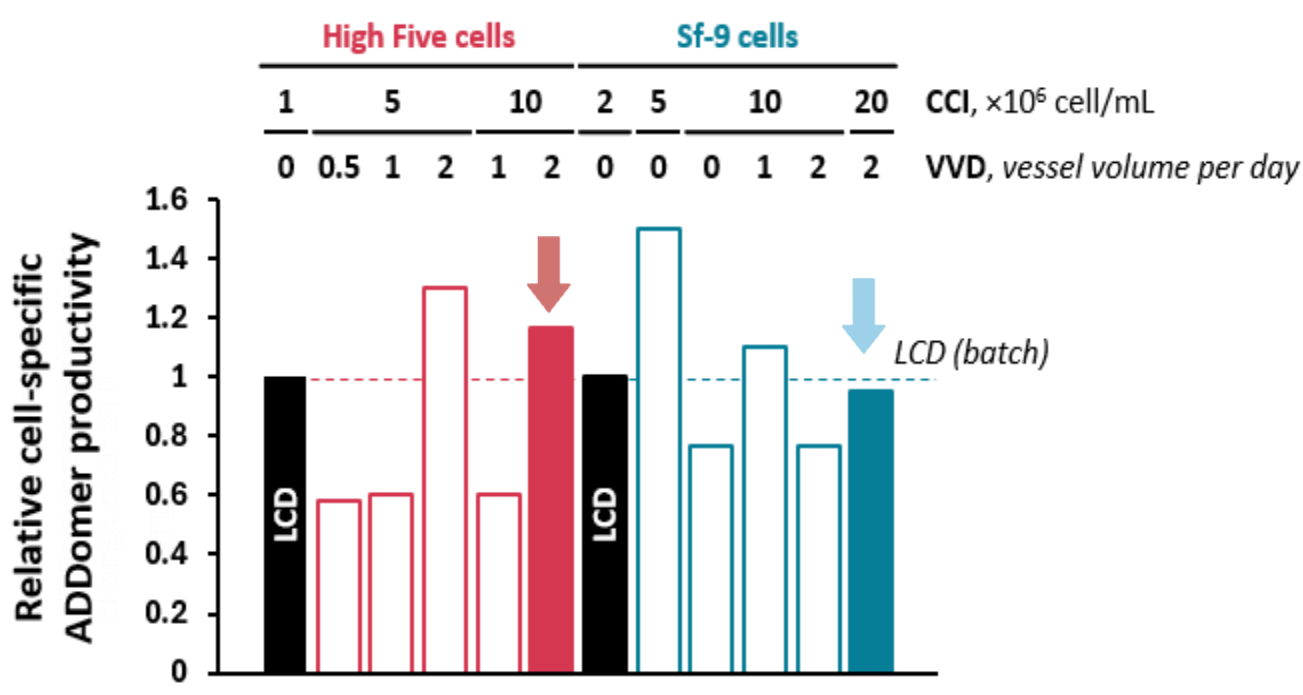
Strategy

Pseudo-perfusion in shake-flask using periodic medium exchange (via centrifugation)

Cell line	Culture medium exchange rate (VVD)	Cell concentration at infection (CCI)							
		1	2	5	10	20	30	40	
High-Five	0	LCD		n.d.	n.d.				
	0.5			-	n.d.				
	1			-	-				
	2			+	+				
Sf-9	0	LCD	+	-	n.d.	n.d.	n.d.		
	0.5				n.d.				
	1			+		n.d.			
	2			-	+				

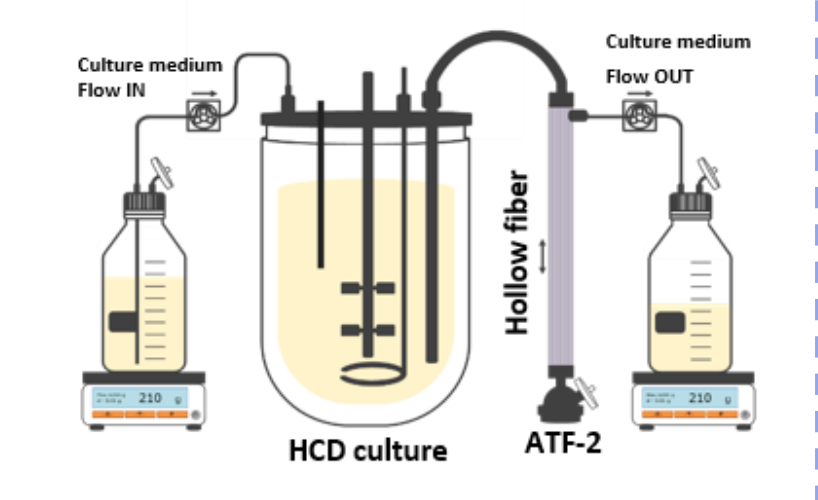
VVD: vessel volume per day
■ Optimal production condition at low cell density (LCD)
□ Conditions not tested
■ Conditions tested, yielding:
(-) lower cell-specific ADDOmer productivity to LCD
(+) similar cell-specific ADDOmer productivity to LCD
(n.d.) not detectable ADDOmer expression

Fine-tuning CPP



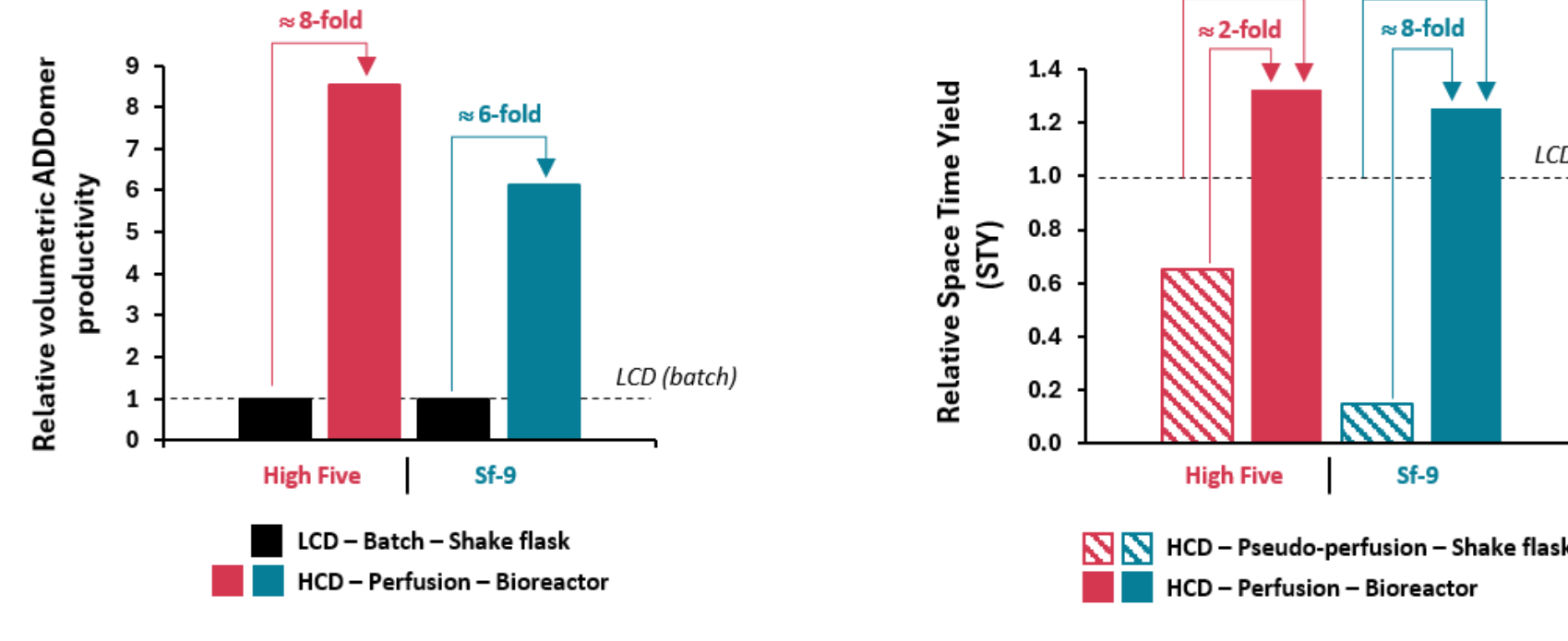
HCD conditions allowing to maximize ADDOmer titer were identified and selected for perfusion bioreactor

Strategy



Perfusion bioreactor (2 L)
• ATF-2 and HF (0.5 µm)
• VVD = 2
• CCI = 10x10⁶ cell/mL (High Five cells) or 20x10⁶ cell/mL (Sf-9 cells)

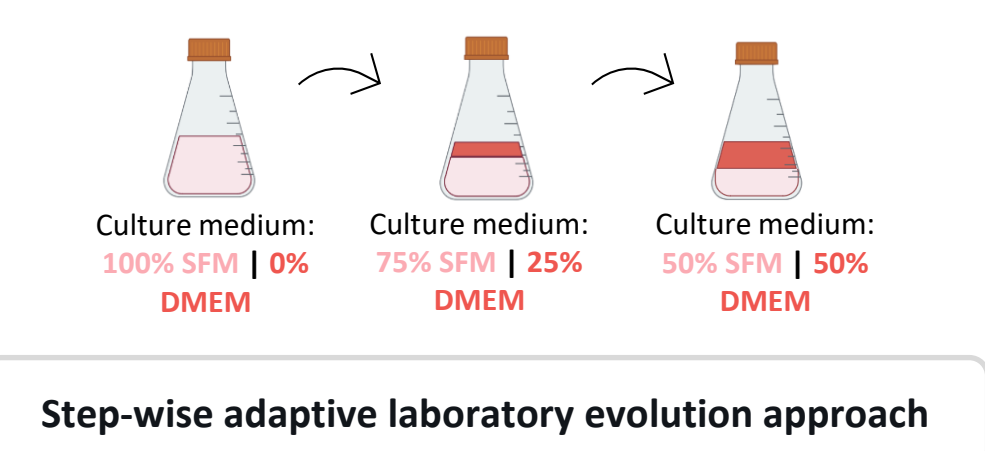
Expression at HCD in perfusion bioreactor



Up to 6-8-fold increase in volumetric ADDOmer titer using perfusion at HCD (vs standard conditions)

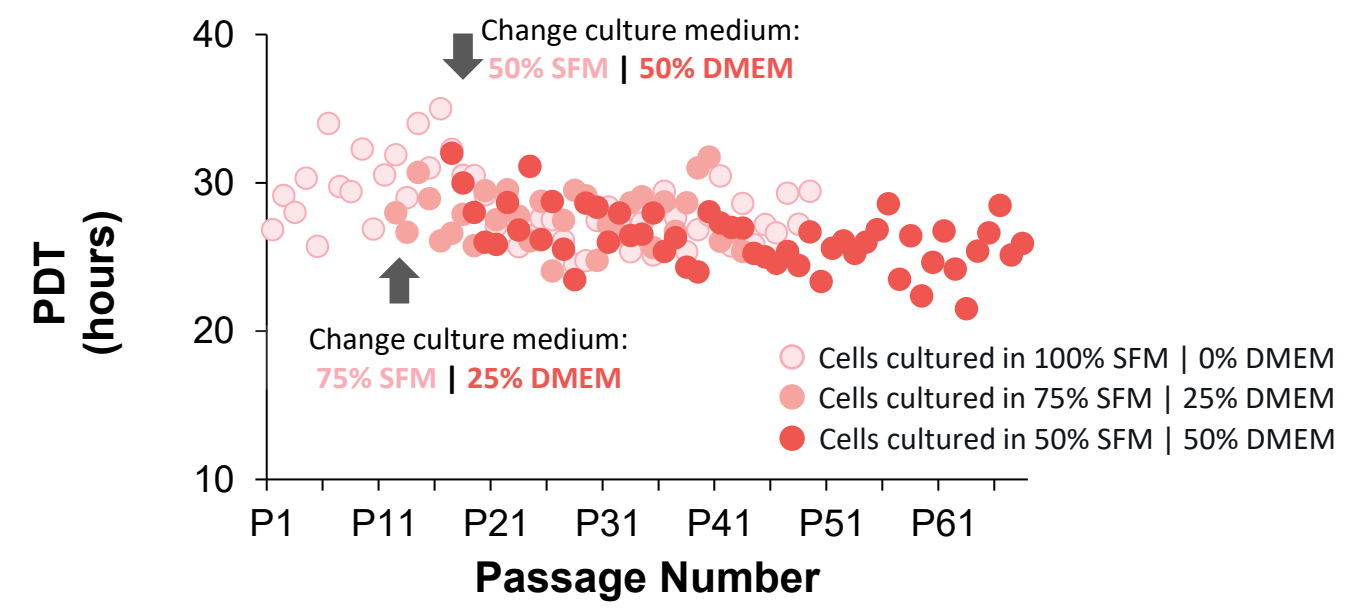
rAAV as gene therapy vector: towards continuous upstream and downstream

Strategy



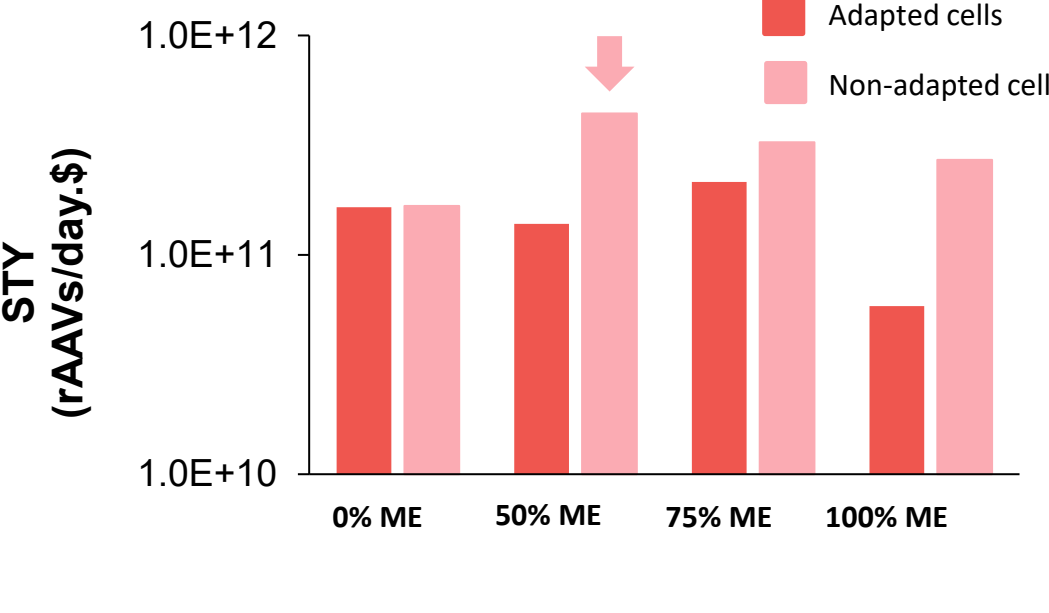
Adaptive laboratory evolution: Adaptation of HeLa cells to grow and produce rAAV in same culture medium

Adaptation process

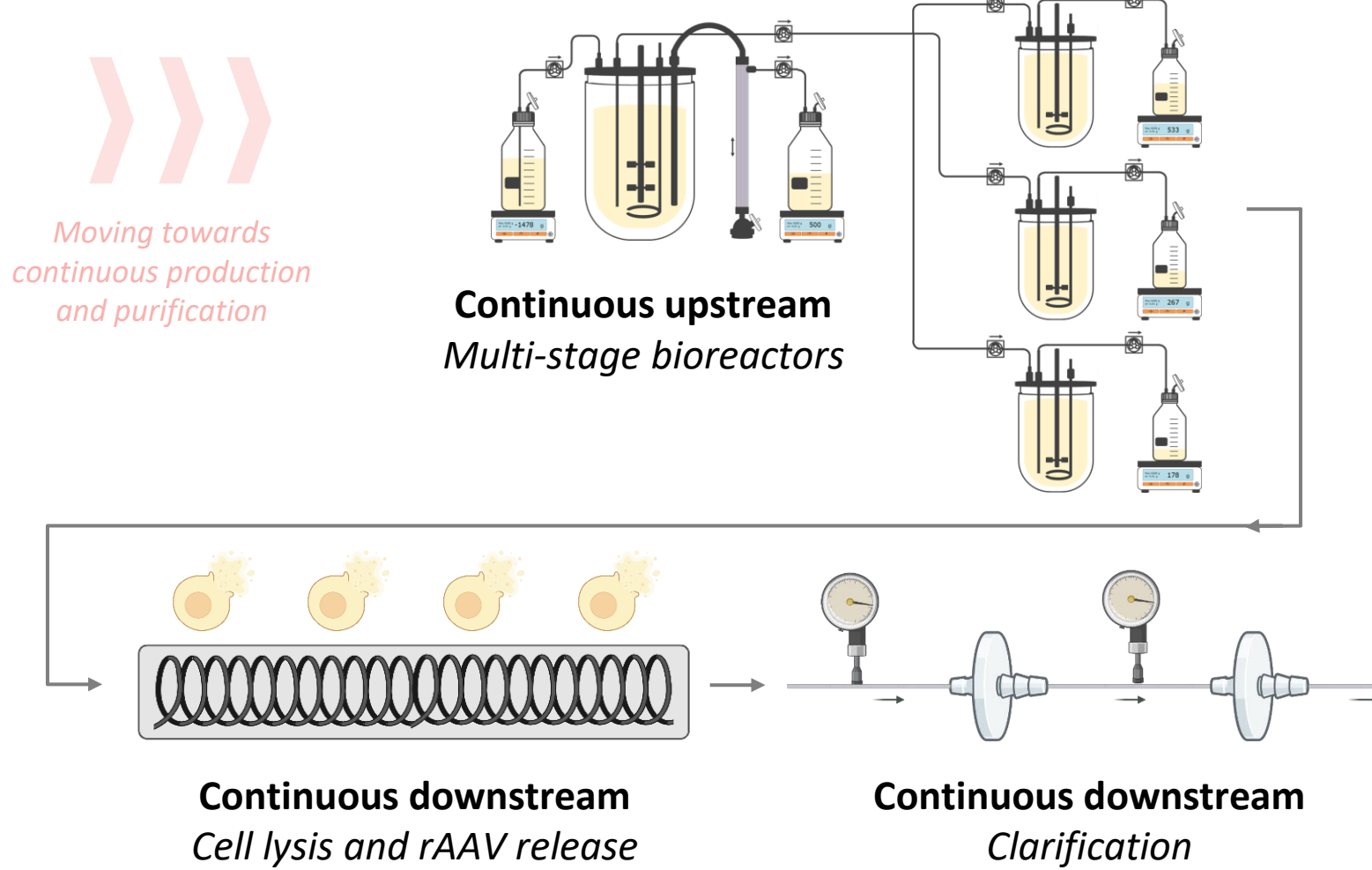


HeLaS3 rAAV PCL adapted to a media formulation compatible with cell growth and rAAV production

Adapted vs non-adapted cells



Most cost-effective approaches for rAAV expression identified
Non-adapted cells outperform adapted cells



CONCLUSION

Process intensification and continuous operation expand the applicability of virus-based expression systems for production of different biopharmaceuticals

Funded by:

