Contribution of Product Sieving to the Passage of High Molecular Weight Species in ATF and TFF Perfusion Cell Cultures

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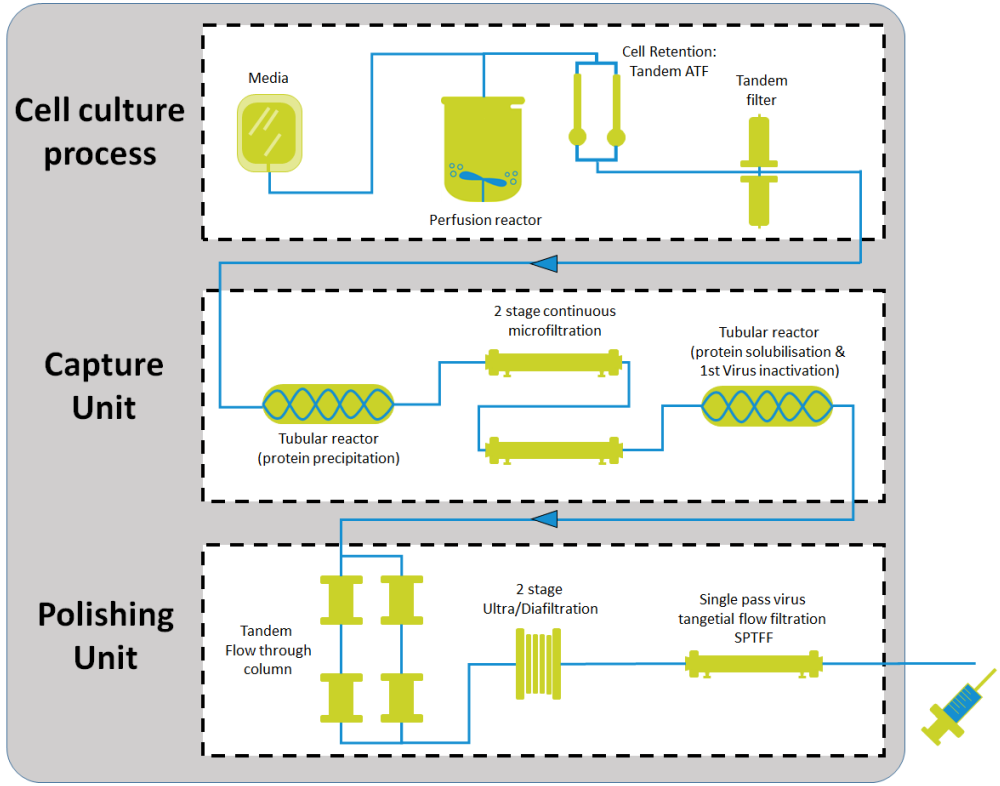
Innovation Management Pharma

New Technologies – ICB-Process skid for manufacturing of bio-based products

The Vision

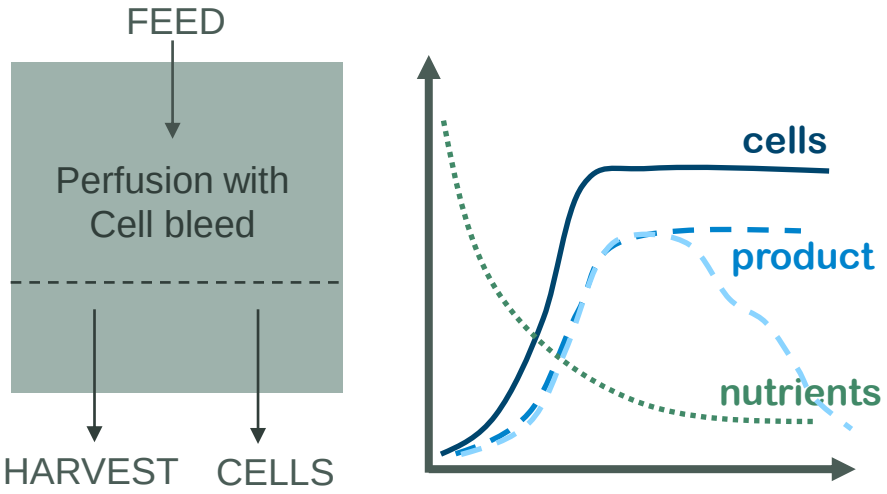
- Construction of an entire End-to-End process train
  - → truly continuous mass flow
  - → Fully automated
  - → smallest possible residence time distribution
- 2- Stage advanced automation concept
  - → independently working modular process entities
  - → interface to APC solutions
- Industrial relevant showcase
  - → 2 to 10 L scale
  - → CHO process producing recomb. mAb



The product sieving challenge

Perfusion hallmarks – Steady state definition

- Constant nutrient concentrations
- Constant cell concentration
- Continuous, constant harvest of target product



Membrane fouling can have impacts on…

Total product yield and consistent purity

Premature termination of bioprocess

Loss of steady state

Undesired complexity for process integration: variable product conc. Feeding to downstream unit

The product sieving challenge

How to address the hallmarks in small-scale perfusion bioreactors (200ml)?

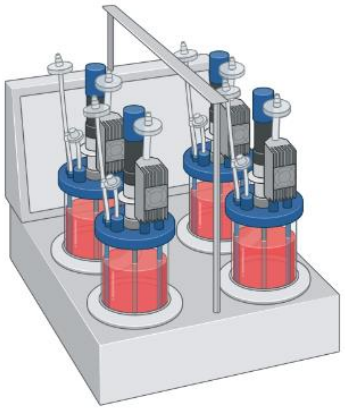
Membrane fouling can have impacts on…

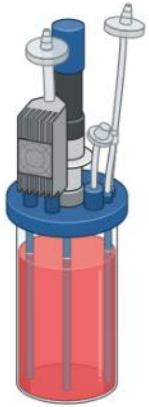
- …oduct yield and consistent purity
- Premature termination of bioprocess
- Loss… steady state
- …plexity for process integration: variable …ng to downstream unit

How can we minimize fouling behavior in TFF systems and at the same time maximize product quality and purity?

Methodology – Perfusion cultures

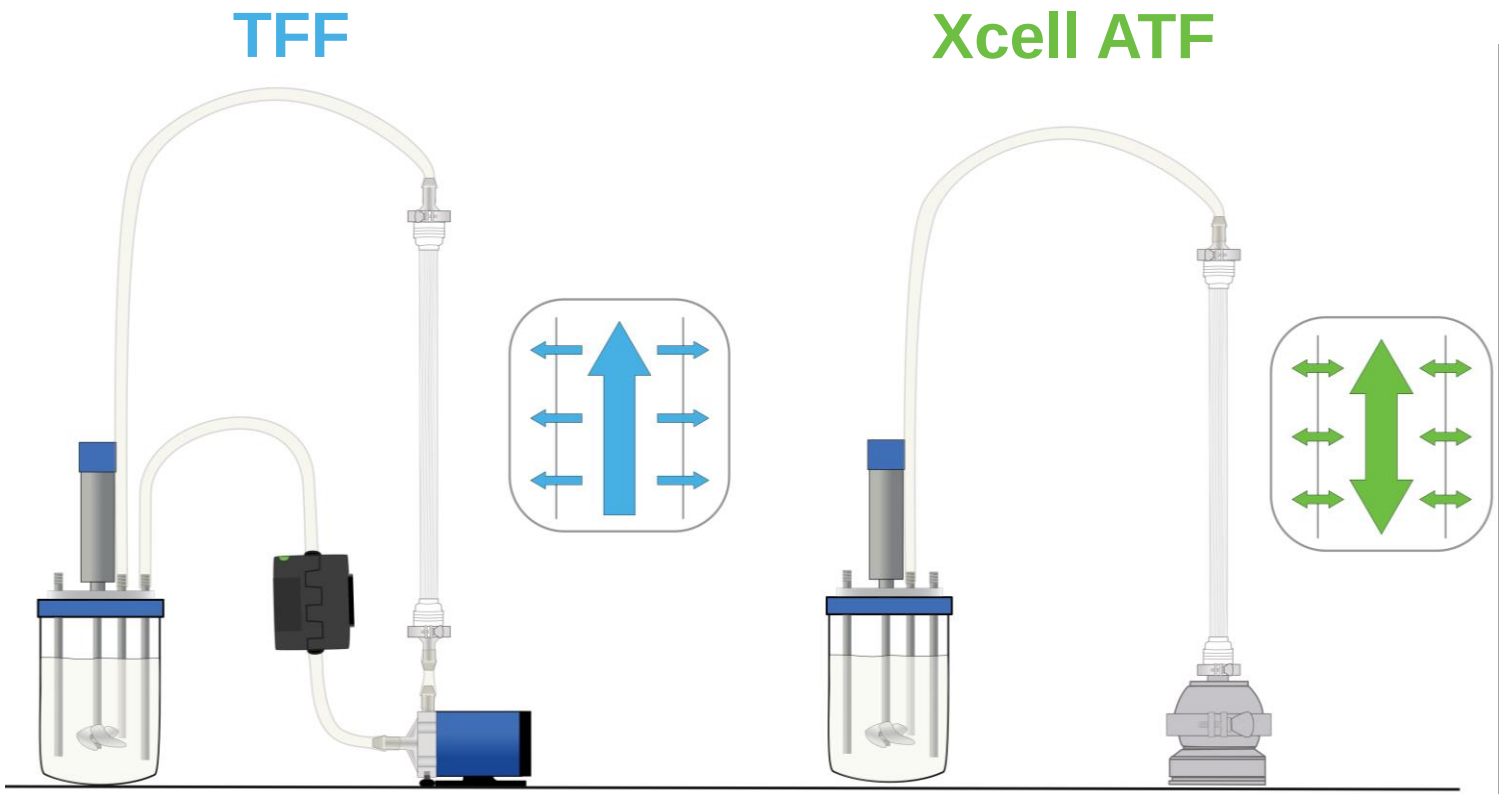
- **200ml** working volume
- HyClone ActiPro media with Cell Boost 7a/7b (Cytiva)
- 110cm^2, 0.2µm, 1mm fiber ID Hollow Fiber (Cytiva)
- 15days+ steady state operation
- Perfusion rate = 1 vvd
- CSPRtarget = 10-20 pL/(cell*day)





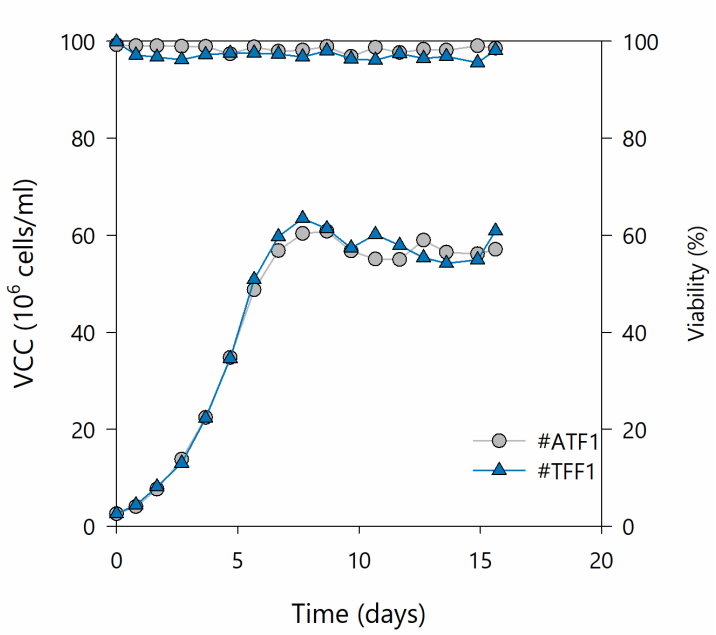
Perfusion run	Flow rate (ml/min)	Shear rate (s^{-1})	Mode of operation
#ATF1	400	5200	static
#TFF1	400	5200	static
#TFF2	200/400	2600/5200	dynamic
#ATF2	200	2600	static
#rTFF1	200/400	2600/5200	dynamic
#rTFF2	200/400	2600/5200	dynamic

State of the art



Head to head comparison ATF and TFF

- Comparison study evolved similar performance in
  - **Cell growth**
  - **Viability**
  - **Cell specific productivity**



Perfusion run	Flow rate (ml/min)	Shear rate (s^{-1})	Mode of operation
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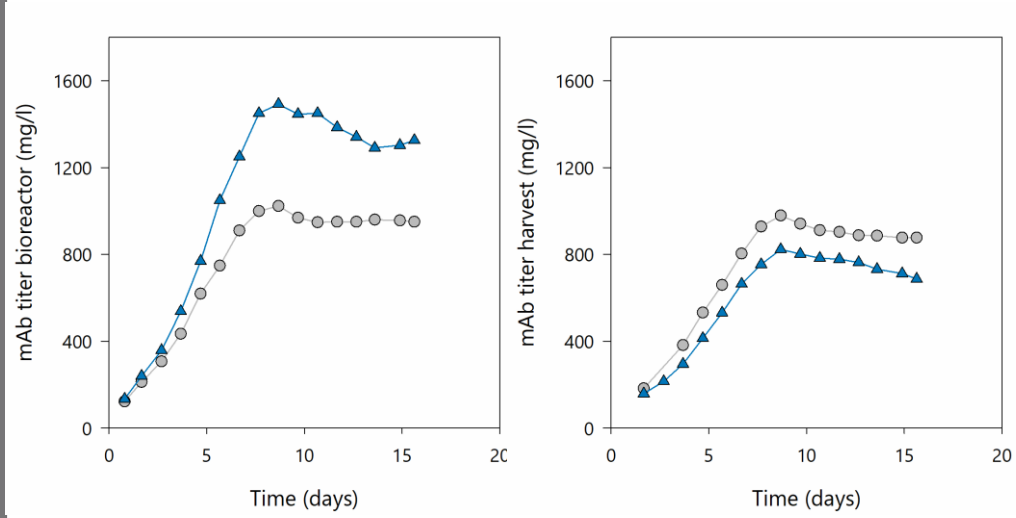
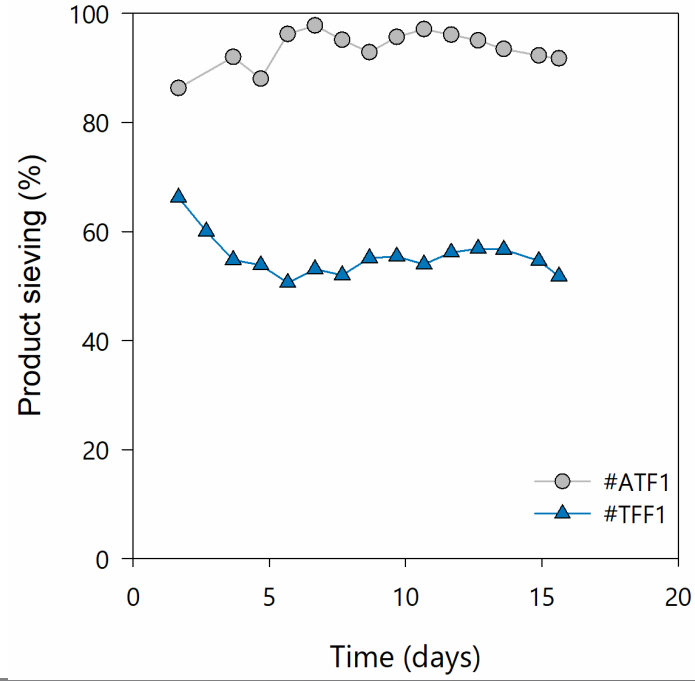
Head to head comparison ATF and TFF

- Difference in mAb titer due to high product retention in TFF bioreactor



- **Product sieving minimum**
  - 85% in #ATF1
  - 50% in **#TFF1**

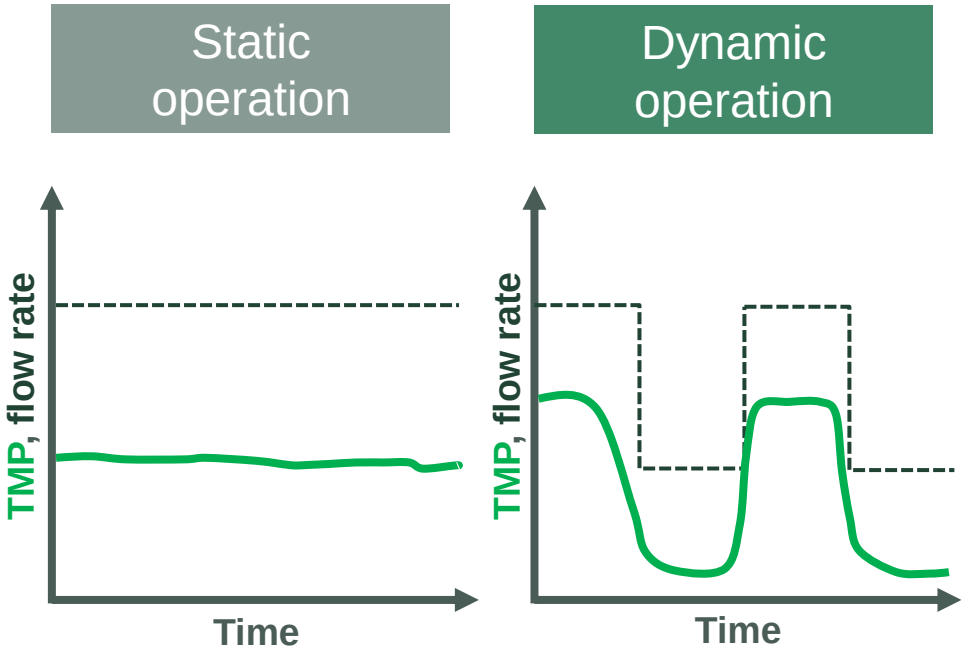


TFF process improvement

- Application of dynamic recirculation flow rate:

TFF recipe control

	Phase 1	Phase 2
Flow rate	200 mlpm	400 mlpm
Time	180 sec	120 sec



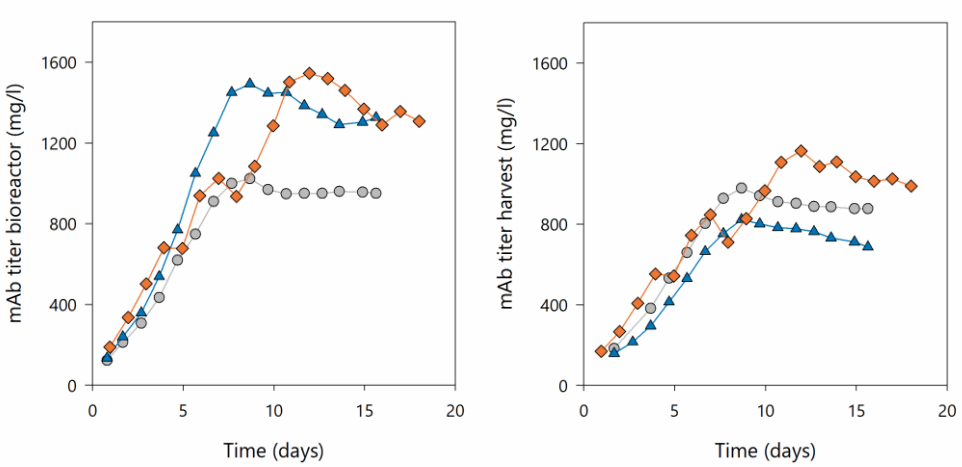
constant flow rate
Constant TMP*

varying flow rate
Pulsating TMP

*ideal state = no membrane fouling

Product sieving improvement through dynamic TFF

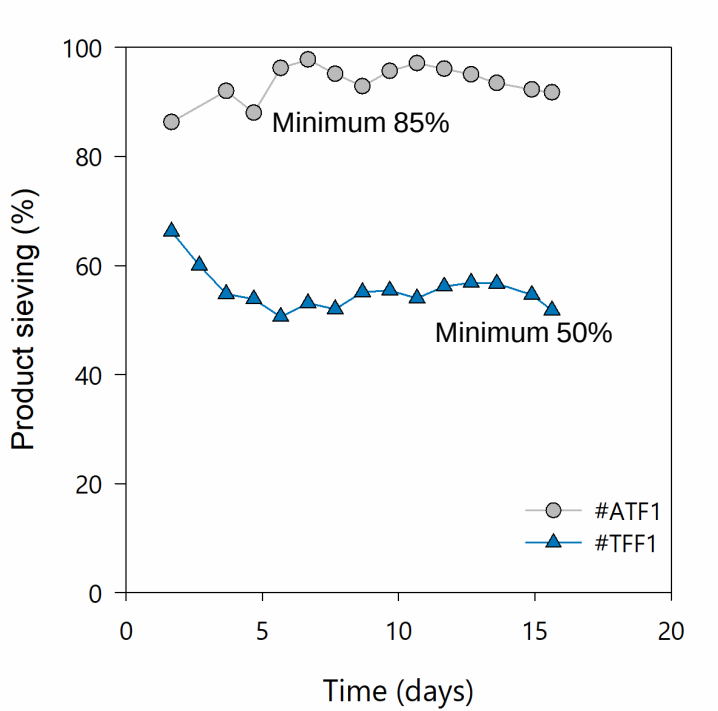
- #TFF2 bioreactor titers similar to #TFF1
- #TFF2 achieved higher harvest titers



Perfusion run	Flow rate (ml/min)	Shear rate (s^{-1})	Mode of operation
#ATF1	400	5200	static
#TFF1	400	5200	static
#TFF2	200/400	2600/5200	dynamic

Product sieving improvement through dynamic TFF

- Pronounced sieving difference between ATF and TFF



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#TFF1	400	5200	static
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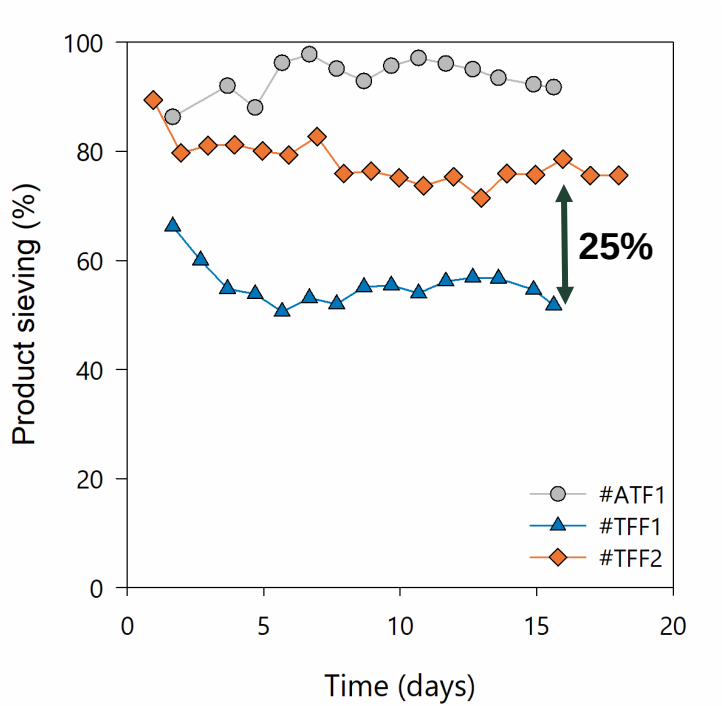
Product sieving improvement through dynamic TFF

- Pronounced sieving difference between ATF and TFF
- Improvement of product sieving in **#TFF2**

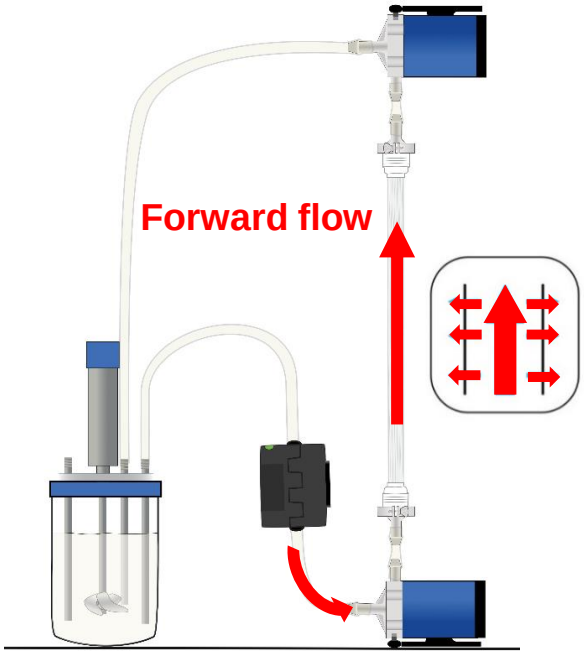


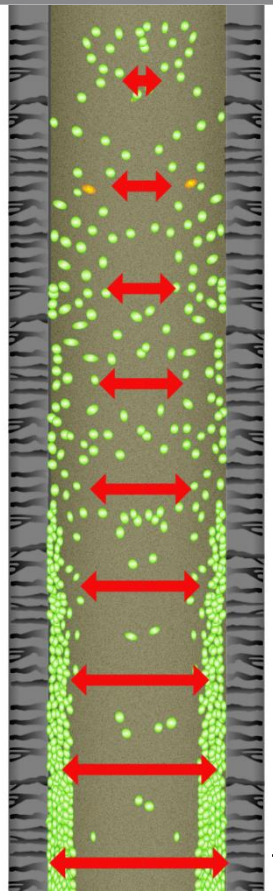
Perfusion run	Flow rate (ml/min)	Shear rate (s^{-1})	Mode of operation
#ATF1	400	5200	static
#TFF1	400	5200	static
#TFF2	200/400	2600/5200	dynamic

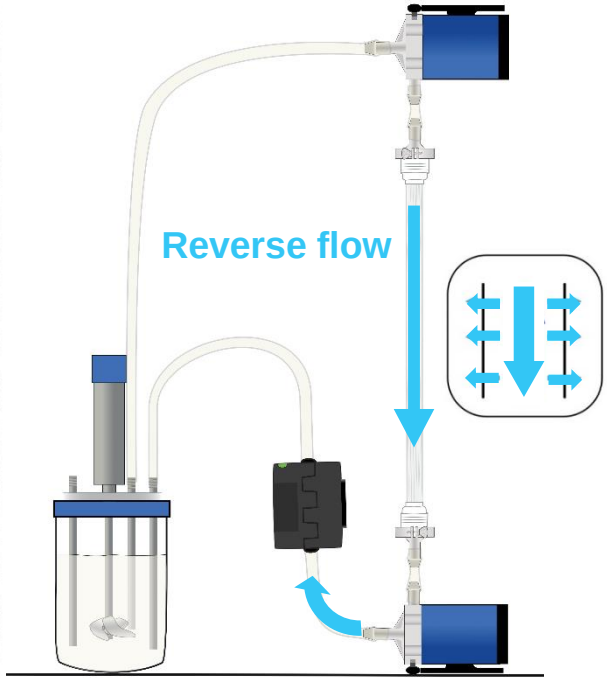
How can we fully address the product sieving issue using magnetic levitating pumps?

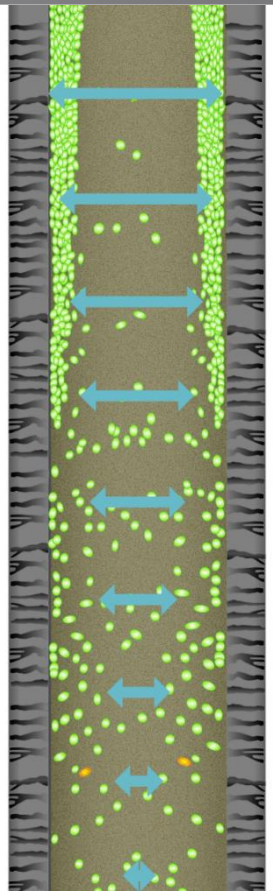
Reverse TFF for uniform TMP distribution

- Novel concept for better product recovery in TFF systems compared to commercial ATF



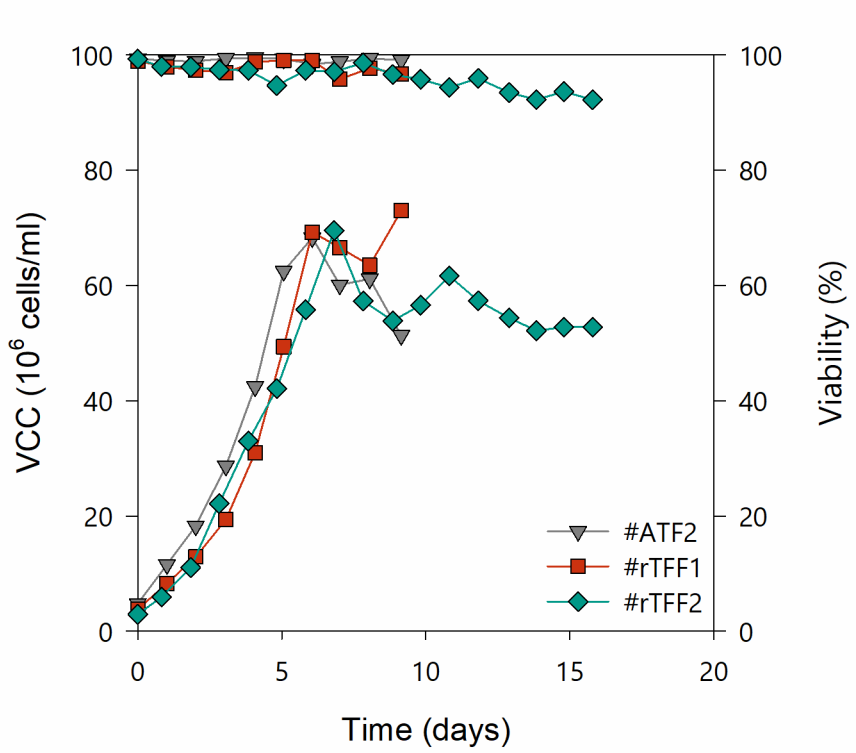






rTFF alleviates product retention

- Reverse TFF modus as alternative to commercial ATF

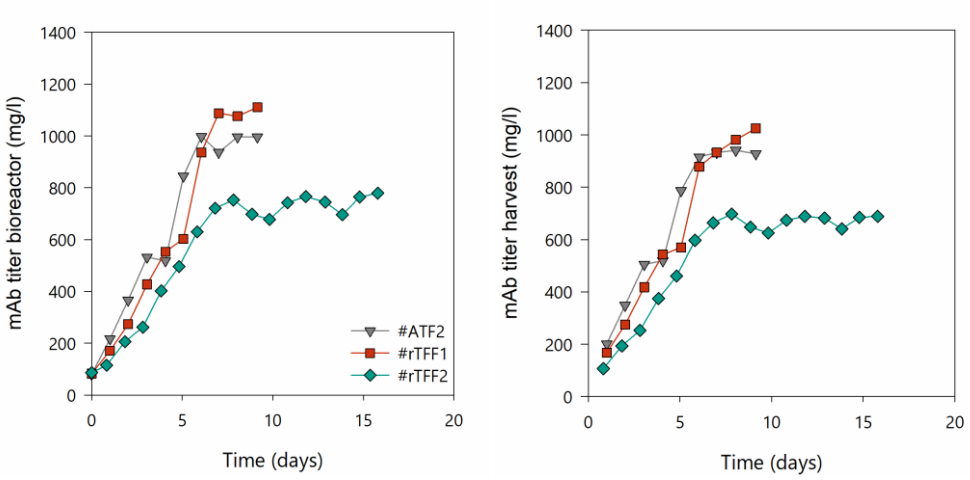


Perfusion run	Flow rate (ml/min)	Shear rate (s^{-1})	Mode of operation
#ATF2	200	2600	static
#rTFF1	200/400	2600/5200	dynamic
#rTFF2	200/400	2600/5200	dynamic

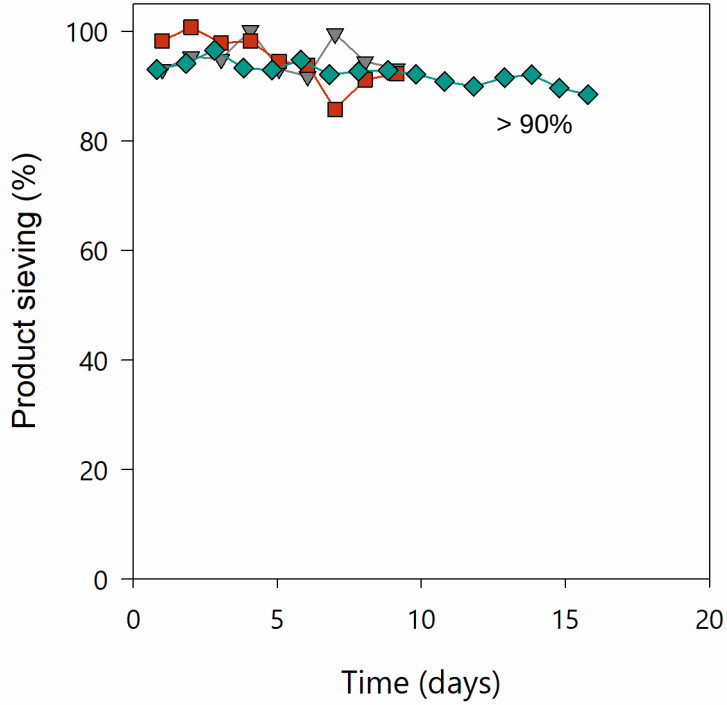
*Cycle time = 30 sec

rTFF alleviates product retention

- Comparable performance between ATF and rTFF
- Productvity lower in **#rTFF2** (higher generation no. of cells)

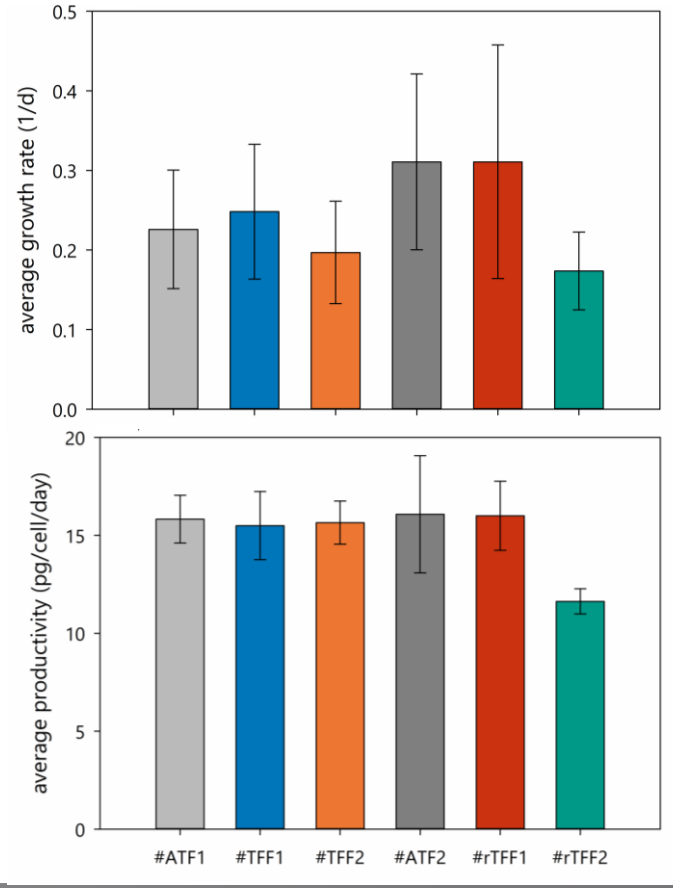


- **Product sieving reduced to a minimum** over long process duration



Lessons learned

- Process performance similar in different modes of operation (ATF, TFF, rTFF)
- Magnetically levitating pumps did not influence cell growth, viability, productivity
- TFF performance comparable to ATF except for product retention
- Dynamic recirculation generates pulsating TMP and is therefore benefical to reduce reversible membrane fouling
- Reverse TFF (rTFF) as a novel concept to alleviate product retention

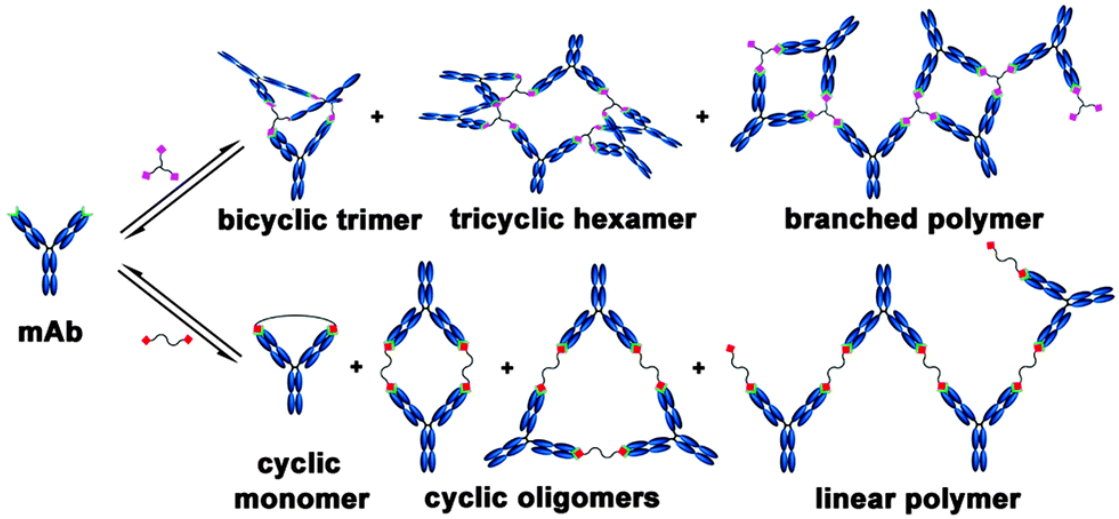


What influence does product sieving have on the purity and product quality of the mAb harvest in a perfusion process?

… and can we monitor or even control it?

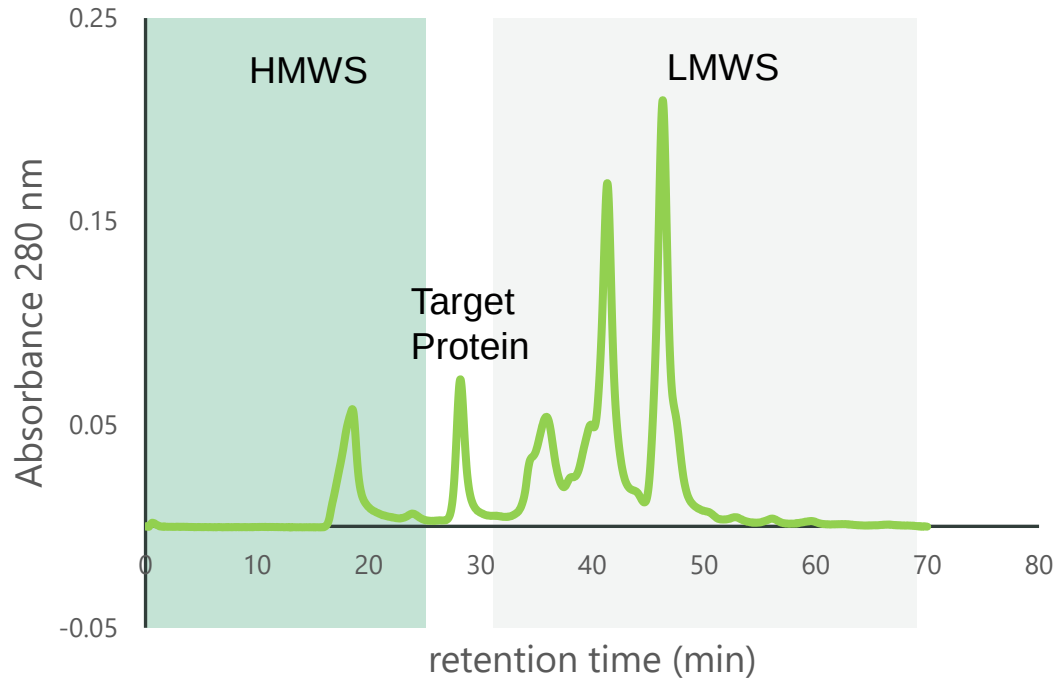
HMWS = high molecular weight species

- Presence of HMWS and aggregates reduces product purity
- Increased HMWS concentration decreases product yield and increases complexity of downstream purification (**especially in an ICB!)**
- Protein aggregation could lead mAbs to stimulate immune responses in patients (**CQA**)



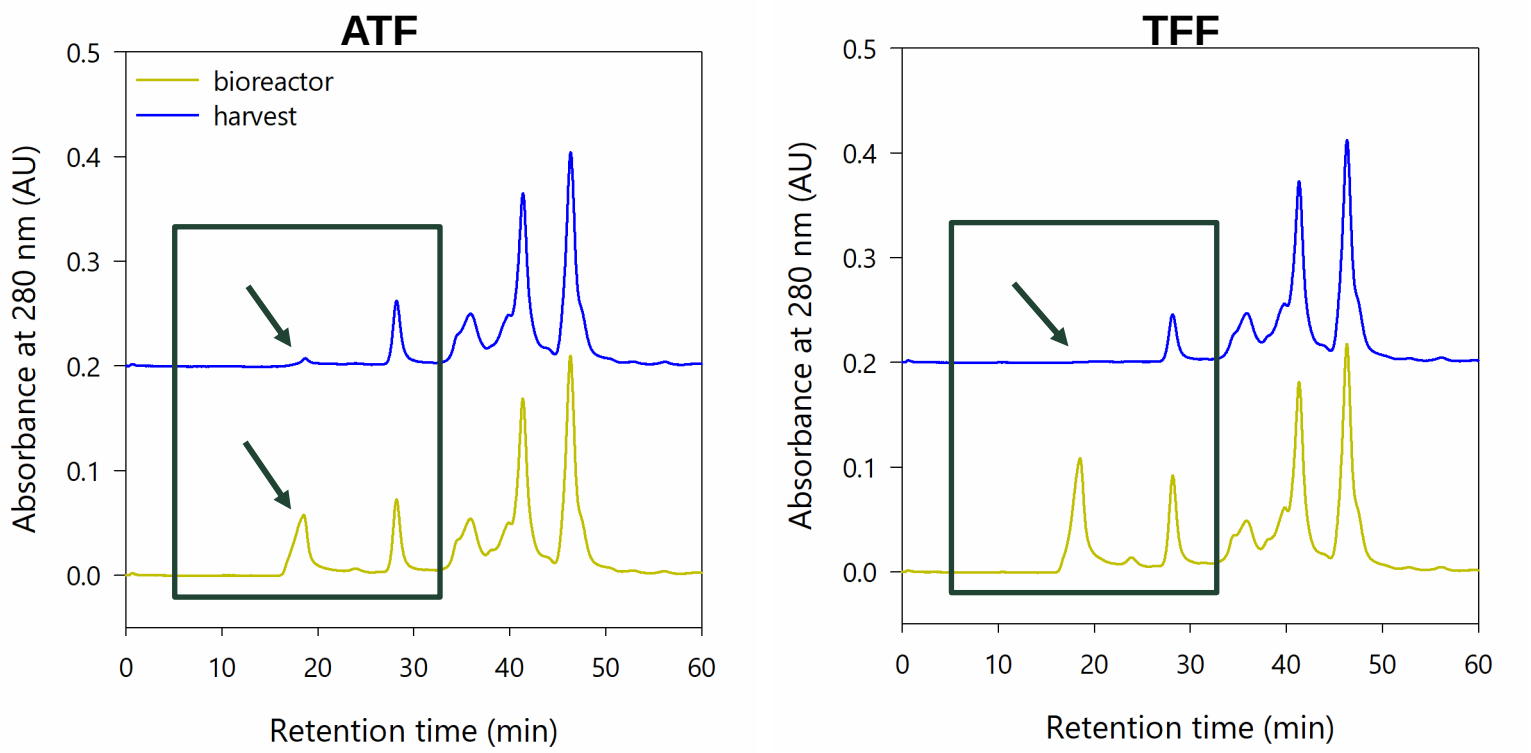
Investigation of protein impurities

- SEC-HPLC allows peak separation between protein monomers, HMW and LMW species



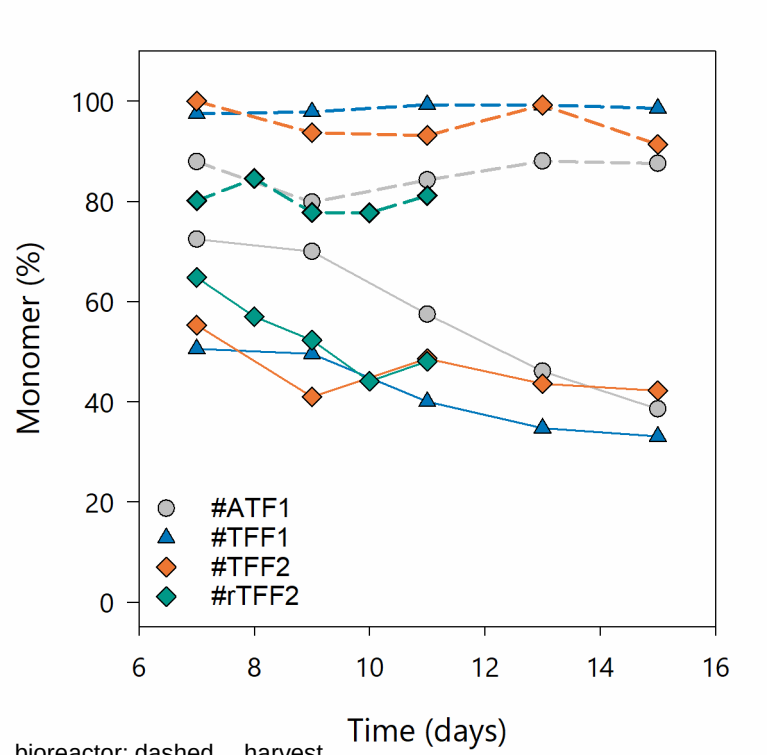
Investigation of protein impurities

Different passage of HMWS in ATF and TFF perfusion systems



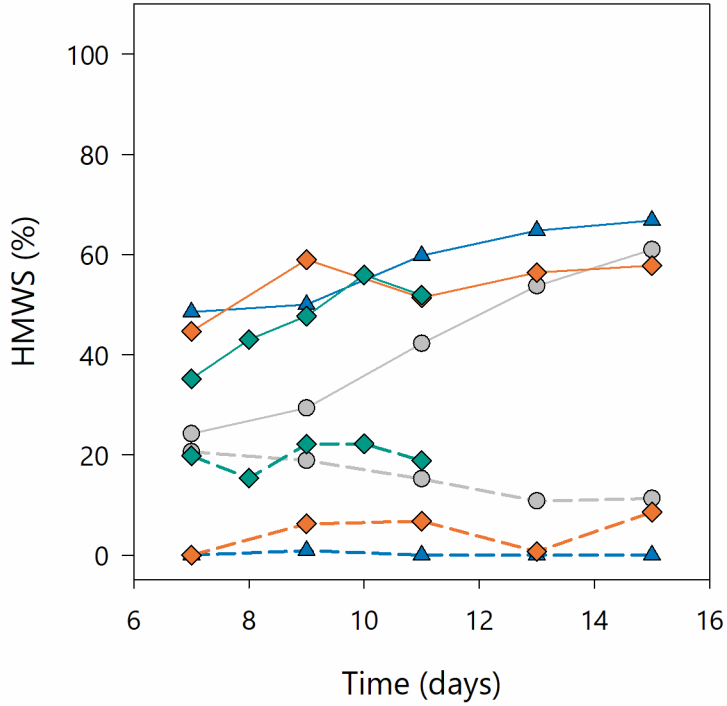
Amount of protein impurities in ATF and TFF

- Purity of TFF harvest larger than ATF and rTFF (>98%)



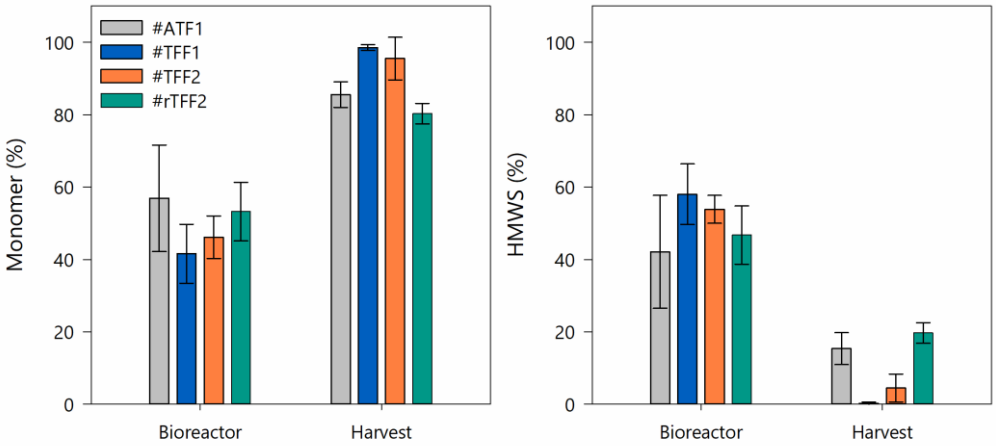
Solid line… bioreactor; dashed… harvest

- HMW species < 5% in harvest using TFF mode
- HMW species approx. 15% in ATF and rTFF

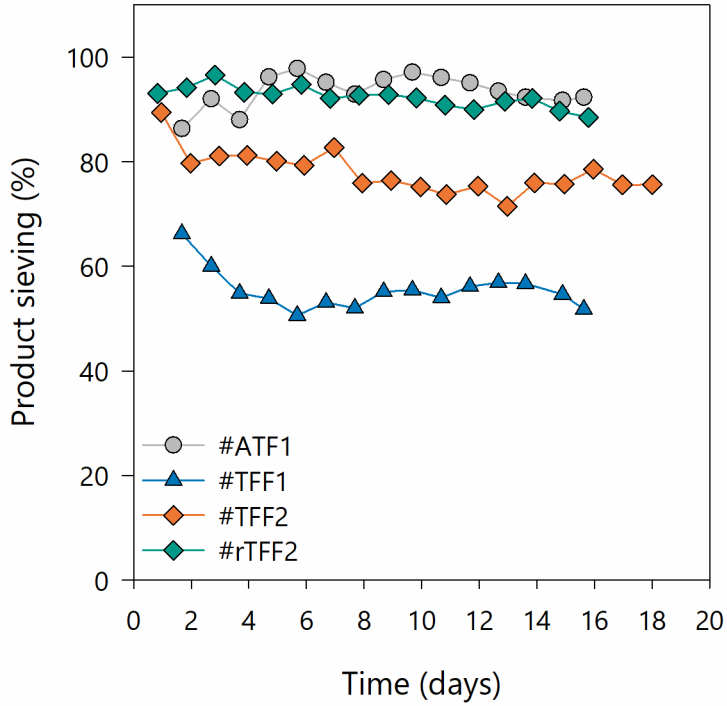


Comparison of steady states

- Highest monomer purity in TFF harvest
- Lowest HMWS passage in TFF mode
- Purity depending on **product sieving and filtration mode**

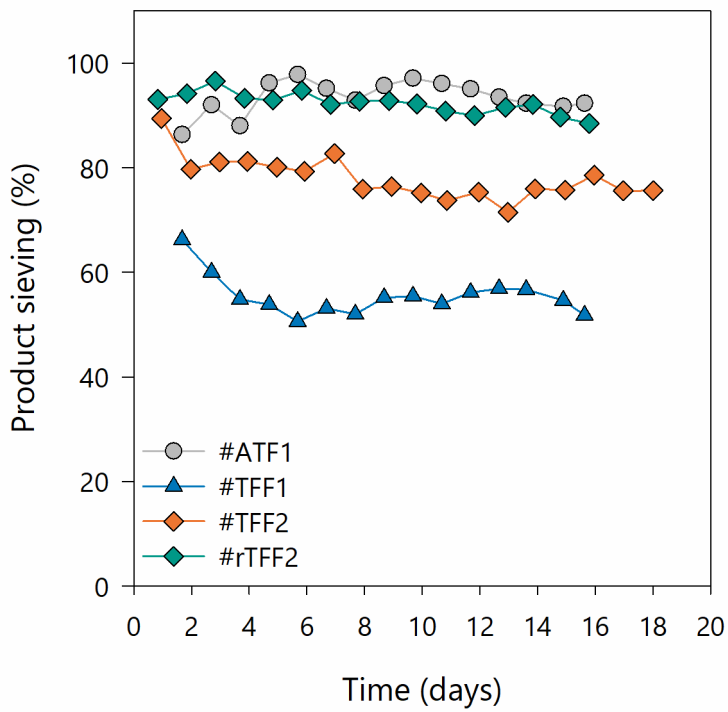


- ATF and rTFF generally results in **higher product yield** (less product retention)

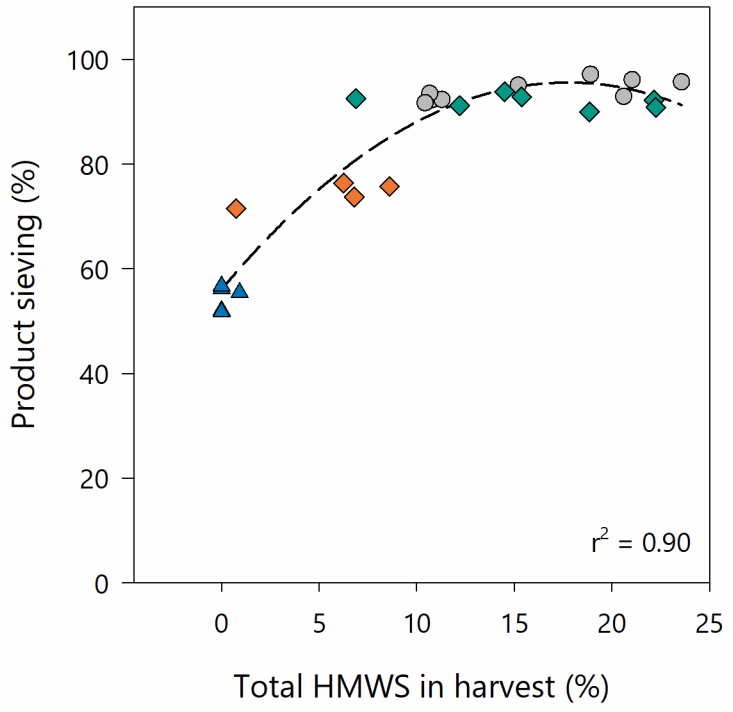


HMWS and product sieving

- Sieving and perfusion mode influences HMWS passage to Harvest



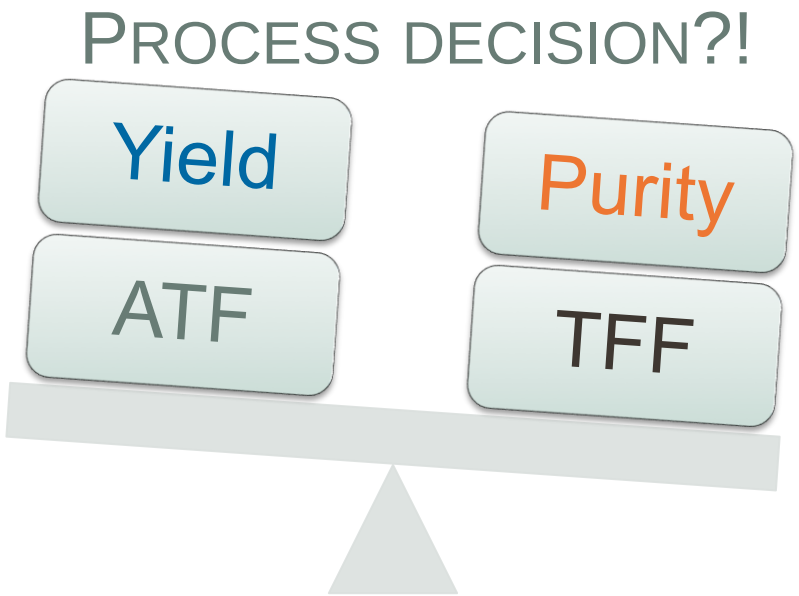
- Correlation between product sieving and total HMWS in harvest
- **Product purity can be estimated via product sieving**



Concluding remarks

- Amount of HMW impurities in perfusion harvest differ from ATF to TFF mode
- Specific HMW species only get retained by TFF mode
- Product sieving correlates with the passage of HMW species into perfusion harvest

- **Higher product yield does not necessarily mean higher product purity!**



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References

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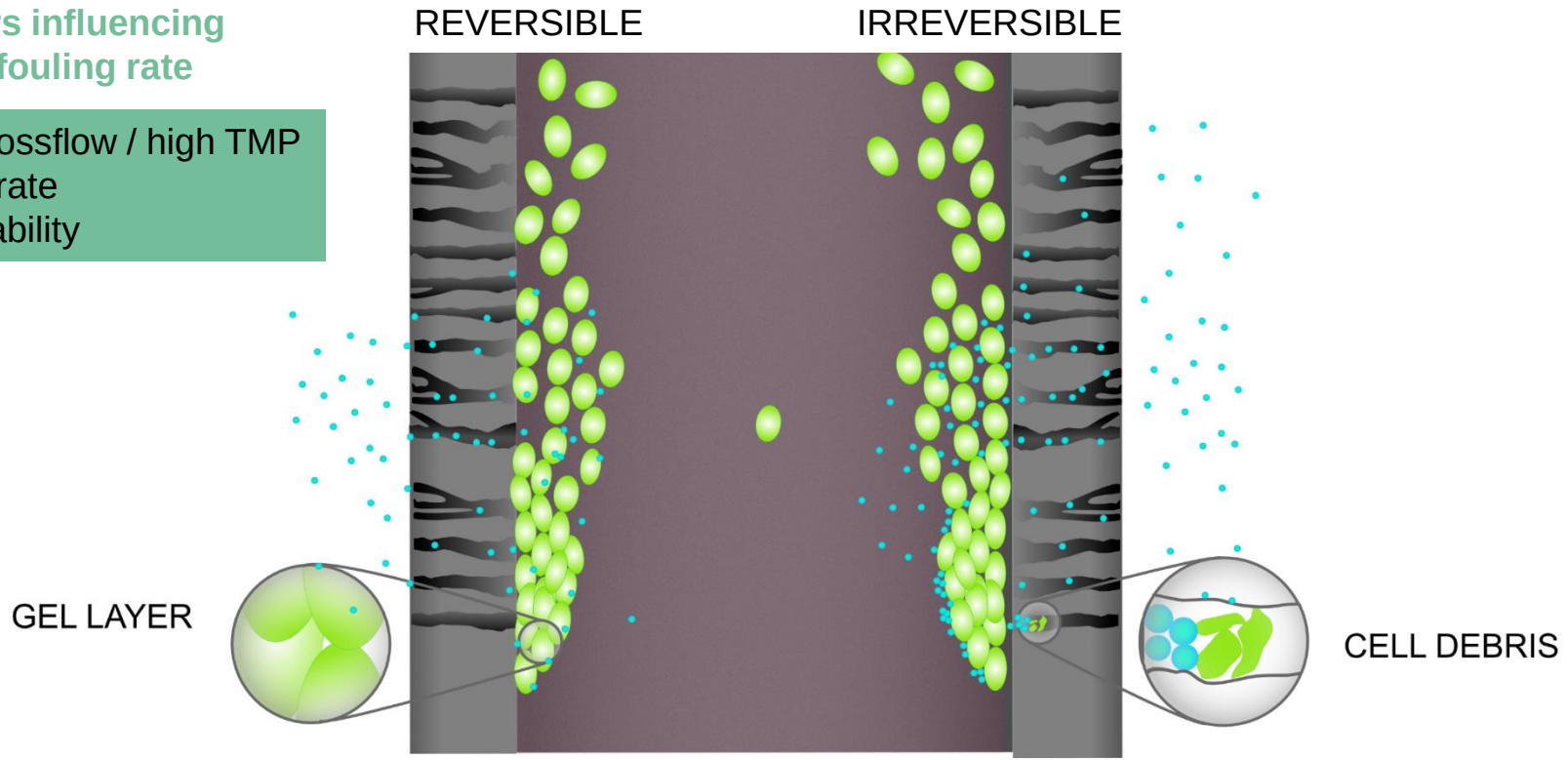
Back up slides

The story of fouling

Factors influencing the fouling rate

1. Low crossflow / high TMP
2. Shear rate
3. Cell viability



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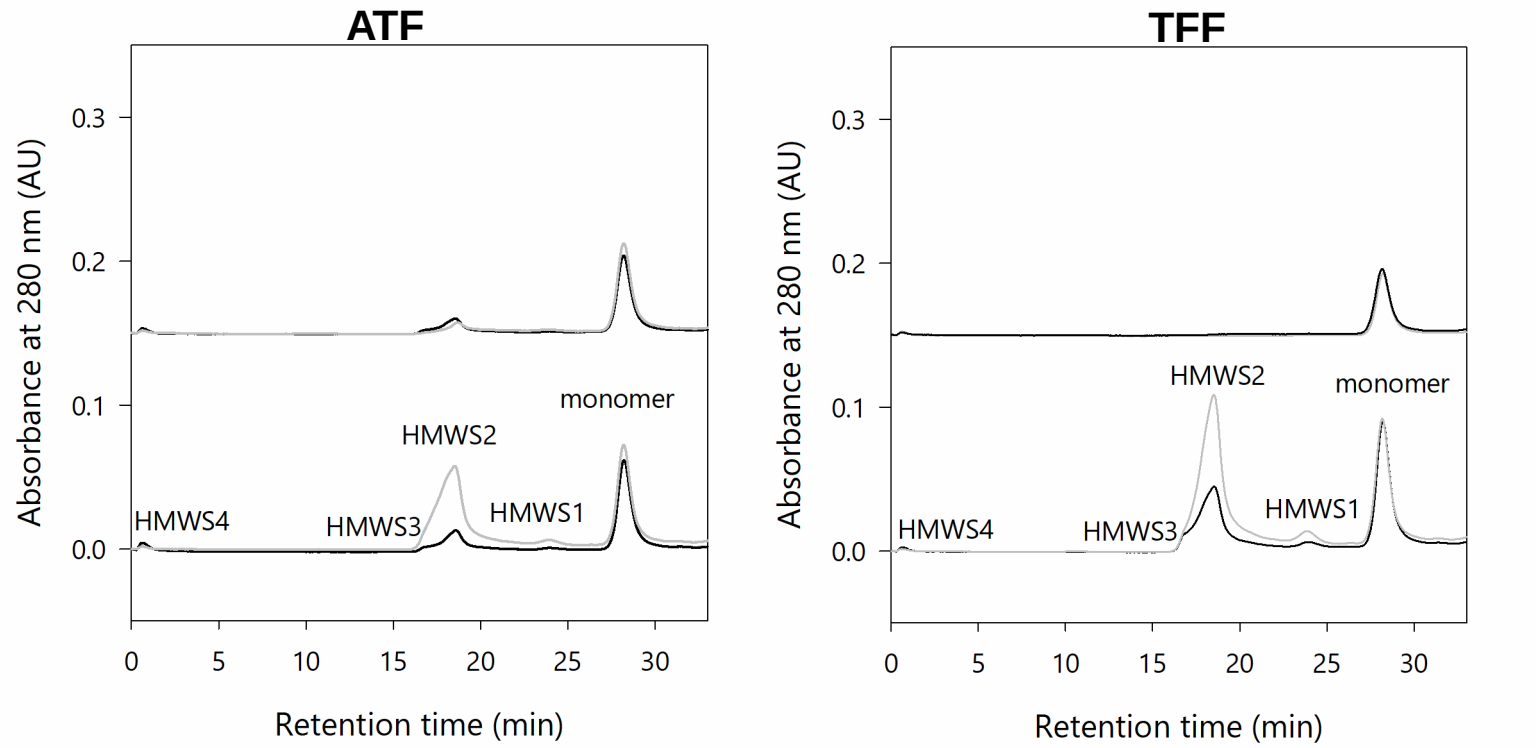

TMP profile dynamic recirculation

REVERSIBLE fouling was the driving factor for product retention

- Hypothesis is that local TMP is continuously changing when applying dynamic recirculation flow rates
- Pulsating TMP influences the deposition of particles
- With dynamic recirculation the deposit layer is loosens and becomes more permeable
- Cell viability was similar in all experiments → amount of cell fragments is the same and not responsible for the difference in sieving profiles