USP Biologics Open Forum:
April 28, 2021, 11am - 12pm EDT

Shaping Tomorrow's Solutions to Today's Biologics Quality Challenges:

Update on USP's Work Supporting Multi-Attribute Methods for Biologics

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Update on MAM Laboratory Studies and Future Plans

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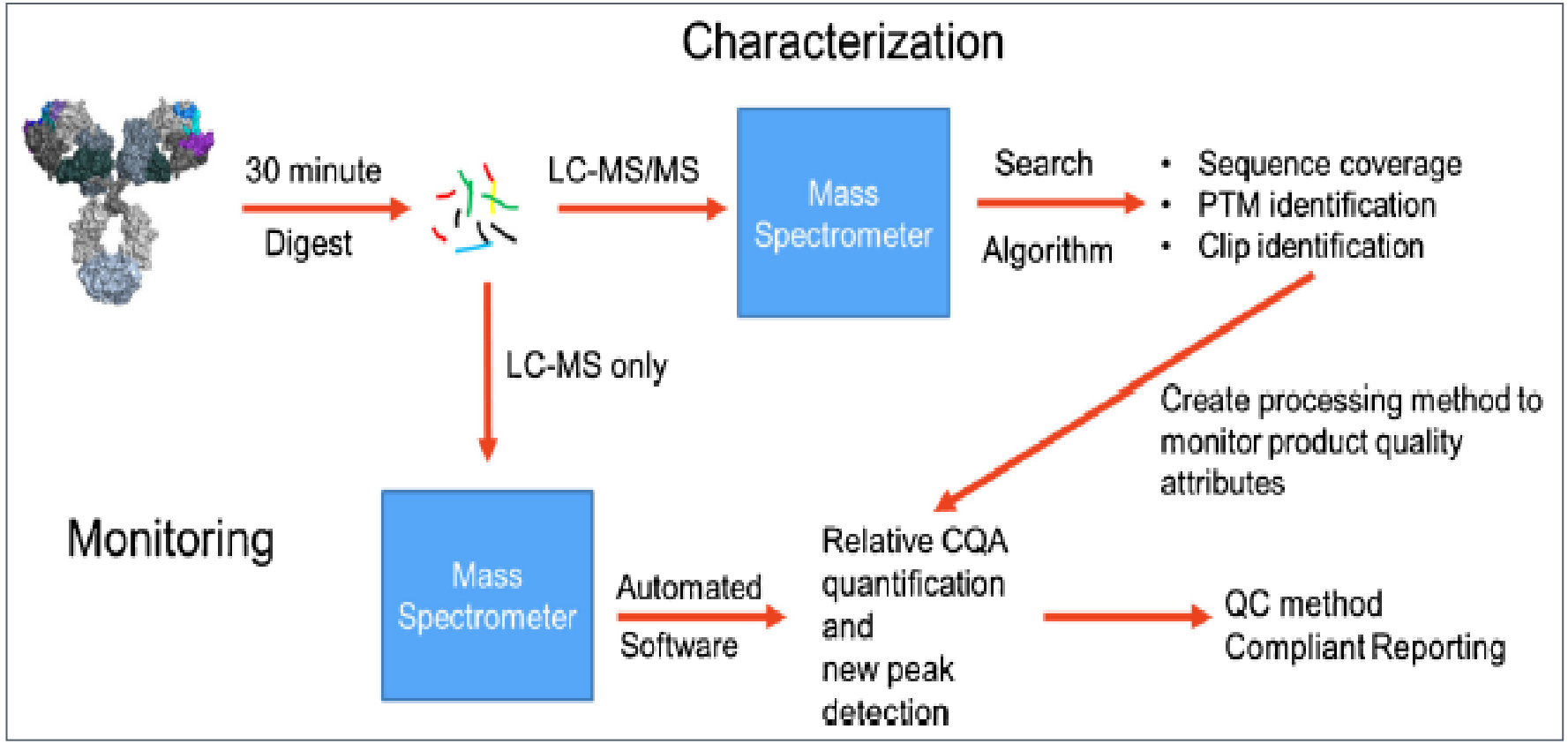
Overview of MAM

Examples of Quality Attributes Assessed with MAM
Deamidation
Oxidation
N- and C-terminal clipping
Pyroglutamate
Glycosylation
Glycation
Phosphorylation
Sulfation
Methylation
Acetylation
Hydroxylation
and more…



Rogers et al (2018) "A View on the Importance of "Multi-Attribute Method" for Measuring Purity of Biopharmaceuticals and Improving Overall Control Strategy" *AAPS J.* 20: 7.

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USP activities related to MAM

- Stakeholder input identified MAM as an area of interest
  - Roundtable on monoclonal antibodies identified post-translational modifications such as deamidation and oxidation as challenges and opportunities for standards
  - 2020 Stakeholder Forum identified a need for both documentary and physical standards to support MAM
  - New USP Expert Panel was created to draft a general chapter on MAM and to advise on additional standard development

- USP initiated work on MAM in 2019 as a method to provide more efficient and comprehensive protein characterization of USP Reference Standards
  - Surveys of stakeholders to understand current practices for MAM/ peptide mapping
  - Collaborations to develop MAM methods and utilize them to evaluate 3 USP mAb reference standards
    - Evaluate mAbs to identify materials and attributes that would be useful for physical standards
    - Focus on sample preparation, which has a big impact on deamidation

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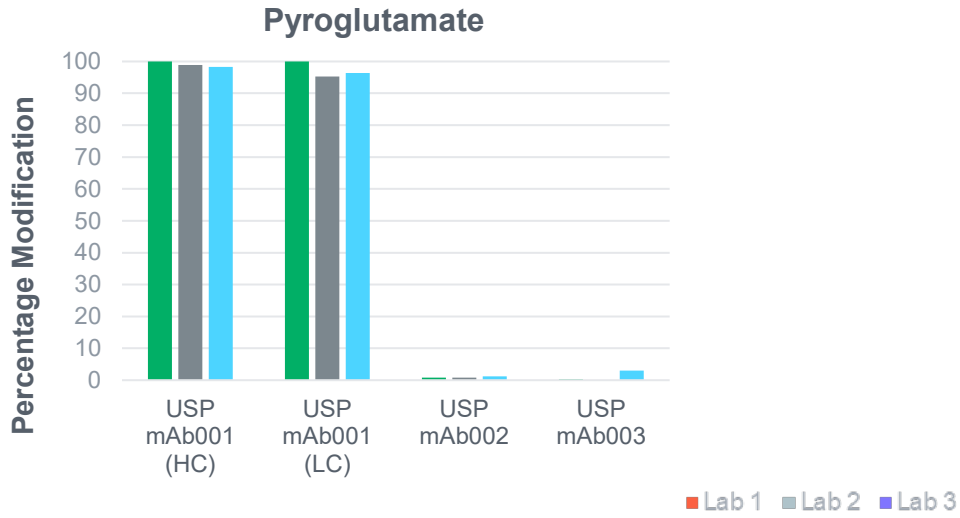
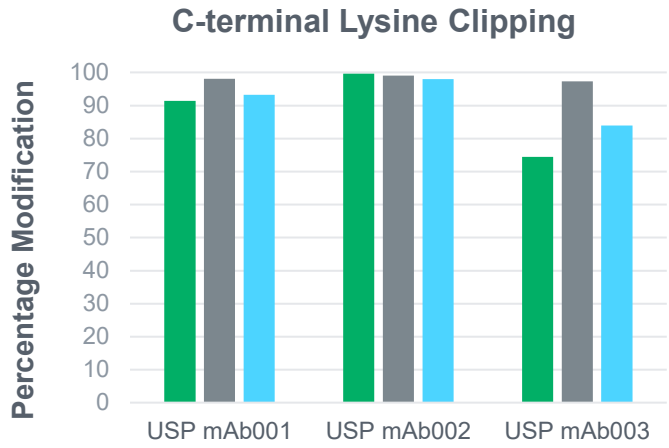
Preliminary MAM results

- Compared data obtained from multiple labs and using different digestion methods
- Most results were consistent across labs and conditions
  - Lysine clipping
  - Pyroglutamate
  - Glycosylation
  - Oxidation





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Deamidation levels varied across labs and methods

Peptide	Modification	Relative % of Modification		
		Lab 1	Lab 2 Method 1	Lab 2 Method 2
Peptide 1		---	---	
	Oxidation	9.60%	9.80%	5.60%
Peptide 2		---	---	
	Deamidation	14.50%	6.60%	ND
	Oxidation	ND*	0.10%	0.20%
Peptide 3		---	---	
	Deamidation	41.80%	28.70%	ND
	Oxidation		0.04%	ND
Peptide 4		---	---	
	Deamidation	ND	9.10%	ND
Peptide 5		---	---	
	Deamidation	36.20%	10.40%	2.80%
Peptide 6		---	---	
	Deamidation	9.40%	8.20%	ND
	Oxidation	ND	1.90%	1.70%

Major differences observed in percentage of deamidation

- Ranged from undetectable to over 40% depending on reduction/alkylation and digestion conditions

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Multiphase study to assess factors that contribute to variable deamidation results

- First Step: Identified variables
  - Reviewed MAM and peptide mapping methods in over 15 publications
  - Talked with MAM experts to get their input on parameters to test

- Designed two phase study to assess impact of variables
  - Assessment of three different mAbs provided insight into molecule-based variability

Phase 1
- Varied reduction/alkylation conditions
- Fixed digestion condition

Phase 2
- Varied digestion conditions
- Fixed reduction/alkylation conditions

- Criteria for comparison included:
  - Sequence Coverage
  - Missed Cleavages
  - % of Trypsin Peptides
  - % of Deamidated Products
  - % Oxidated Products
  - % Non-specific cleavages

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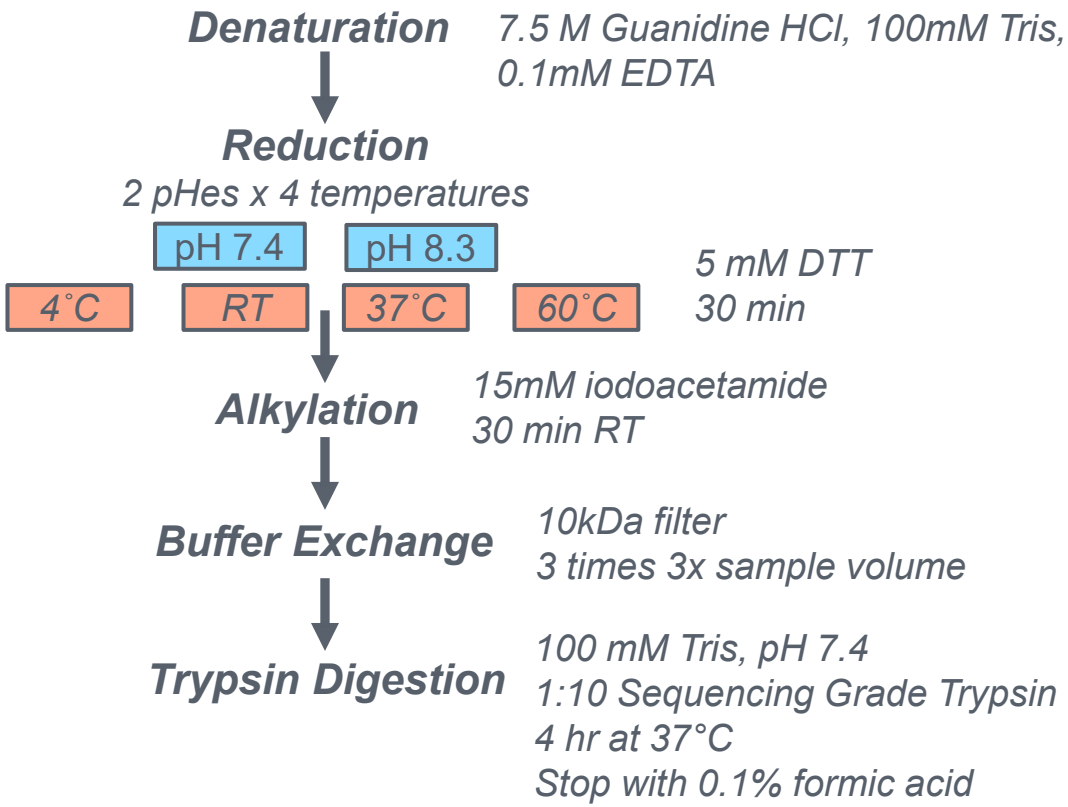
Phase 1 study design

- Focused on reduction and alkylation step
- Key variables included
  - pH
    - 7.4
    - 8.3
  - Temperature
    - 4 °C
    - Room temperature
    - 37 °C
    - 60 °C



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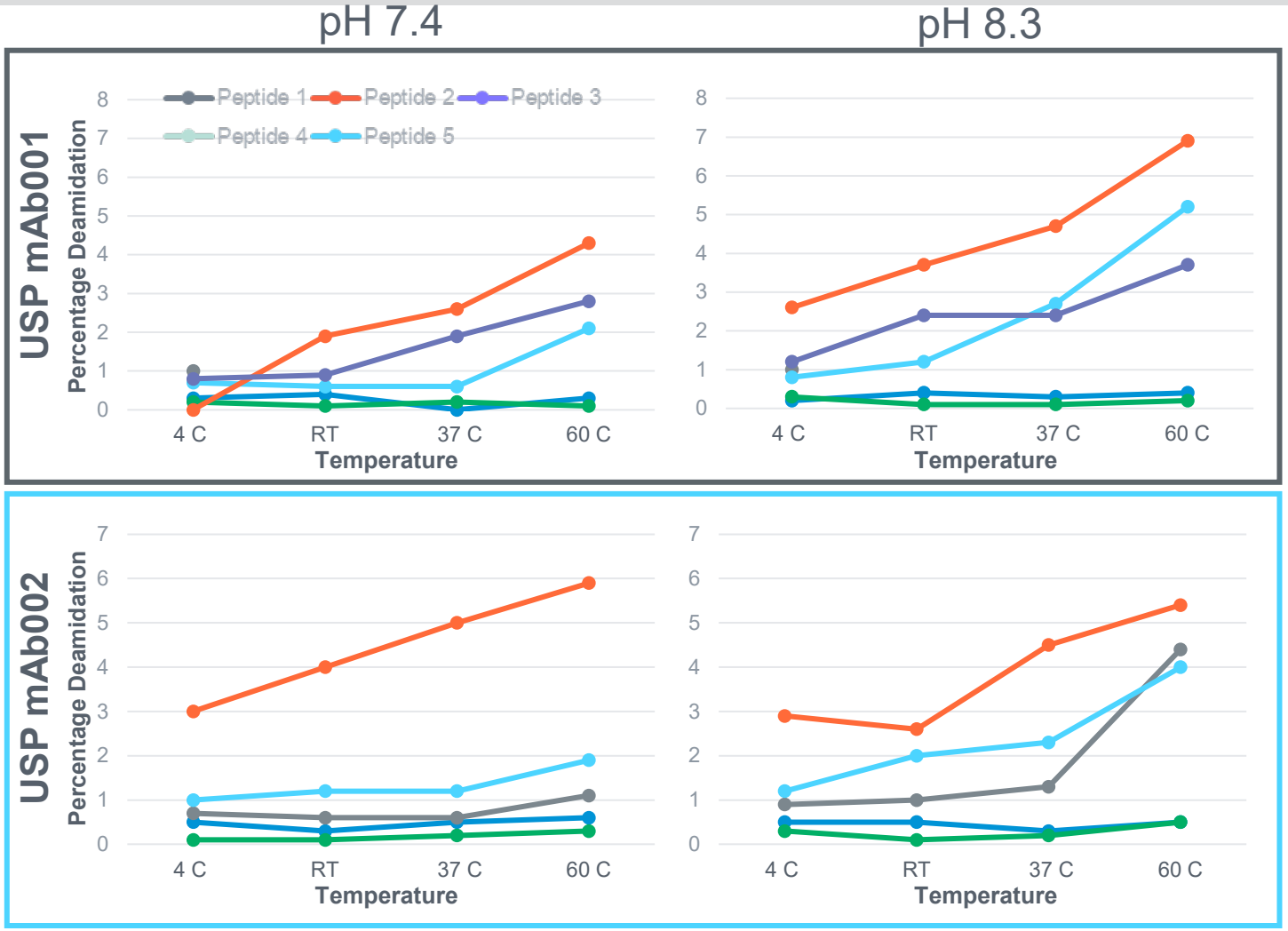
Phase 1 results: influence of pH and temperature



- Deamidation increased with
  - Increased temperature
  - Increased pH
- Changes in deamidation were highly peptide specific
- Changes in pH and temperature did not have a significant effect on overall digest as measured by
  - Coverage
  - Missed or nonspecific cleavages
  - Missing carboxyamidomethylation

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Phase 2 study design

- Based on Phase 1 Study, selected pH 7.4 and room temperature for reduction and alkylation in Phase 2 study
- Phase 2 study varied
  - Trypsin source
  - Enzyme: substrate ratio
  - Digestion time

pH and Temperature	Enzyme: Substrate Ratio	Trypsin Source	Time (hours)
pH 7.4 37 °C	1:50	Trypsin Gold	1, 2, 4, 6, 16
	1:10	Trypsin Gold	1, 2, 4, 6, 16
		Sequencing Grade	1, 2, 4, 6, 16
		Roche Trypsin	1, 2, 4, 6, 16

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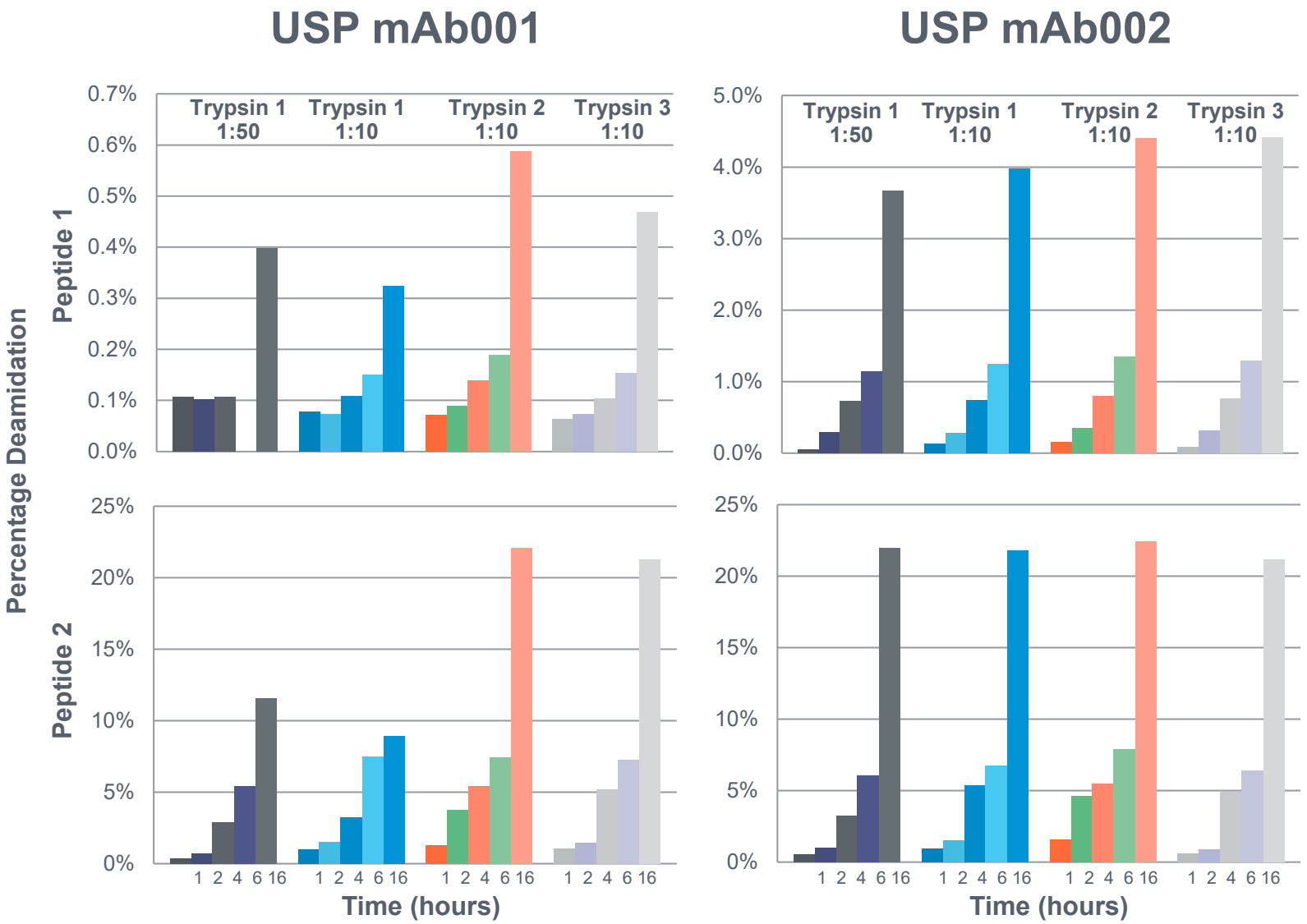
Phase 2 results



- All digestion conditions yielded good coverage
- Digestion time had the greatest impact on deamidation
- Trypsin source had little impact

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Summary of results

- Deamidation rates were highly context specific
- Study identified several key factors that can lead to sample preparation induced modifications, with a focus on deamidation
  - Lower temperatures and pH during the reduction and alkylation step reduced deamidation
  - Digestion time had the greatest impact on deamidation in the digestion step
- Outcomes and next steps
  - Method developed in this study will be used to replicate the results in an independent lab
  - Work also provided the foundation for developing pre-digested mAb reference standards
    - Pre-digested mAb standards, coupled with the existing USP mAbs, will provide users the ability to assess both the instrumentation and assay itself (including sample preparation) for peptide mapping and MAM applications.

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USP Stakeholders

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- Other Stakeholders

USP

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Evolution of MAM at USP

Stakeholder Engagement

2017/18

- *Roundtables on monoclonal antibodies identified analysis of PTMs as a challenge*

2019

- Initiated characterization of USP mAbs
- *Conducted surveys of stakeholders to understand current practices*
- *Solicited feedback from Mass Spec Peptides working group and other stakeholders on physical standards*

2020

- *Stakeholder Forum on MAM and additional survey on best practices*
- *Recruited and initiated MAM Expert Panel*
- Published MAM article in GEN
- Explored new collaborations to support standard development

2021

- Established collaboration to expand MAM work in independent laboratory
- Initiated work to produce pre-digested mAb standards

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