Using the ambr® crossflow system, a UF/DF high throughput screening tool, to predict pilot-scale TFF performance during monoclonal antibody processing

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Session 2: Case studies – Monoclonal antibodies and antibody-like molecules

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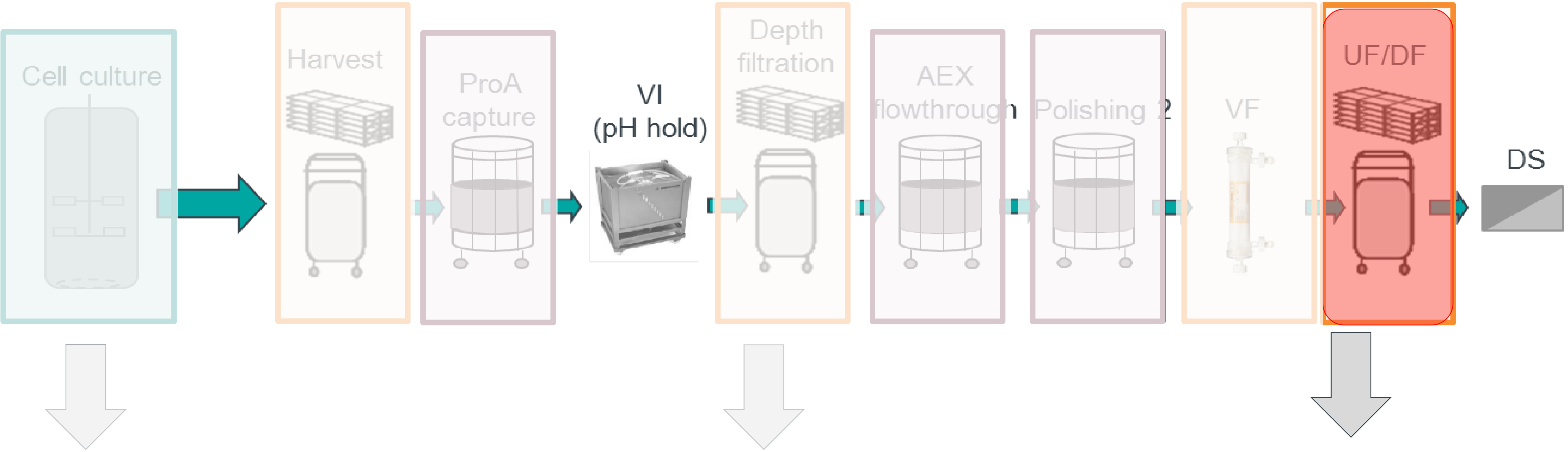
High-throughput systems for biologics process development



- **High throughput scale-down system:** Ambr® 15 and 250 bioreactors
- **Volume requirements:** 15-250 mL per condition
- **Established in literature** (Manahan et al., 2019; Hsu et al., 2012)

- **High throughput scale-down system:** Robocolumns, 4 or 7 mL columns
- **Volume requirements:** 600 µL – 7mL per condition
- **Established in literature** (Evans et al., 2017; Welsh et al., 2014)

- **High throughput scale-down system:** n/a
- **Smallest available format:**

Harvest: e.g. 23 cm^2 for A1HC filter
VF: e.g. 10 cm^2 for Planova
UF/DF: 88 cm^2 for flat-sheet Pellicon

- **Established in literature** (-)

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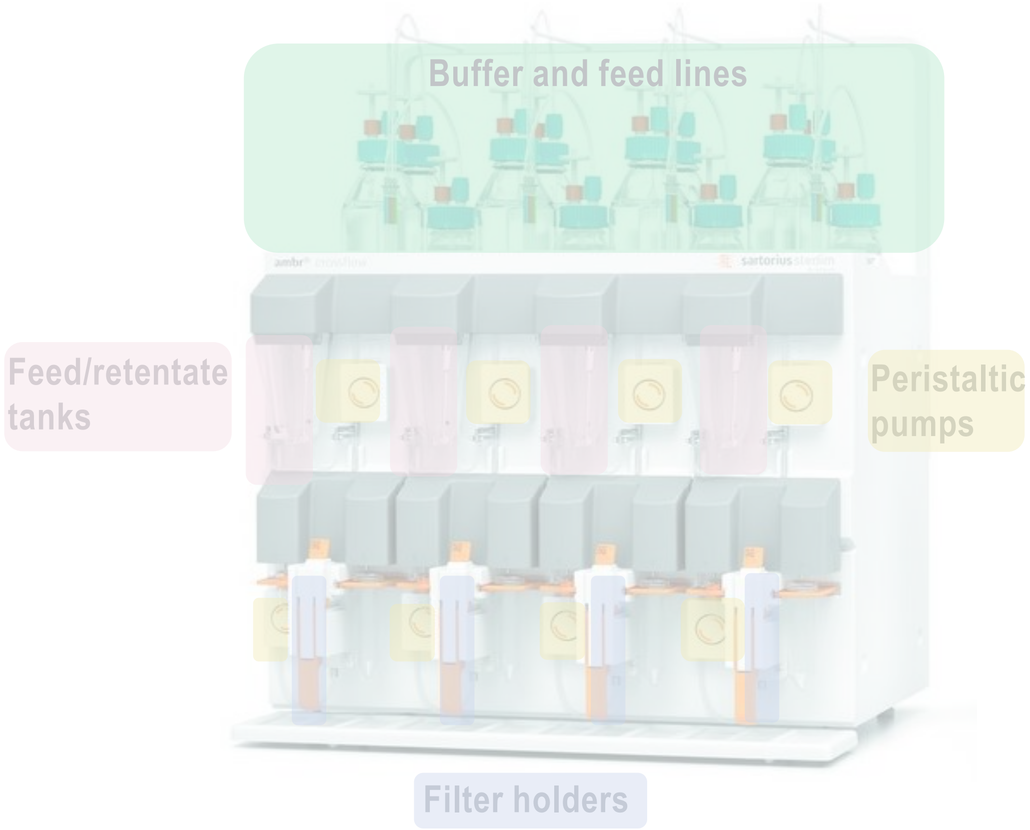
Filling the gap with the *ambr® crossflow:*
a high throughput UF/DF screening tool



System dimensions per channel	
Membrane area (cm^2)	10
System hold-up volume (mL)	3
Minimum final retentate volume (mL)	5
Typical material requirements per channel (mL)	Protein: 0.1 – 1.0 g (~ 100 – 1000 gm^{-2}) Buffer: ~300 mL (Equilibration, diafiltration & product flush) 0.1 M NaOH: ~150 mL (Filter storage & flush lines)

Advantages and limitations of the ambr® crossflow system

Advantages	Limitations
+ Excellent automation + Can run up to 4 conditions at a time (scope up to 16) + Easy data collection + Online data viewing of key parameters (pressures, flux, pH, conductivity, etc) + Flexible recipe method builder	- The four channels are not totally independent for specific commands - Limited filter choice: • Screen: custom-made (no choice of screen) • Composition: Only regenerated cellulose • Pore size: 10 and 30 kDa - Designed for formulation applications using high viscosity solutions • Typical incoming concentration for UF/DF ~ 2 – 10 g/L which has a water-like viscosity characteristic

Part 1: Proof-of-concept screening experiments with ambr ® crossflow

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Screening studies:
using the ambr® crossflow to identify molecule-specific behavior early on

Input screened Parameters:

- Transmembrane pressure
- Loading
- Diafiltration concentration
- Feed composition

Output metrics:

- Membrane performance (permeate flux, J)
- Product quality (Change in high molecular weight, ΔHMW by UP-SEC)

number of UF/DF/UF runs: 11

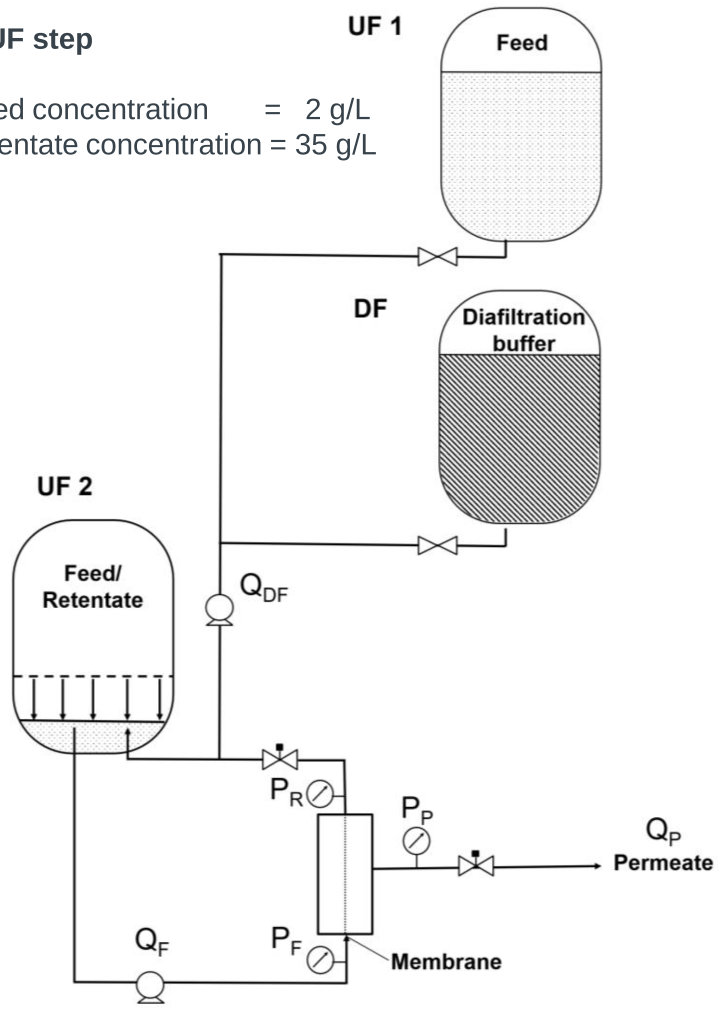
Total protein required: 3.2 g

Total buffer needed: ~1.3 L

Total resources required: ~ 40 hrs

UF/DF/UF step

Initial feed concentration = 2 g/L
Final retentate concentration = 35 g/L



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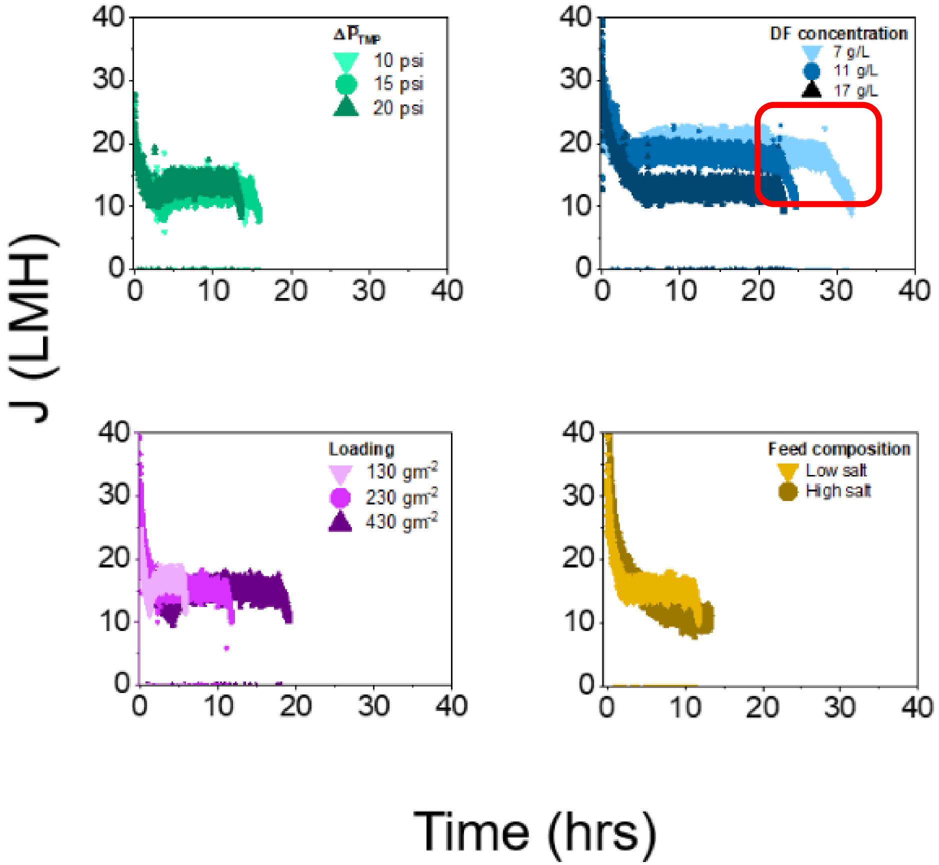
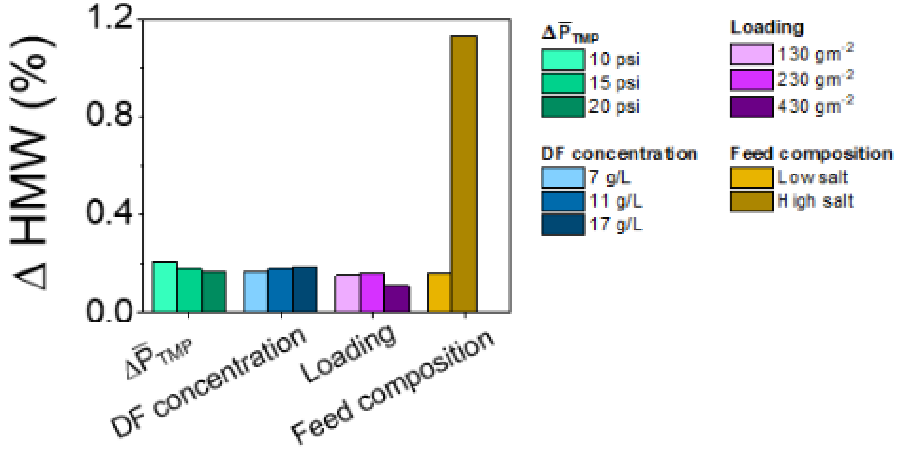
https://doi/10.1002/btpr.2929

Screening studies:
This data can help inform early on process development decisions





- Only loading and DF concentration > 11 g/L significantly impact permeate flux (J)
- Only feed composition affects product quality (% ΔHMW)

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Part 2: How scalable is the ambr® crossflow data?

Scale translation with traditional TFF system

Method: Compare performance between ambr® crossflow and qualified TFF system

Conditions Loading : 230 g of protein per m^2 of membrane area

Q_F : 7 LMM

ΔP_{TMP} : 15 psi

2 g/L (initial) → 25 g/L (diafiltration) → 35 g/L (final) concentration

	TFF	Ambr® crossflow
Area (cm^2)	176 cm^2 (2 x 88cm^2)	10 cm^2
Type of pump (-)	Quaternary Diaphragm Quattroflow™	Peristaltic ambr®-proprietary
System hold-up volume (mL)	50	3
Volume required per run (mL)	2500	140
Volumetric loading (L/m^2)	142	
Scale-down ratio (-)	1:18	

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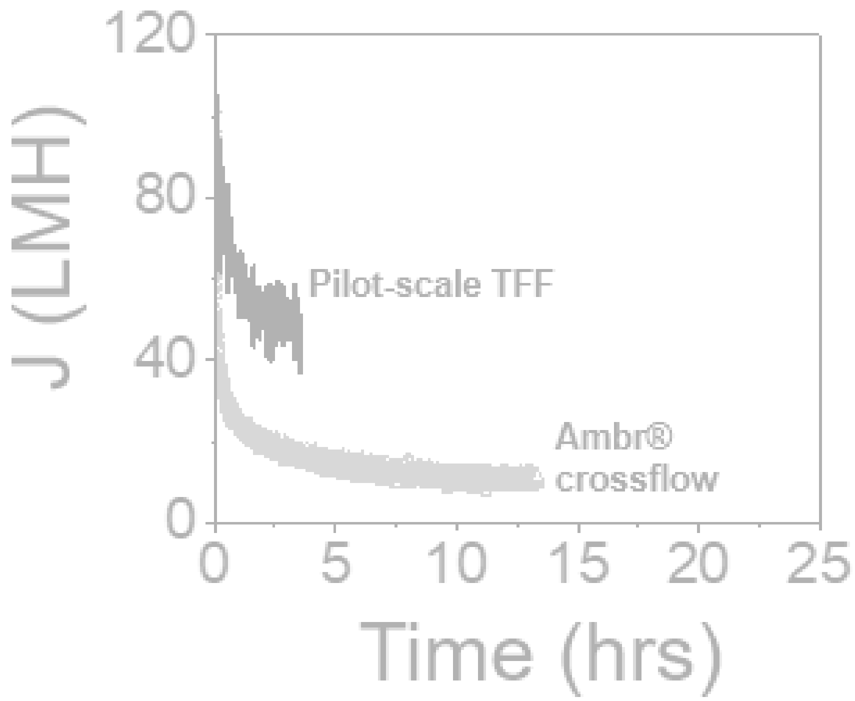
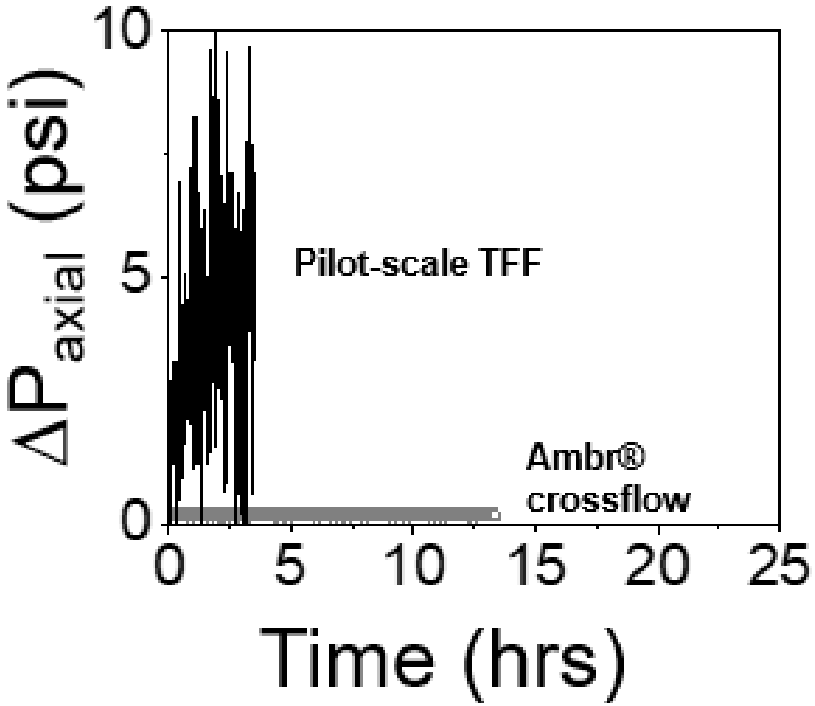
Scale comparison:
There is a mismatch between scales



- Ambr® crossflow data underpredicts flux by 3-fold (t_{total} = 14 vs. 4 hrs)



- Axial pressure drop, ΔP_{axial} ($P_{feed} - P_{retentate}$) does not match either.
  - ✓ It is not a typical scale-down parameter, but it should be within a similar range to obtain comparable membrane performance

230 gm^{-2} , Q_F = 7 LMM, ΔP_{TMP} = 15 psi
Initial feed concentration = 2 g/L
Diafiltration concentration = 25 g/L
Final retentate concentration = 35 g/L

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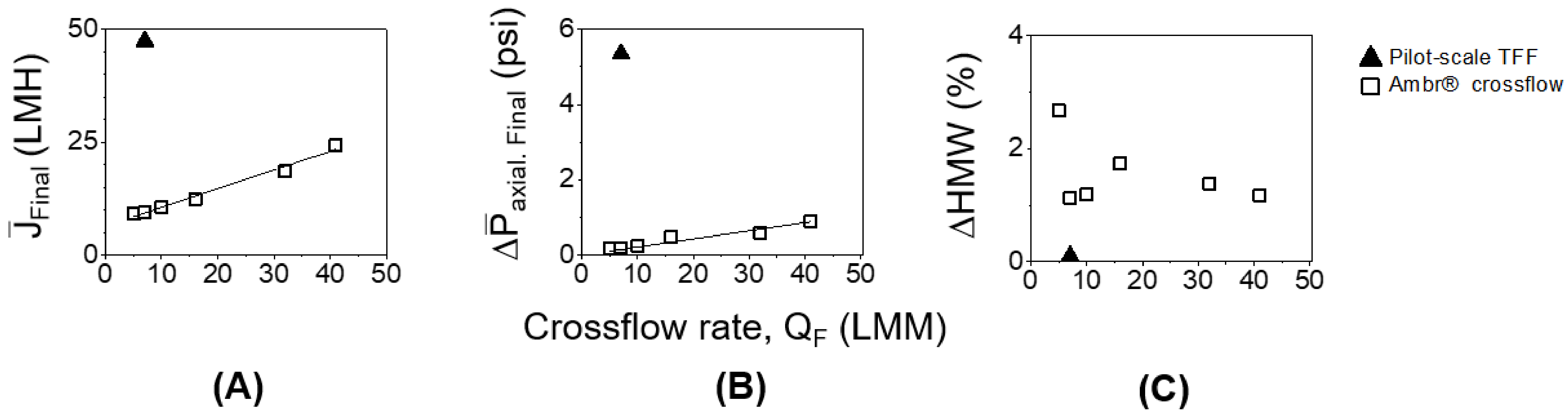
Part 3: Evaluating different strategies to improve the match between scales

Strategy I:
Increase crossflow rate in the ambr® crossflow



- Expected behavior of ambr® crossflow with increasing crossflow rate (5 - 42 LMM)
- However, no progress in match between systems even with the 42 LMM ambr® crossflow run

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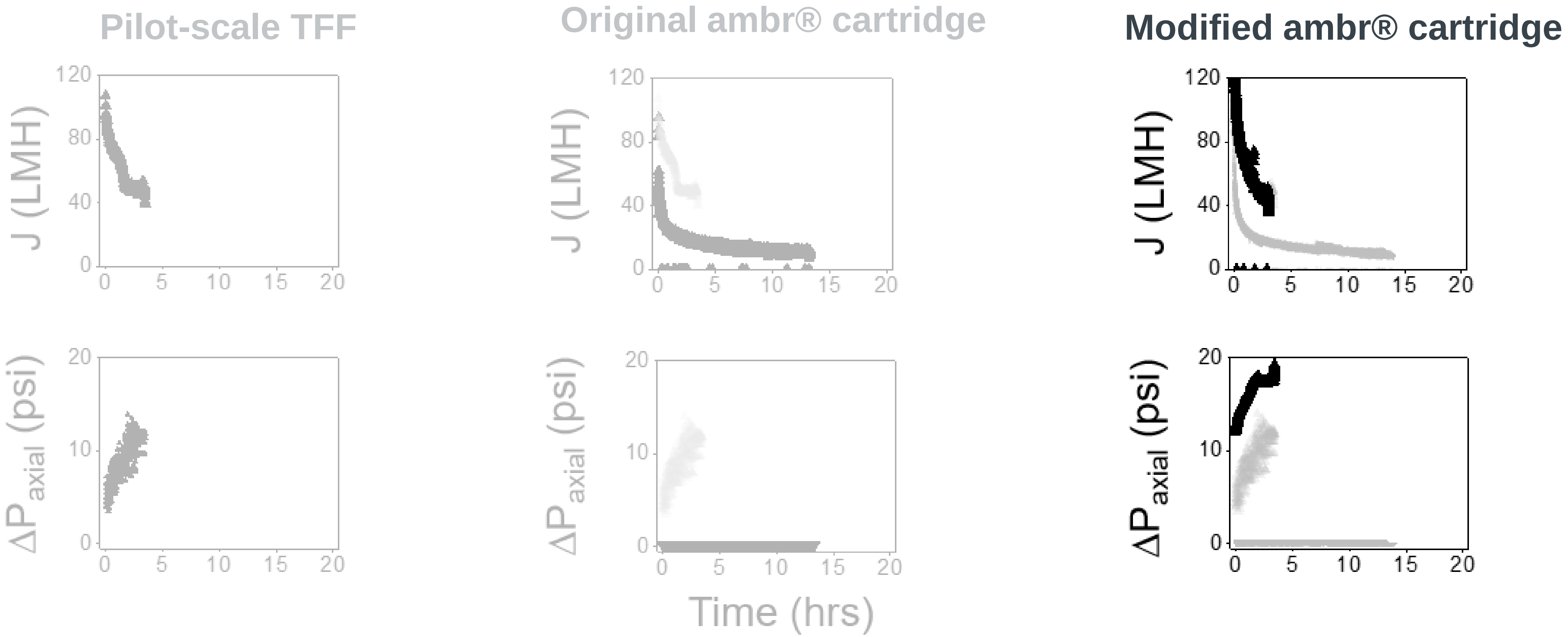
Strategy 2:
Modify the ambr® cartridge to reproduce flat-sheet behavior



ΔHMW (%) +0.10% ΔHMW (%) +1.13% **ΔHMW (%)** +0.06%

- Significant improvement between original ambr® and modified ambr® cartridge
- Match in flux and product quality, and similar range ΔP_{axial}

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Part 4: Testing the modified ambr® cartridge across a typical DoE space

Comparing ambr® crossflow with qualified TFF system

Method: Compare performance between ambr® crossflow and our qualified TFF system

Conditions: Using 2^3 full factorial central composite design (CCD) design of experiments (DoE)

Loading: 100 – 230 – 600 gm^{-2} (+ 823 gm^{-2})

ΔP_{TMP} : 12 – 15 – 18 psi

Crossflow rate: 5 – 7 – 10 LMM

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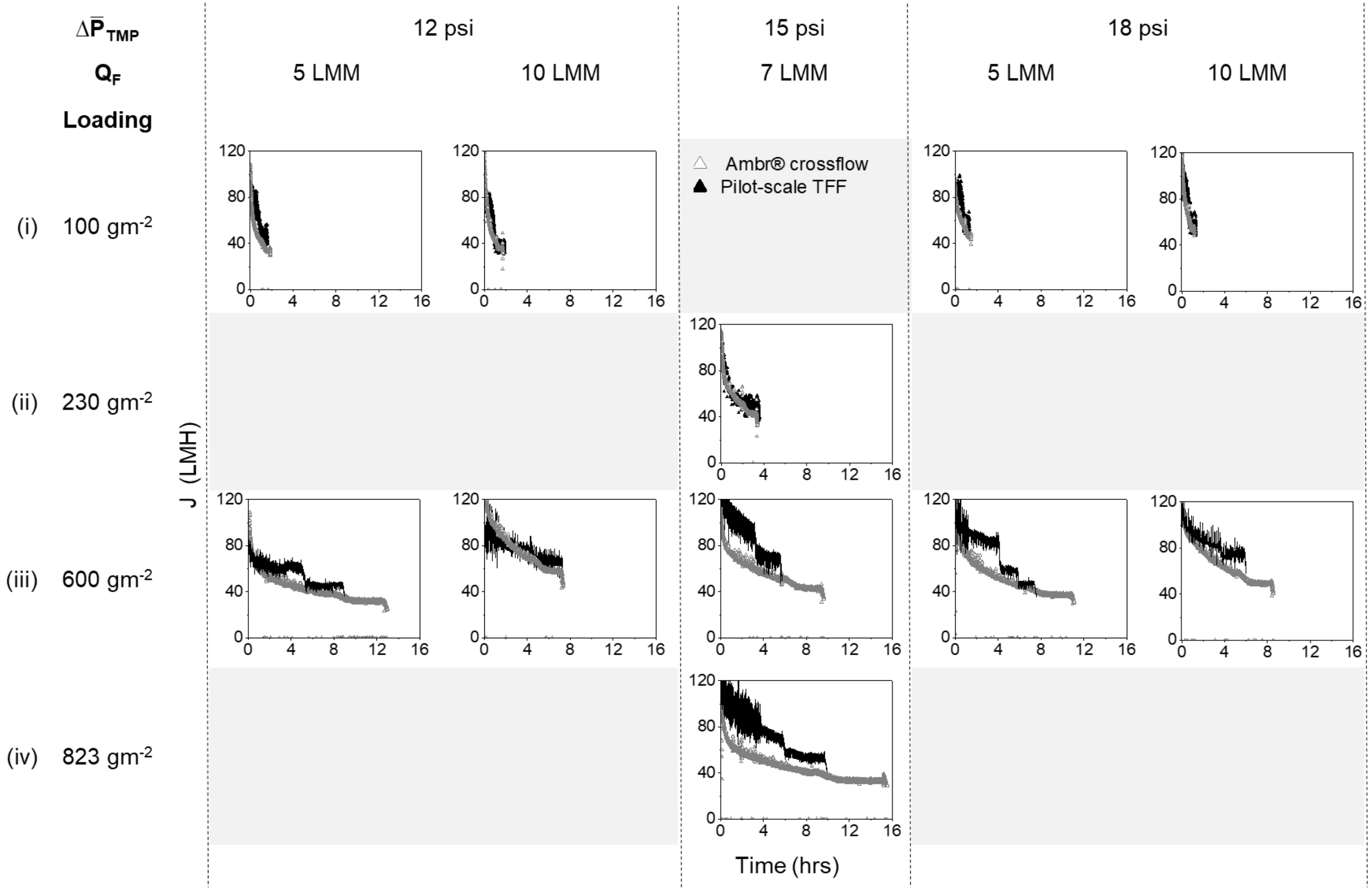
Comparing performance across scales:
Good agreement across the DoE conditions tested



- Increased overall comparability with modified ambr® cartridge runs

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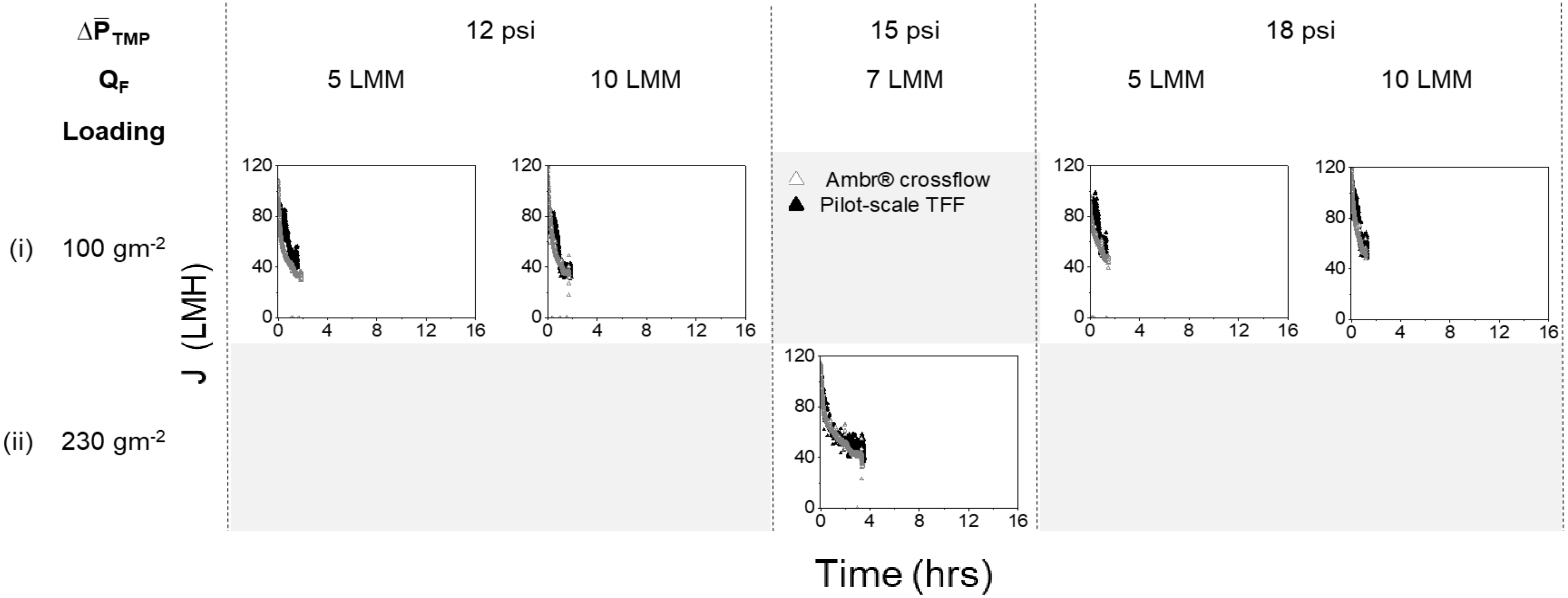
Comparing performance across scales:
Flux matches well at low loadings



- Good agreement at 100 and 230 gm^{-2} conditions. At both scales:
  - ✓ 100 gm^{-2} runs take < 2 hrs
  - ✓ 230 gm^{-2} take ~4 hrs

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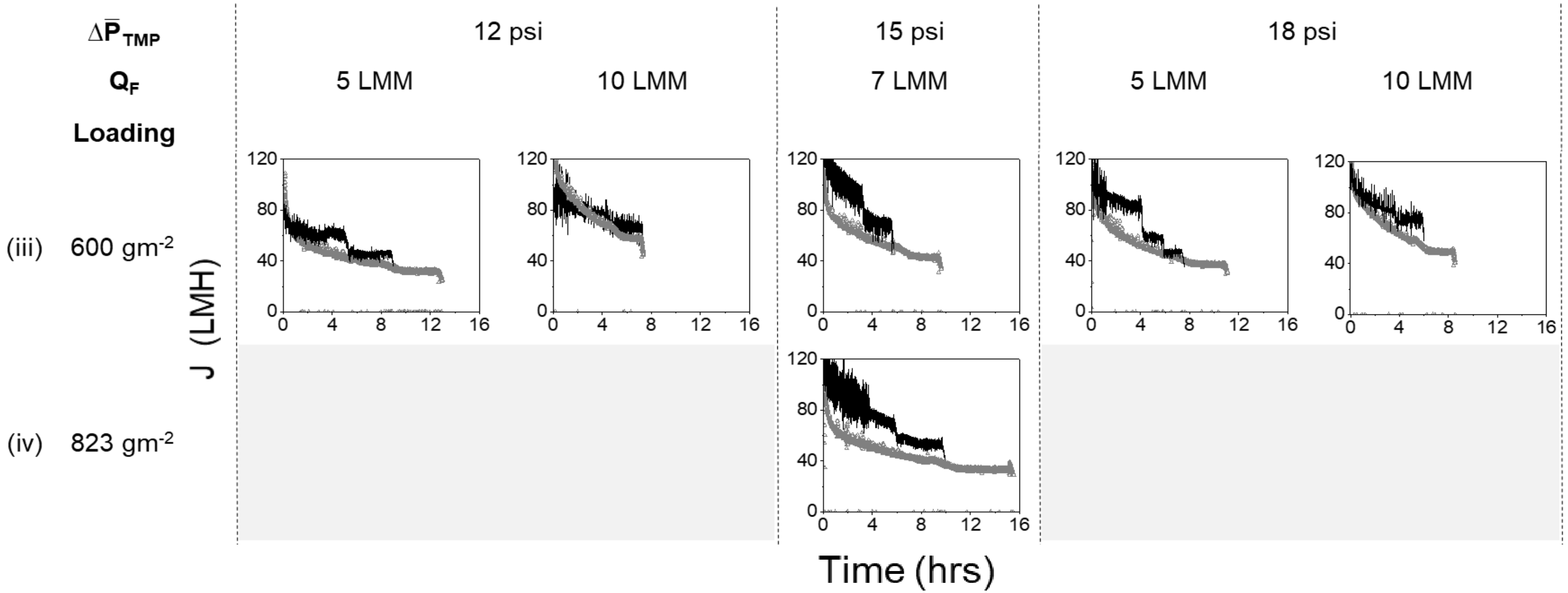
Comparing performance across scales:
Flux starts deviating at higher loadings



- Disparity in flux starts to appear at higher loadings.
- Possible differences that could explain this:
  - ✓ Membrane path length across scales
  - ✓ System set-up (i.e. pump type, system hold-up volume, shear rate)

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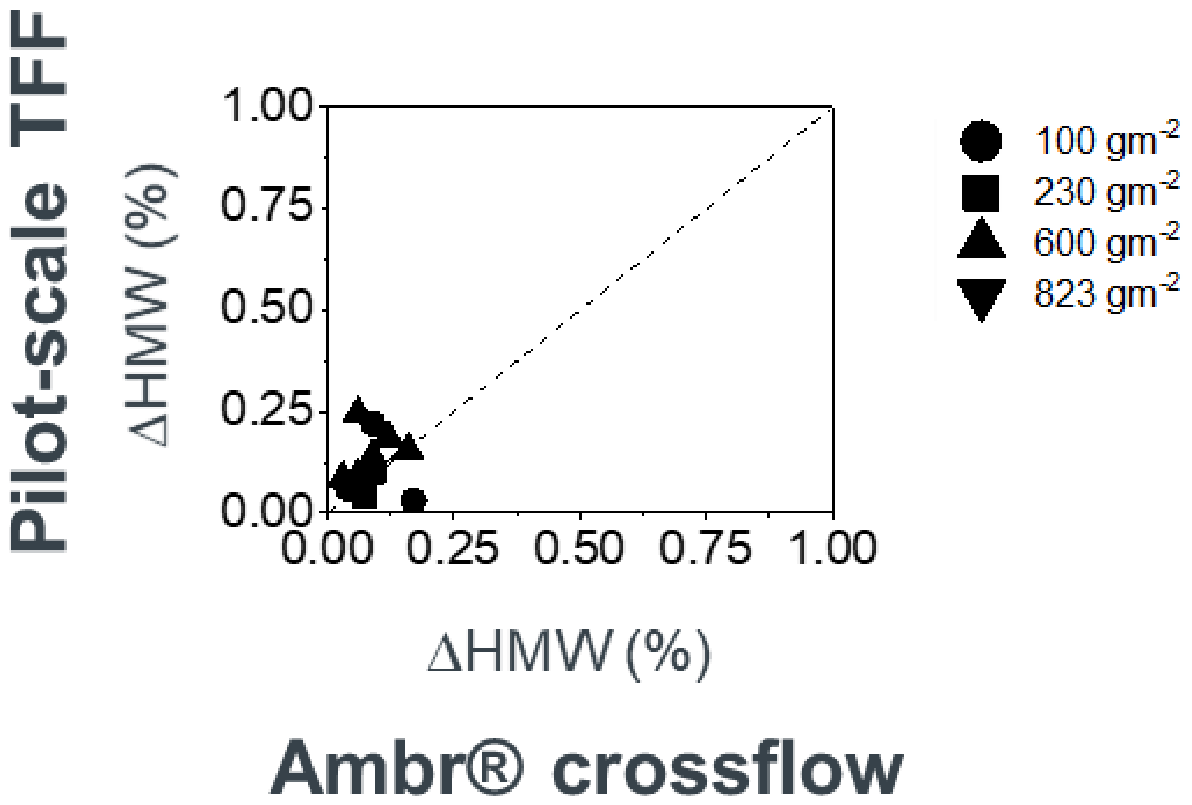
Comparing performance across scales:
Product quality is similar across DoE in both scales



- All conditions at both scales result in < 0.25% ΔHMW (%) during UF/DF/UF step

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Way forward

Summary

1. Proof-of-concept screening ambr® crossflow studies
   - Only a fraction of resources needed

2. Side-by-side comparison with a qualified TFF system
   - Differences in the membrane performance were attributed to the ambr® cartridge configuration.

3. Modification of the ambr® cartridge
   - With a view to better reproduce the flat-sheet cassette performance

4. Comparable product quality and fluxes across DoE study at both scales
   - Better agreement found at low loadings

What's next?

- Work towards establishing ambr® crossflow system as a process characterization tool by:

    ✓ Qualify as scale-down model by investigating key outputs – HMW (%), flux, and/or yield?

    ✓ Evaluating additional mAb molecules

- Work with vendor to develop manufacturing capabilities of different ambr® cartridges to suit a wider range of bioprocessing applications

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THANK YOU.

QUESTIONS?

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