A Fast and Efficient Arginine-Specific Protease for Proteomic Applications

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1. Introduction

In bottom-up proteomic studies, poor digestion due to low specificity and efficiency complicates LC-MS/MS analysis by increasing sample complexity and reducing sensitivity. Aside from Trypsin and Lys-C, most commonly used proteases suffer from either poor efficiency or specificity.

Here we utilize an arginine-specific protease which is both highly efficient and specific, unlike the commonly used Arg-C protease which cleaves extensively at lysine residues. The improved protease, called Arg-C Ultra, was successfully used in a variety of applications including characterization of human cell extracts, antibodies and post-translational modifications of histones.

2. Arg-C Ultra has Significantly Improved Specificity

C-Terminal Cleavage Specificity
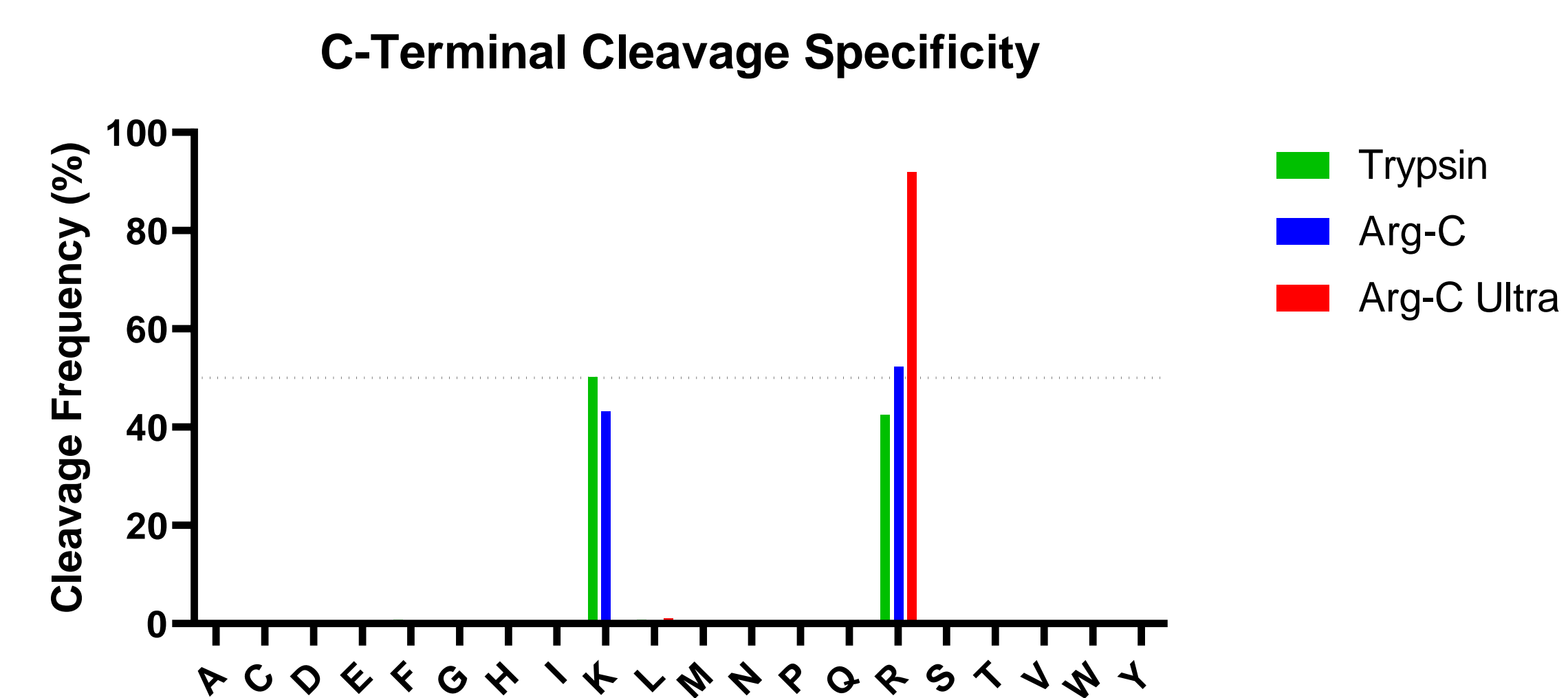
- Human K562 cell extract was digested overnight at 1:50 enzyme:substrate ratio.
- Data were analyzed on an Orbitrap Exploris 240 and searched with Byonic (no enzyme specified)

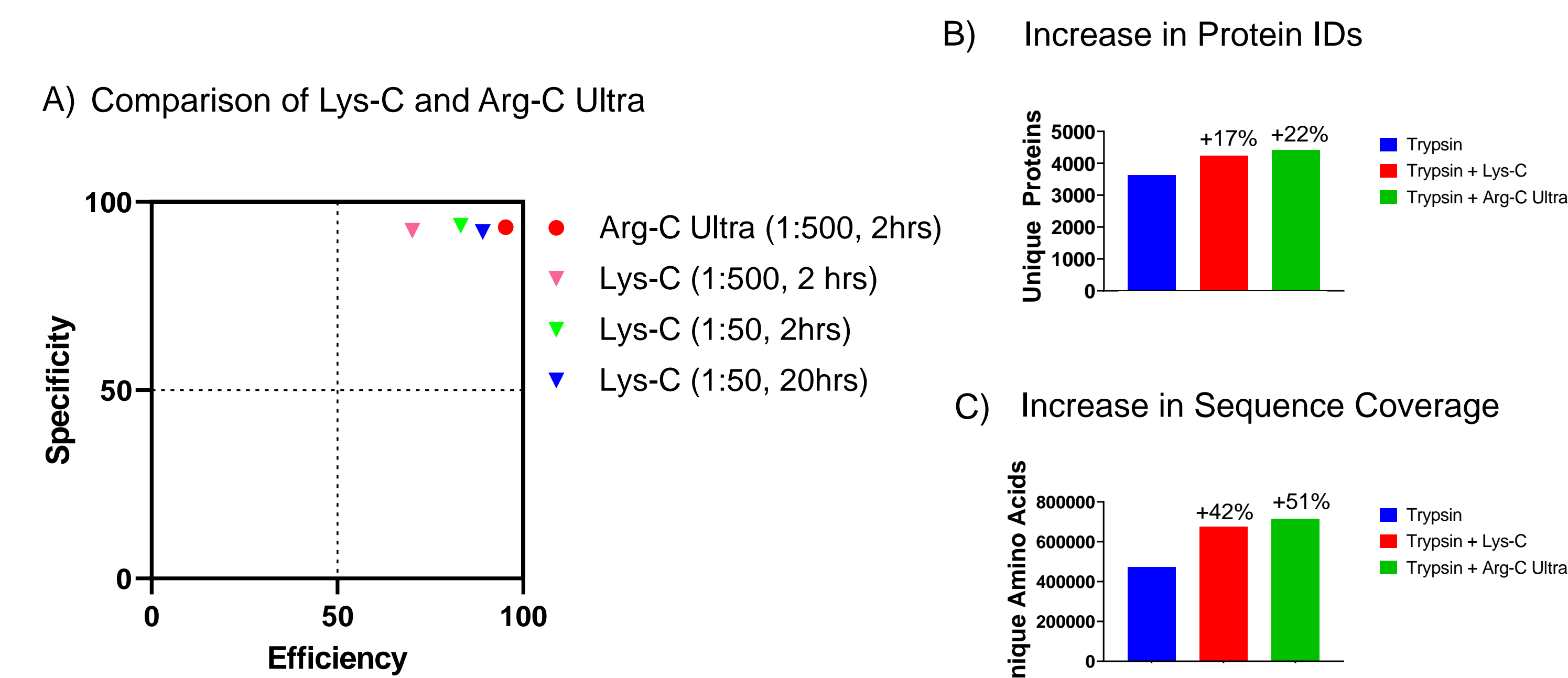➤ The digestion specificity of Arg-C Ultra is far superior to standard Arg-C.

3. Arg-C Ultra is a Fast and Efficient Protease

A) Specificity-Efficiency for Arg-C Proteases
B)
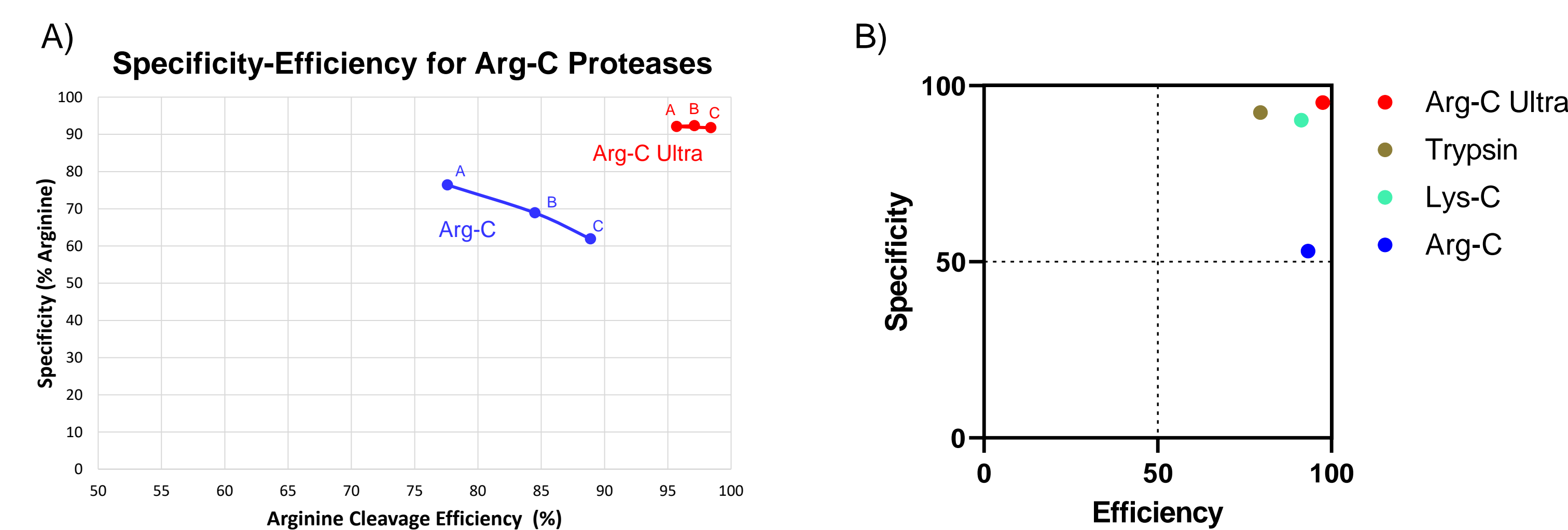
A) Human K562 extract was digested with Arg-C or Arg-C Ultra for 2 hours at 1:500 (A), 1:150 (B) and 1:50 (C). B) K562 extract was digested 1:50 overnight with various proteases.
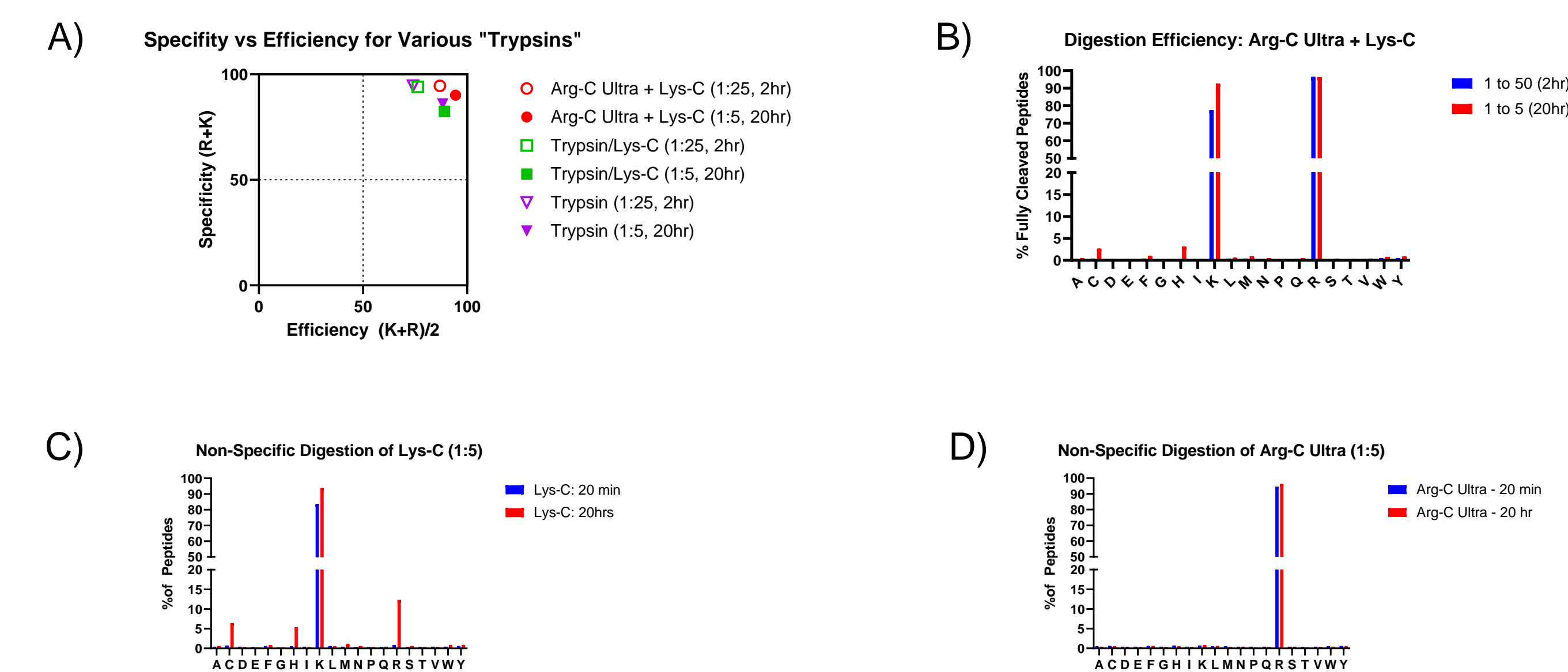
➤ Arg-C Ultra has exceptional digestion efficiency even with short digests using little enzyme.
➤ Arg-C Ultra has the best combination of efficiency and specificity of MS-grade proteases.

4. Arg-C Ultra Outperforms Lys-C

A) Comparison of Lys-C and Arg-C Ultra
- Arg-C Ultra (1:500, 2hrs)
- Lys-C (1:500, 2 hrs)
- Lys-C (1:50, 2hrs)
- Lys-C (1:50, 20hrs)

B) Increase in Protein IDs
C) Increase in Sequence Coverage

➤ Arg-C Ultra achieves higher digestion efficiency than Lys-C, even with 10X less enzyme and digestion time.
➤ Arg-C Ultra improves protein IDs and sequence coverage relative to Trypsin to a greater extent than Lys-C.

5. Arg-C Ultra + Lys-C = "Trypsin Ultra"

A) Specificity vs Efficiency for Various "Trypsins"
B) Digestion Efficiency: Arg-C Ultra + Lys-C
C) Non-Specific Digestion of Lys-C (1:5)
D) Non-Specific Digestion of Arg-C Ultra (1:5)

Human K562 extract was digested with either trypsin, Trypsin/Lys-C or a mixture of Lys-C and Arg-C Ultra. Data were searched with Byonic (No enzyme specified)

➤ Combining Arg-C Ultra with Lys-C results in better performance than with Trypsin or Trypsin/Lys-C.
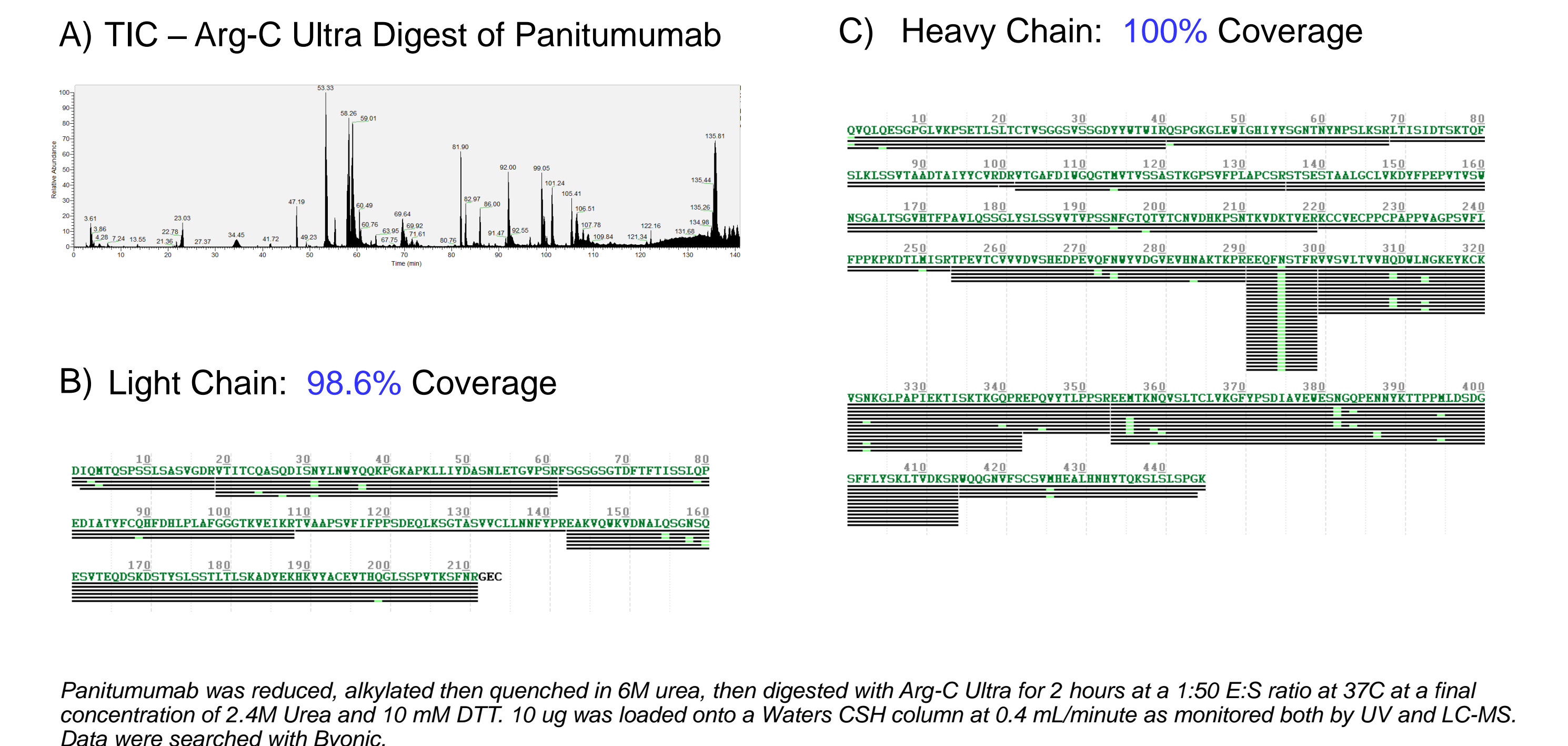➤ Arg-C has higher digestion specificity than Lys-C.

6. High Digestion Efficiency both at Low pH or High Urea

A) Effect of Urea on Digestion Efficiency
B) Effect of pH on Digestion Efficiency
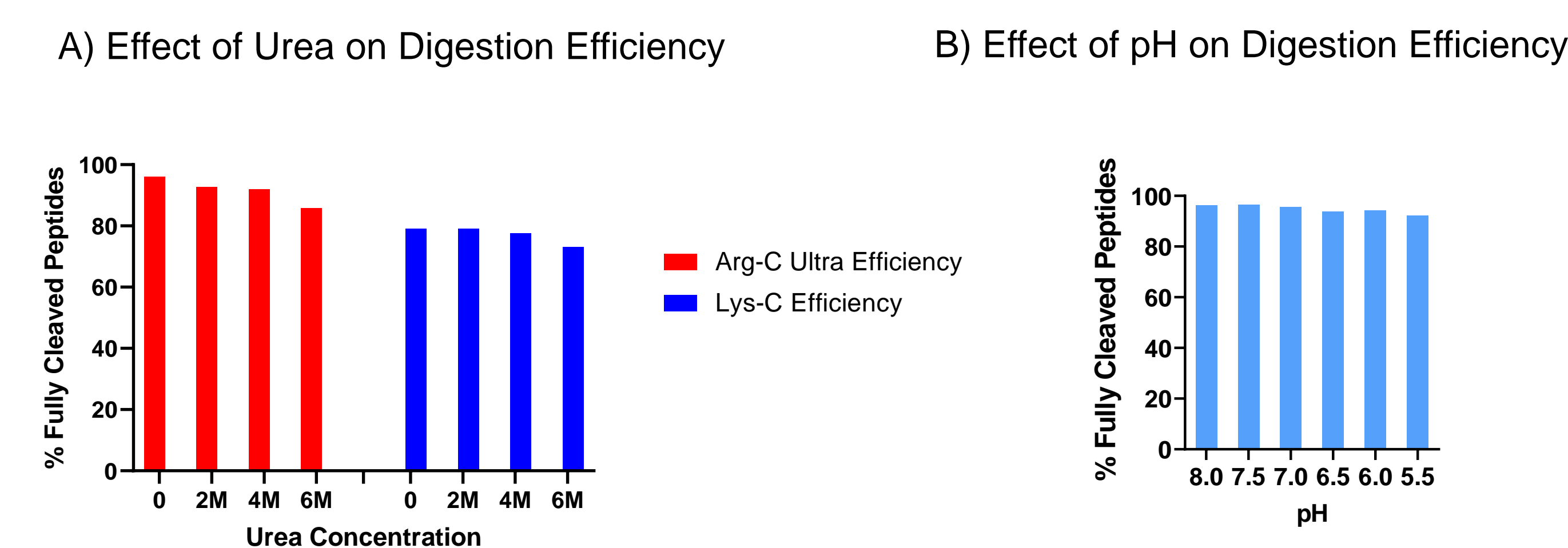
Human K562 extract was digested with Arg-C or Lys-C at 1:100 for 2 hours at 37C at various urea concentrations or pH values. Data were searched with Byonic (No enzyme specified)

➤ Arg-C Ultra is more efficient in 6M urea than Lys-C.
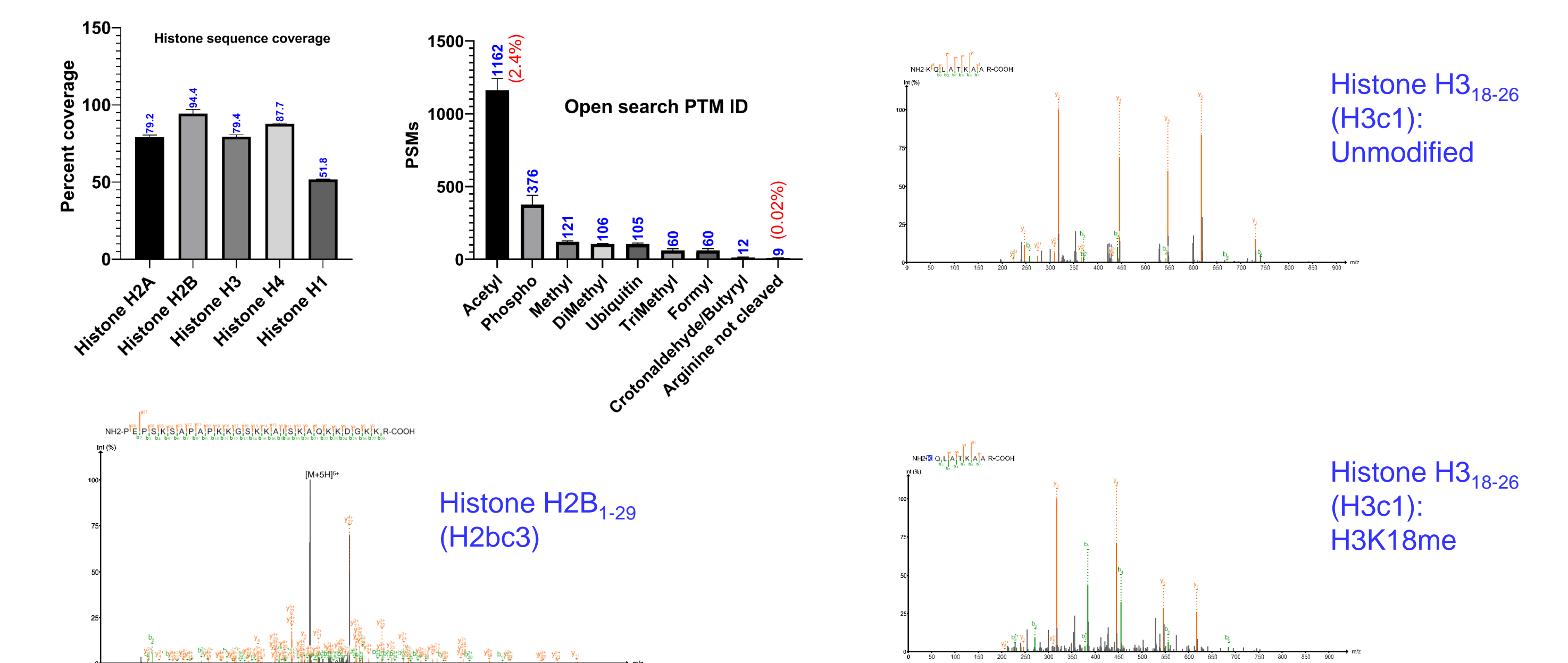➤ Arg-C Ultra is highly efficient over a wide pH range (~5-9).

7. Highly Specific Peptide Mapping of Therapeutic IgGs

A) TIC – Arg-C Ultra Digest of Panitumumab
B) Light Chain: 98.6% Coverage
C) Heavy Chain: 100% Coverage

Panitumumab was reduced, alkylated then quenched in 6M urea, then digested with Arg-C Ultra for 2 hours at a 1:50 E:S ratio at 37C at a final concentration of 2.4M Urea and 10 mM DTT. 10 ug was loaded onto a Waters CSH column at 0.4 mL/minute as monitored by both by UV and LC-MS. Data were searched with Byonic.

➤ High sequence coverage of Panitumumab obtained after digestion with Arg-C Ultra for 2 hours.

8. Histone Analysis: High Sequence Coverage and PTM ID

Histone sequence coverage
Open search PTM ID
Histone H3[18-26] (H3c1): Unmodified
Histone H2B[1-29] (H2bc3)
Histone H3[18-26] (H3c1): H3K18me

Histones were extracted from mouse microglia with the EpiQuik Total Histone Extraction Kit, digested with ArgC-Ultra, and analyzed via DDA-PASEF on a timsTOF Pro. MS Fragger search results show high sequence coverage (spanning the N-terminal tail) and PTM ID.

➤ ArgC-Ultra digestion allows detection of PTM-containing and unmodified cognate histone peptides for accurate histone PTM quantitation.

9. Advantages of Arg-C Ultra

➤ Highest performing MS-grade protease
  - Specific for arginine.
  - Efficient: < 5% missed cleavages even with 1:500, 2hr digest.
➤ When combined with Lys-C, Arg-C Ultra + Lys-C is superior to trypsin.
➤ Superior to Lys-C:
  - Higher digestion efficiency despite requiring less enzyme.
  - Higher specificity.
  - More orthogonal to Trypsin for protein coverage in K562 digest.
  - More efficient even in 6M urea.
➤ Wide pH range, potentially useful for peptide mapping at low pH.
➤ Useful for producing high coverage maps of therapeutic proteins.
➤ Useful for comprehensive PTM mapping as evidenced by Histone analysis.
  - Particularly helpful for monitoring PTMs on or around Lysine residues.