

# Investigations in virus filter process performance at different positions in the downstream process

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# Content



INTRODUCTION



MABS



MASKED MABS



CONCLUSIONS

# Breakthrough Solutions for Unmet Needs in Oncology

*Making hope real for patients and their families*



## *Our Vision*

Create **meaningful advances in cancer care** that break through the limitations of current treatments to turn hope into reality for patients

## *Our Mission*

Deliver a **new generation of antibody-drug conjugates & antibody cancer therapeutics** that address significant unmet needs for patients

## *Our Strategy*

Leverage our **fully-integrated discovery, development and manufacturing capabilities** to efficiently advance our proprietary pipeline of novel antibody-drug conjugates and antibody cancer therapeutics that deliver **selectivity** and **diversity** for more effective treatment options.

# A Diverse Pipeline of Proprietary Next-Generation ADCs and mAb Therapeutics


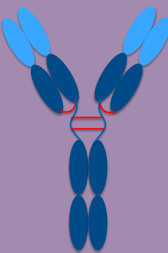
CANDIDATES/ PLATFORMS	MODALITIES	TARGETS	INDICATIONS	DISCOVERY	PRECLINICAL	CLINICAL
BYON4228	Antibody	SIRP $\alpha$	Oncology & Heme Malignancies	[Progress bar spanning Discovery, Preclinical, and Clinical phases]		
BYON4413	ADC	CD123	Heme Malignancies	[Progress bar spanning Discovery, Preclinical, and Clinical phases]		
Platform: Masked Therapeutics	Antibodies/ADCs	undisclosed	Oncology	[Progress bar spanning Discovery and Preclinical phases]		
Platform: Novel Immune-Stimulating ADCs	ADCs	undisclosed	Oncology	[Progress bar spanning Discovery and Preclinical phases]		
Platform: Novel Cytotoxic ADCs	ADCs	undisclosed	Oncology & Immunology	[Progress bar spanning Discovery and Preclinical phases]		
Novel Platforms and Targeting Modalities	undisclosed	undisclosed	Oncology & Immunology	[Progress bar in Discovery phase]		



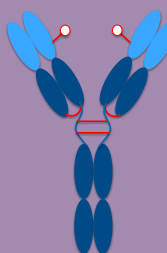
# Introduction

# Overview of the Byondis ADC toolbox

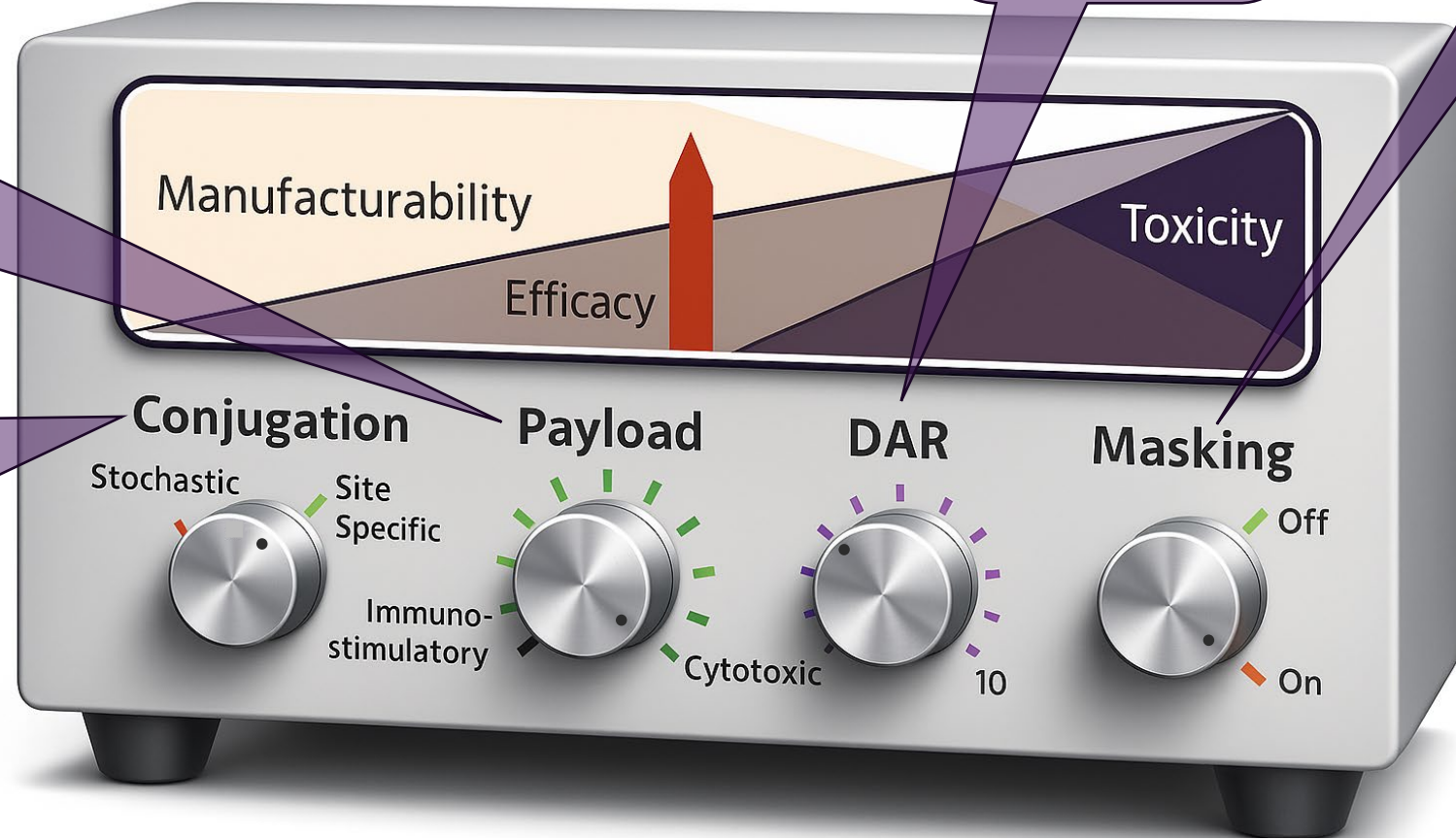
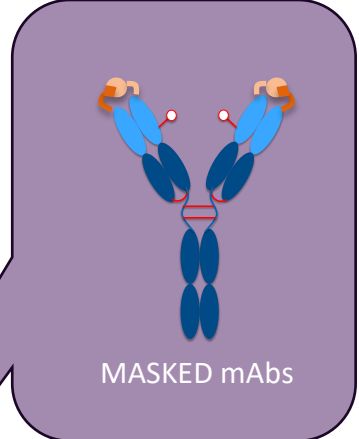
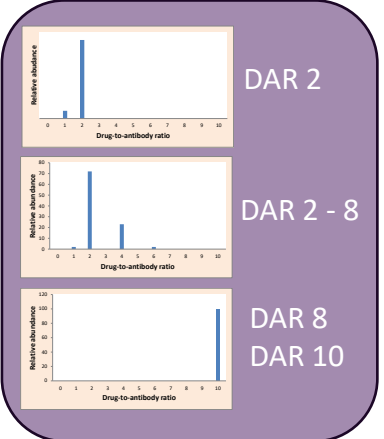
Duocarmycin (SYD980)  
 Drug B  
 Drug C  
 Drug X  
 Drug Z

Random (ByonNative®)



Site-specific (ByonShieLD®)



# The current situation functions, but is suboptimal

## Planova 15N used

- +: very high virus removal, no breakthrough
- -: low maximum load, long process duration, Gold Particle Test (GPT)

## Challenge:

- USP titers increase → process fit decreases as large filter area needed

## GOAL:

- Evaluate other Planova filters on process fit and viral clearance capability

# Other filters available

## 1. 20N

- + : Industry standard, proven concept
- : GPT, suitability higher mAb concentrations unknown

## 2. BioEX

- + : Fit for higher mAb concentrations, no GPT
- : PVDF (in our experience)

## 3. S20N

- + : Fit for higher mAb concentrations, higher flux, no GPT
- : New filter, unknown behavior

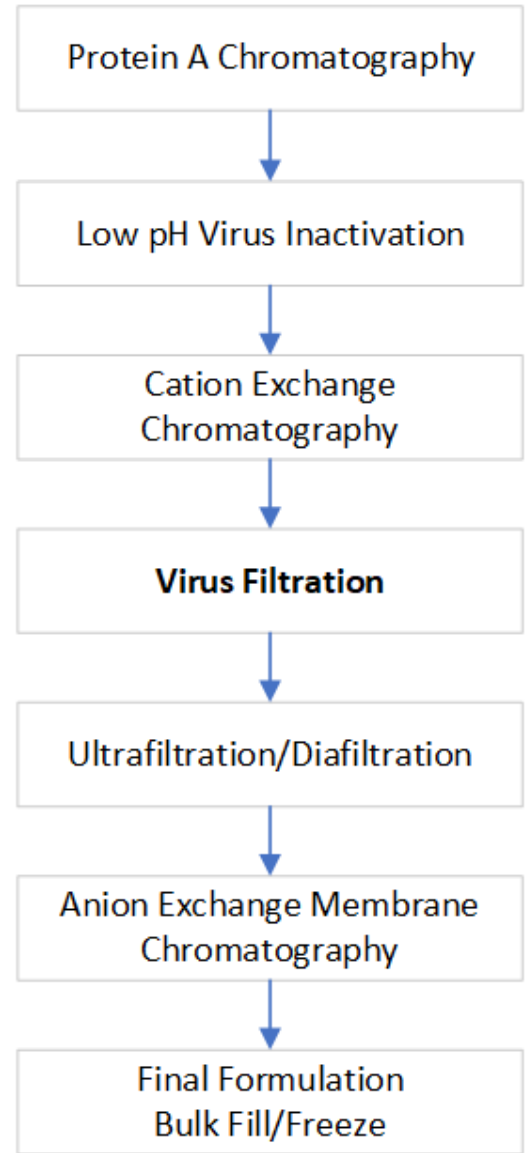
## 4. FG-1

- + : Fit for higher mAb concentrations, higher flux, no GPT
- : New filter, unknown behavior, but PES (+ in our experience)



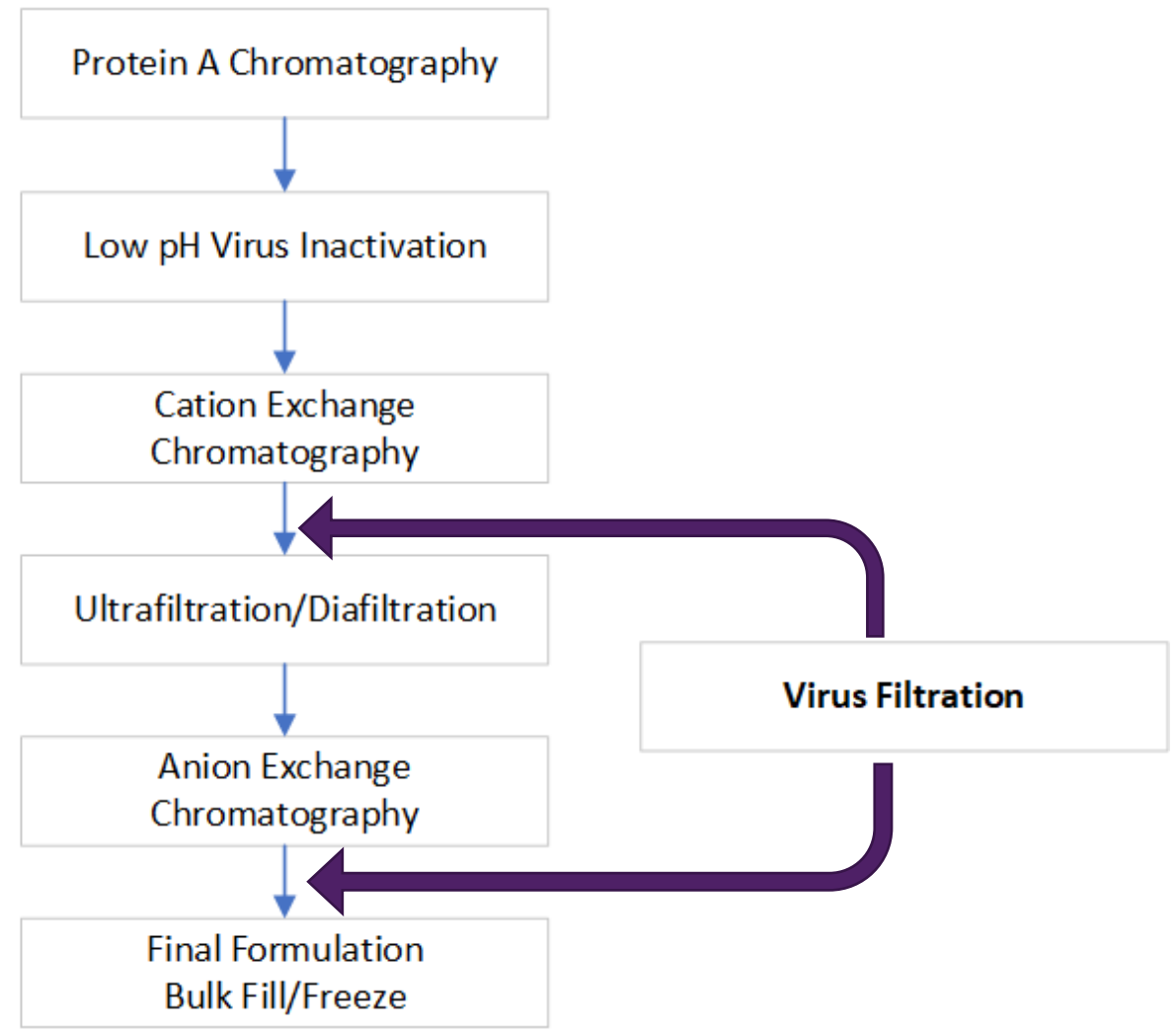


## Current platform



15N filter used

## Alternative approach



## The mAb process



# VF position @Byondis

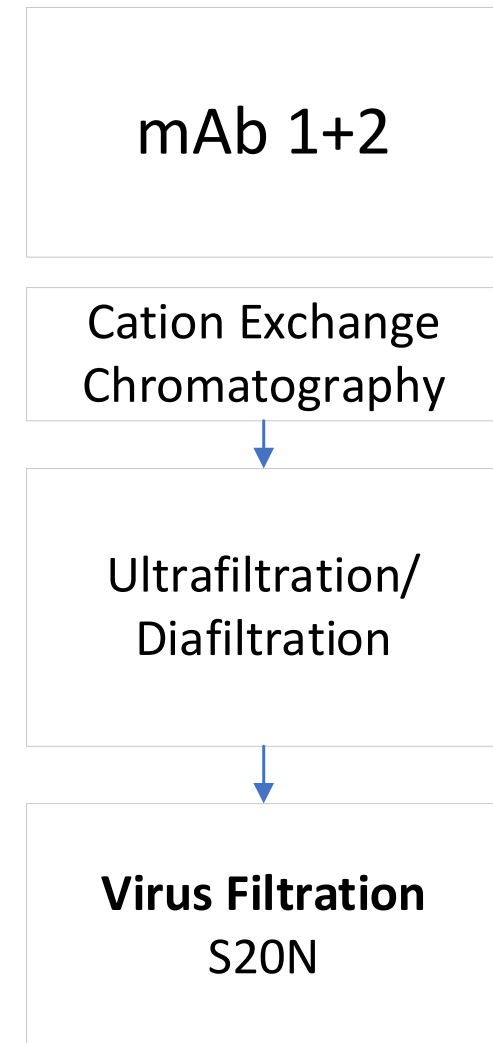
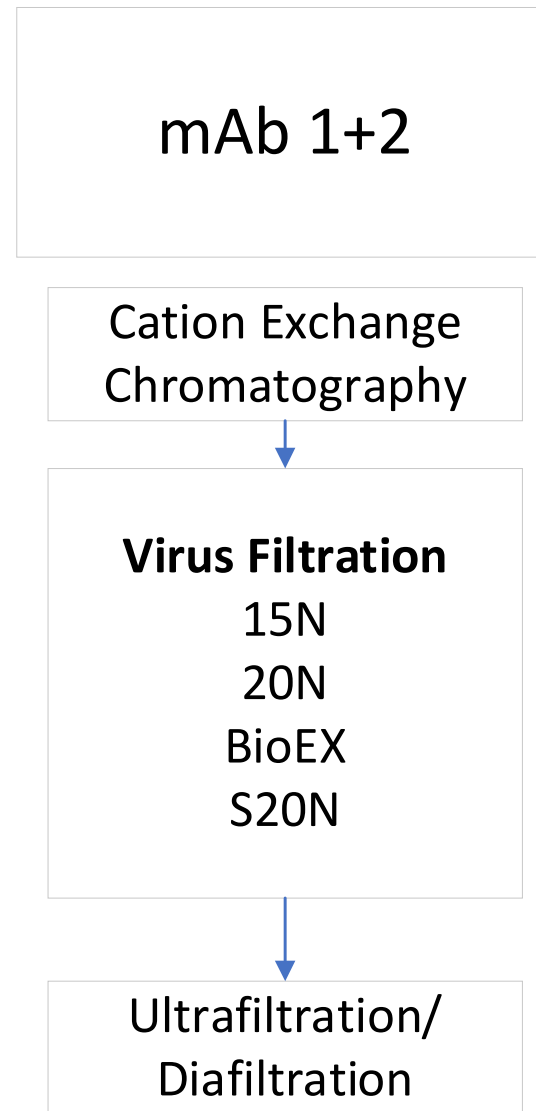
		Advantages	Disadvantages
Before UF/DF	Process	<ul style="list-style-type: none"> <li>• Current process fit</li> <li>• Knowledge standard mAb process</li> <li>• Lower [mAb] → VF flux</li> </ul>	<ul style="list-style-type: none"> <li>• Large volumes → longer duration?</li> <li>• Variation in [mAb] → more process variation</li> <li>• Increase mAb amount and concentration</li> </ul>
	Compliance	-	<ul style="list-style-type: none"> <li>• Suboptimal viral segregation</li> </ul>
After UF/DF	Process	<ul style="list-style-type: none"> <li>• Less variation in [mAb] → 'Fixed' volume</li> <li>• Low volumes</li> </ul>	<ul style="list-style-type: none"> <li>• Higher [mAb] → VF flux</li> <li>• Impact excipients</li> </ul>
	Compliance	<ul style="list-style-type: none"> <li>• Adventitious virus removal/Segregation</li> <li>• Smaller bags/equipment</li> <li>• Dedicated space for Final Formulation/Fill</li> </ul> <ul style="list-style-type: none"> <li>• New virus filter needed → better performance</li> </ul>	<ul style="list-style-type: none"> <li>• New virus filter needed → new Critical Material Attributes</li> </ul>



# Virus filtration of mAbs

# Study set-up

1. Filtration conditions before UFDF
  - NaOAc + 200-300 mM NaCl pH 4.5
  - 12-16 mg/mL mAb
2. Filtration conditions after UFDF
  - His + Trehalose pH 5.5-6.0
  - 30 mg/mL mAb



# Why the need to study frozen/fresh material?

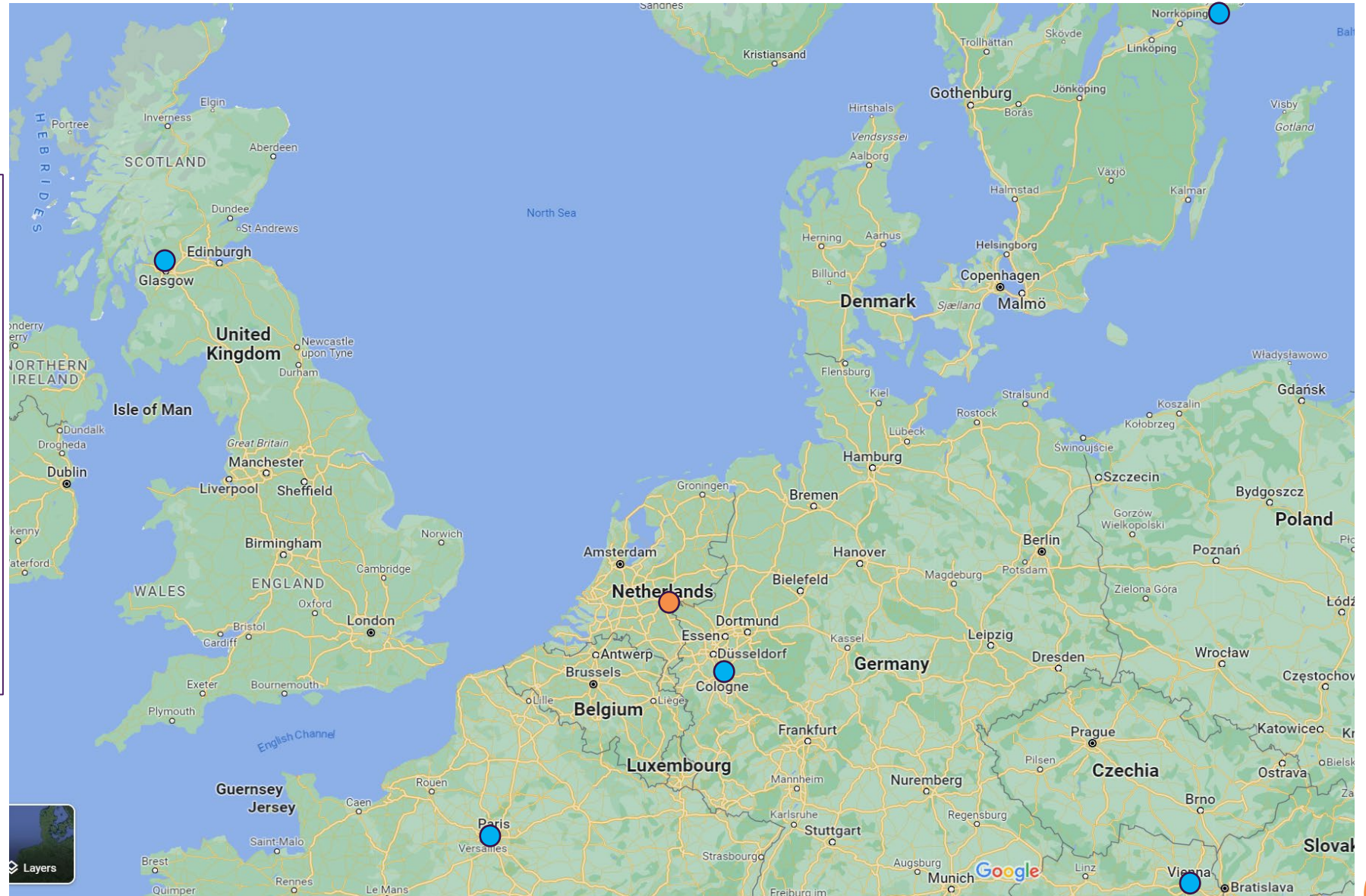
Byondis has no VC test lab

- CRO needed
- Transport is an issue
- Ease of frozen samples transport

Frozen samples

Experience with blocked VF filters

mAb dependent



# Testing of 15N, (S)20N, BioEX – Frozen vs non-frozen

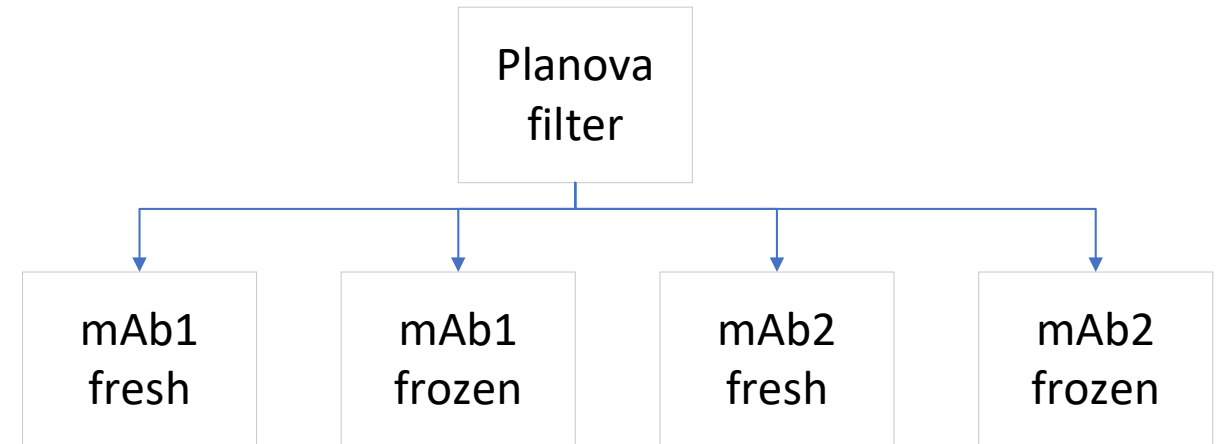
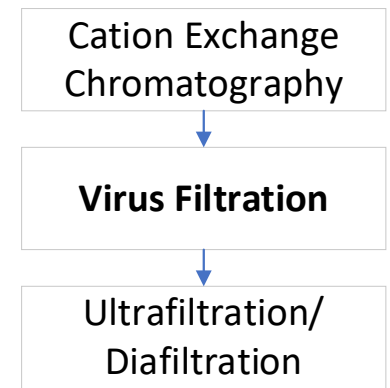
# Filtration set-up

## Set-up

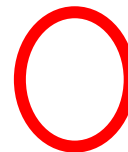
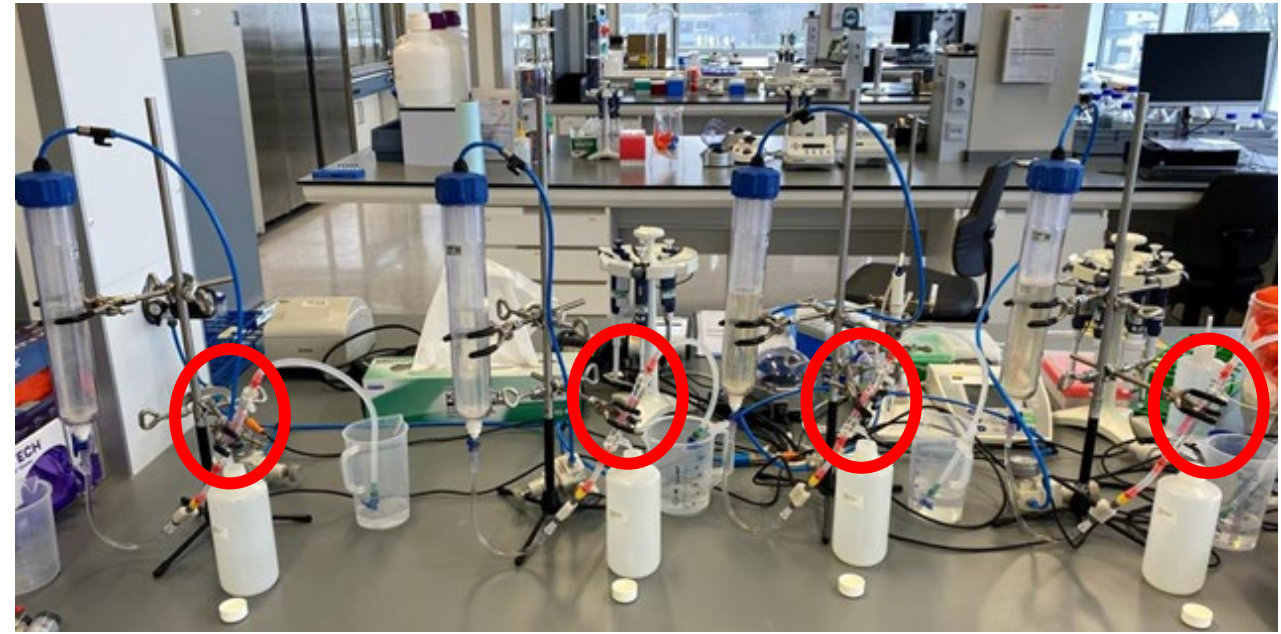
- 4 filtrations at once for each filter
- 0.2  $\mu\text{m}$  pre-filter used

## Goals

1. Assess Process Performance
  - Flux
  - Maximum load
2. Test Viral Clearance
  - Maximum load
  - Pressure breaks



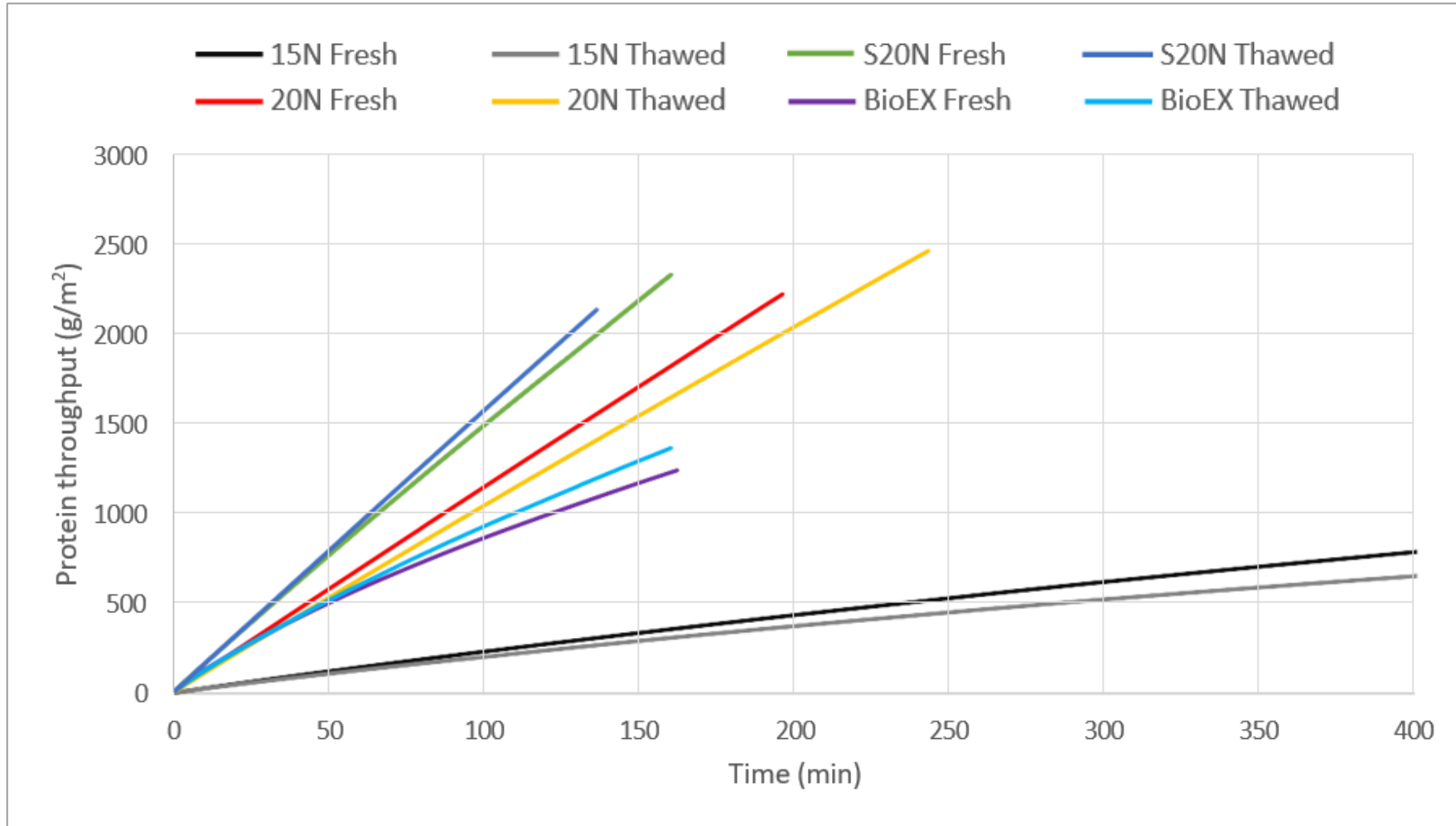
# Asahi Planova Filtration set-up



Planova filter location

# Results – Mass throughput mAb1

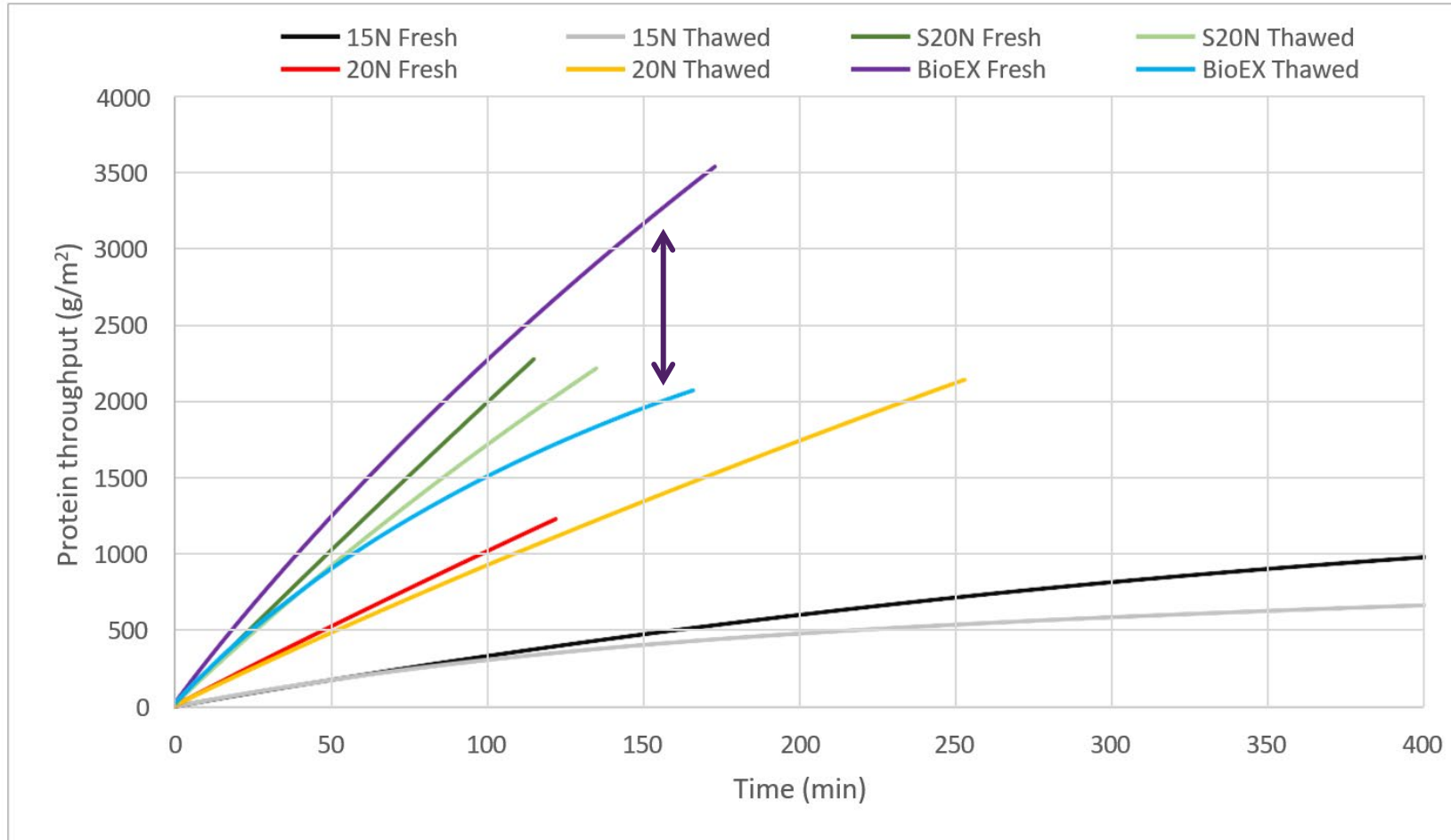
- High loads reached for **S20N, 20N, BioEX**
- **S20N>20N>BioEX>15N**



Filter	TMP (kPa)
15N	75
20N	98
BioEX	343
S20N	216

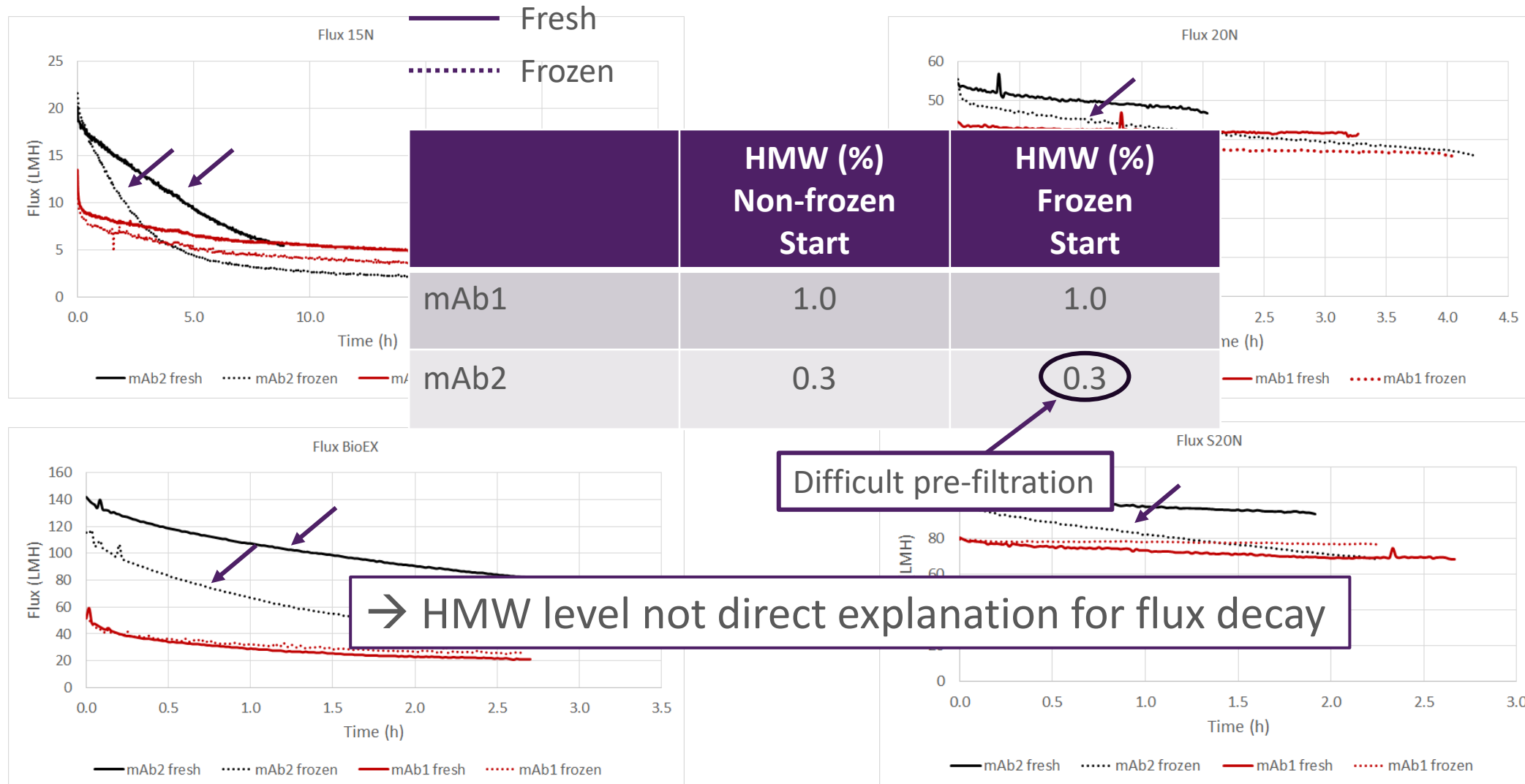
# Results – Mass throughput mAb2

- High loads reached for fresh **BioEX** and **S20N**
- BioEX=S20N>20N>15N
- Non-Frozen > Frozen (not attributable to HMW)

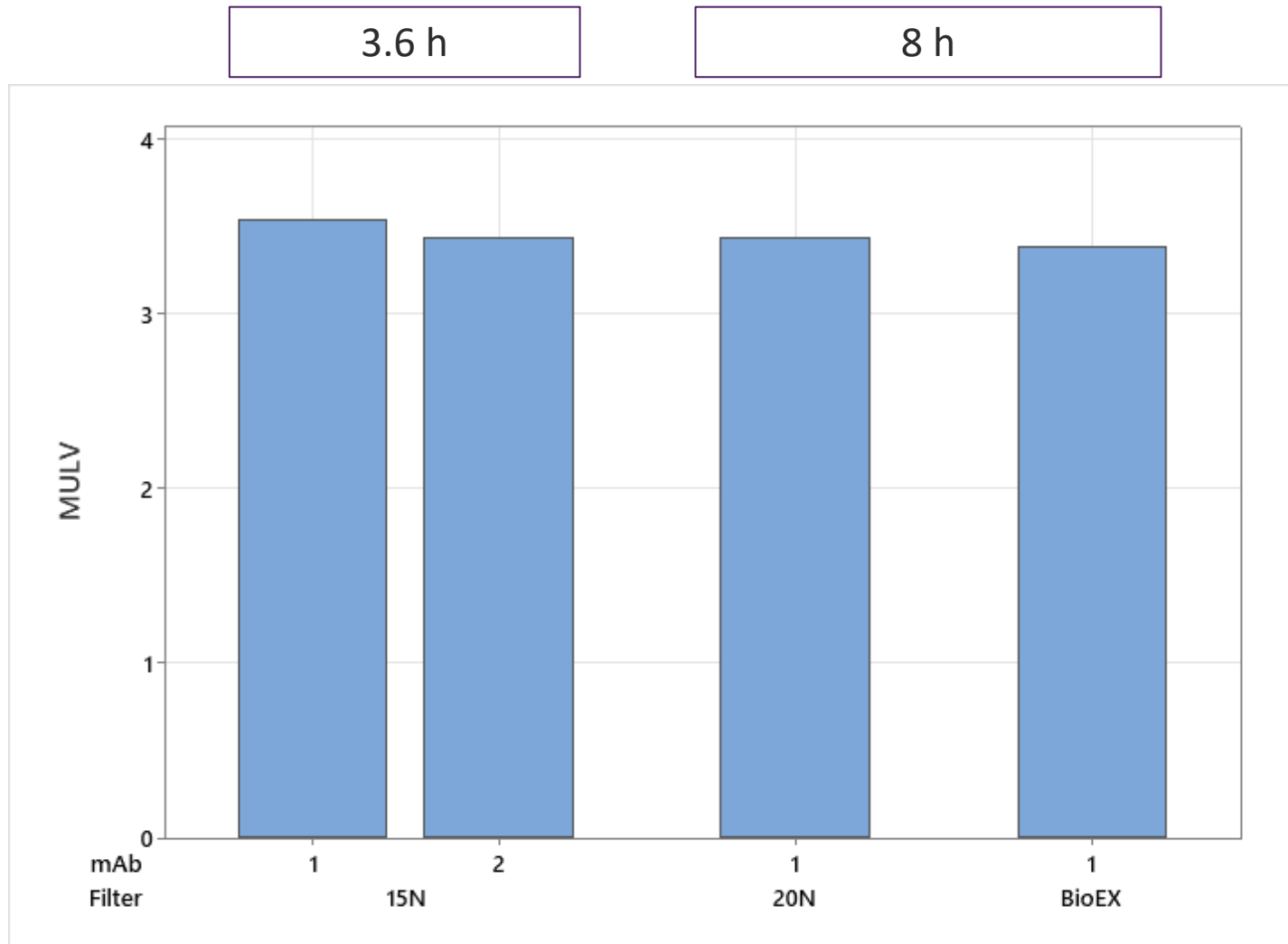


Filter	TMP (kPa)
15N	75
20N	98
BioEX	343
S20N	216

# Results – Flux decay observed for mAb2, frozen material, BioEx



# Viral Clearance – MuLV + mAb1 used (non-frozen)



## Loads reached:

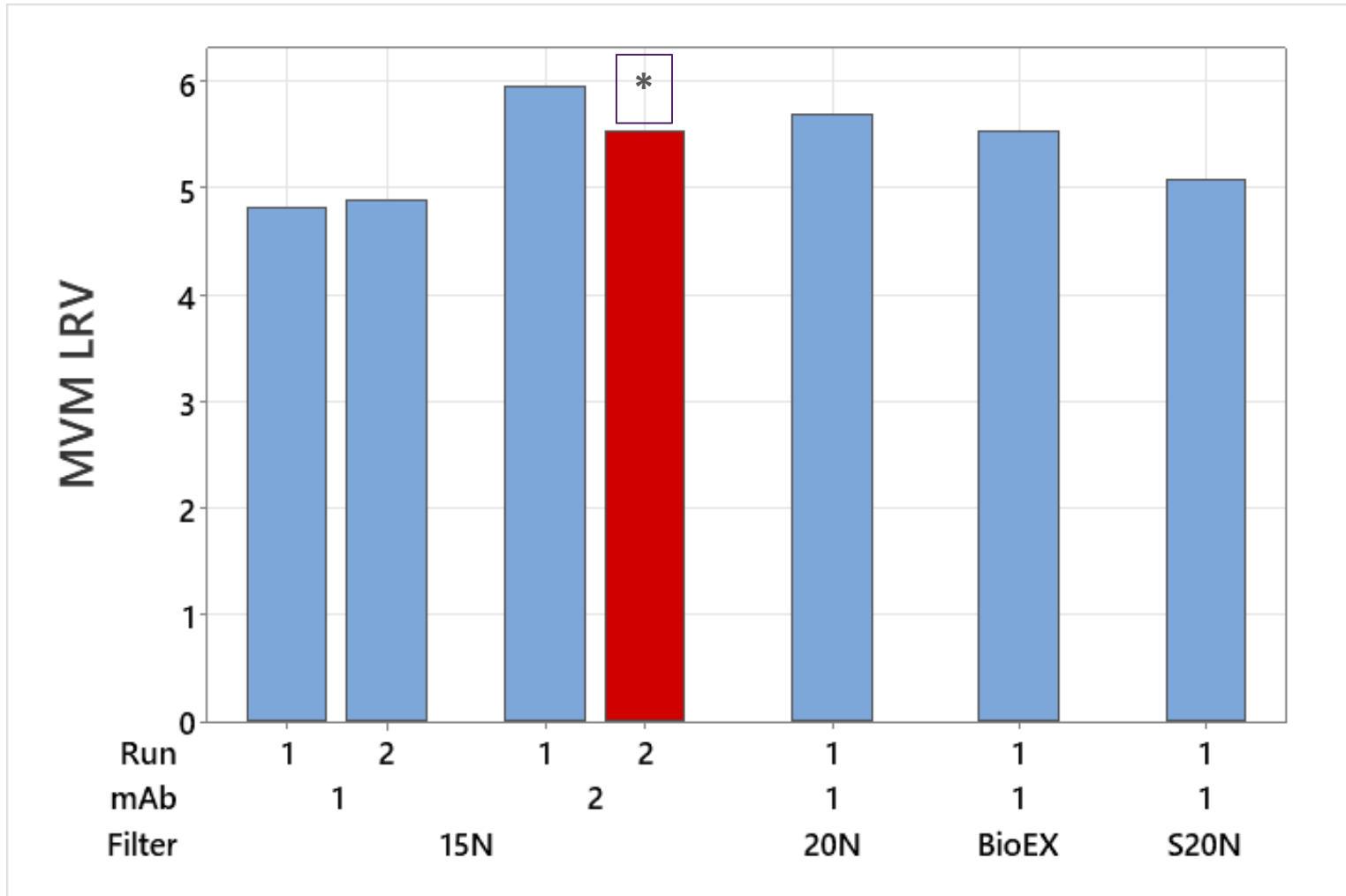
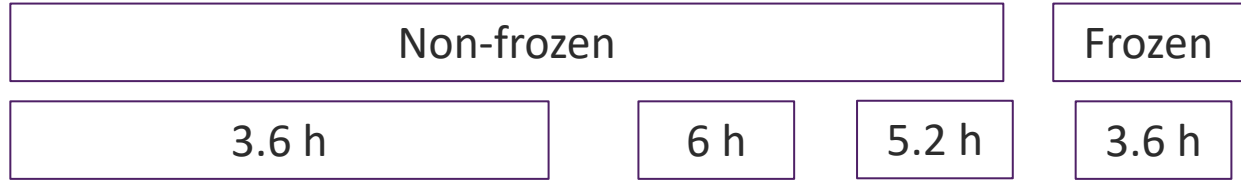
- 15N: 600 g/m<sup>2</sup>
- 20N: 4300 g/m<sup>2</sup>
- BioEX: 3300 g/m<sup>2</sup>

## MuLV LRV:

pH 4.5 during VF

→ MuLV inactivation affects LRV VF

# Viral Clearance – Comparison filters **before** UF/DF – MVM – mAb1 + mAb2



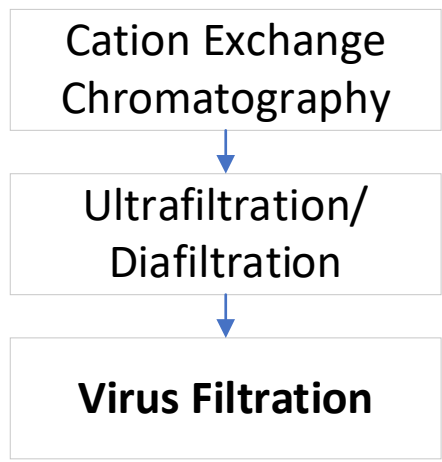
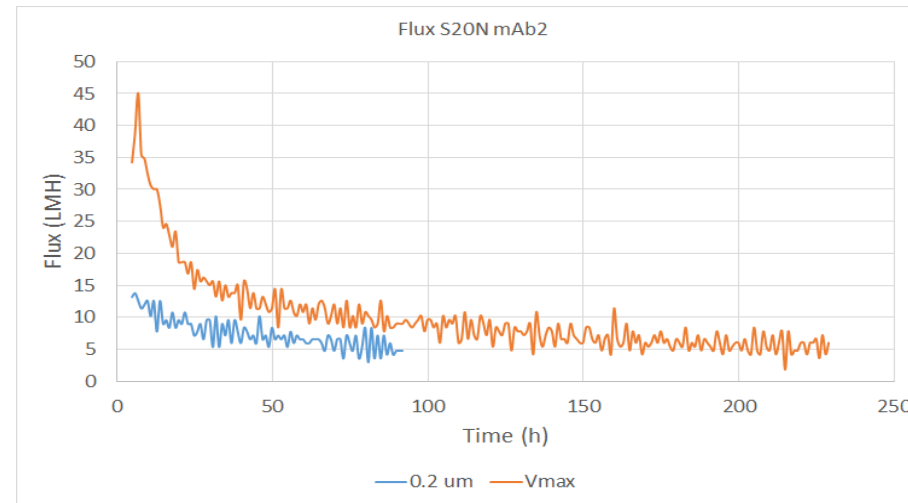
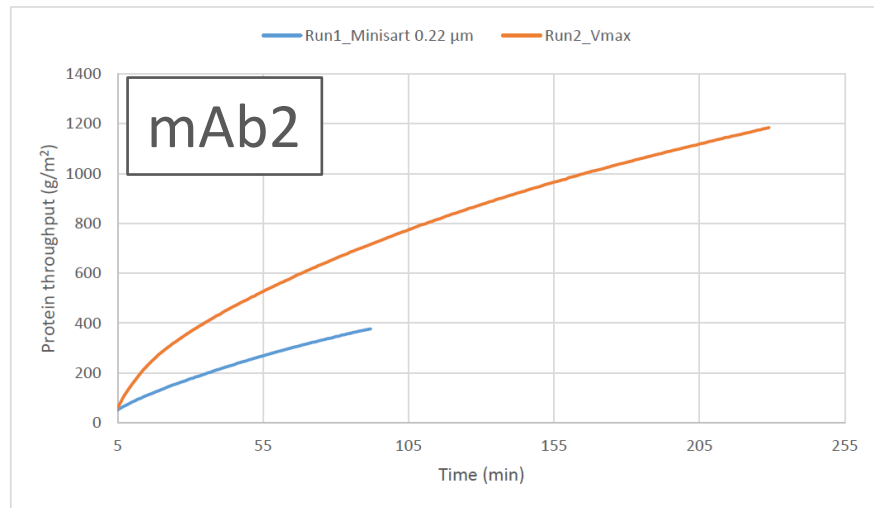
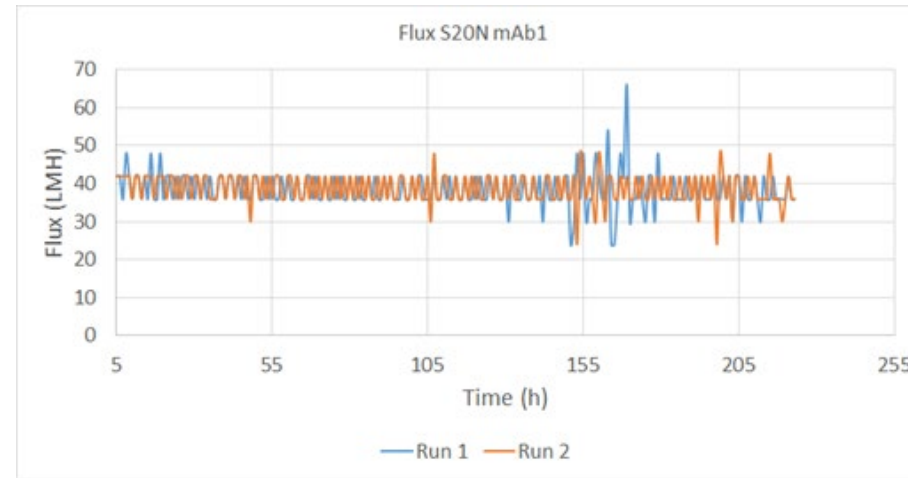
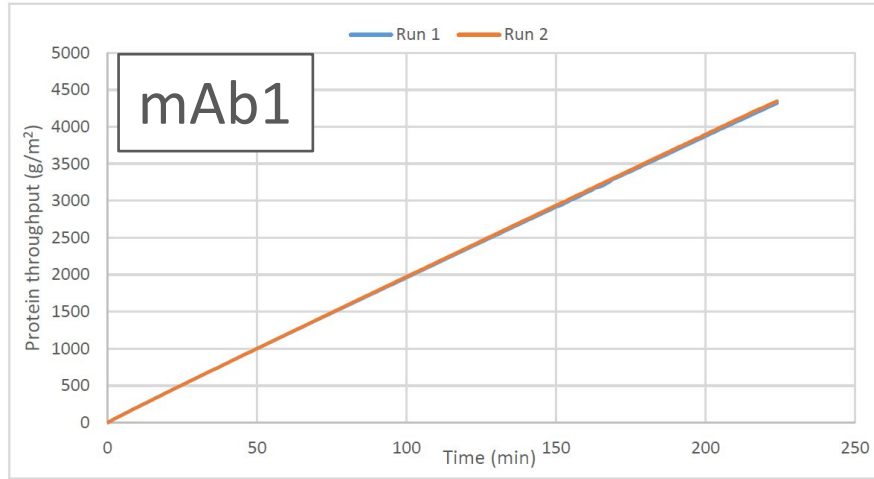
- Loads reached:**
- 15N: 600 g/m<sup>2</sup>
  - 20N: 4300 g/m<sup>2</sup>
  - BioEX: 3300 g/m<sup>2</sup>
  - S20N: 4400 g/m<sup>2</sup>

\*: Breakthrough



# Virus Filtration at end of mAb process

# S20N – mAb2 again shows flux decay



# VC study using S20N and frozen mAb1

## mAb concentration:

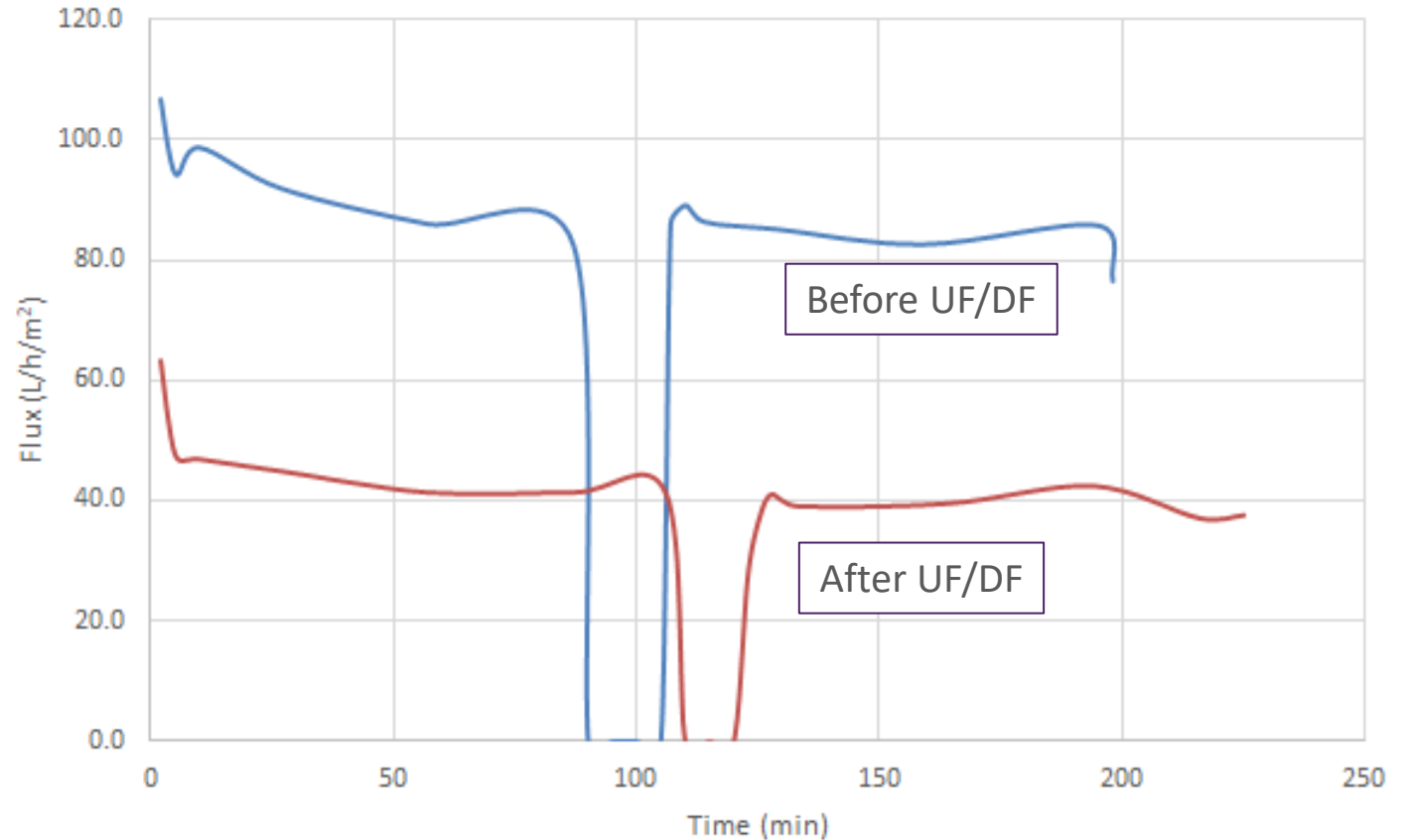
- Before UF/DF: 16 mg/mL
- After UF/DF: 30 mg/mL

## Results:

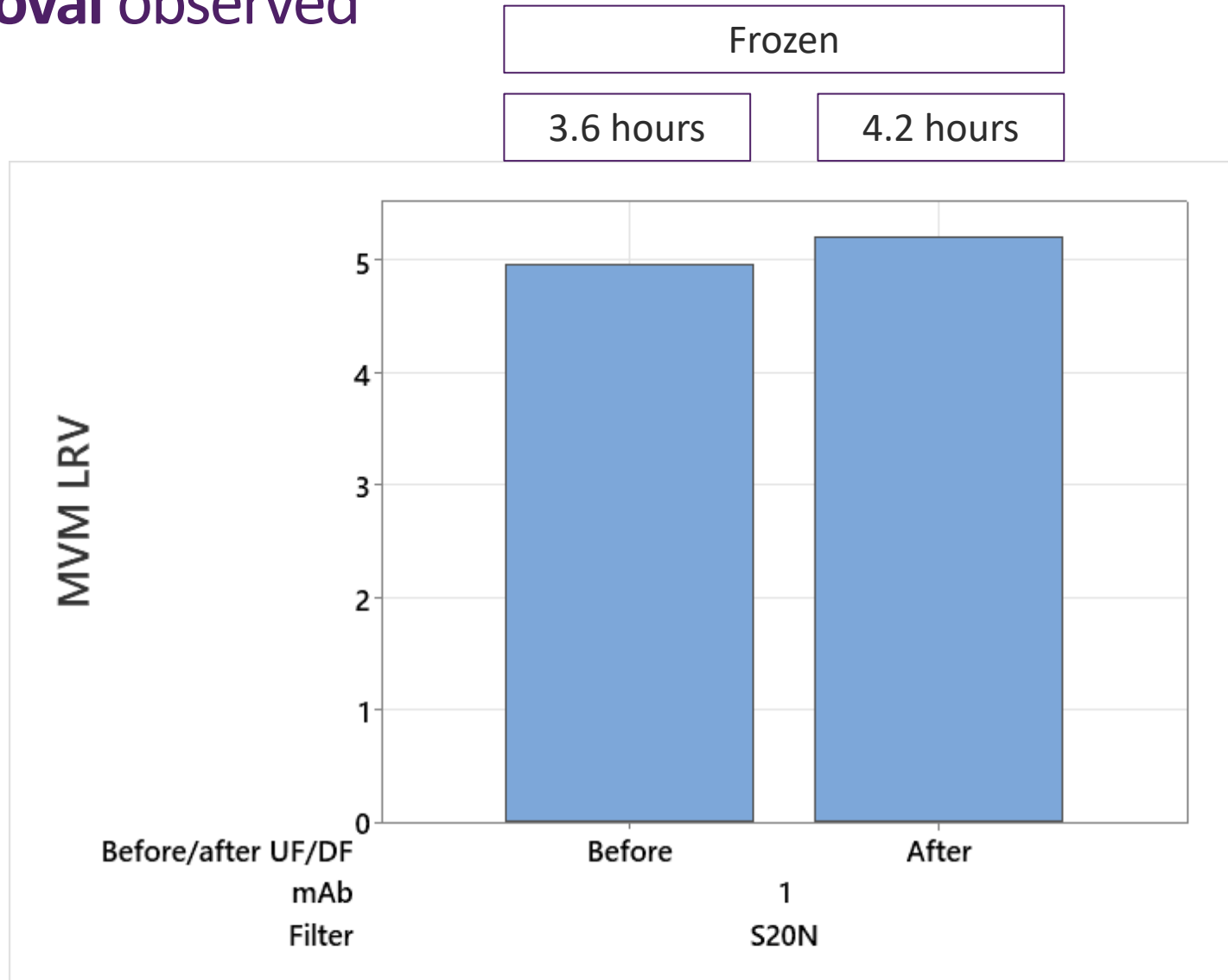
Minor flux decay @high load (4400 g/m<sup>2</sup>)  
→ Higher load possible

Flux lower with higher concentrated mAb

Flux similar to test without virus spike



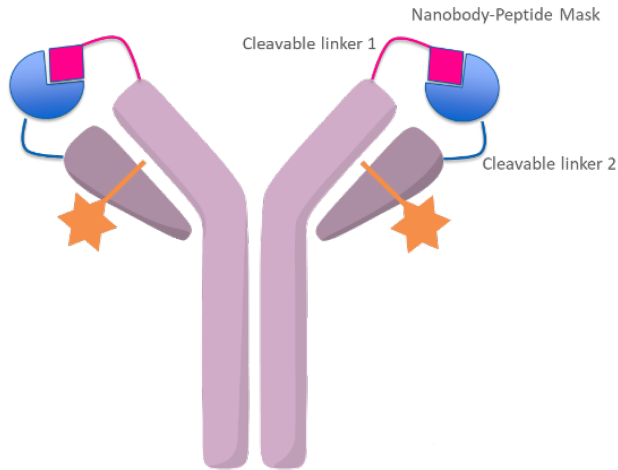
# Comparison S20N before vs after UF/DF – load: 4400 g/m<sup>2</sup> similar removal observed





# Virus filtration of masked mAbs

# MASKED ADCs and mAbs – The Concept



## Process challenges VF:

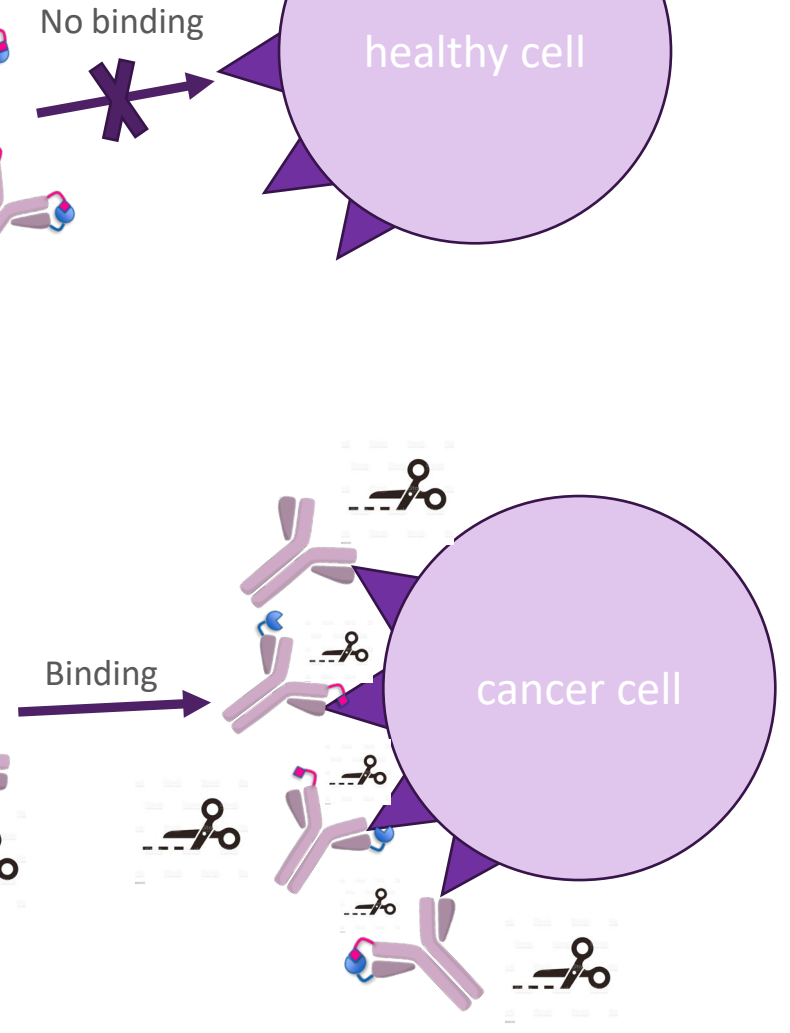
- larger mol weight
- more susceptible to shear stress

## Performance evaluation:

- S20N (regenerated cellulose)
- BioEx (PVDF)
- FG-1 (PES)

## Two different process locations:

- Before UF/DF
- After UF/DF



## Goal

Create an antibody or ADC that only binds to the tumor and not to healthy tissue

## AFTER UF/DF:

S20N gives acceptable flux @22 g/L, not @32 g/L

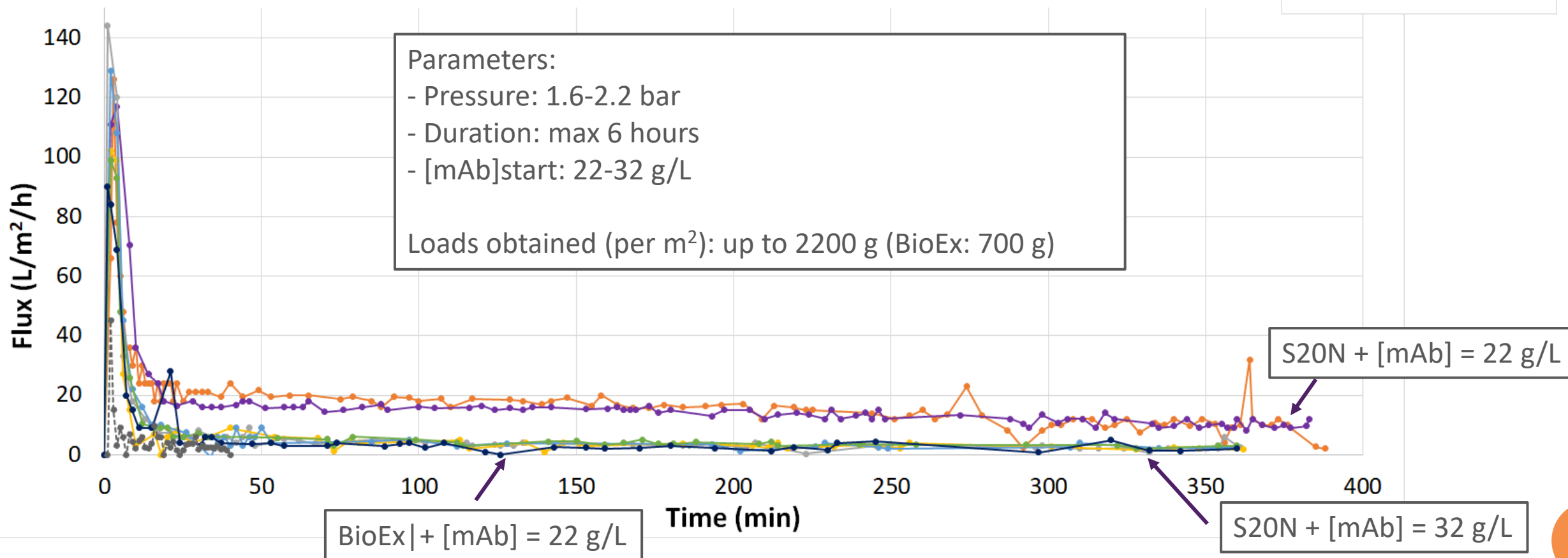
BioEx not suitable for high masked mAb concentrations

Cation Exchange  
Chromatography

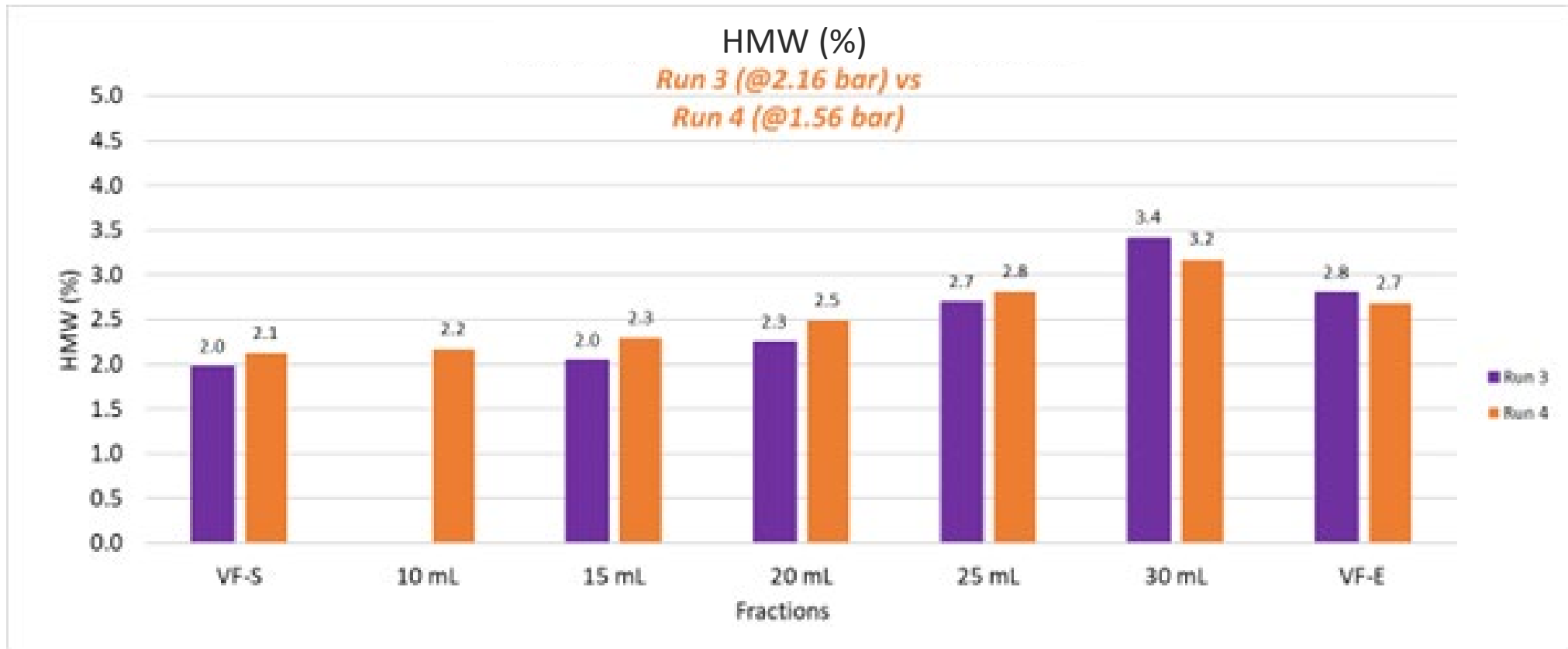
↓  
Ultrafiltration/  
Diafiltration

↓  
**Virus Filtration**

S20N/BioEX Flux of masked mAb

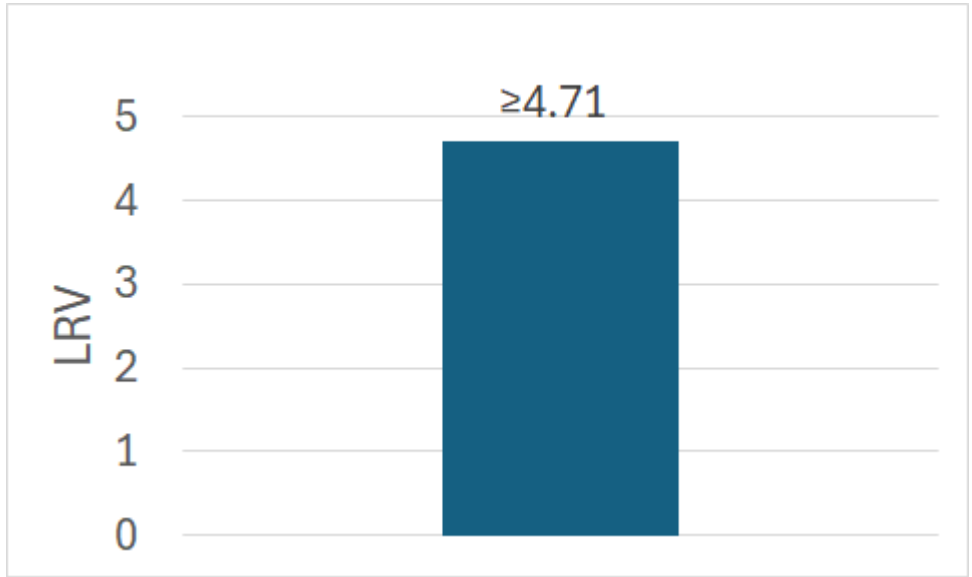
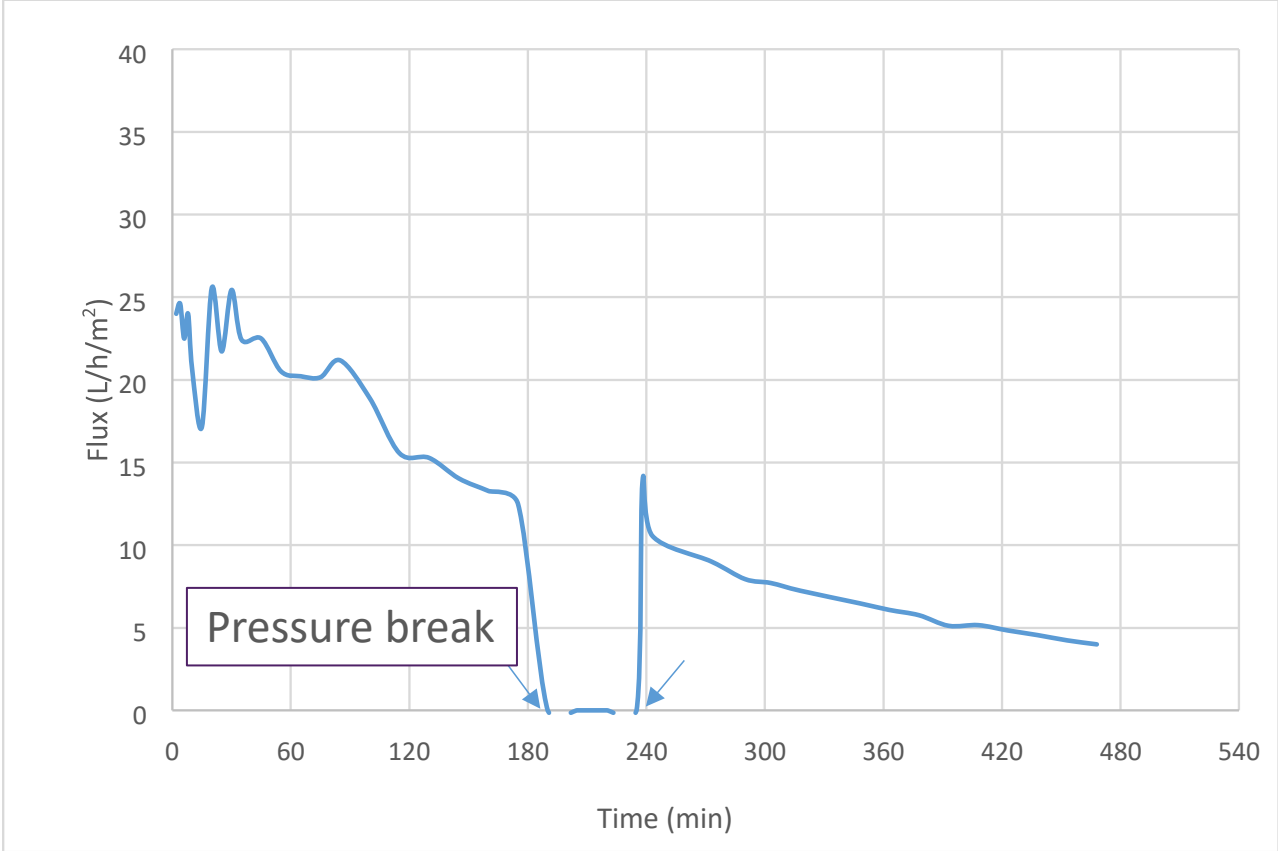


# Analysis shows some HMW increase, partially caused by the hold time



# S20N: Viral Clearance using **non-frozen** samples: Similar flux decay pattern with 1% virus spike Effective virus removal

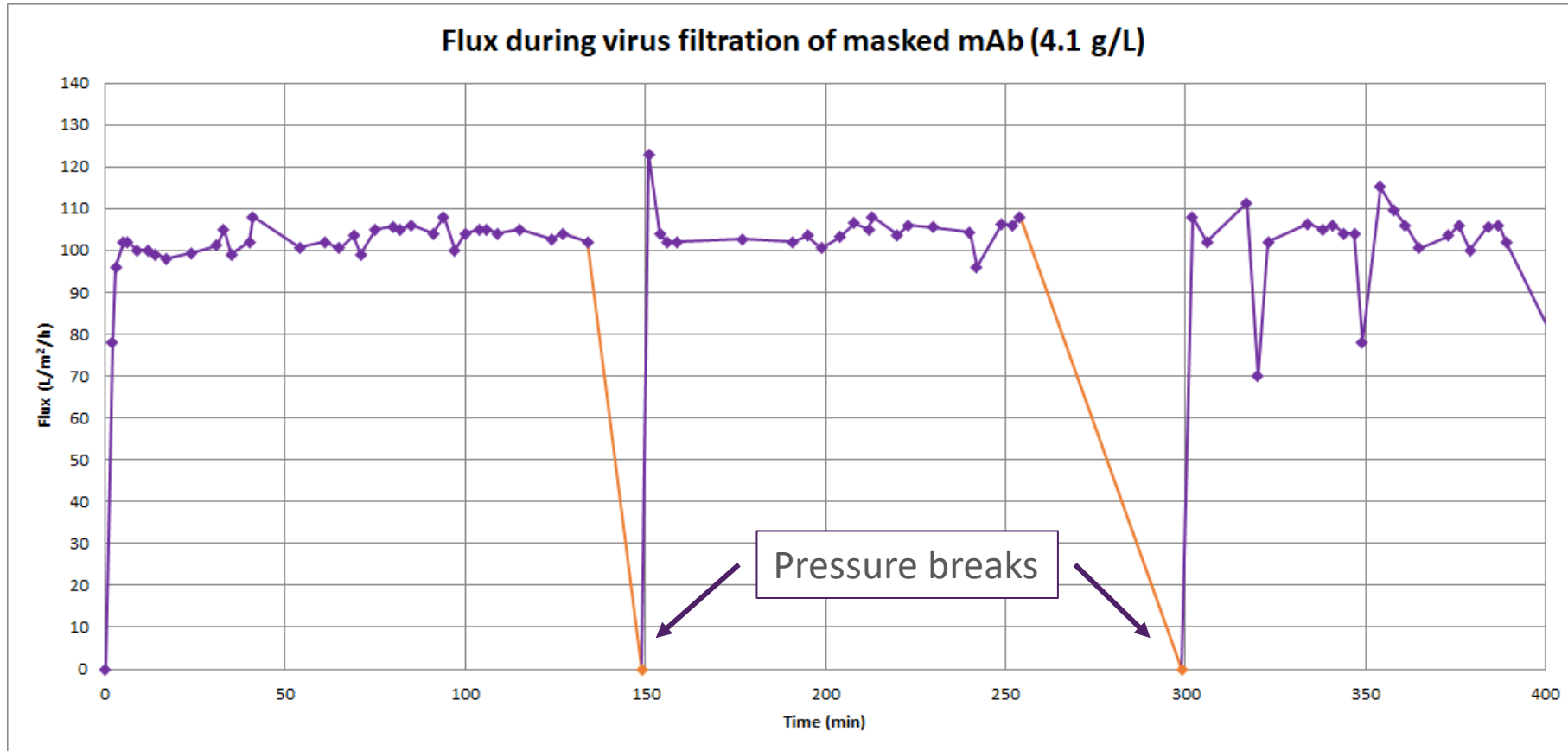
Conditions:  
[mAb]: 22 g/L  
Pressure: 2.2 bar  
Duration: 7 h + 1 h pause  
Mass throughput: 1730 g/m<sup>2</sup>



## Before UF/DF:

S20N: high flux and 2500 g/m<sup>2</sup> in 6 hours

→ S20N suitable, but high masked conc unfavorable



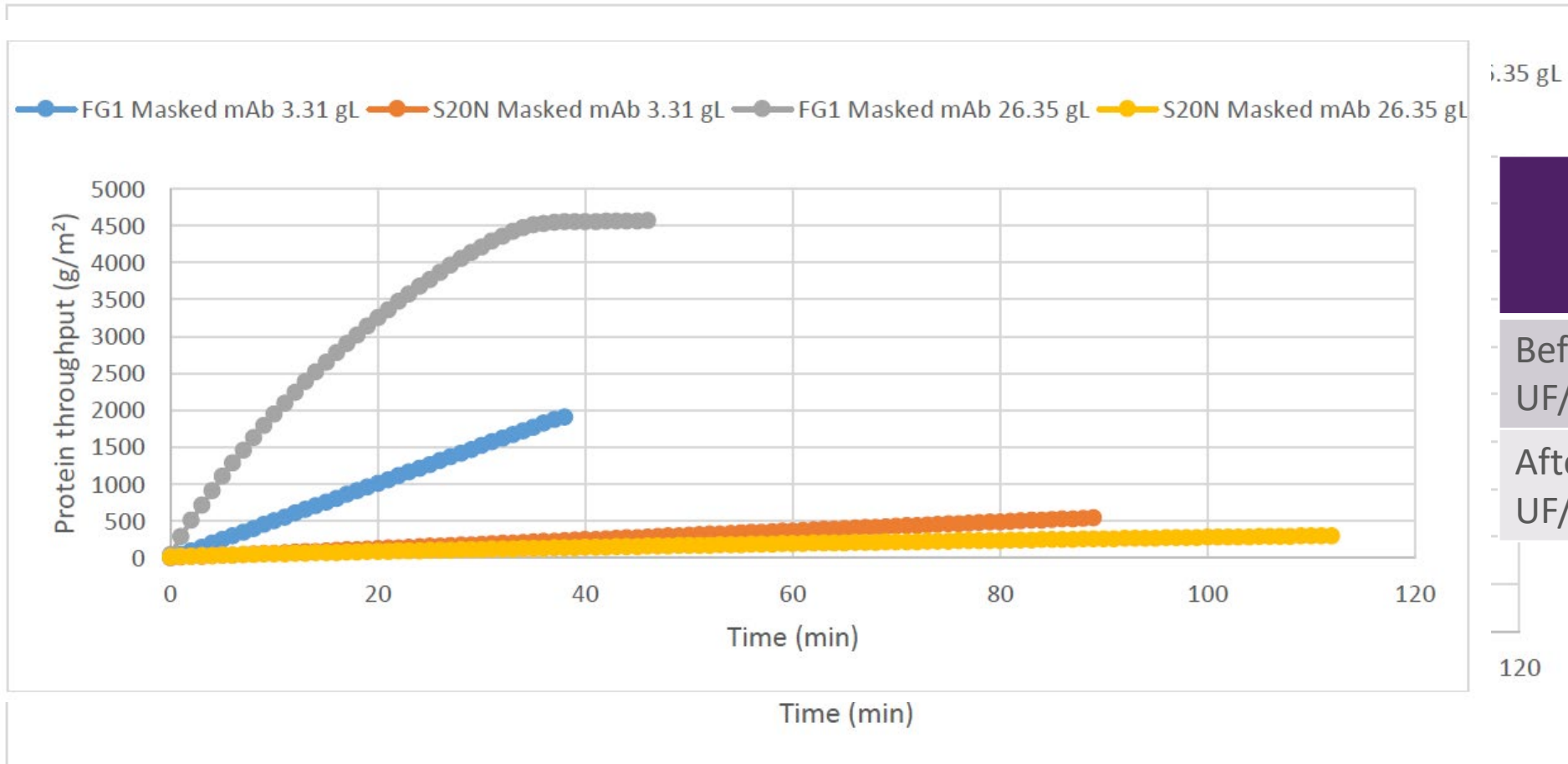
Cation Exchange  
Chromatography

Virus Filtration

Ultrafiltration/  
Diafiltration

## FG-1 before and after UF/DF:

- Superior flux compared to S20N; no decay @low conc
- Flux decay at higher conc, but mass throughput higher
- HMW level does not increase



	[mAb] (g/L)	HMW (%) @start
Before UF/DF	3.31	0.6
After UF/DF	26.35	1.8



# Final Conclusions

## Final Conclusions

- FG-1 shows superior filtration capacity, also with masked mAbs
- Virus Filtration at high mAb concentrations possible, but challenging
- Filter behavior is mAb dependent
- Perform VC studies with fresh material to avoid clogging

# Acknowledgments

Byondis

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Roya Dayani

Akika Futamura

Hiroki Fukutomi

# Questions

