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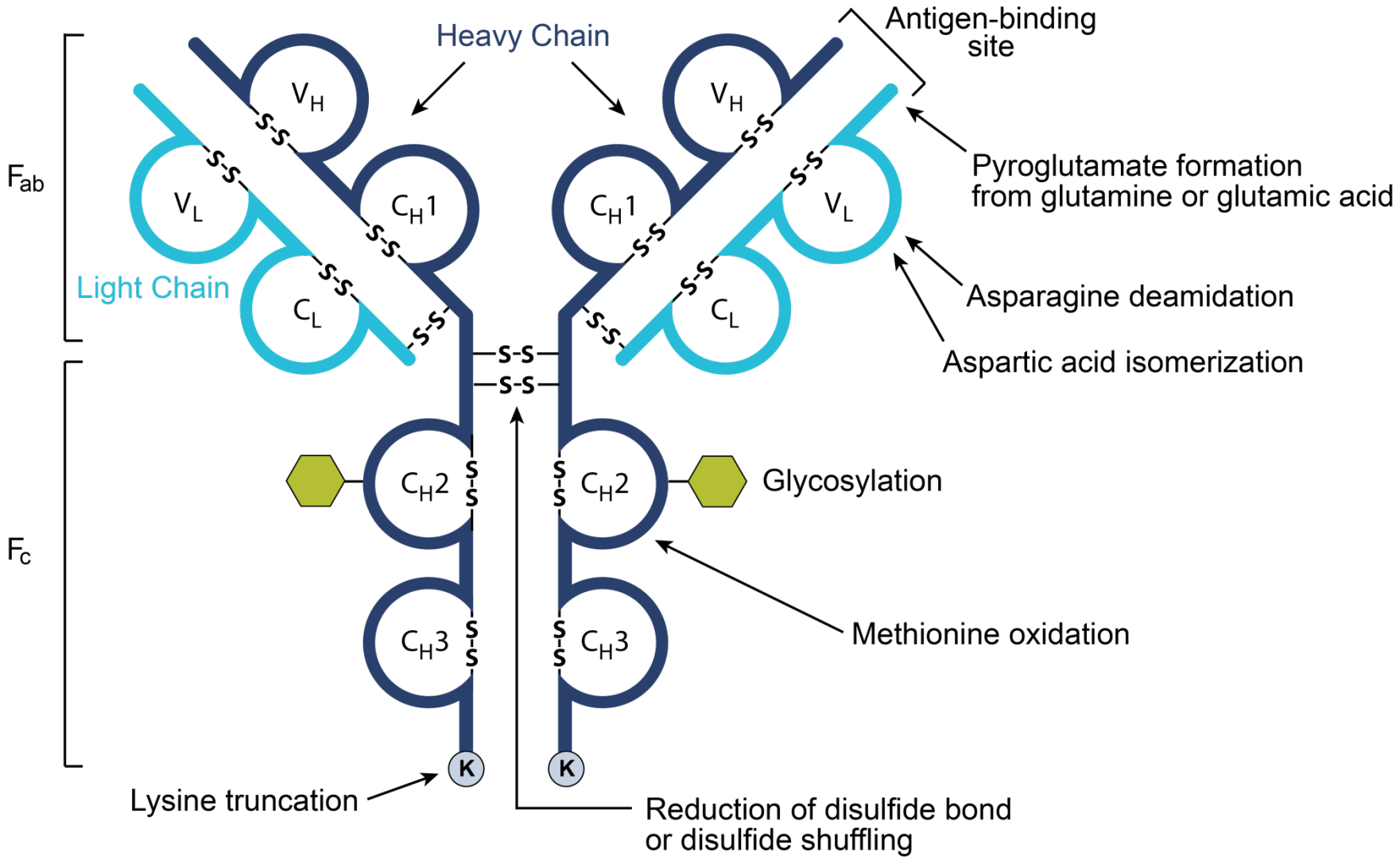
mAb and ADC Analysis

Shanhua Lin, Ph.D.
R&D Manager
Bioseparation, Chromatography Consumables

Structure of IgG and Typical Forms of Heterogeneity



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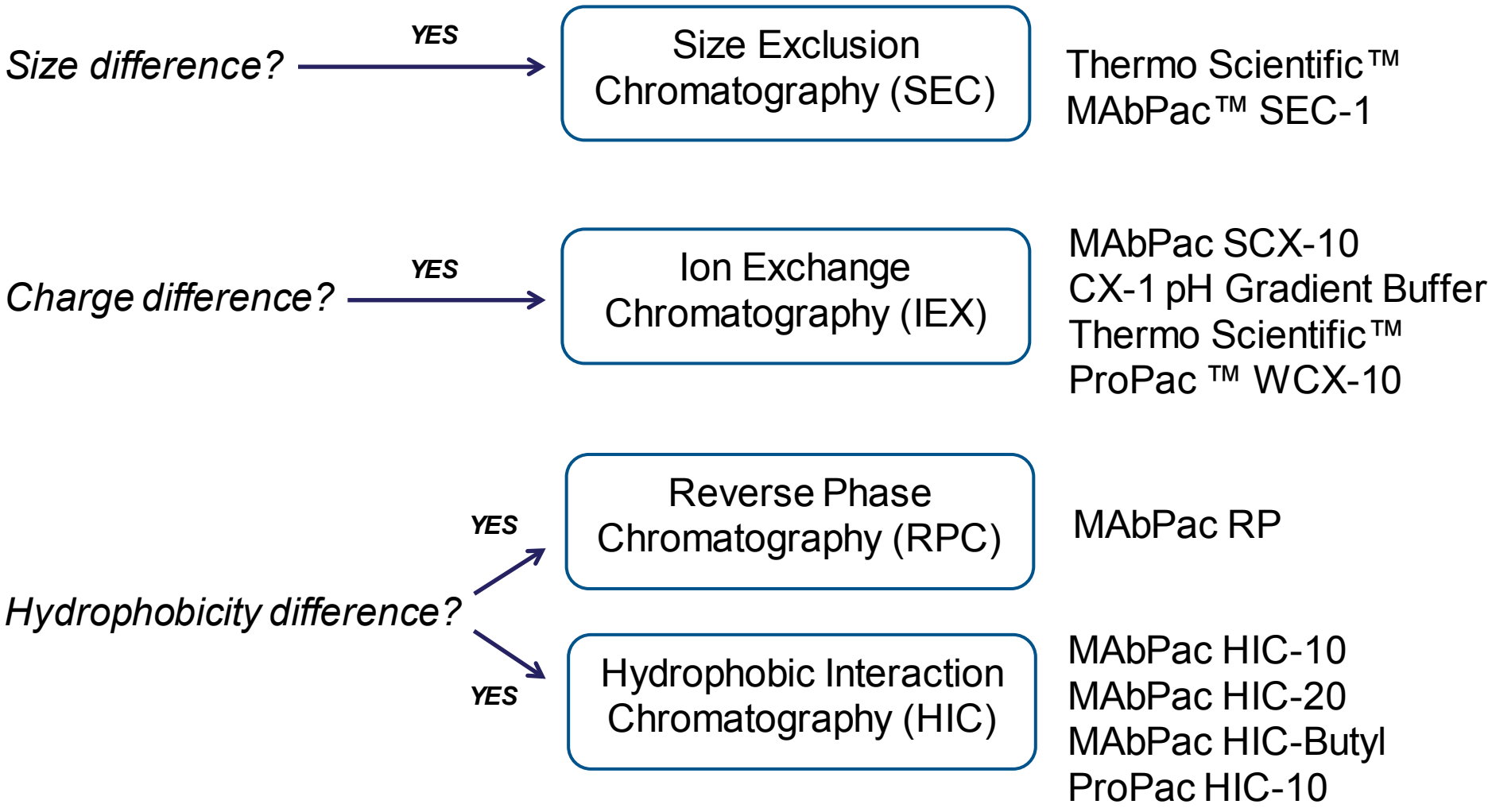
Protein and mAb Separation by HPLC

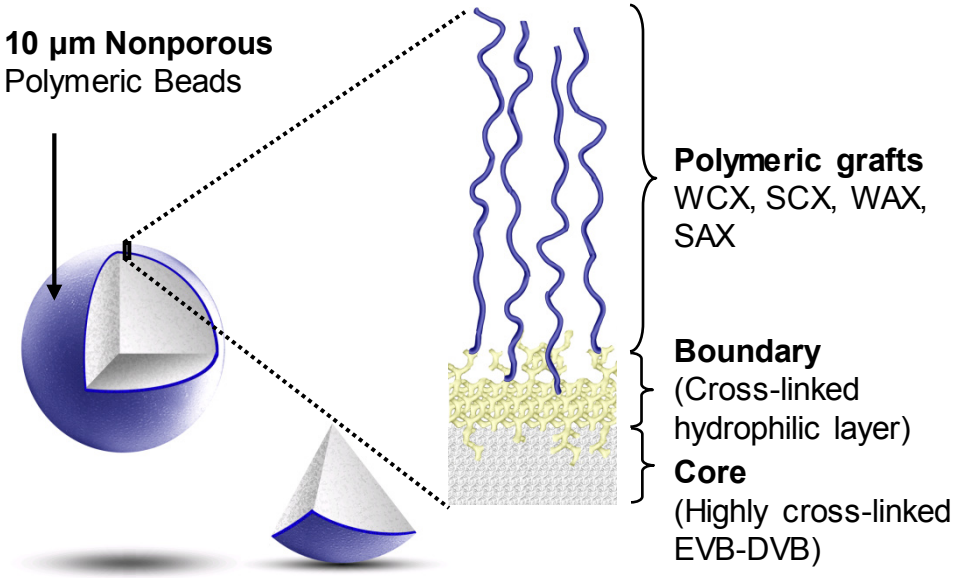


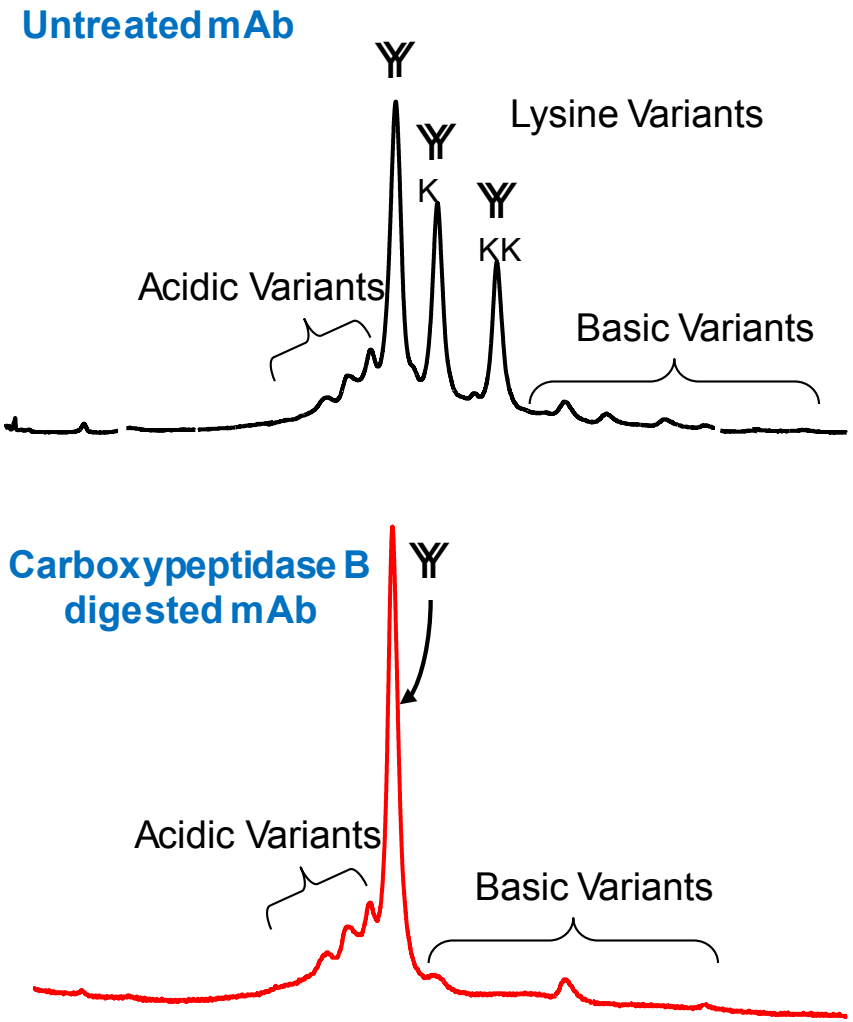
IEC Analysis for Charge Variant

- ProPac WCX-10 and MAbPac SCX-10 columns
- Separate acidic and basic variants
- CX-1 pH Gradient Buffer Kit provides a platform solution

Charge Variant Analysis

ProPac WCX-10 Column





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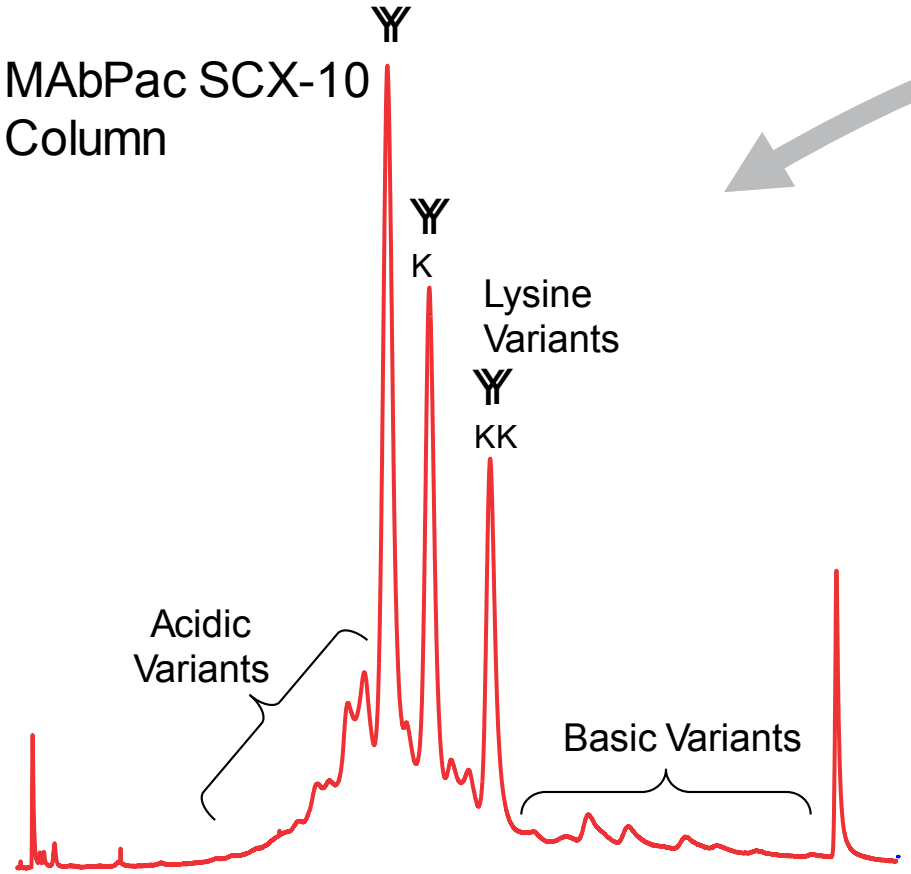
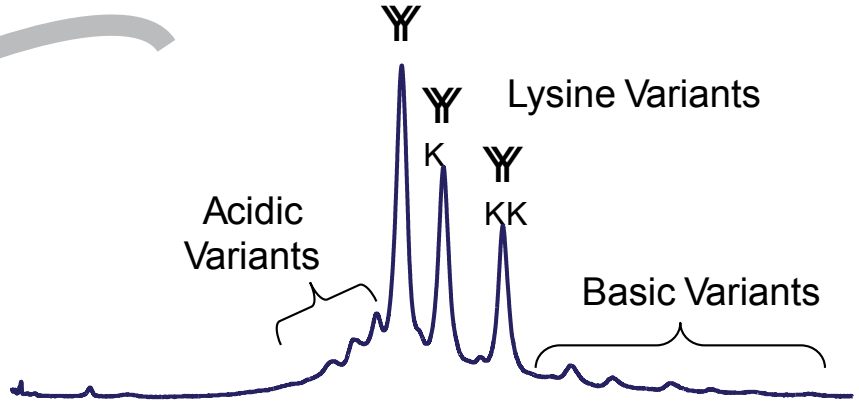
Next-generation CEX Column

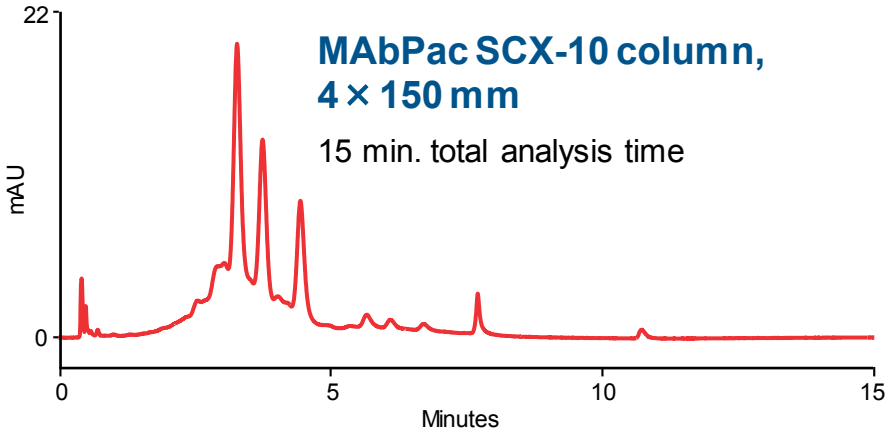


MAbPac SCX-10 column, 4×250 mm

60 min. total analysis time



ProPac WCX-10 column, 4×250 mm



MAbPac SCX-10 column, 4 × 150 mm

15 min. total analysis time

Improve resolution or sample throughput through column chemistry

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Thermo Scientific CX-1 pH Gradient Buffers

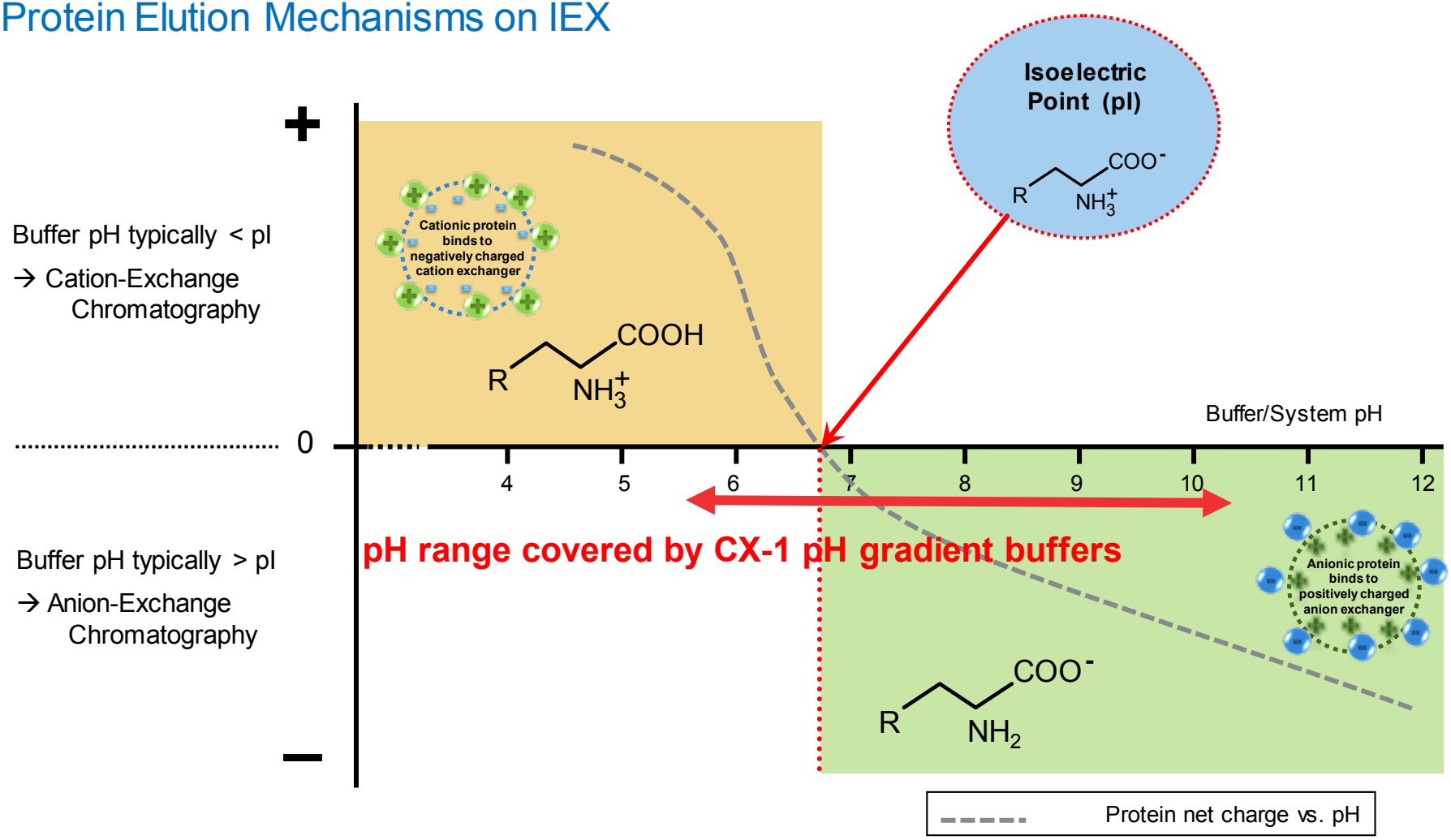
- Dilute buffers 10-fold with DI water
- A linear pH gradient (pH 5.6 - 10.2) is generated by running a linear pump gradient from 100% Buffer A to 100% Buffer B
- It is platform, fast, and high-res!

	Buffer A	Buffer B
pH	5.6	10.2
Form	Liquid	Liquid
Concentrate	10X	10X
Shipping condition	Room Temp	Room Temp
Storage condition	4 ~ 8 °C	4 ~ 8 °C

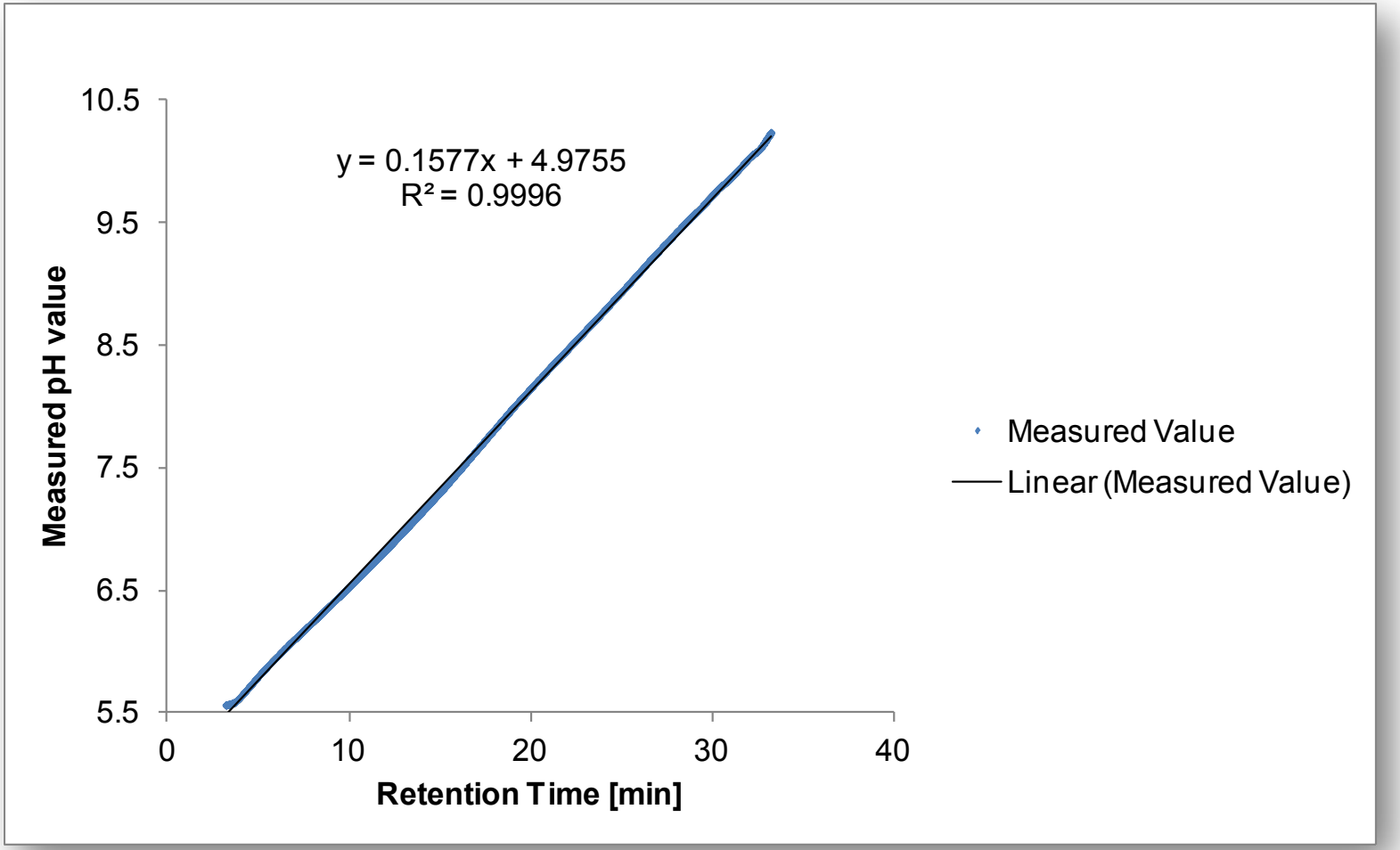
US 8921113 B2: Buffer kit and method of generating a linear pH gradient

pH Gradient Buffers – How Do They Work?

Protein Elution Mechanisms on IEX



Linear pH Gradient by Zwitterionic Buffer Cocktail



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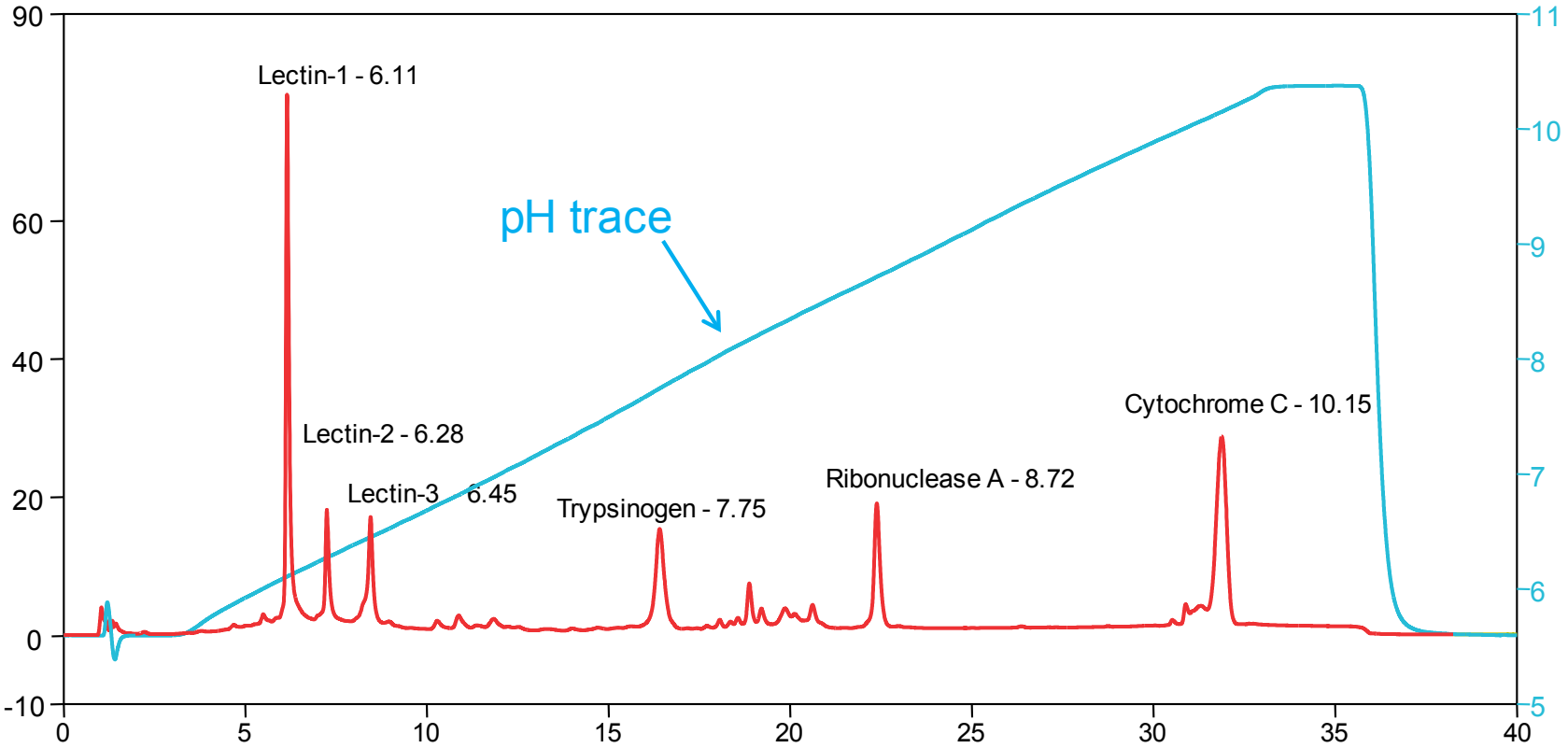
Protein Standard – CX-1 pH Gradient Buffer

MAbPac SCX-10 Column, 4 × 250 mm

Peak label: protein name – elution pH



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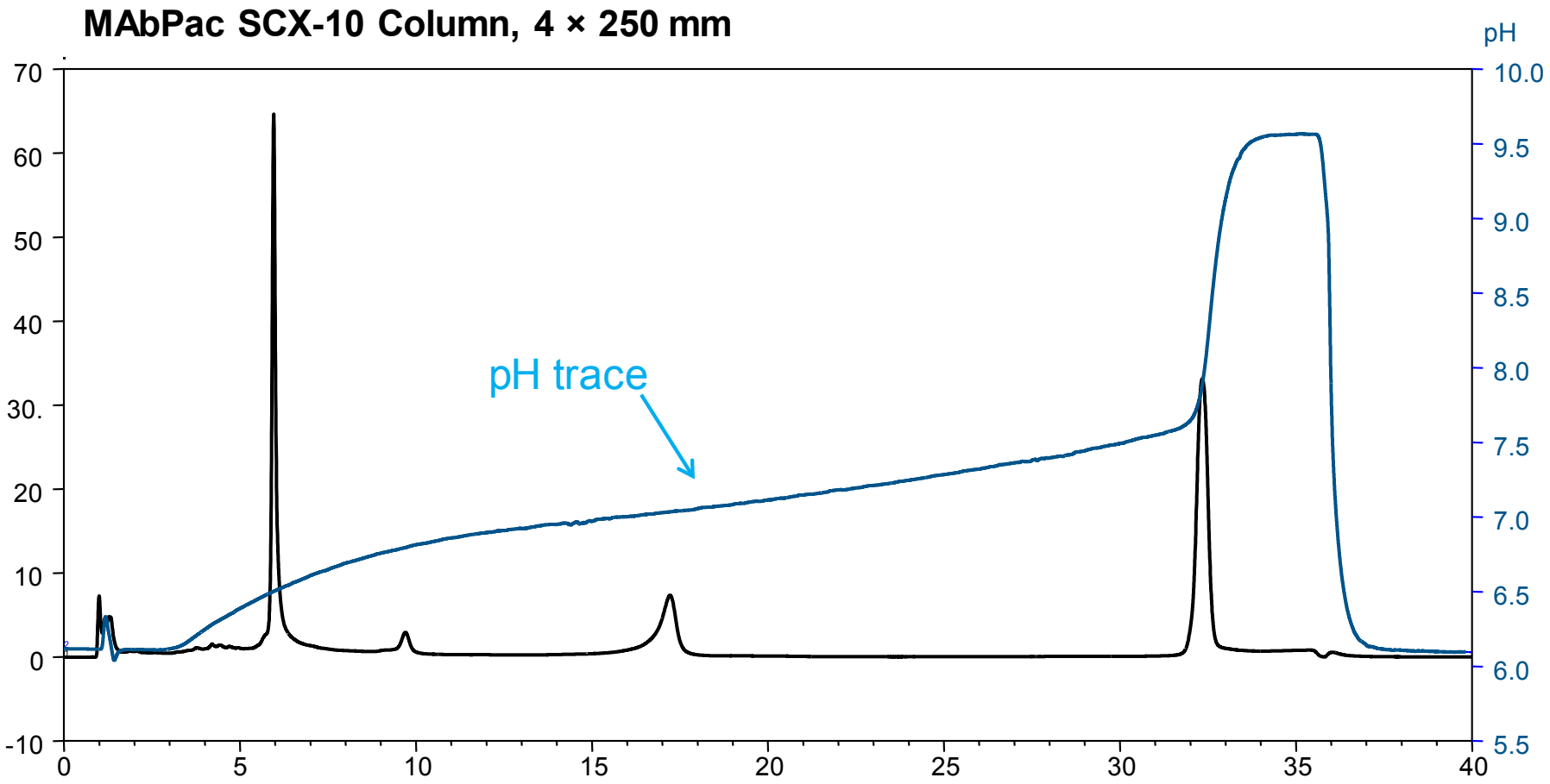
Protein Standard – Phosphate-based pH Gradient



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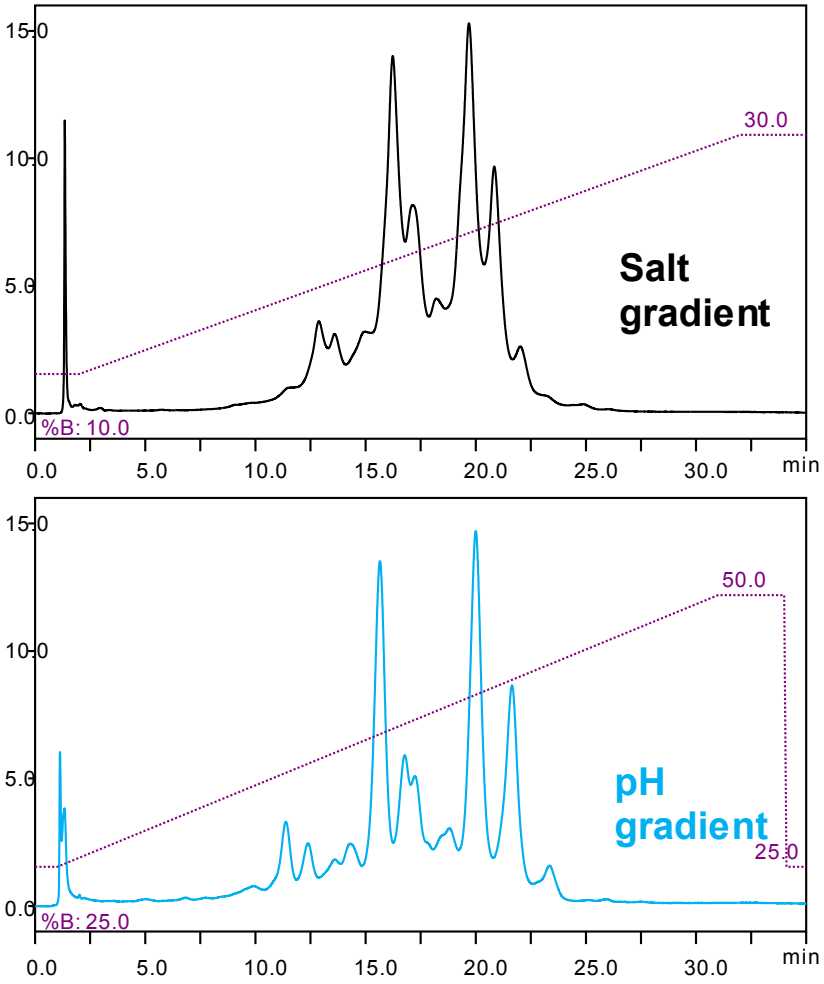
Why pH Gradient Buffers?

- Traditional salt gradient using ion exchange chromatography
  - Needs to be tailored for individual charge variants
- Patented pH buffer formulations
  - Fast, robust and reproducible pH gradients
  - Ready to use with existing CEX columns and systems
  - Simple to optimize and easily automated
  - Applicable to the majority of mAbs



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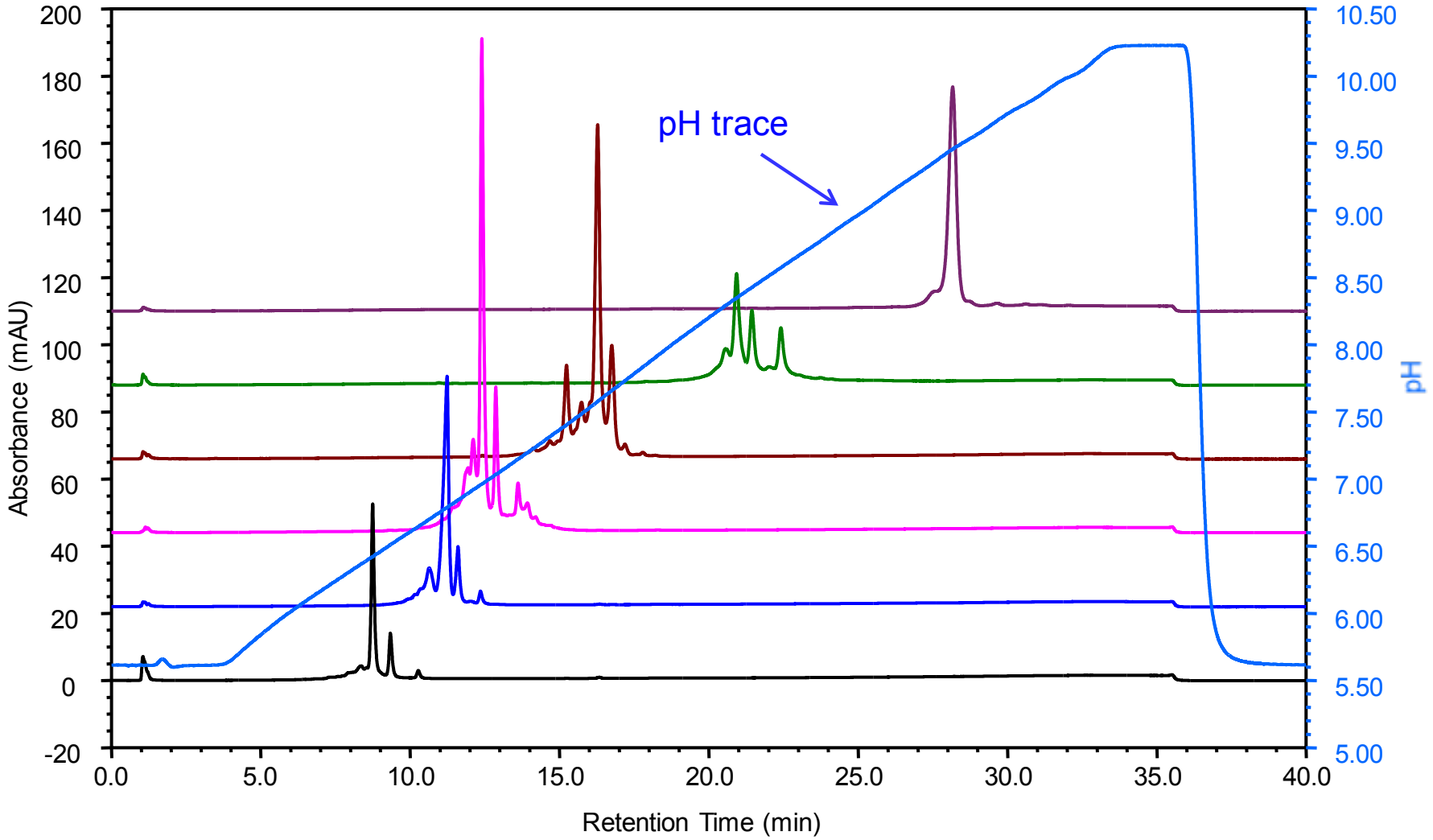
Benefit of Linear pH Gradient: Platform Approach

- A platform approach for charge variant analysis, covering the pH range 5.6 to 10.2
  - The same pH gradients is applicable to majority of mAb charge variants with pI value between 6-10
  - pI value of the unknown mAb can be predicted from the correlation curve

mAb Standards Using Linear pH Gradient



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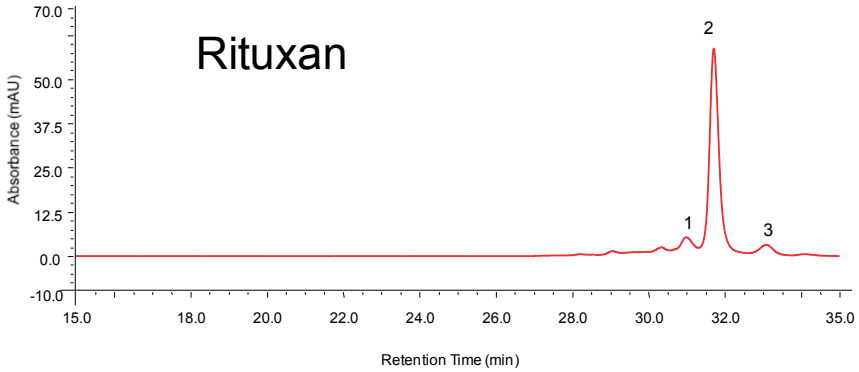
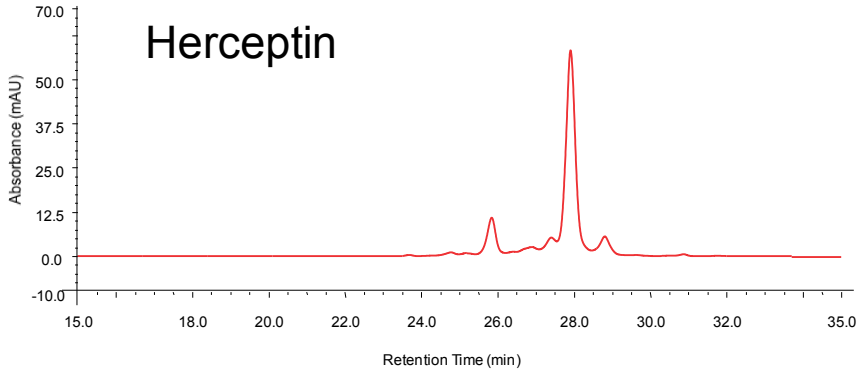
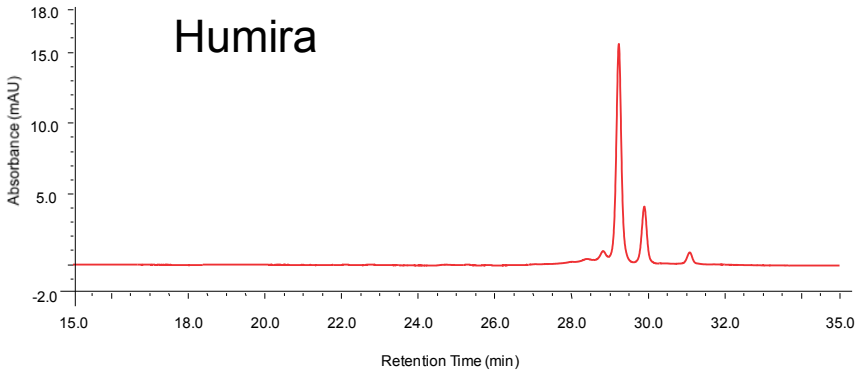
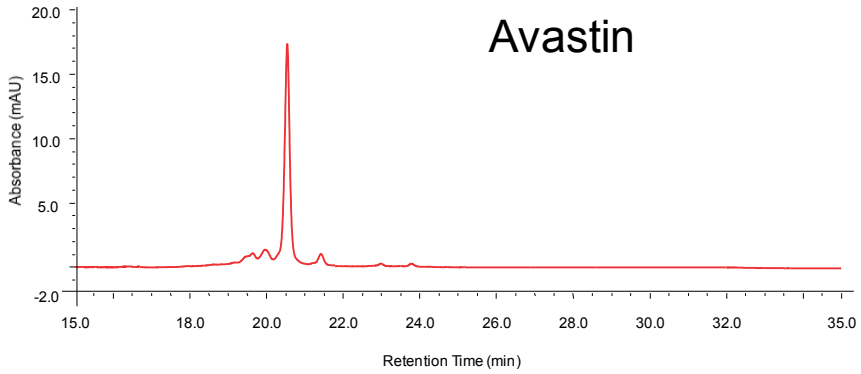
Top-selling mAbs Analyzed by pH Gradient Method









Platform method for mAb charge variant analysis

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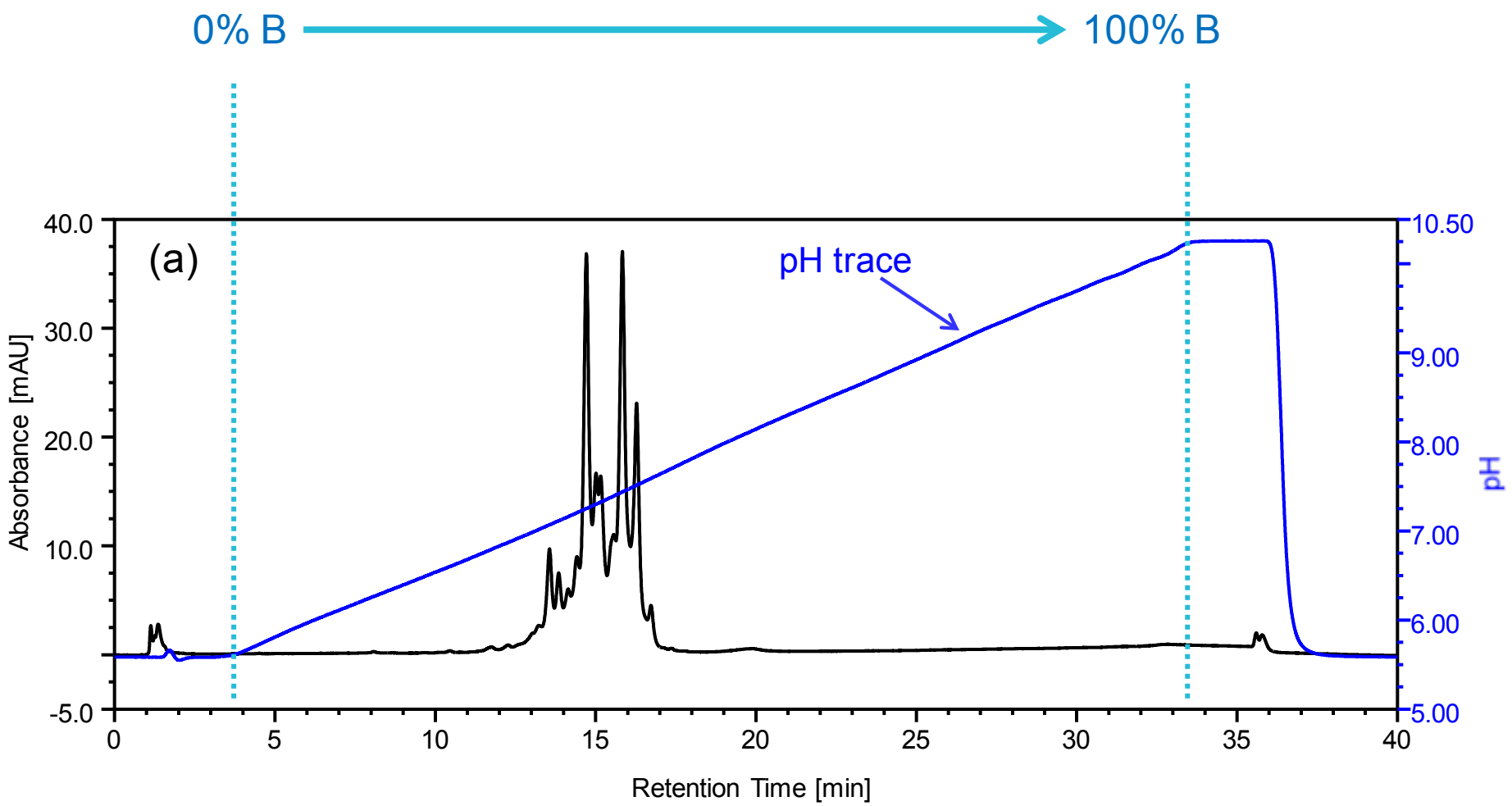
Benefit of Linear pH Gradient: Simple Optimization

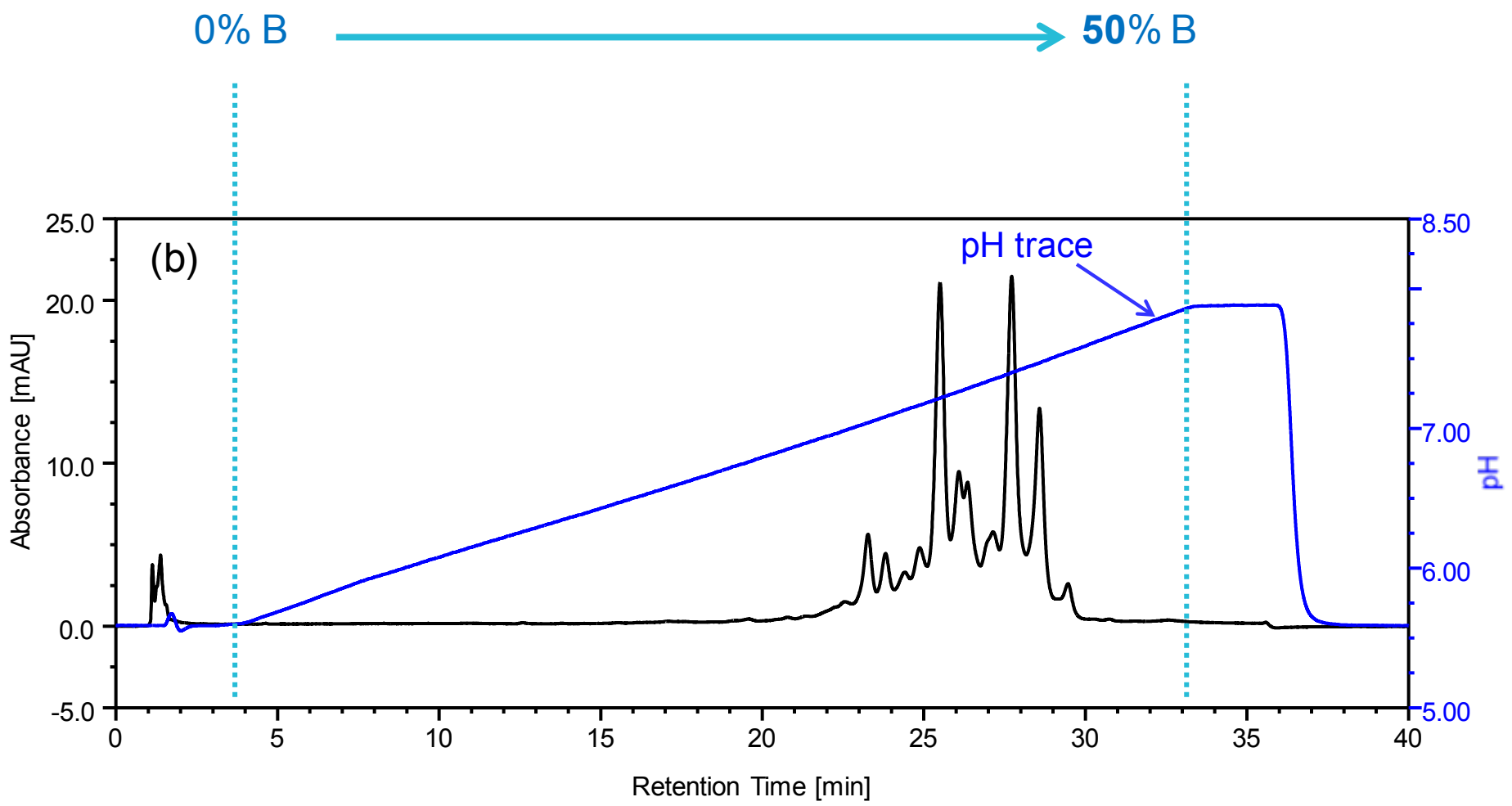
- The method can be simply optimized
  - By running a **shallower pH gradient** a higher resolution separation is obtained (e.g. 50-100%, rather than 0-100%B)

mAb Charge Variant Separation, 0–100% B



*The pH trace at elution was obtained with the Thermo Scientific™ Dionex™ UltiMate™ 3000 pH and Conductivity Monitoring Module (PCM-3000)

mAb Charge Variant Separation, 0–50% B



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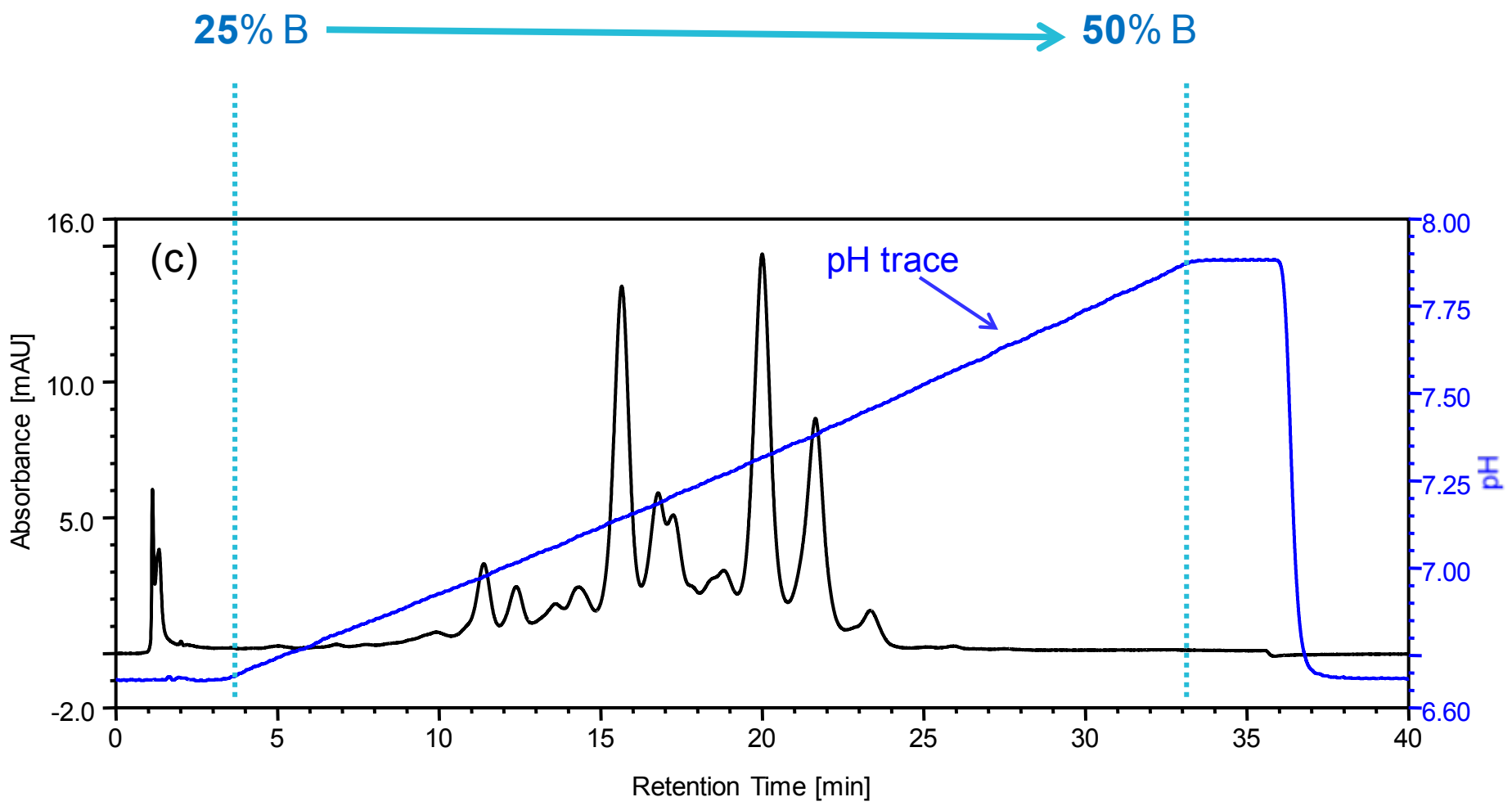
mAb Charge Variant Separation, 25–50% B



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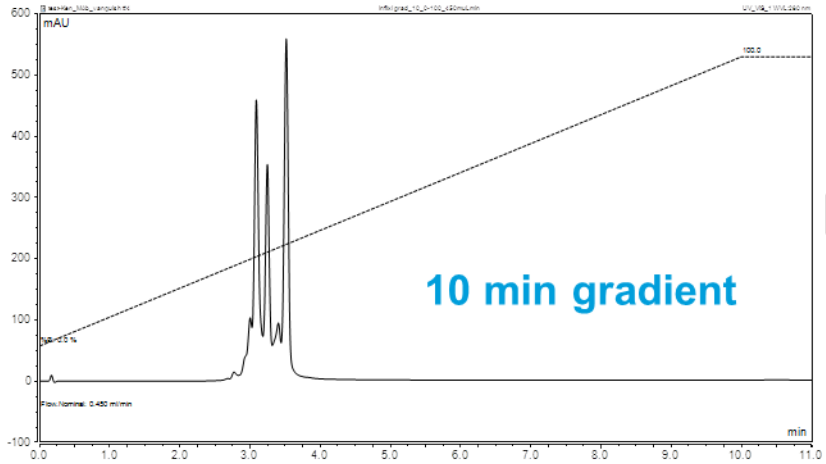
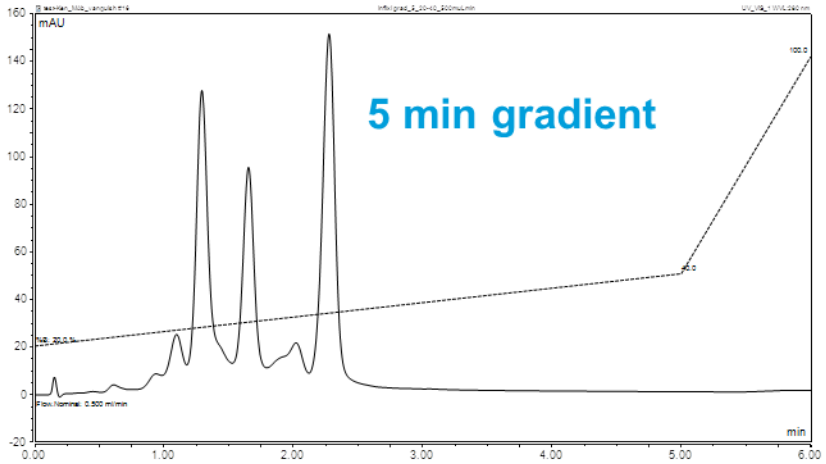
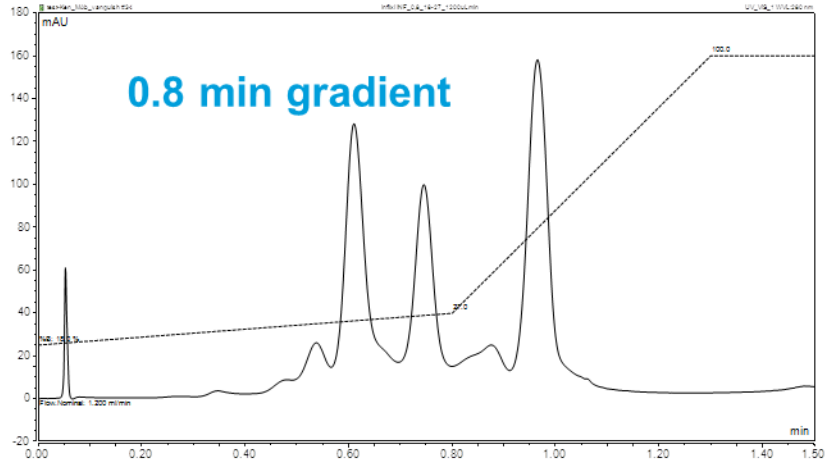
Ultra Fast Method Development







3 steps method development

1. 10 minutes 0→100 % B in 10 minutes
2. 20→40 %B in 5 minutes
3. 18→27 %B in 0.8 minutes

Number of charge variants and resolution maintained for sub-minute gradient

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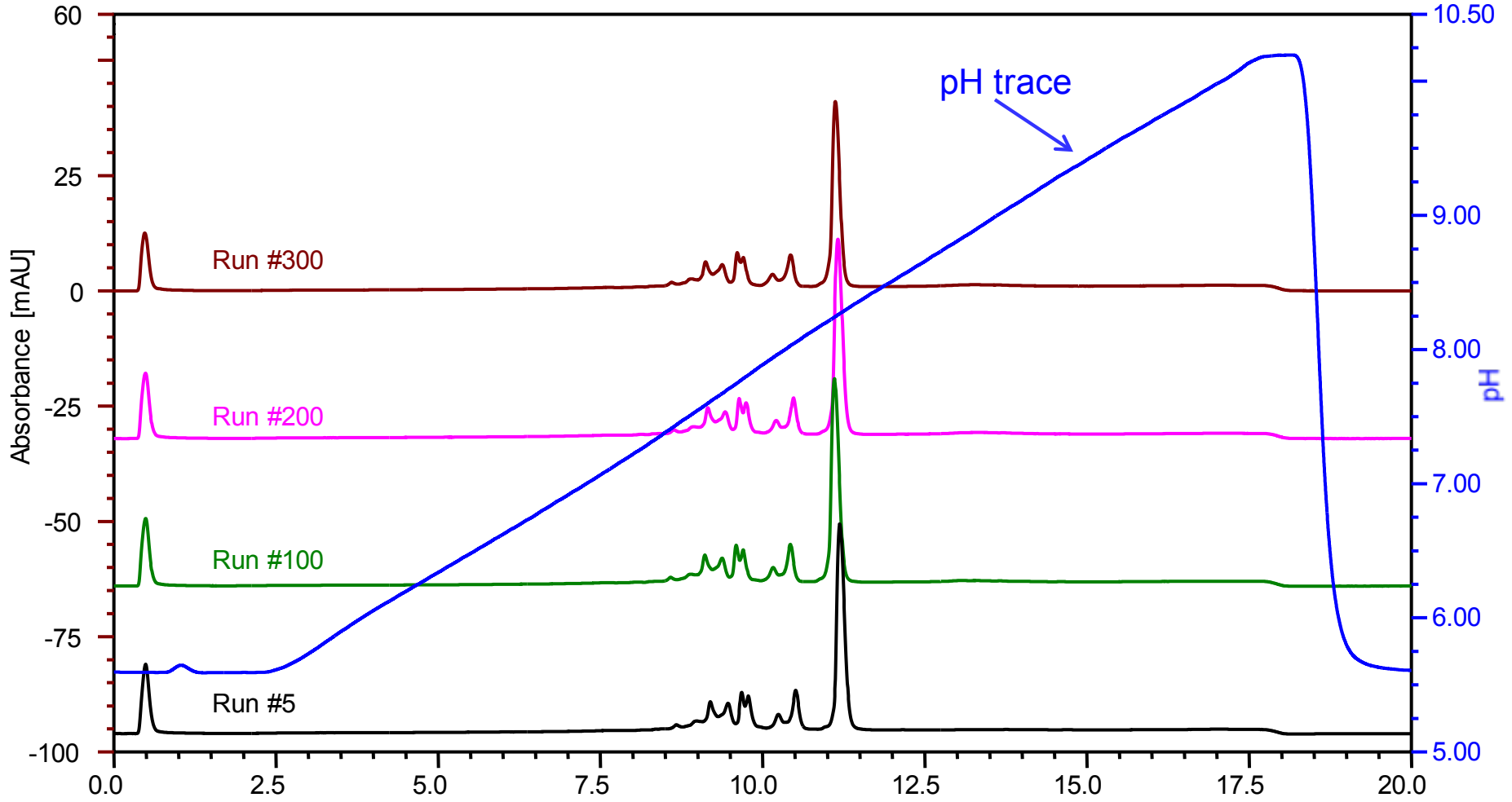
Repeat Injections of Ribonuclease A: Over 300 Runs



Retention time reproducibility <0.8% RSD

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CX-1 pH Gradient Buffer Kit (10X): Lot to Lot Consistency

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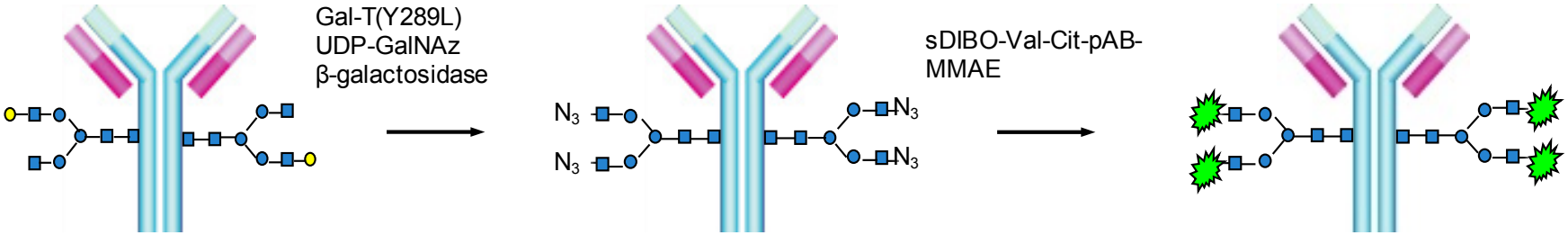
RP LC/MS Analysis for Intact, mAb Fragments, and ADCs

- MAbPac RP column
- Separate mAb, mAb fragments (LC, HC, Fc, Fab, scFc, and $F(ab')_2$) and ADC DAR forms
- Often coupled with MS instrument for high resolution accurate mass determination

MAbPac RP columns

Column Chemistry	Phenyl
Substrate	Spherical, polymer, wide-pore (1,500 Å)
Particle size	4 µm
Pore size	1,500 Å
Formats	100 mm: 3.0 + 2.1mm ID 50 mm: 3.0 + 2.1mm ID 10mm Guard Cartridges: 3.0 + 2.1mm ID

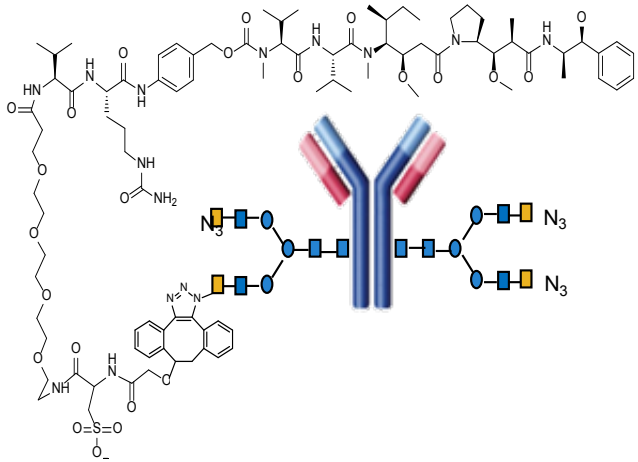
SiteClick™ Enzyme-based N-glycan Labeling of Antibody



Antibody
- **Polyclonal**
- **Monoclonal**
- **Recombinant**

Azide-Activated Antibody

Site-Specifically Labeled Antibody



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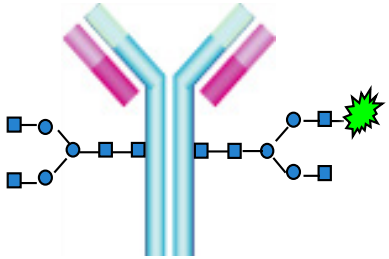
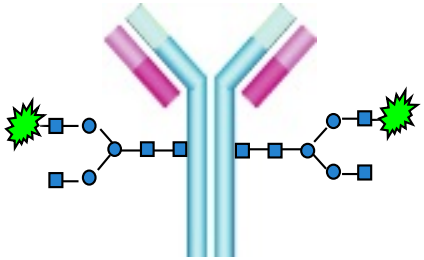
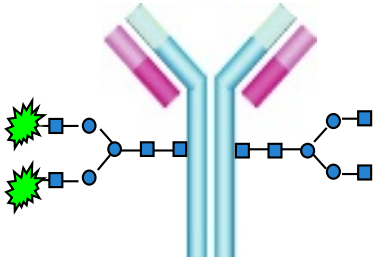
Site-selective Antibody-drug Conjugates (ADCs) DAR Forms

DAR 1

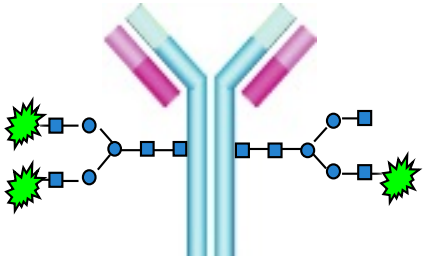


DAR 2

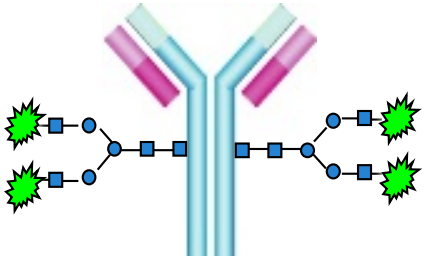




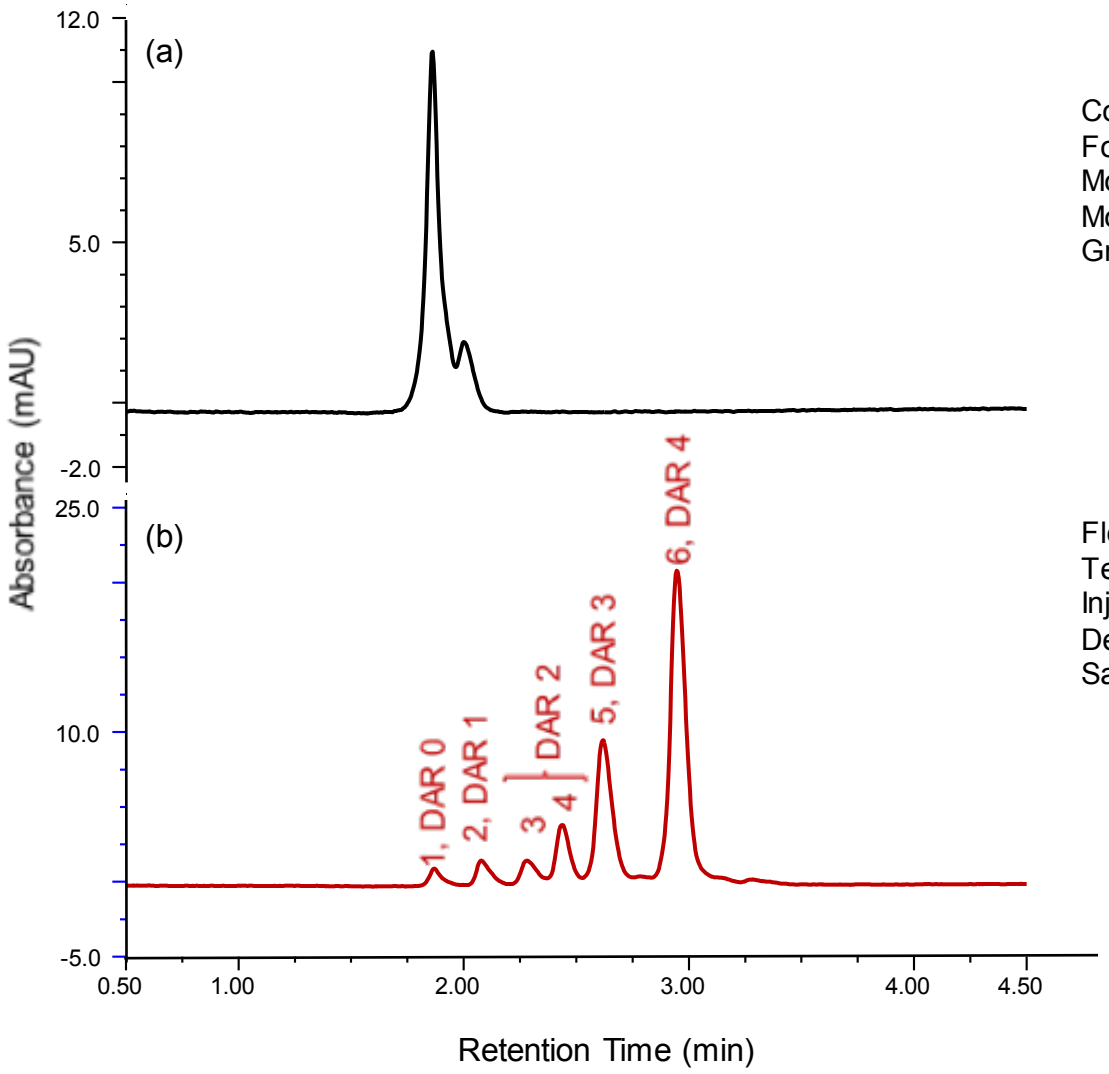
DAR 3



DAR 4



LC/UV Analysis of ADC



Column:	MAbPac RP, 4 µm
Format:	2.1 × 50 mm
Mobile phase A:	H_2O/TFA (99.9 : 0.1 v/v)
Mobile phase B:	MeCN/ H_2O/TFA (90: 9.9 :0.1 v/v/v)
Gradient:	

Time (min)	%A	%B
0.0	65	35
0.5	65	35
4.5	45	55
5.0	45	55
5.5	65	35
6.0	65	35

Flow rate:	0.6 mL/min
Temperature:	80 ºC
Inj. volume:	2 µL
Detection:	UV (280 nm)
Sample:	a. Trastuzumab
	b. Trastuzumab-MMAE

LC/MS Analysis of ADC

(a) Peak 1, DAR 0

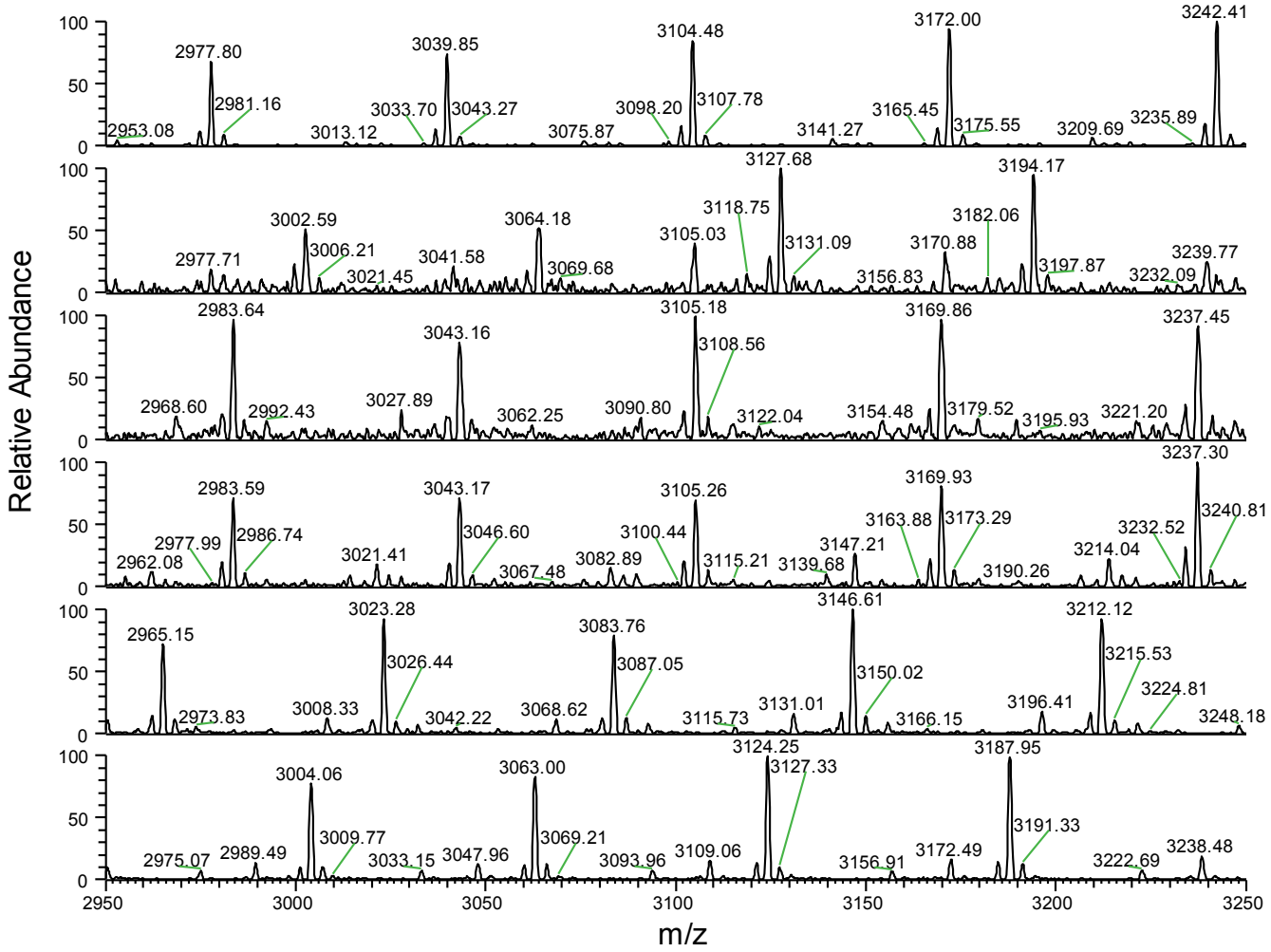
(b) Peak 2, DAR 1

(c) Peak 3, DAR 2

(d) Peak 4, DAR 2

(e) Peak 5, DAR 3

(f) Peak 6, DAR 4



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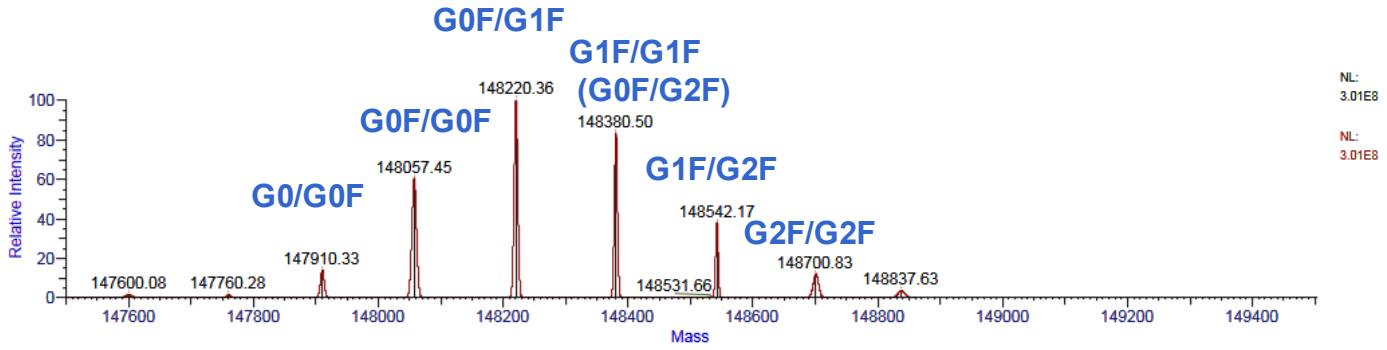
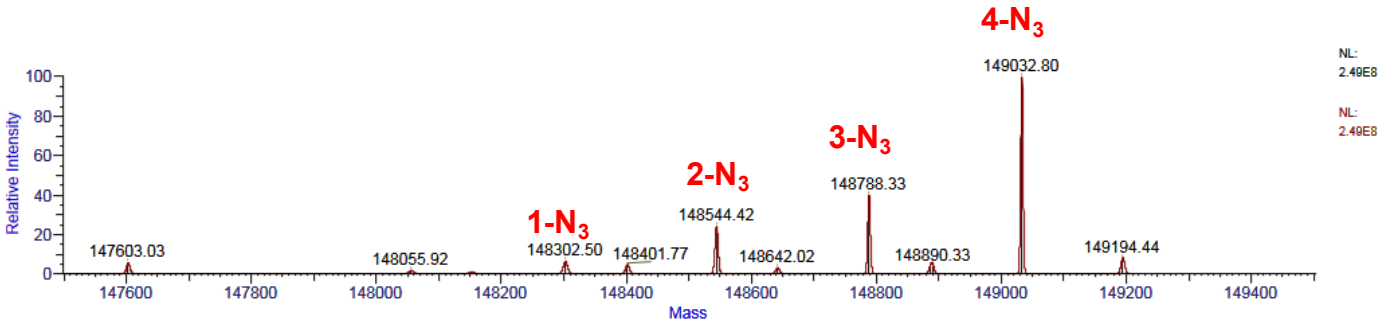
Deconvolution of mAb and Intermediate

(a) Trastuzumab



(b) Trastuzumab-azide



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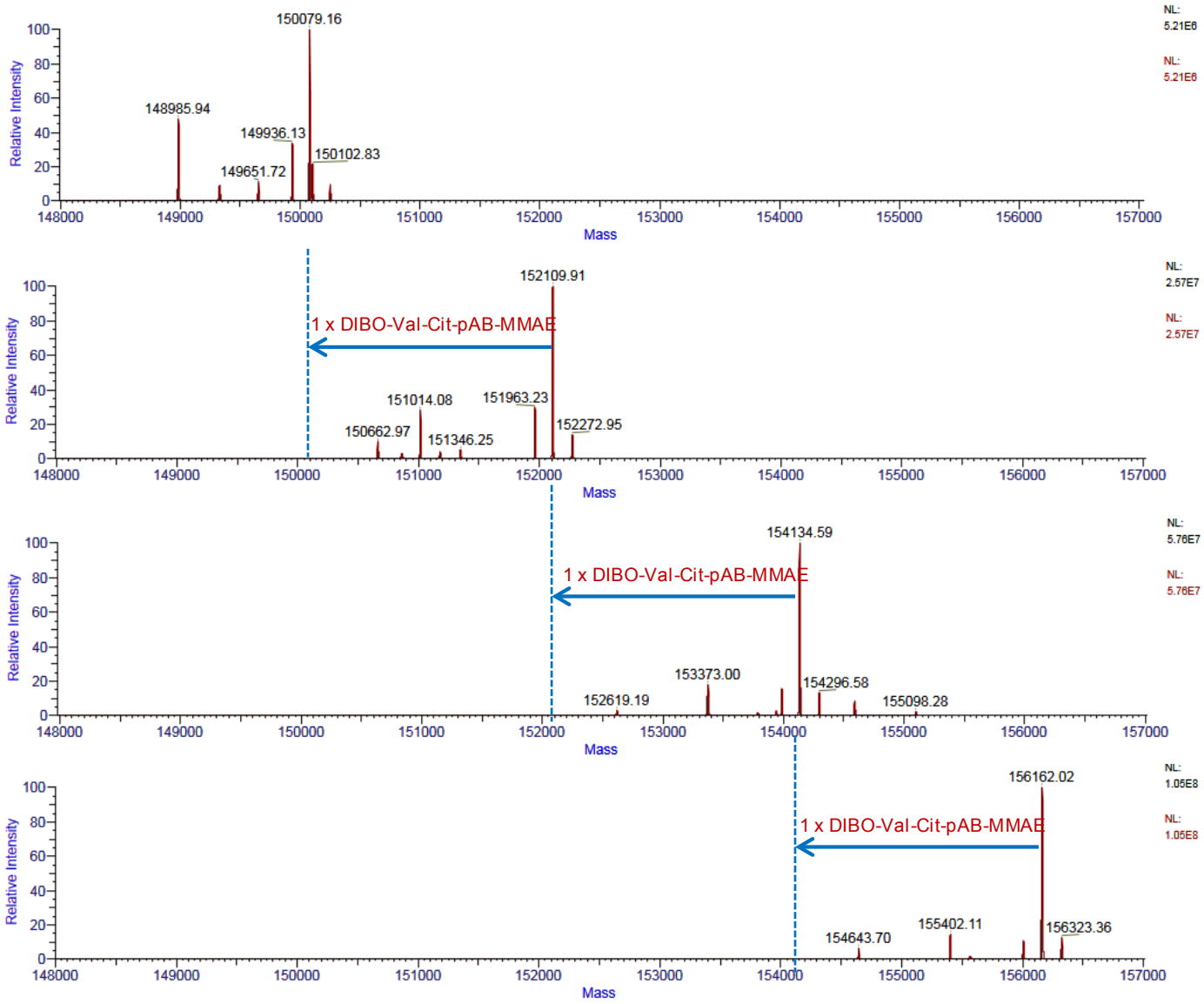
Deconvolution of Multiple DAR Forms

(a) DAR 1

(b) DAR 2

(c) DAR 3

(d) DAR 4



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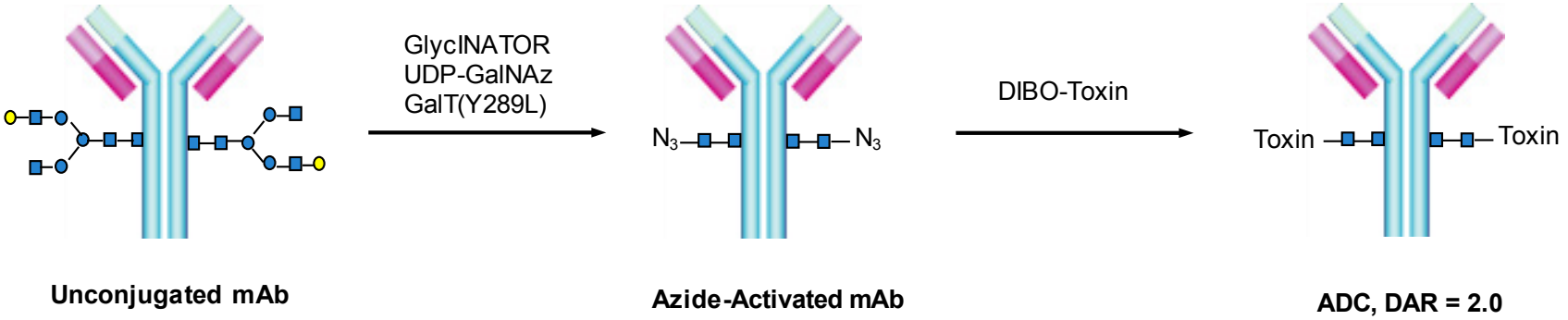
SiteClick™ Enzyme-based N-glycan Labeling of Antibody



Poster number: P-202-TH
Presenting: Thursday, January 26 from 7:30am – 8:30am.
Title: High Resolution LC/MS Separation and Characterization of Chemoenzymatic Site-specific Engineered Antibody-drug Conjugates (ADCs)

Thank you!

- Chris Pohl
- Xiaodong Liu
- Julia Baek
- Yoginder Singh
- Brian Agnew
- Terry Zhang
- Jonathan Josephs