A Probability-Based Modeling Approach for Characterization of ADC Charge Variants Separated by icIEF that Leverages Bottom-Up Mass Spectrometry Datasets

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Table of Contents

AGENDA

Introduction - rationale

- Challenges of indirect and direct charge variant (CV) characterization
- Addressing characterization challenges with models of CV separations

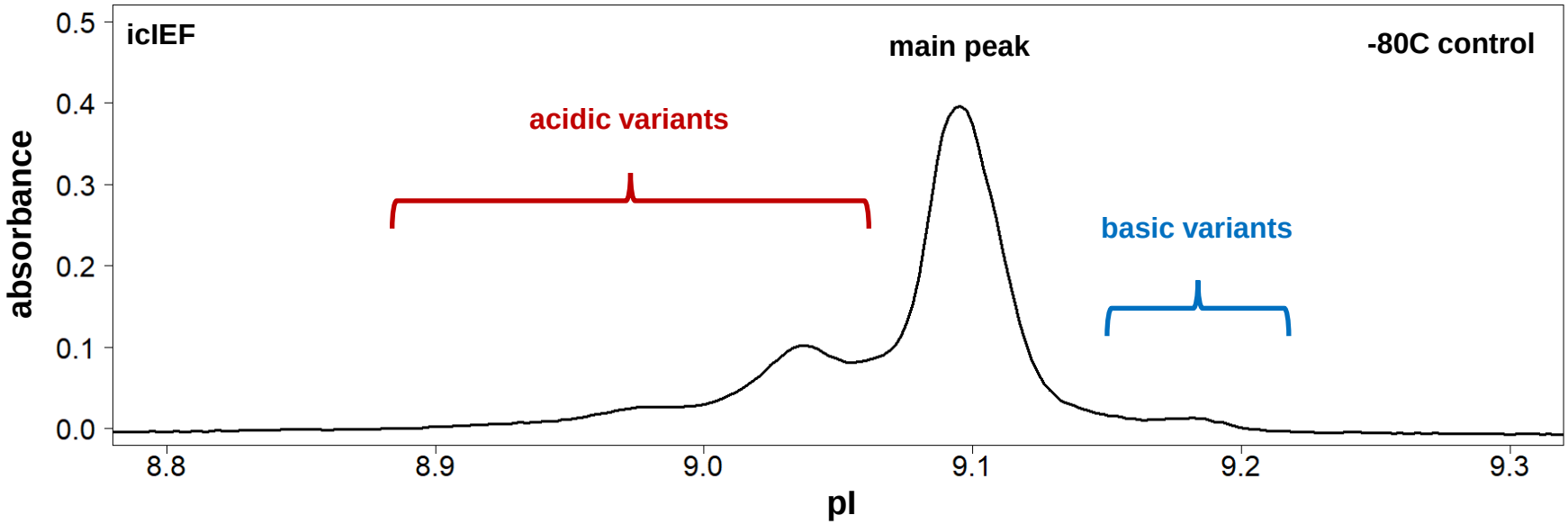
Comparison between empirical and modeled CV distributions

- Uncharged and charged ADC drug-linker models
- Chemical modifications of mAb backbone and drug-linkers (DL)

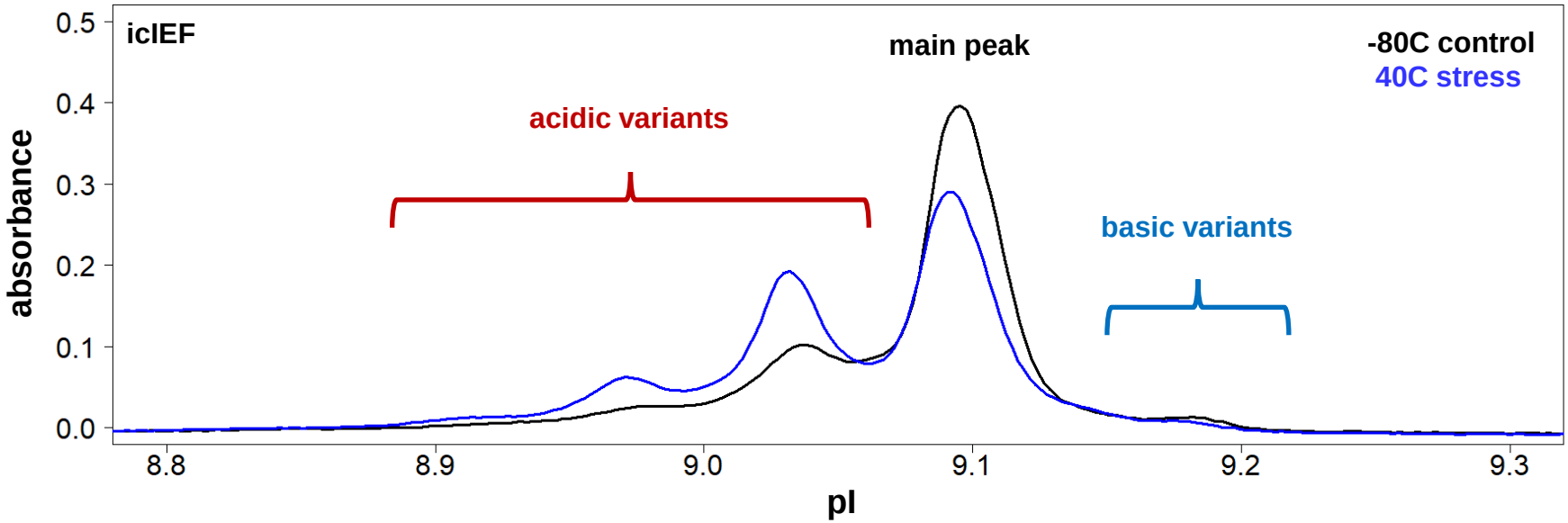
CV modeling applications

- Conversion of CV models to *in silico* intact MS
- Using modeling to enhance understanding of CE-MS and CEX-MS data

Charge variant assay interpretation



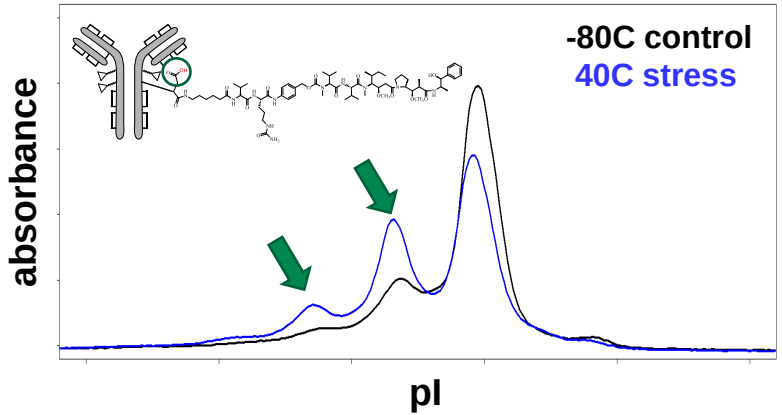
Charge variant assay interpretation



What do we do with this observed difference?

Analytics to insights approach

icIEF

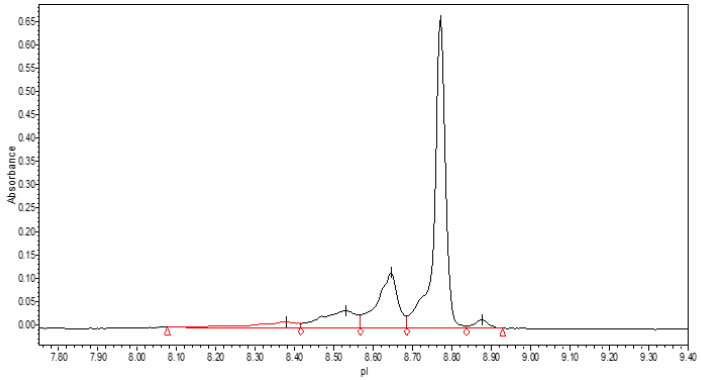


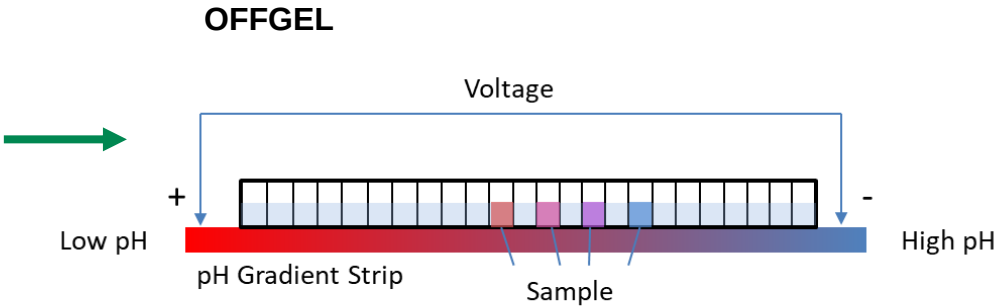
Peptide map

	-80 control	40C stress
deamidation	1.1%	1.4%
DL hydrolysis	~2%	~9%

- What is it?
  - What are we observing in the assay?
- Why did it happen?
  - What is driving the change?
    - Deamidation or other PTM
    - DL hydrolysis
- Should we care?
  - Where does the change occur?
  - What is the impact for patients?
- What should we do?
  - Tailor control strategy to presumed criticality of the attributes that are changing

Direct characterization of CVs by OFFGEL fractionation

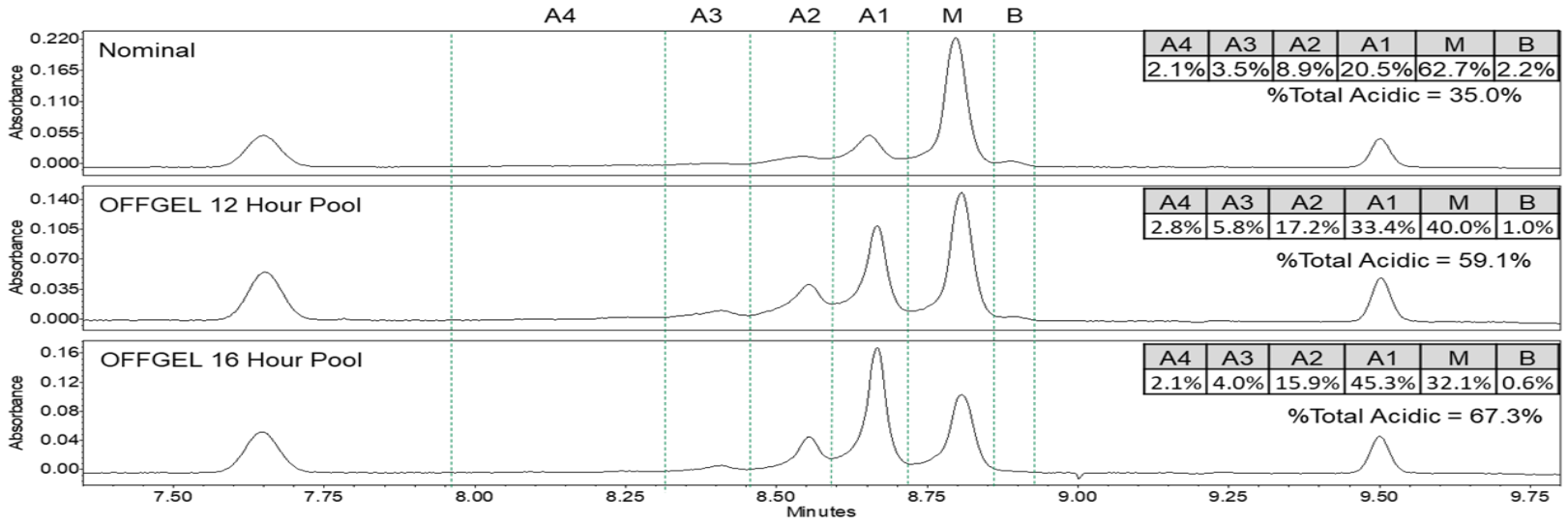




Size-based Characterization:	MS-based Characterization:	Functional Assay Characterization:
• R CE-SDS • NR CE-SDS • SE-UPLC	• Subunit • NR Mass • PTM Analysis	• Binding • Potency

OFFGEL as an ADC charge variant isolation strategy is artifactual

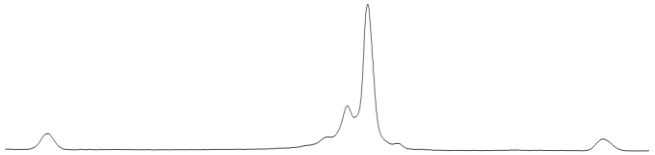
- OFFGEL: Direct characterization, but not viable for all ADCs
  - Observed assay-induced artifactual hydrolysis of drug linkers over duration of separation



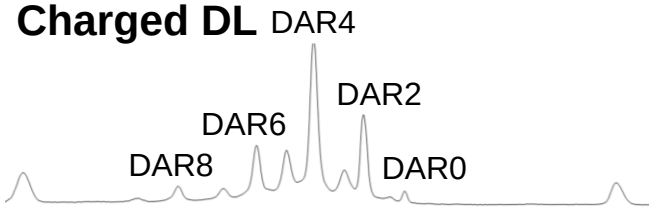
CV profiles are complex and charged DLs will increase complexity

- Understanding of charge variants (CVs) is essential for developing ADC process and product knowledge
- The coming challenge: charged drug-linkers
  - Partially-loaded ADC species separate on the basis of drug-load
  - Additional complexity makes it very difficult to indirectly characterize and understand what is causing CV differences
- ***For all biologics*** there is a need for a holistic strategy that does not rely on fractionation and direct characterization of CVs

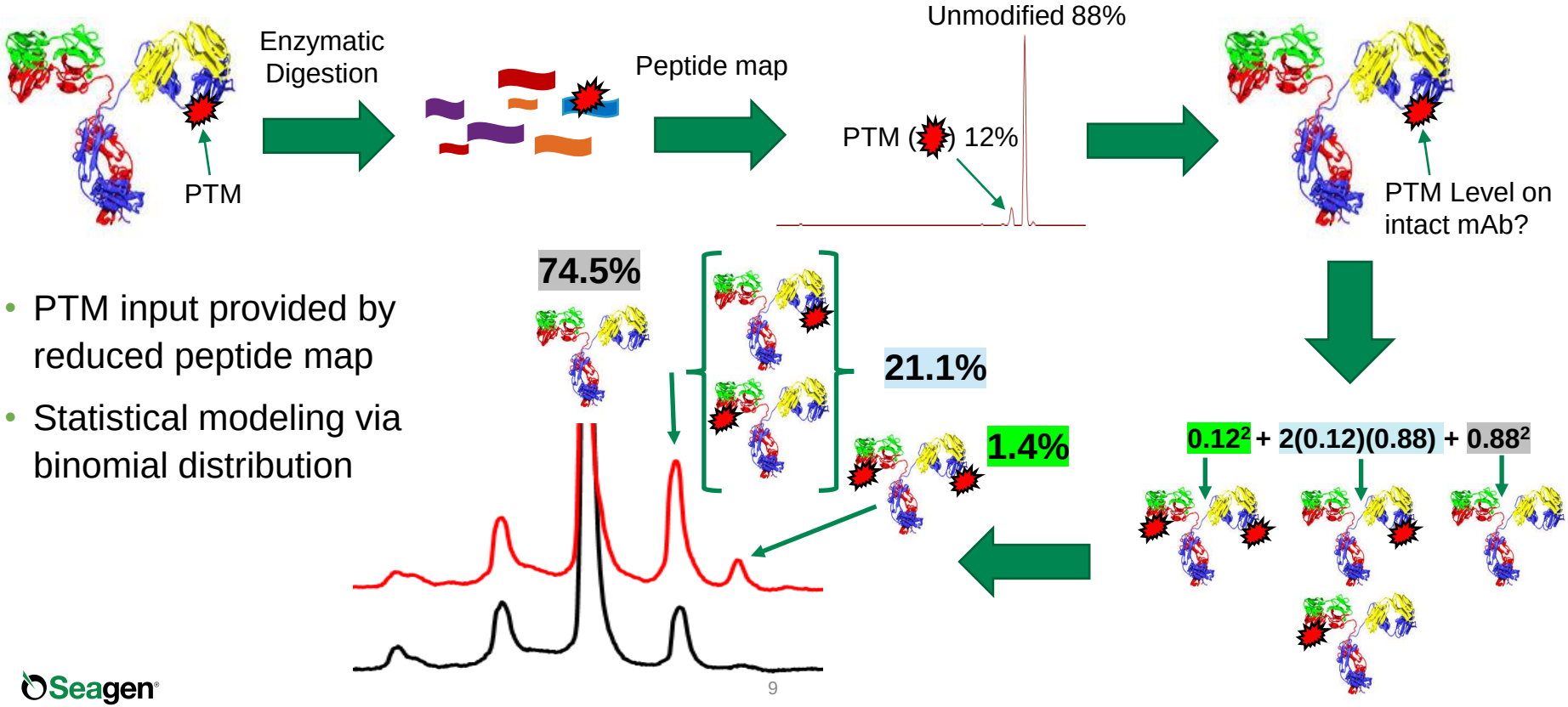
Uncharged DL



Charged DL



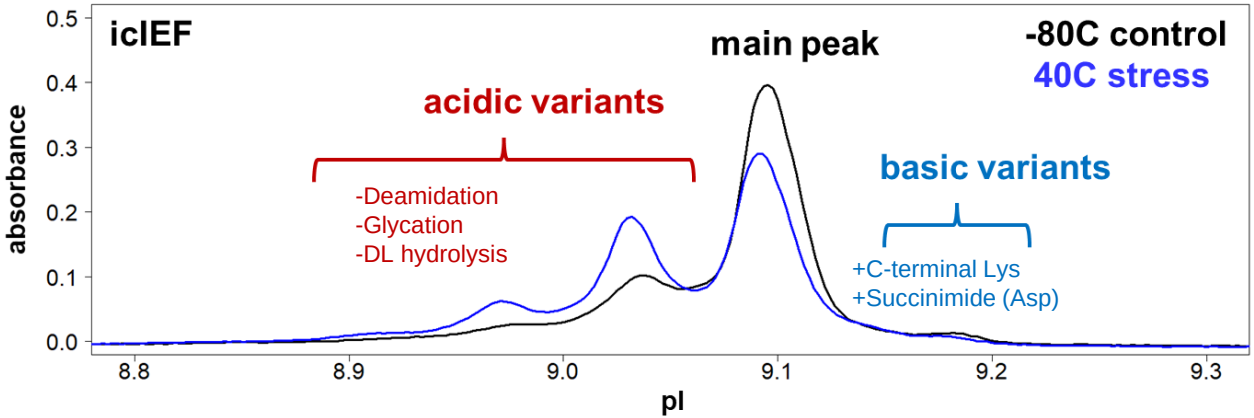
Binomial distributions are used to model CV profiles



- PTM input provided by reduced peptide map
- Statistical modeling via binomial distribution

Model (expected) CV separation is generated from known molecular properties and direct PTM quantitation

- What do we know based on PTM molecular properties



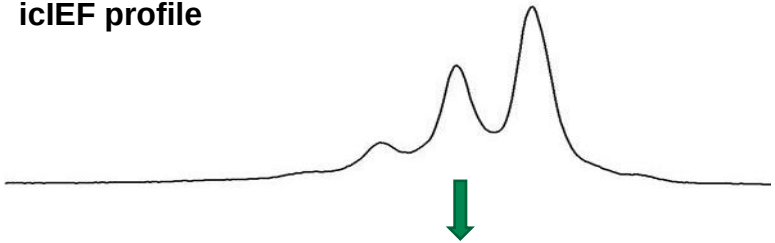
- Apply basic probabilities and charge shift multiplier to all PTMs in peptide map data

PTM	Assay	Location	Net charge shift	Statistical model
Deamidation	Peptide map	Protein backbone	-1	Simple binomial
Hydrolysis	**Peptide map, LCMS**	**Drug-linker**	**-1**	**Weighted simple binomial**
Clip	LCMS	Protein backbone	Unknown	Simple binomial
Oxidation	Peptide map	Protein backbone	0	Simple binomial
Succinimide	Peptide map	Protein backbone	1	Simple binomial
Glycation	LCMS	Protein backbone	-1	Simple binomial

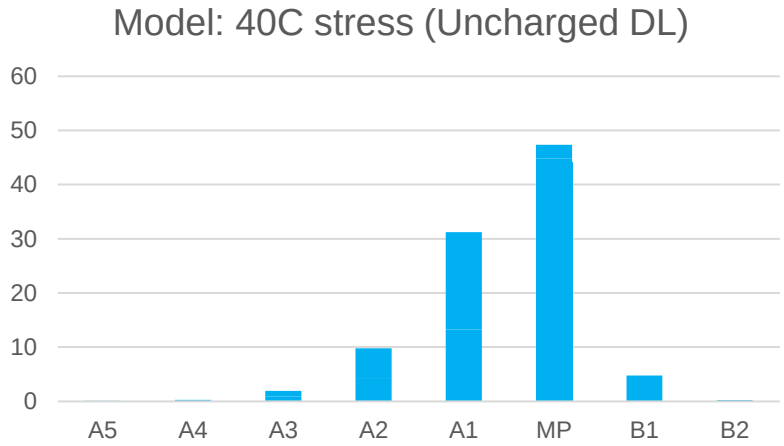
Combined model output is charge sorted and compared to icIEF

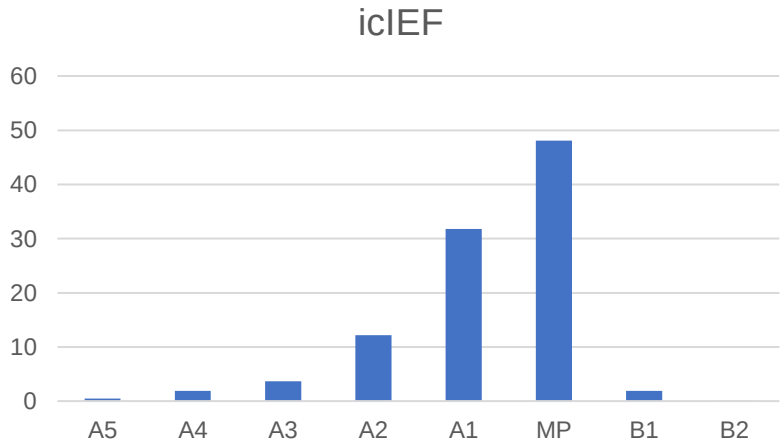
- Binomial modeling parameters
  - PTMs
    - Deamidation, Succinimide, N-term cyclization, C-term Lys processing, Glycation
  - DL hydrolysis

icIEF profile

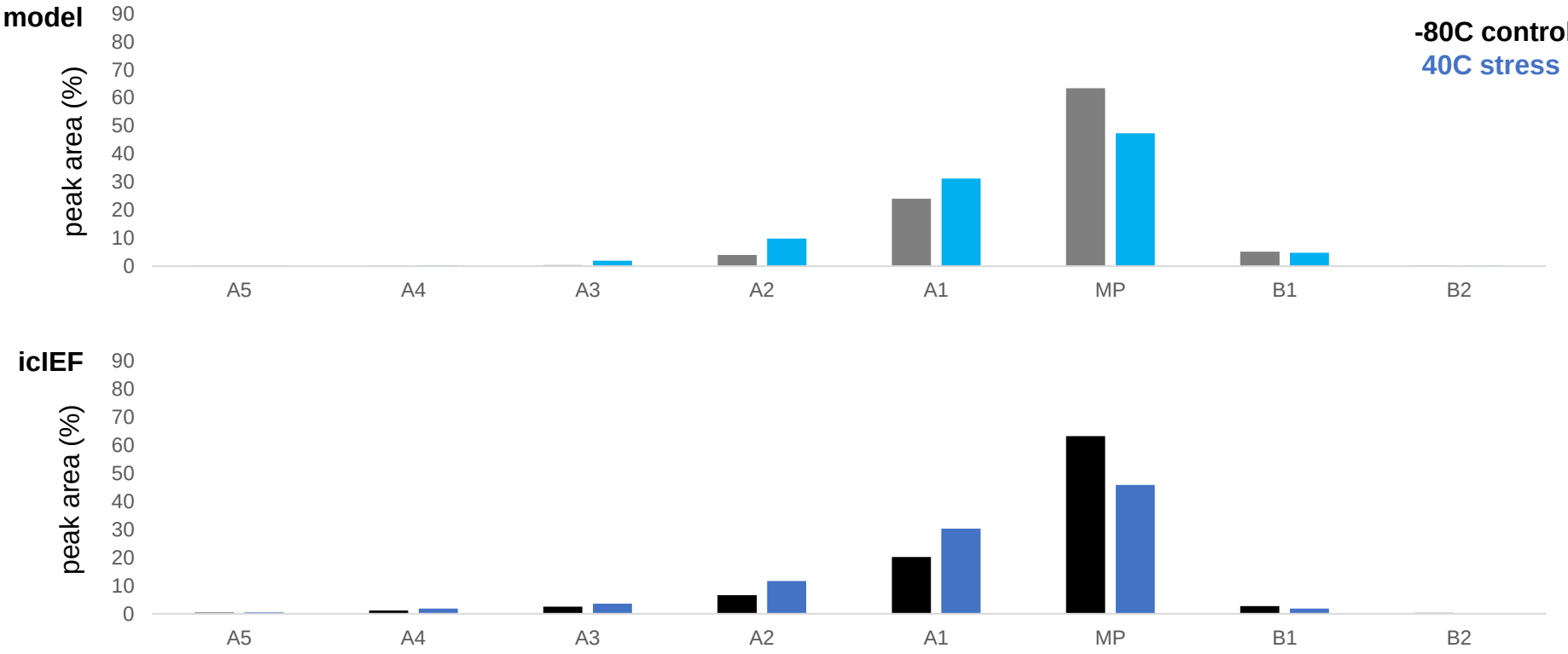


Convert to discrete bars and compare with model

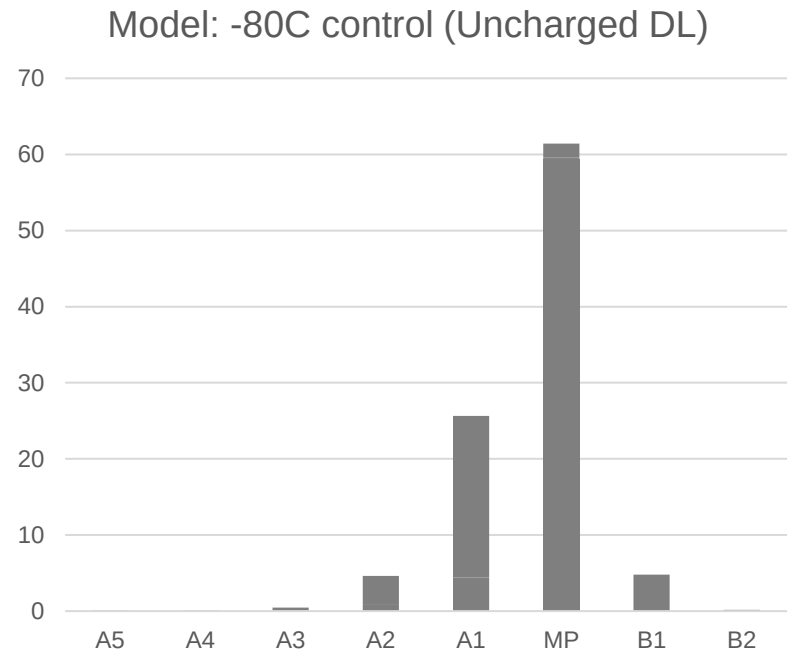


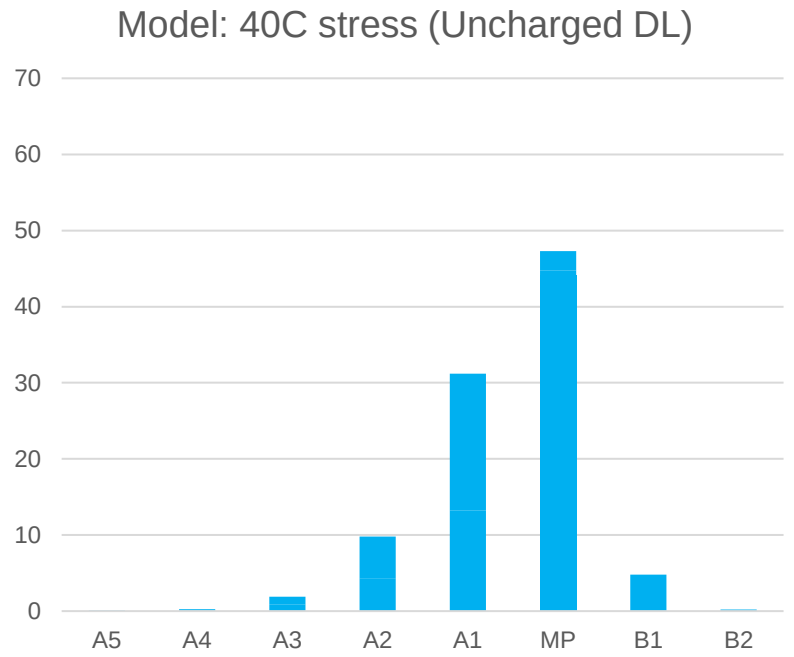


Binomial modeling tracks well for control and stress samples

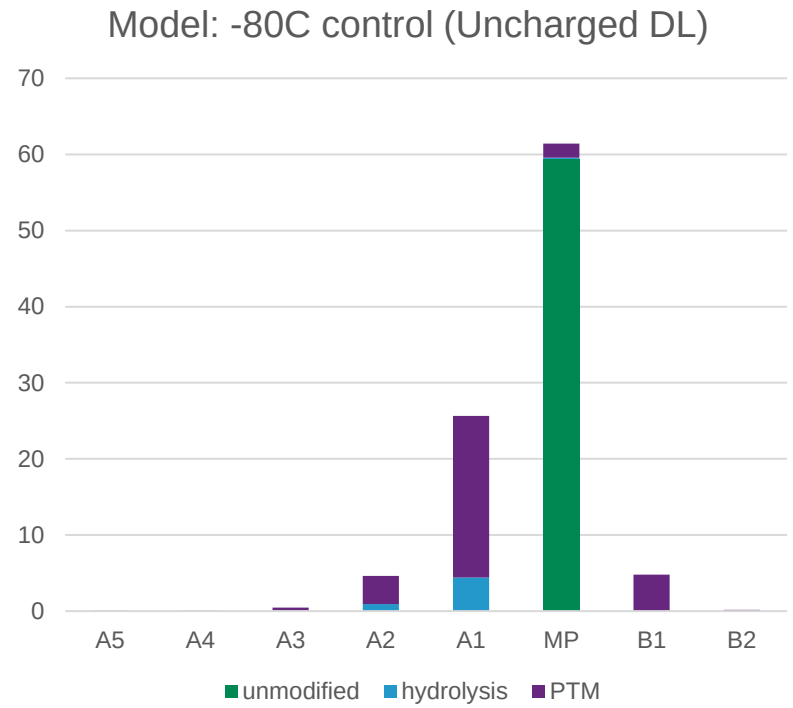


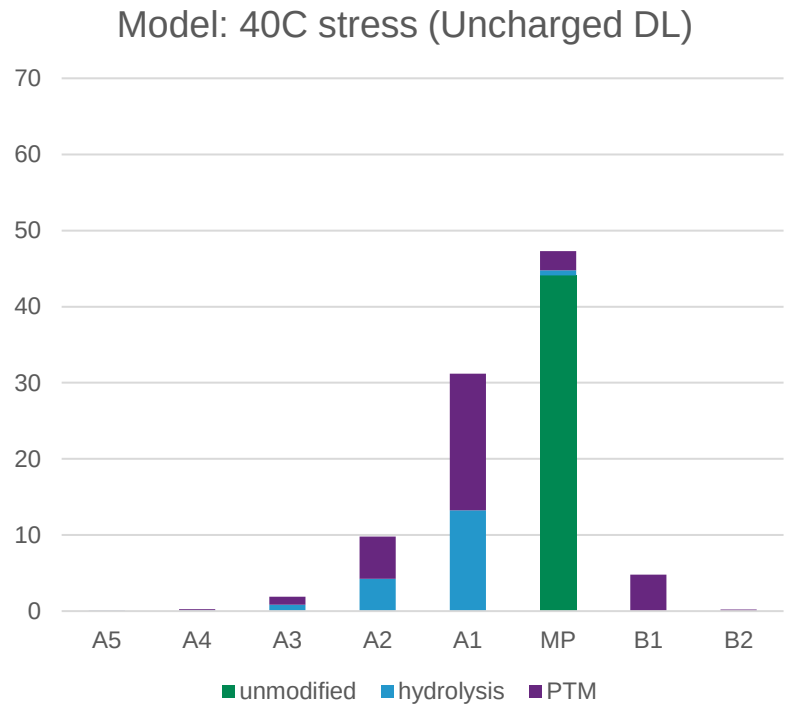
PTM differences between control and stressed material underly the profile changes observed in the CV model



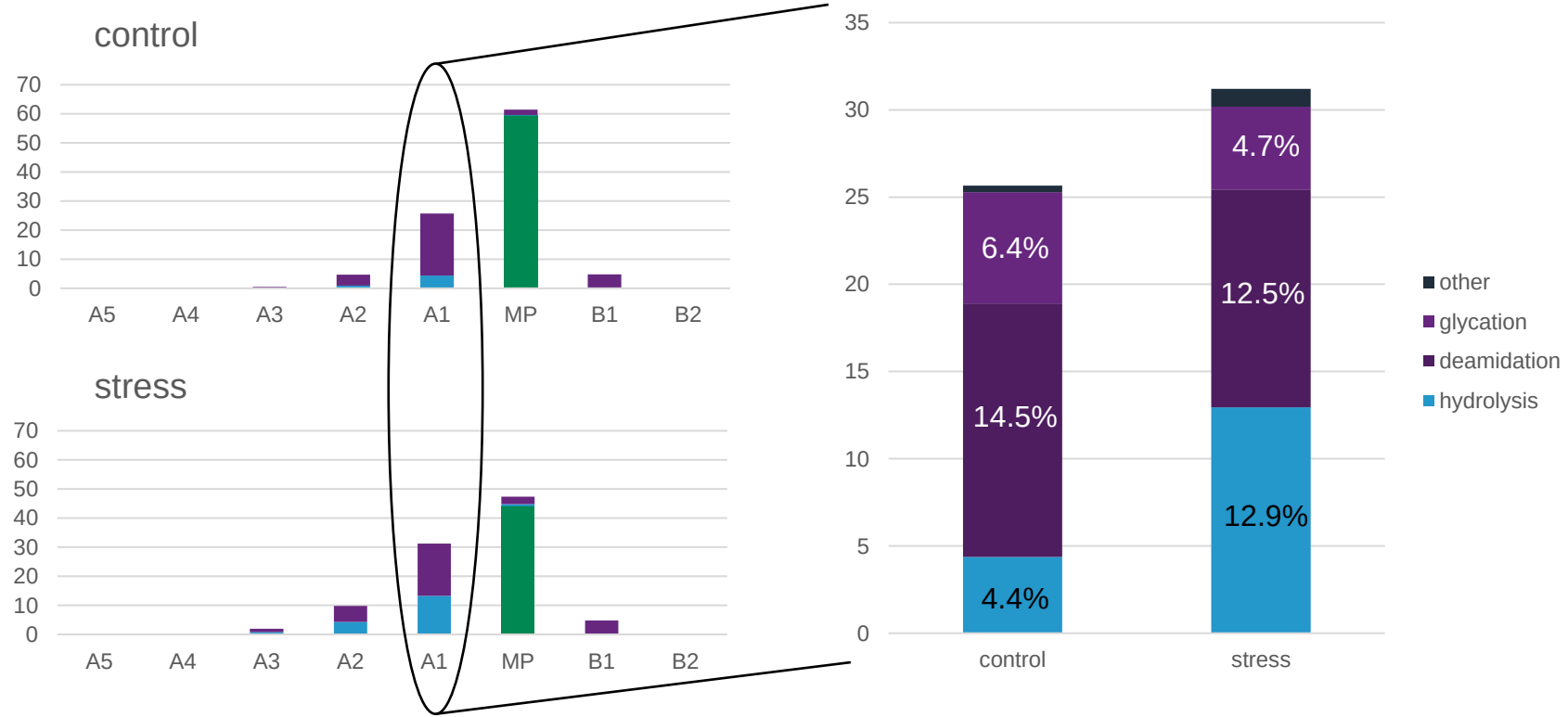


The impact of stress induced increases in PTMs and DL hydrolysis on CV separations can be abstracted

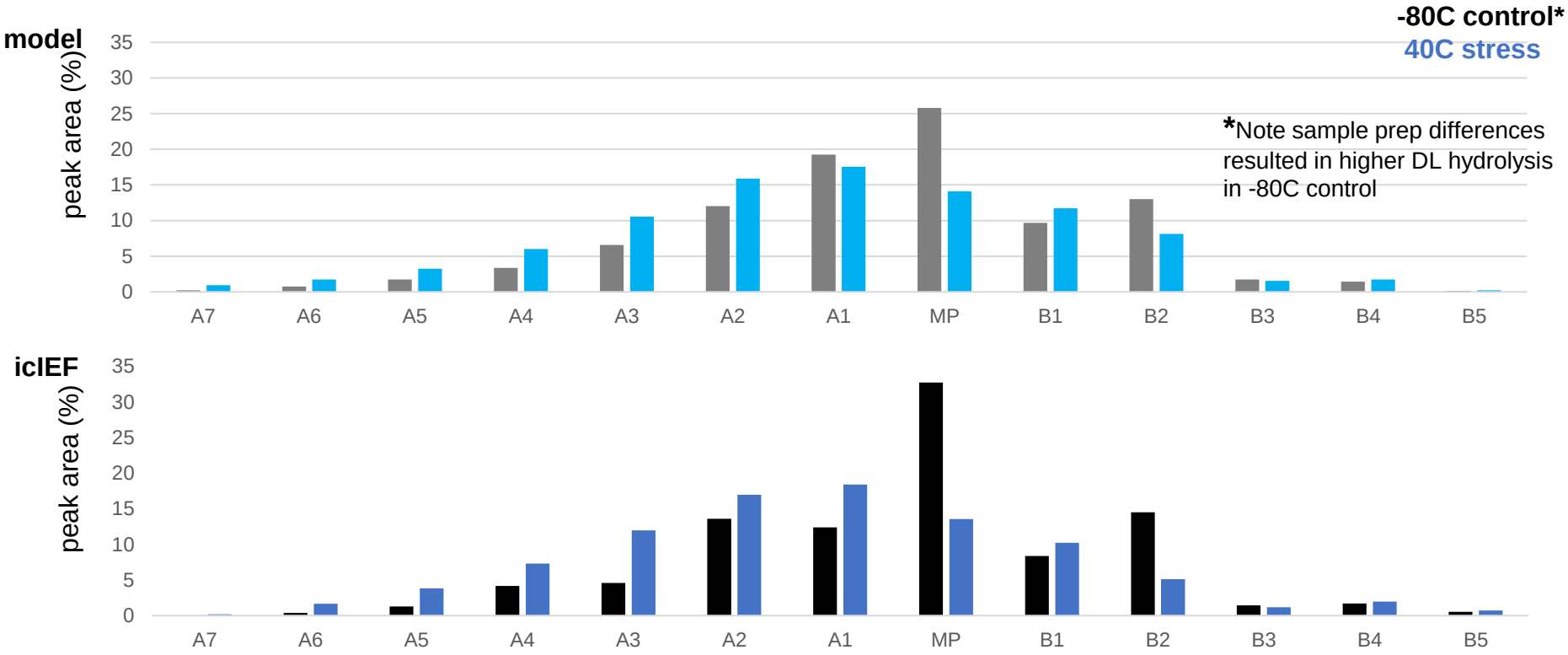




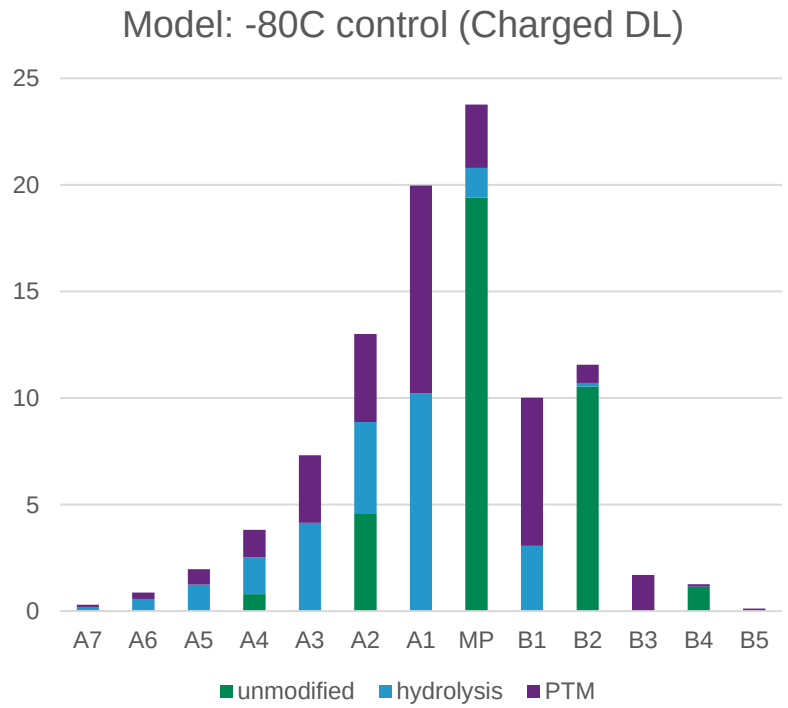
Additional level of detail such as composition of PTMs in particular peaks can be inferred from modeled data

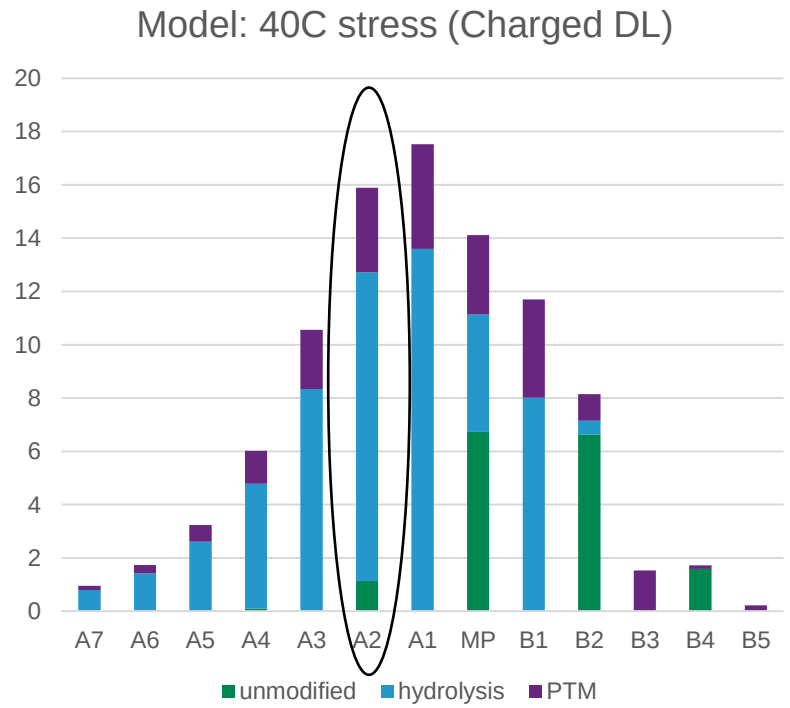


Charged DL model to icIEF shows good agreement for stressed material

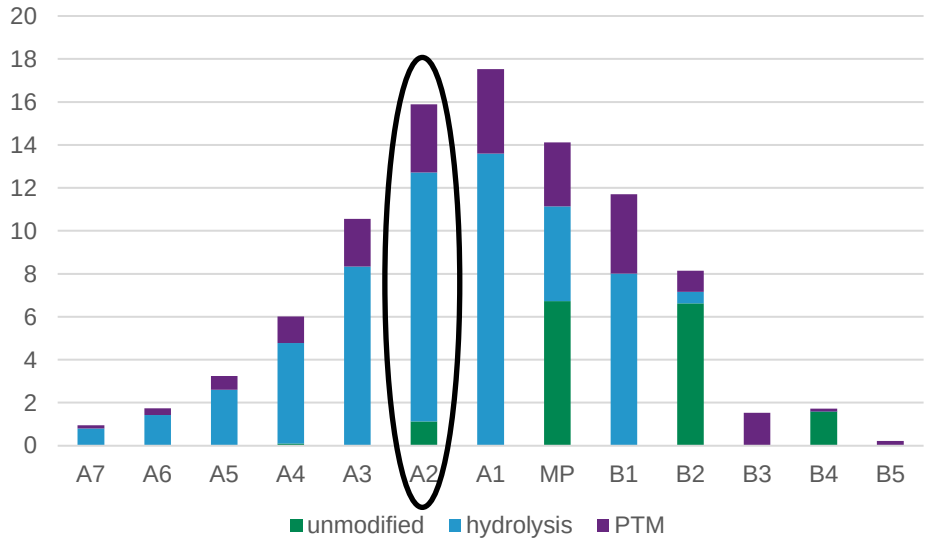


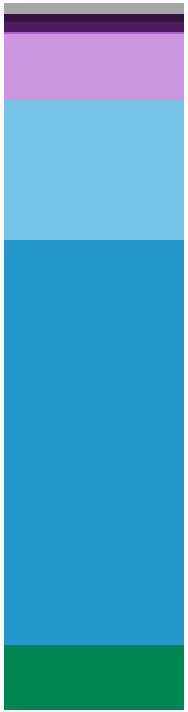
DL hydrolysis is the primary driver for CV profile change and increase in acidic species

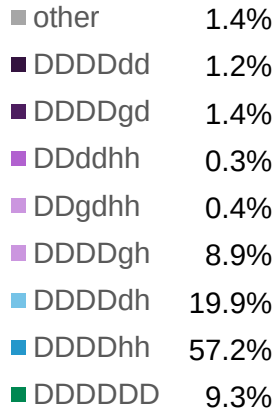




The modeling approach provides granularity into changes in specific molecular populations





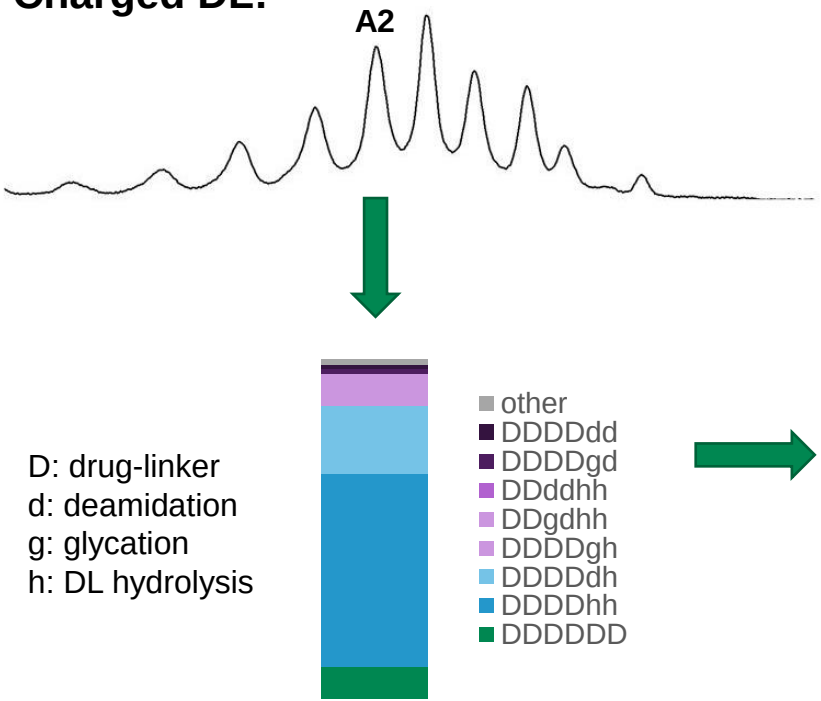


D: drug-linker
d: deamidation
g: glycation
h: DL hydrolysis

- Categorical view for broad understanding
  - Model provides greater granularity with enumeration of species with combinations of modifications

In silico mass spectrum generation from PTM-based CV model

Charged DL:



D: drug-linker
d: deamidation
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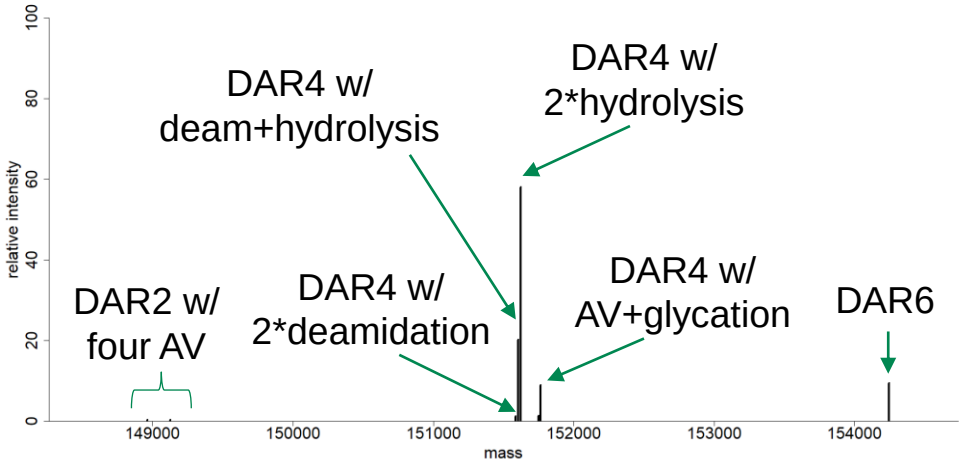
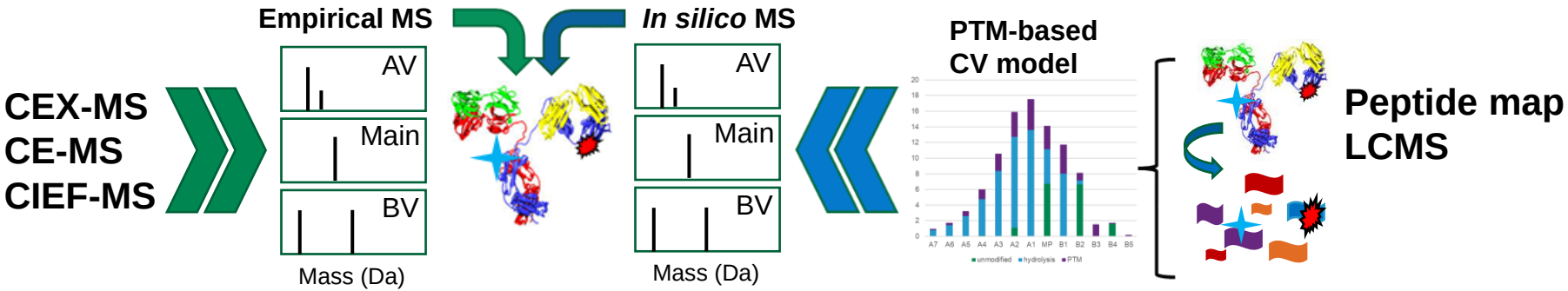
- Compositional characterization allows for extended modeling opportunities
  - icIEF → CV profile model → theoretical mass spectrum



Theoretical mass spectrum based on statistical species distribution

Merging intact CV-MS approaches with PTM-based CV modelling

- The development of MS compatible charge variant separations such as CEX-MS[1], CE-MS[2] and CIEF-MS[3] enhances understanding of separated proteoforms
- PTM-based CV modelling is an orthogonal approach that can be leveraged to add complimentary, site-specific PTM information



1. F. Fussl, K *et al.*, **Charge Variant Analysis of Monoclonal Antibodies Using Direct Coupled pH Gradient Cation Exchange Chromatography to High-Resolution Native Mass Spectrometry**, Anal Chem 90(7) (2018) 4669-4676.
2. M. Han *et al.*, **Intact mass analysis of monoclonal antibodies by capillary electrophoresis-Mass spectrometry**, J Chromatogr B Analyt Technol Biomed Life Sci 1011 (2016) 24-32.
3. S. Mack *et al.*, **A novel microchip-based imaged CIEF-MS system for comprehensive characterization and identification of biopharmaceutical charge variants**, Electrophoresis 40(23-24) (2019) 3084-3091.

Advantages of utilizing PTM-based CV modelling

- Addresses the knowledge gap that exists when CV separations are not amenable to direct characterization through fractionation
- Provides a means to rapidly infer identities of new and changing peaks in analytical assays in a rigorous and quantitative manner
- Can be leveraged to better understand if a CV change is impactful to patients
  - Is the change due to a PTM in a mAb CDR potentially impact binding/activity

Acknowledgements

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