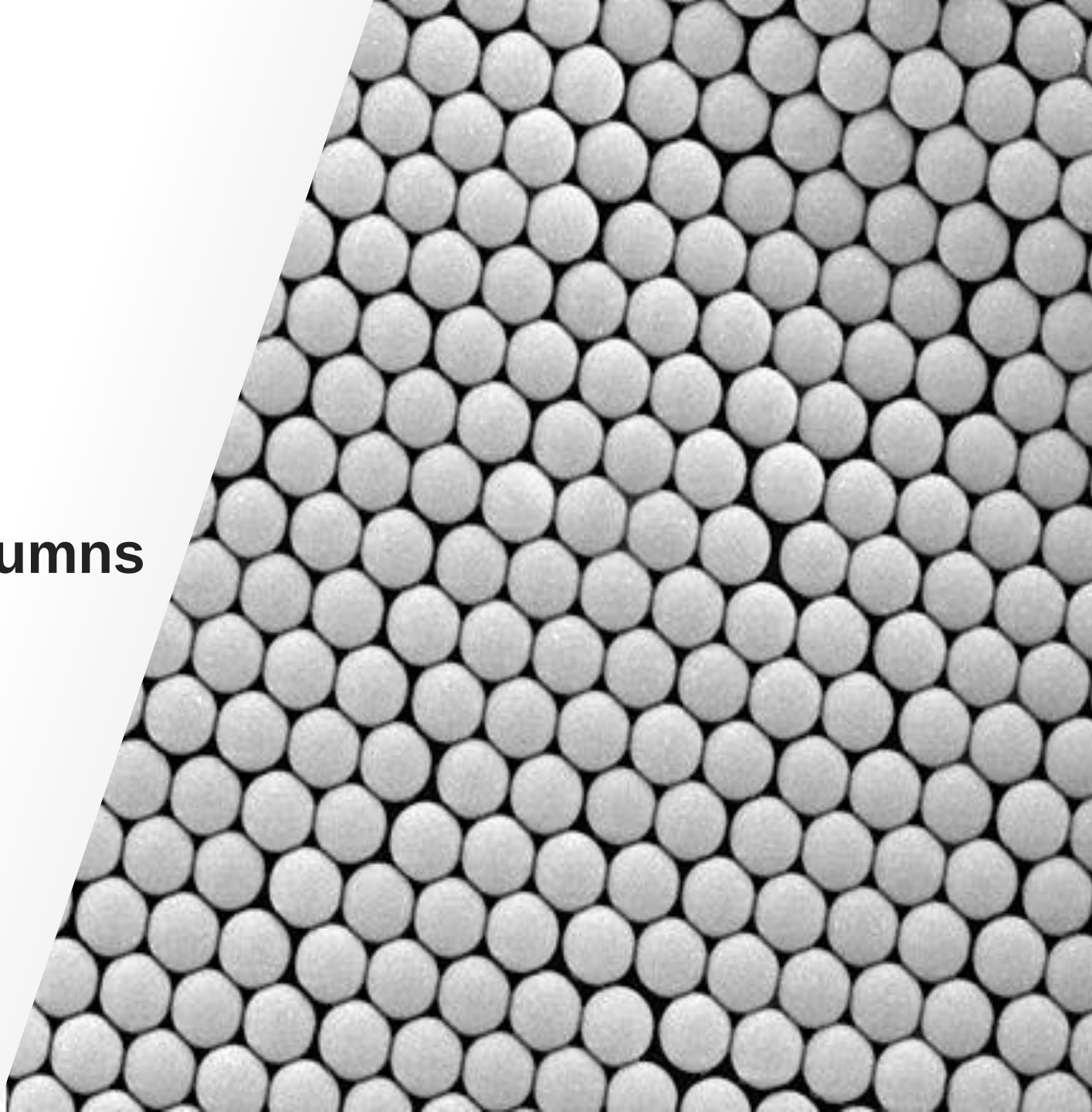


Evolution of Ion-Exchange columns for biologics characterization

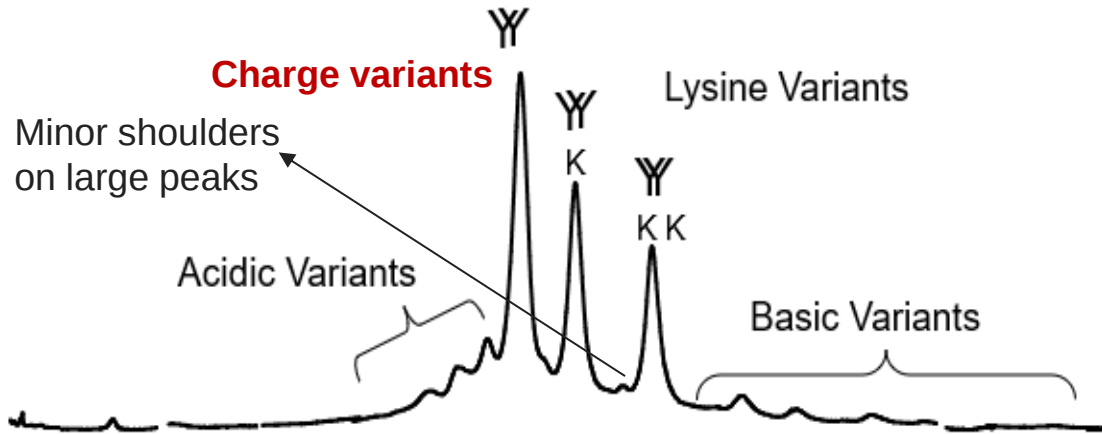
Shanhua Lin

Senior Director, Research and Development
Thermo Fisher Scientific

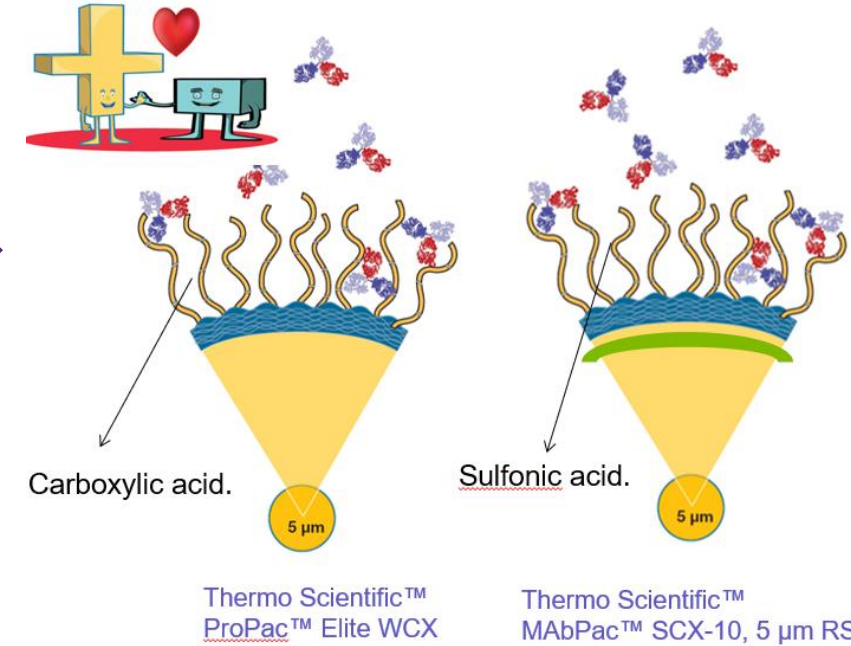
 The world leader in serving science



What are charge variants?



Thermo Fisher
Scientific answer



Post Translational Modifications (PTMs) of the protein that directly effect the charge or indirectly effect the charge by causing a change in conformation

Deamidation, succinimide, lysine truncation, oxidation etc.

Can cause:

- Immune reaction
- Change of structure
- Half-life variation
- Change of function

Critical Quality Attributes that are essential to be monitored

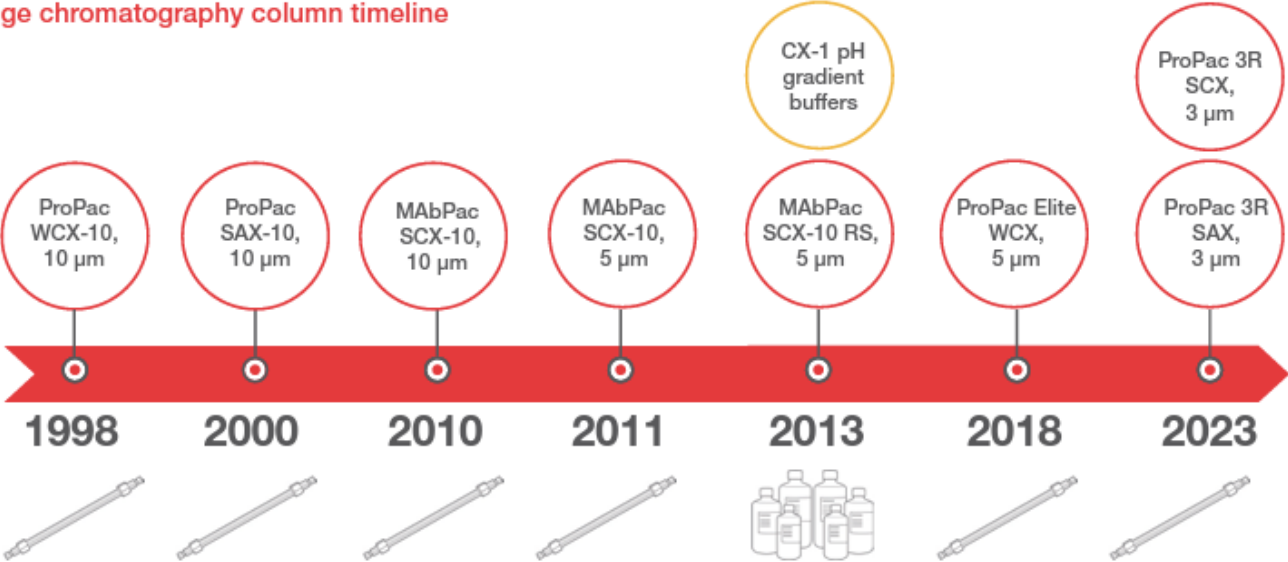
Will this run reliably for the next **ten** years?

QC and R&D

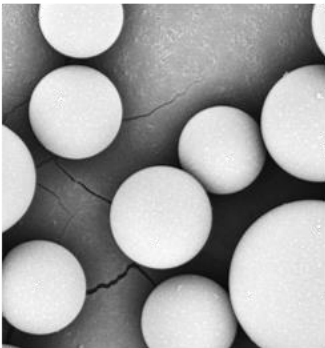
How to get the best optimisation for a CVA analysis – high resolution

Evolution of the Ion-exchange columns

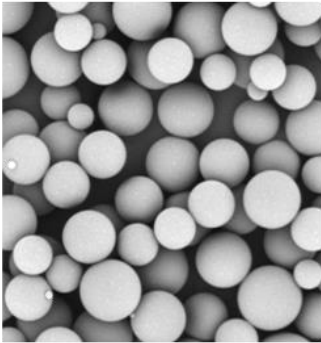
Ion-exchange chromatography column timeline



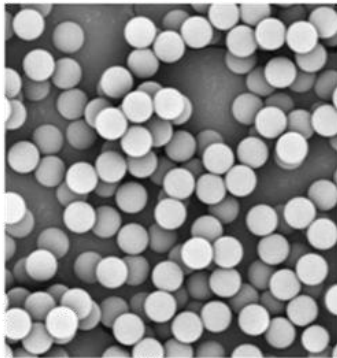
10 μm



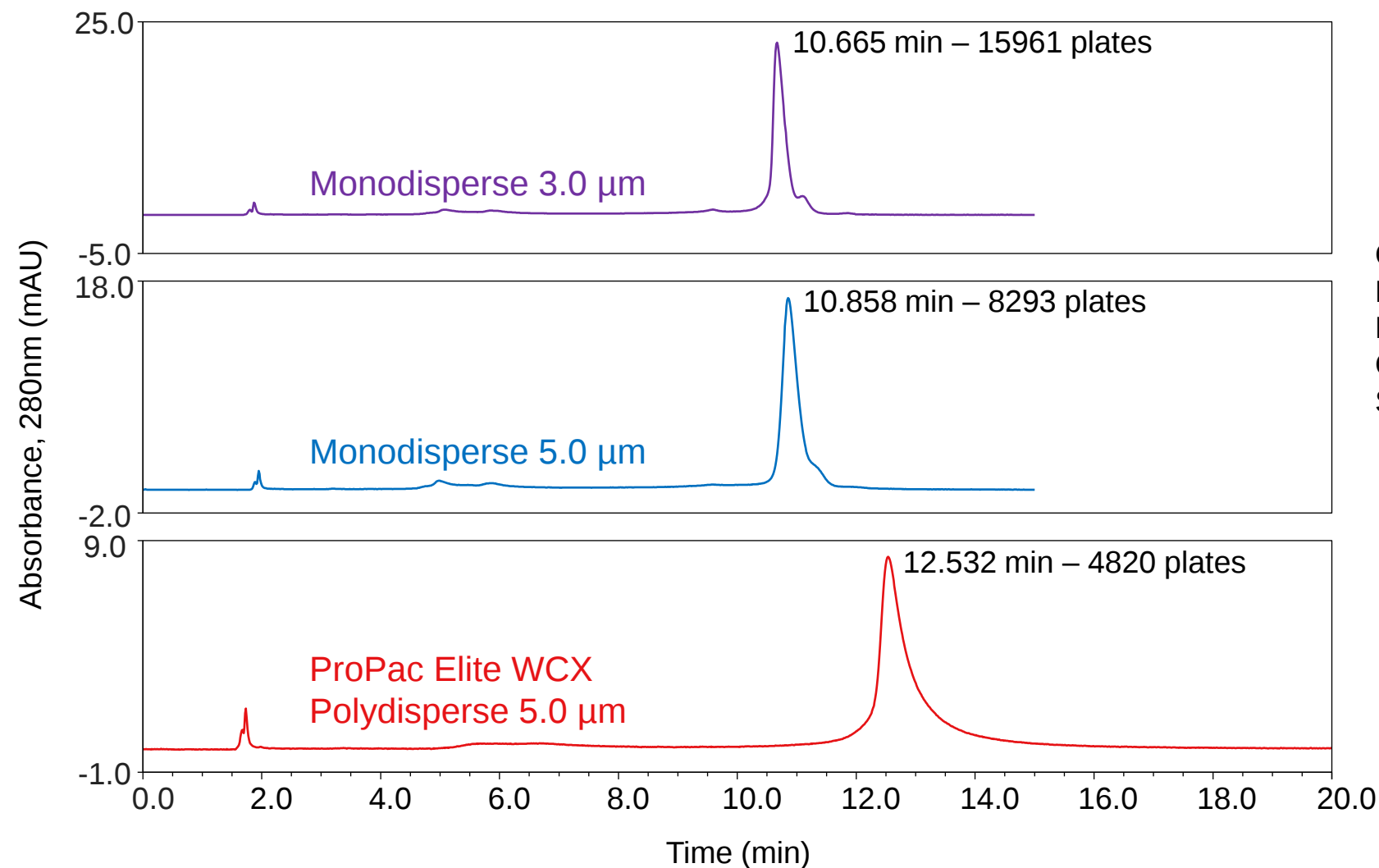
5 μm



3 μm



Impact of particle size and uniformity on efficiency



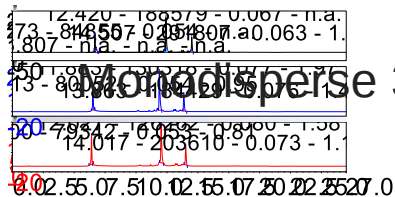
Column: 4 x 150mm, Prototype WCX
Buffer: 20 mM MES + 180 mM NaCl pH 6.5
Flow rate: 0.4 mL/min
Col Temp: 30°C
Sample*: 8 μg Cytochrome C

*Comparison of isocratic cytochrome C separation using prototype WCX chemistry on 3 μm monodisperse (top) and 5 μm monodisperse (middle) particles and a polydisperse 5 μm (bottom) particle.

Advantage of monodisperse particle in gradient analysis



Impact varies depending on size of the analytes

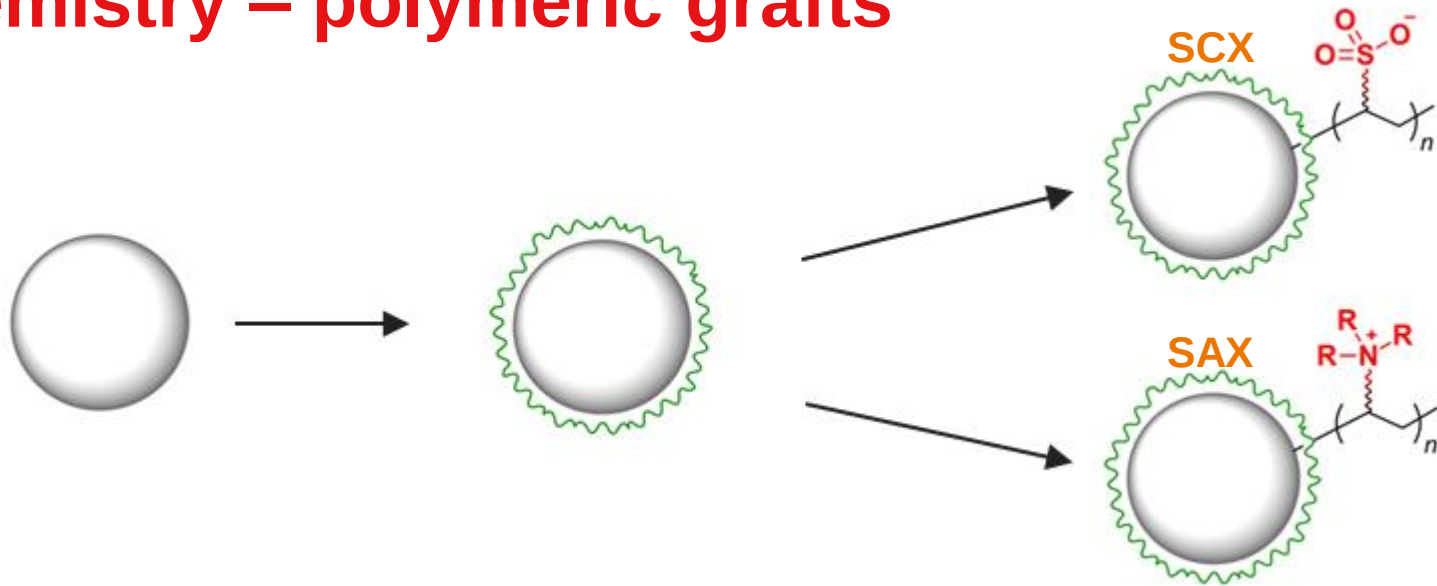


Monodisperse 3.0 μm

Monodisperse 5.0 μm

ProPac Elite WCX
polydisperse 5.0 μm

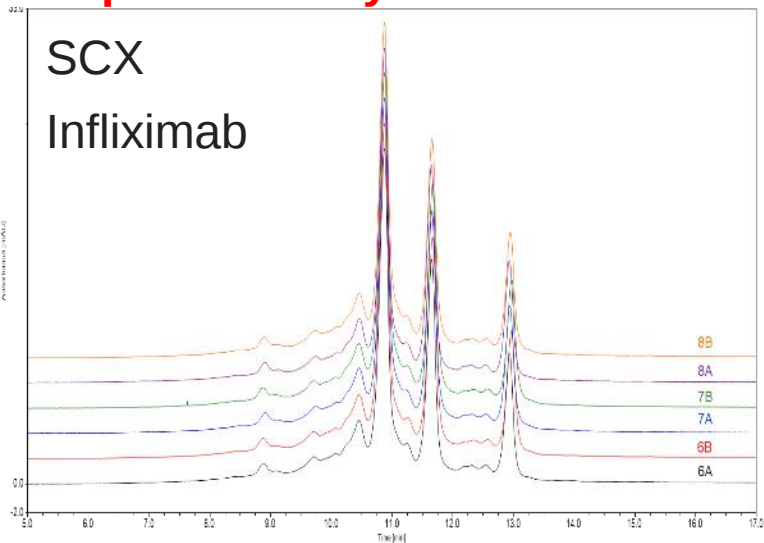
Chemistry – polymeric grafts



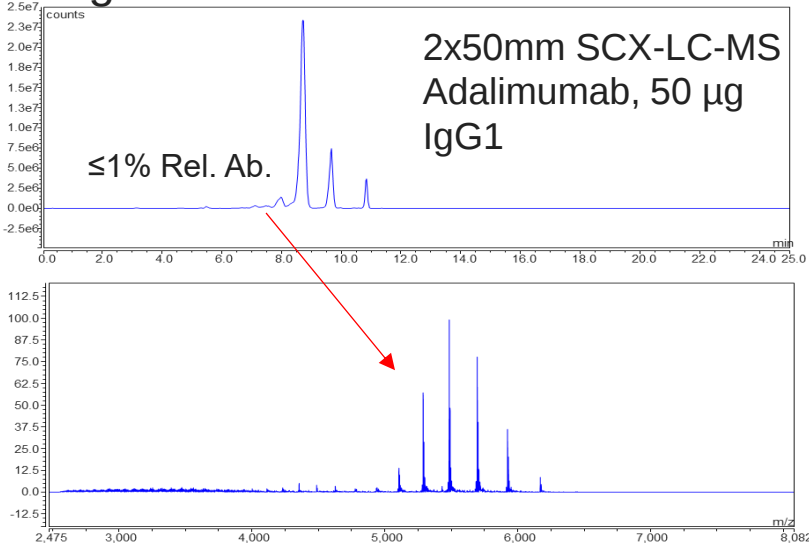
mAb characterization and QC

Acidic therapeutic protein,
AAV characterization and QC

Reproducibility Lot-to-Lot



High Resolution



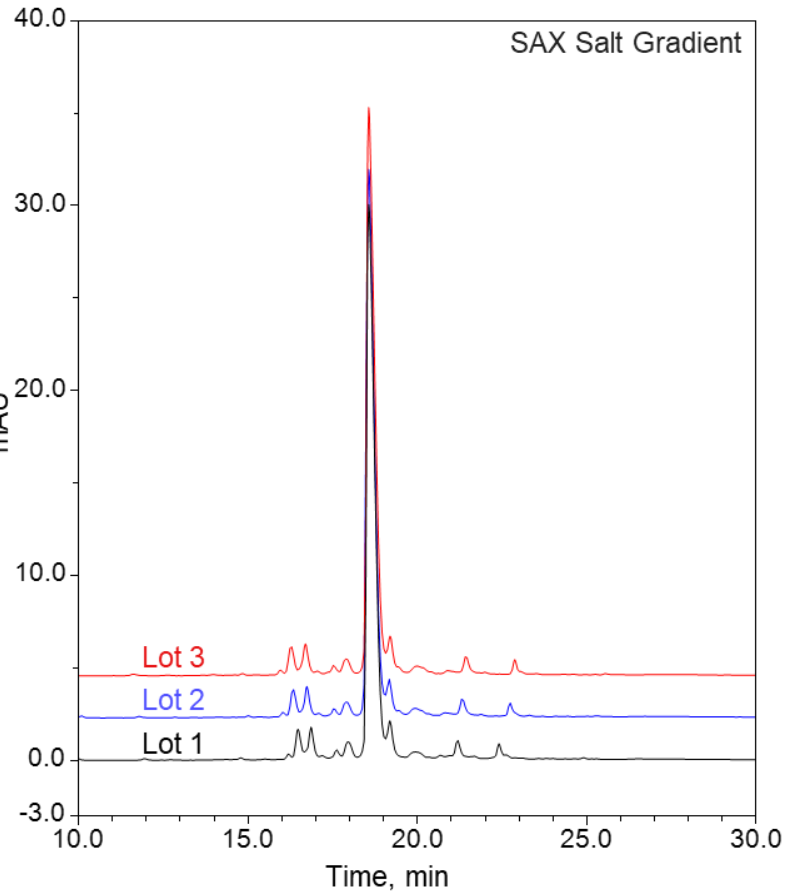
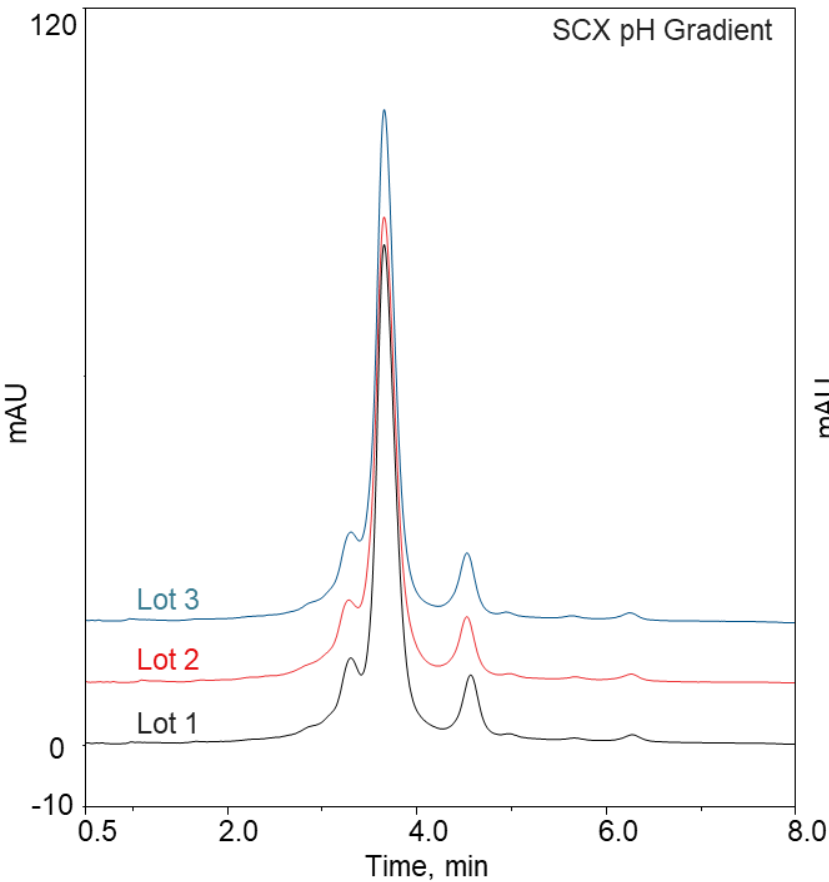
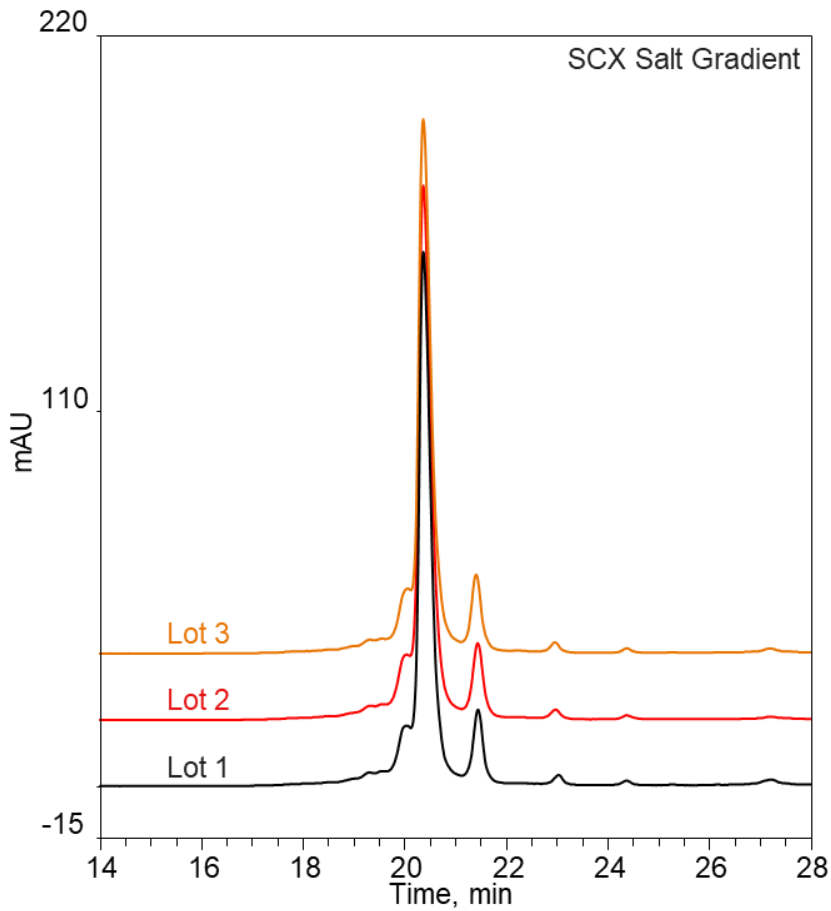
Robust Operating Range



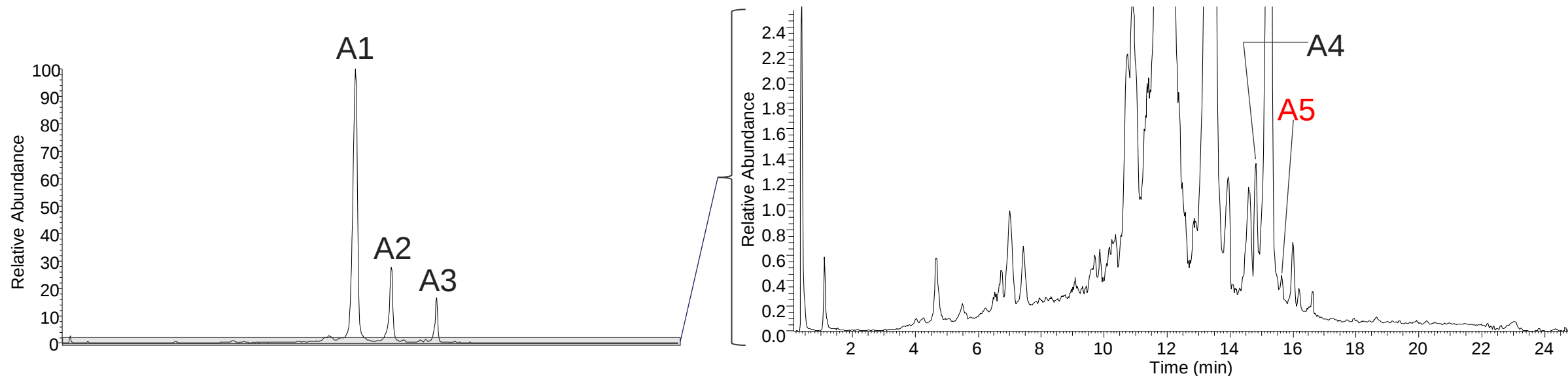
SAX

Rapid QC
15 min Method

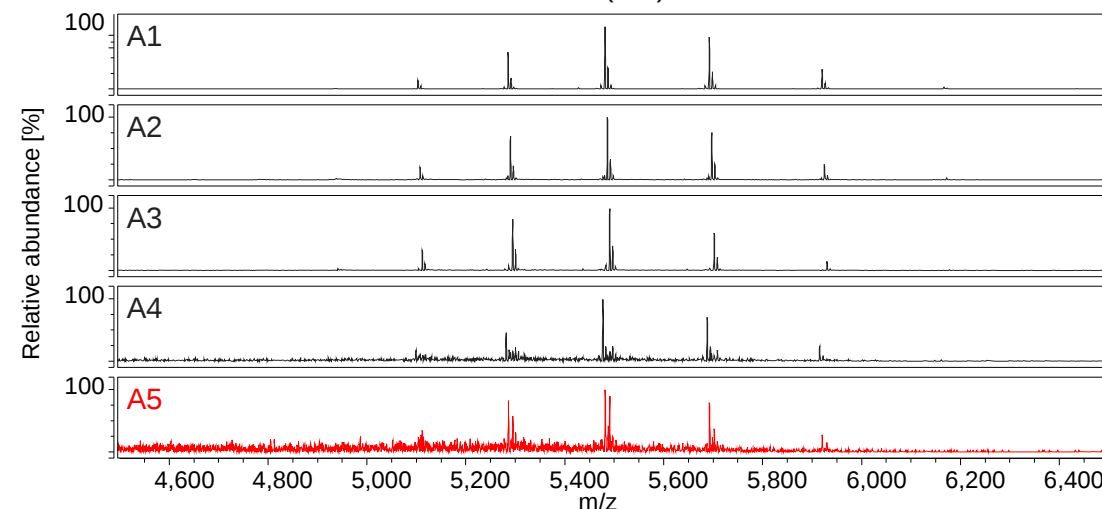
Thermo Scientific™ ProPac™ 3R SCX & SAX columns – lot-to-lot reproducibility



The resolution power of monodisperse particle



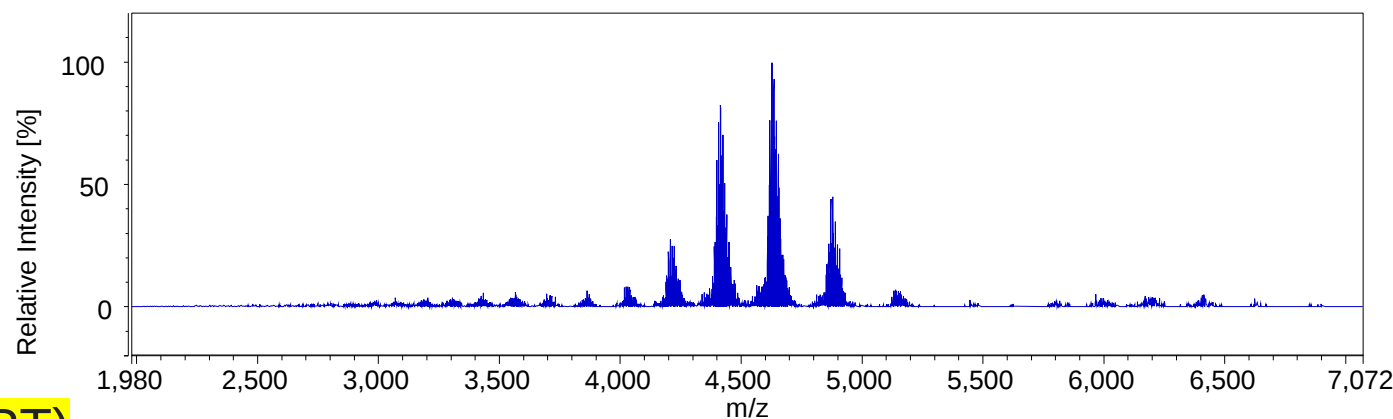
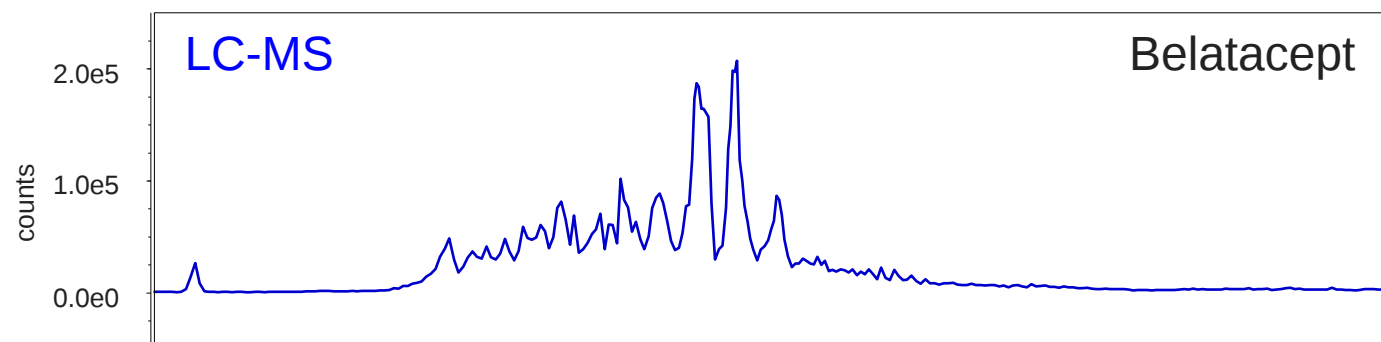
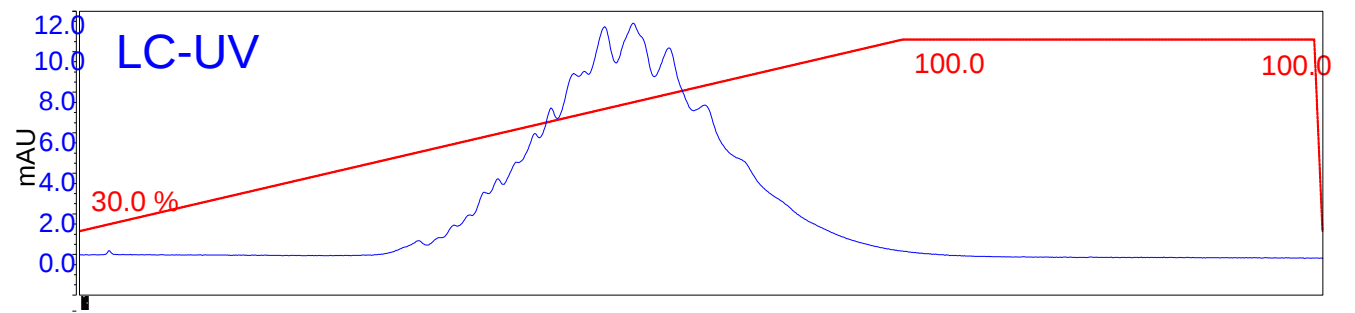
- Excellent MS sensitivity, 50 μg loading.
- Peaks clearly distinguished even <1% relative abundance, clear mass spectra obtained for all major peaks.
- Even peaks with a relative abundance of 0.4% (A5) show spectral quality sufficient for deconvolution.



Data are provided by Dr. Jonathan Bones (NIBRT)

Separation of low pI protein on ProPac 3R SAX column

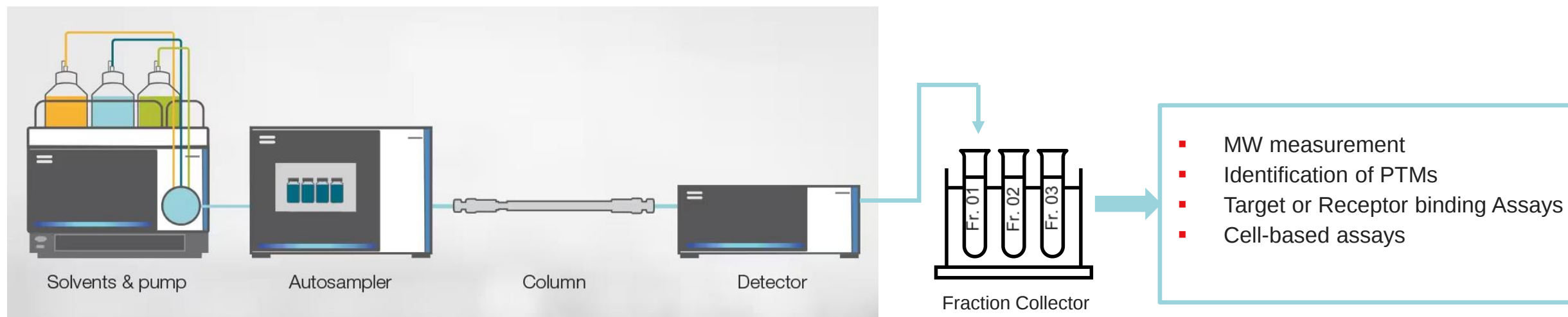
- Separation of multiple charge variants of **Belatacept** on anion exchange column.
- Most Fc-fusion proteins contain both N-linked and O-linked glycosylation sites. All N-linked sites and multiple O-linked sites are occupied with typical mammalian oligosaccharide structures. These sugar structures are partially terminated with negatively charged sialic acid residues (sialylation).
- Reduction of the complexity resulted in well resolved charge envelope.



Data are provided by Dr. Jonathan Bones (NIBRT)

Characterization of product related variants

Downstream analysis



Analytical to semi-prep: hardware availability

Analytical Column

PEEK Hardware
Low Pressure Bio Inert



<https://www.thermofisher.com/order/catalog/product/43103-052068>

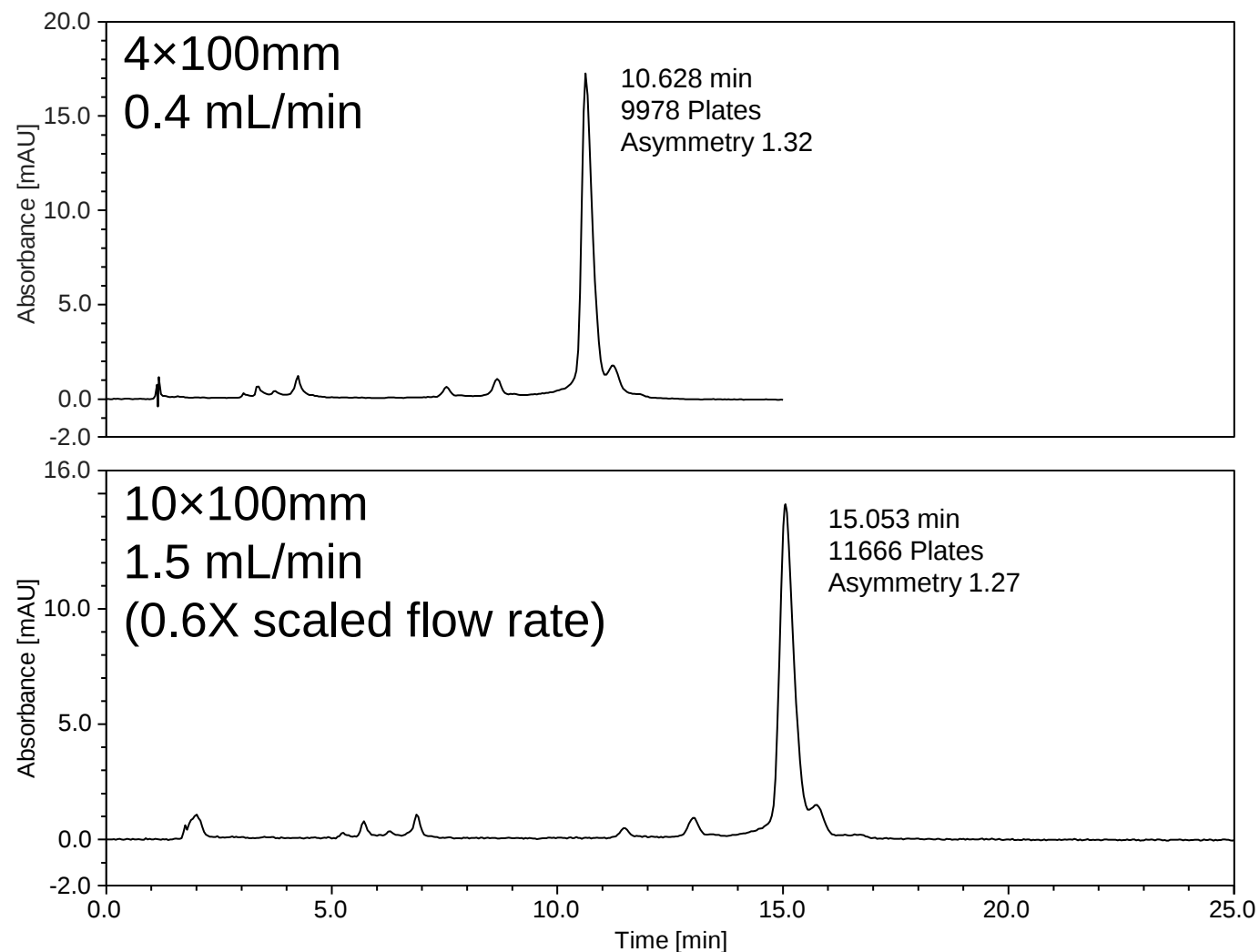
Preparative Column

Coated Stainless Steel Hardware, including frits



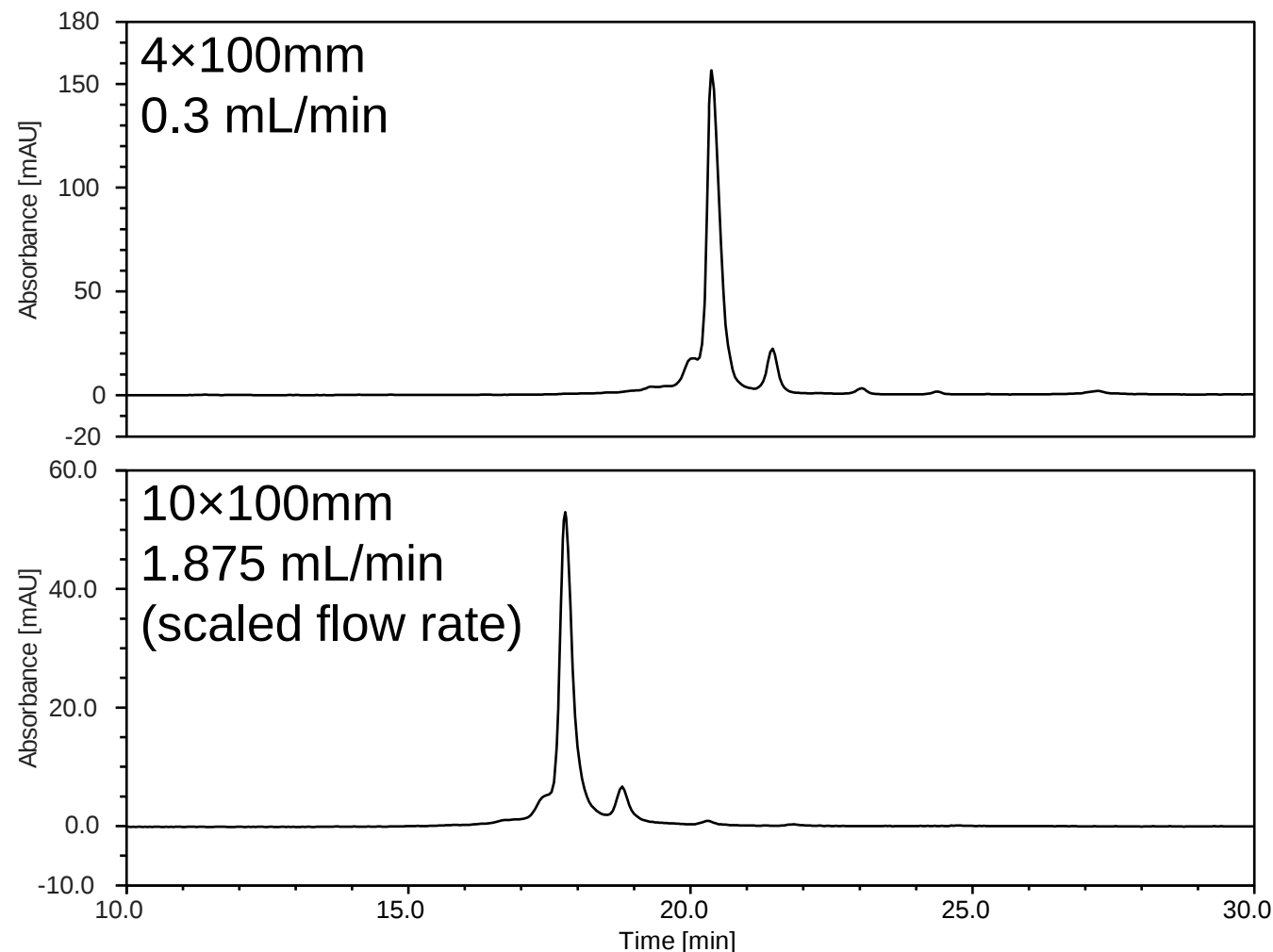
10x100mm ProPac 3R SCX column – Isocratic testing

- Isocratic testing of Cytochrome C protein used to QC all analytical formats for packing quality
- Excellent translation from 4x100mm to 10x100mm at 0.6X scaled flow rate demonstrates
 - Quality of packing
 - Absence of secondary interactions



10x100mm ProPac 3R SCX column – NISTmAb testing

- Gradient separation performance consistent from 4mm to 10mm ID with scaled flow rate
- Dynamic loading capacity ~118 $\mu\text{g}/(\text{mL bed volume})^*$ based on NISTmAb
- 10x100mm loading capacity ~930 μg based on 4x100mm



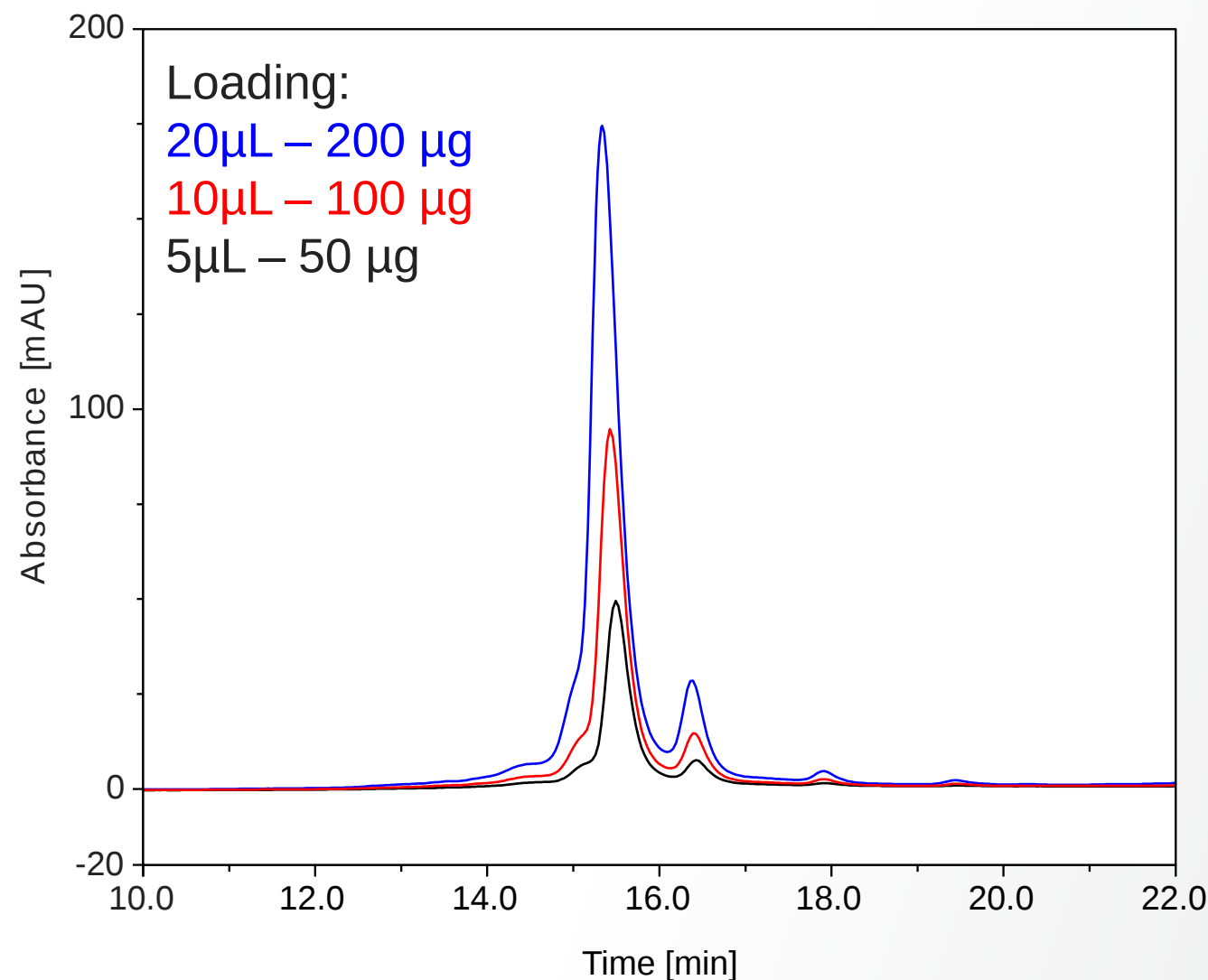
*See Application Note 001694: Salt gradient analysis of monoclonal antibodies using a 3 μm monodisperse SCX chromatography column

Format:	See chromatogram	Gradient:	Time (min)	%A	%B
Eluents:	A: 20 mM MES		0.0	90	10
	B: 20 mM MES + 0.5 M NaCl		25.0	70	30
Flow Rate:	See chromatogram		25.1	20	80
Inj. Vol:	2 μL		27.0	20	80
Temp:	35 $^{\circ}\text{C}$		27.1	90	10
Detection:	UV, 280 nm		37.0	90	10
Sample:	NISTmAb – 10 mg/mL				

ProPac 3R SCX 10x100mm column – NISTmAb loading



Consistent acidic-main-basic peak separation performance with increasing loading

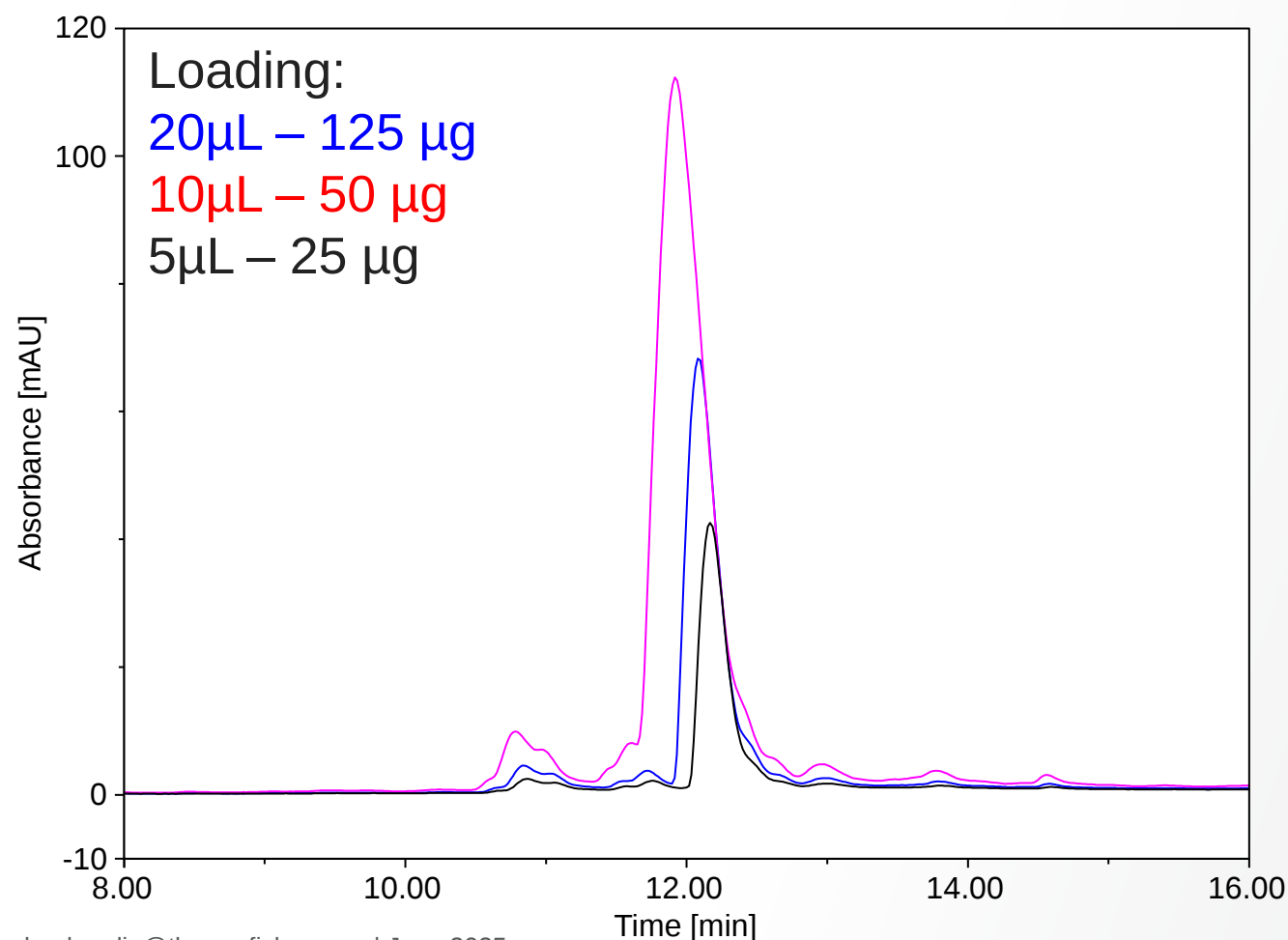


Eluents: A: 20 mM MES
B: 20 mM MES + 0.5 M NaCl
Flow Rate: See chromatogram
Gradient: Time (min) %A %B
0.0 90 10
25.0 70 30
25.1 20 80
27.0 20 80
27.1 90 10
37.0 90 10
Inj. Vol: See chromatogram
Temp: 35 °C
Detection: UV, 280 nm
Sample: NISTmAb – 10 mg/mL

ProPac 3R SAX 10x100mm – Sample Loading

Siltride Coated High Pressure SST Hardware

- Protein G – ~28 kDa protein
- No observed issues with the SAX product using full SST coated hardware



Eluents: A: 20 mM TRIS pH 8.5
B: 20 mM TRIS pH 8.5 + 0.5 M NaCl

Flow Rate: 1.875 mL/min

Gradient: Time (min)	%A	%B
0.0	88	12
1.0	88	12
21.0	58	42
21.1	0	100
23.0	0	100
23.1	88	12
35.0	88	12

Inj. Vol: See chromatogram

Temp: 35 °C

Detection: UV, 280 nm

Sample: Protein G – 10 mg/mL

Summary

When it comes to employing a 3 μm particle for the IEC column

Resolution: Direct
LCMS analysis
identifies variants <1%
abundance

Scalability: prep scale
column of the same
particle retains similar
separation power

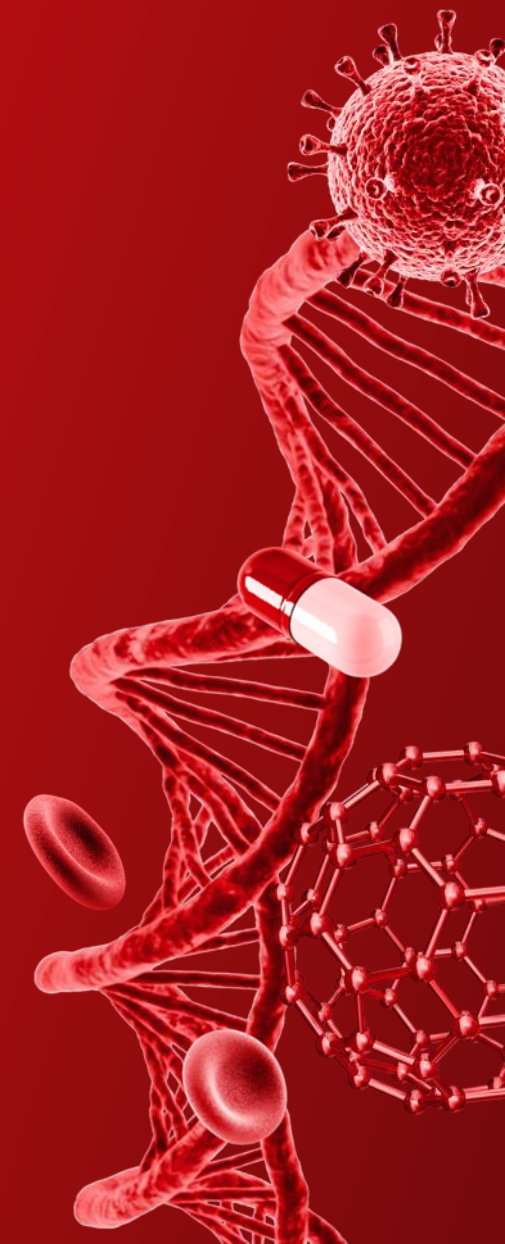
Robustness: Column
achieves 1,000+
injections



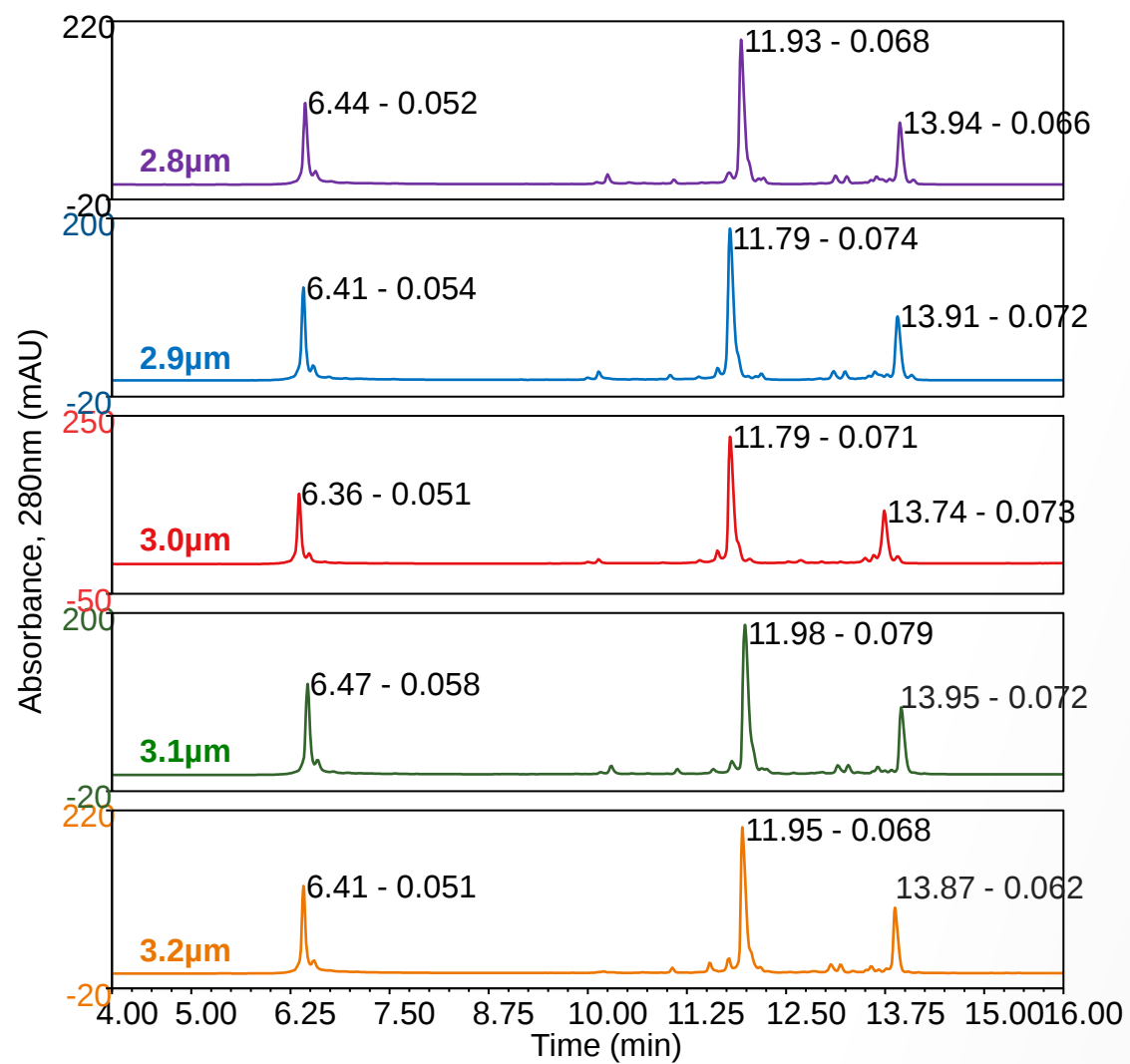
Acknowledgements

- Shane Bechler
- Ryan Cowley
- Brandon Robson
- Ke Ma
- Binalkumari Mistry
- Ken Cook
- Jonathan Bones (NIBRT)
- Sara Carillo
- Florian Füssl

Questions



Particle range and gradient analysis



Column: 4 x 150mm, Prototype WCX
MP A: 20 mM MES pH 6.5
MP B: 20 mM MES + 500 mM NaCl pH 6.5
Flow rate: 0.5 mL/min
Gradient:

Time, min	%A	%B
0.0	90	10
15.0	20	80
18.0	20	80
18.1	90	10
27.0	90	10

Temp: 30°C
Sample*: 4 µg NISTmAb
8 µg Cytochrome C
16 µg Ribonuclease A

Figure 4. Comparison of gradient separation of a 3-protein mixture using prototype WCX chemistry on monodisperse particles sized 2.8, 2.9, 3.0, 3.1, and 3.2µm.