

# Post-Translational Modifications on HLA Class I Presented Peptides

---

Caron Laboratory

---

## Study Overview

---

This analysis characterizes post-translational modifications (PTMs) identified on HLA class I peptides from the JY cell line using data-dependent acquisition mass spectrometry. All analyses are restricted to peptides of length 8-14 amino acids.

**HLA Alleles:** A<sup>\*</sup>02:01, B<sup>\*</sup>07:02, C<sup>\*</sup>07:02

---

## Data Summary

Dataset	Peptides
Background (unmodified)	77,100
**Total Modified**	**8,804**
Cysteinylation	2,355
Deamidation	2,081
Oxidation	1,640
Phosphorylation	702
Acetylation	510
Methylation	475
Ubiquitination	426
Dimethylation	319
Citrullination	250
N-Glycosylation	42
SUMOylation	4

## PTM Categories

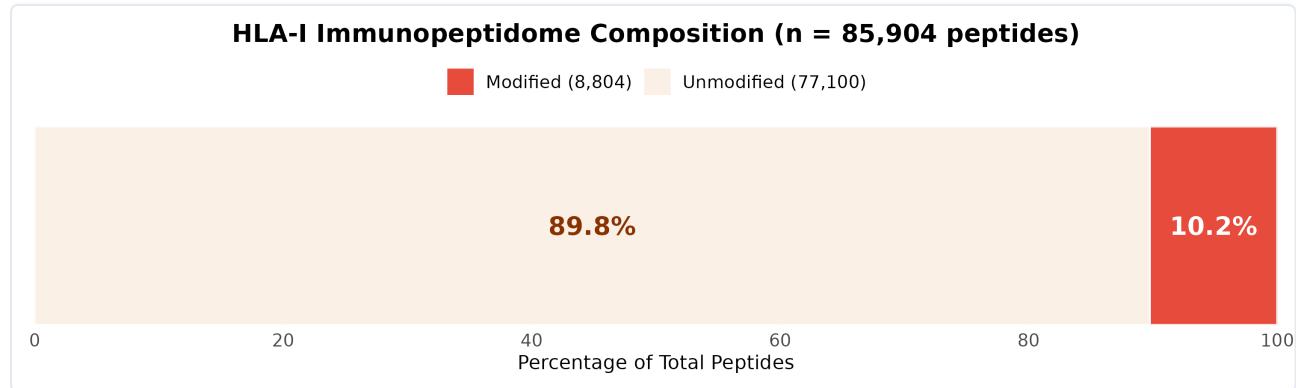
PTM Type	Target Residues
Phosphorylation	S, T, Y
Acetylation	K, N-terminus
Cysteinylation	C
Methylation	K, R
Dimethylation	K, R
Deamidation	N, Q
Biological Oxidation	P, I, L, Q, S, T, V, C, D, E, N, Y, G, K, R
Citrullination	R
Ubiquitination	K
N-Glycosylation	N
SUMOylation	K

## HLA Binding Classification

Category	EL Rank Threshold
Strong binder	< 0.5%
Weak binder	0.5% – 2%
Non-binder	≥ 2%

## Figure 1: PTM Composition

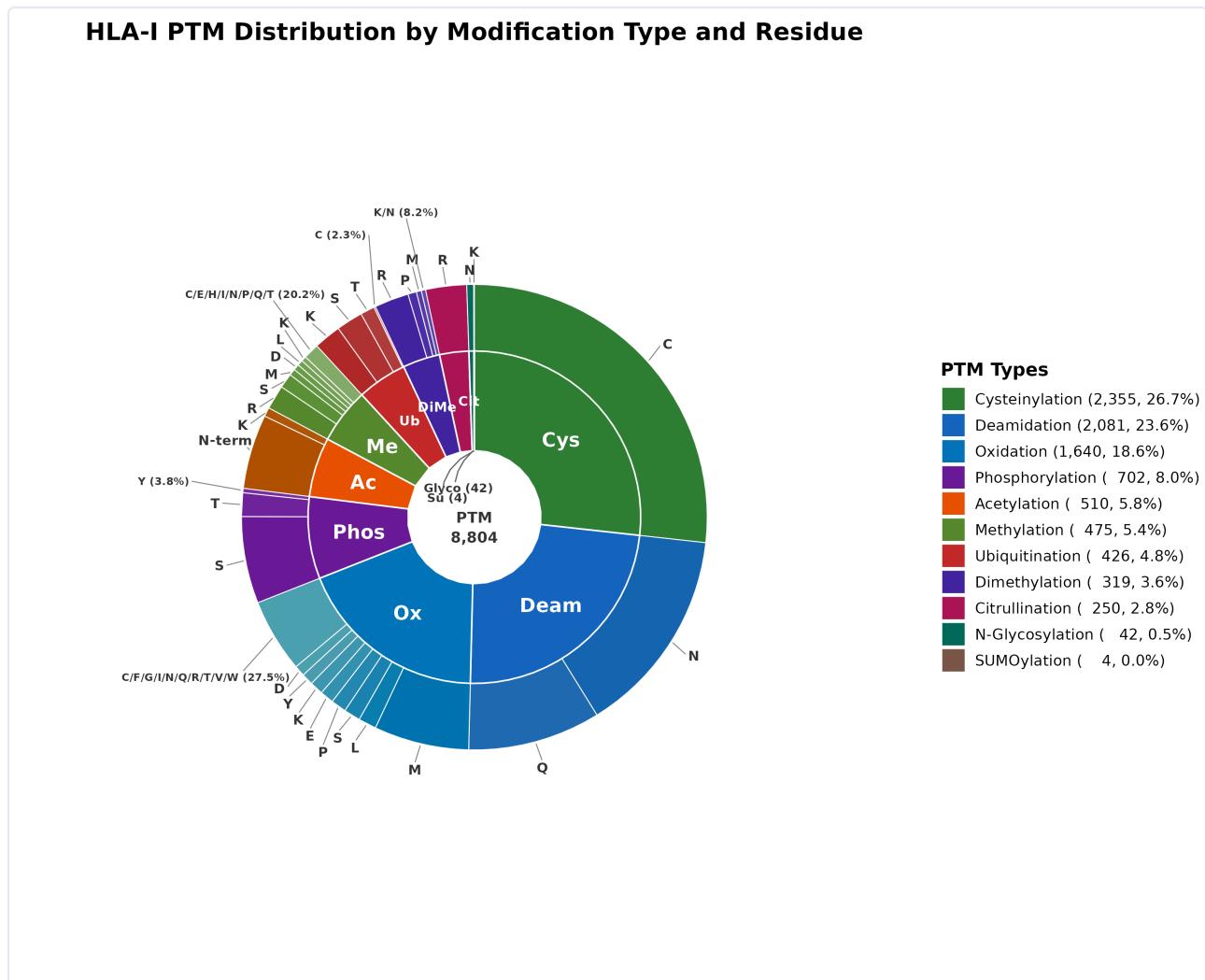
## Figure 1A: PTM Distribution



*![Figure 1A](figure\_panels/Figure1A\_BarChart.png)*

Distribution of unique modified peptides by PTM type (length 8-14 amino acids).

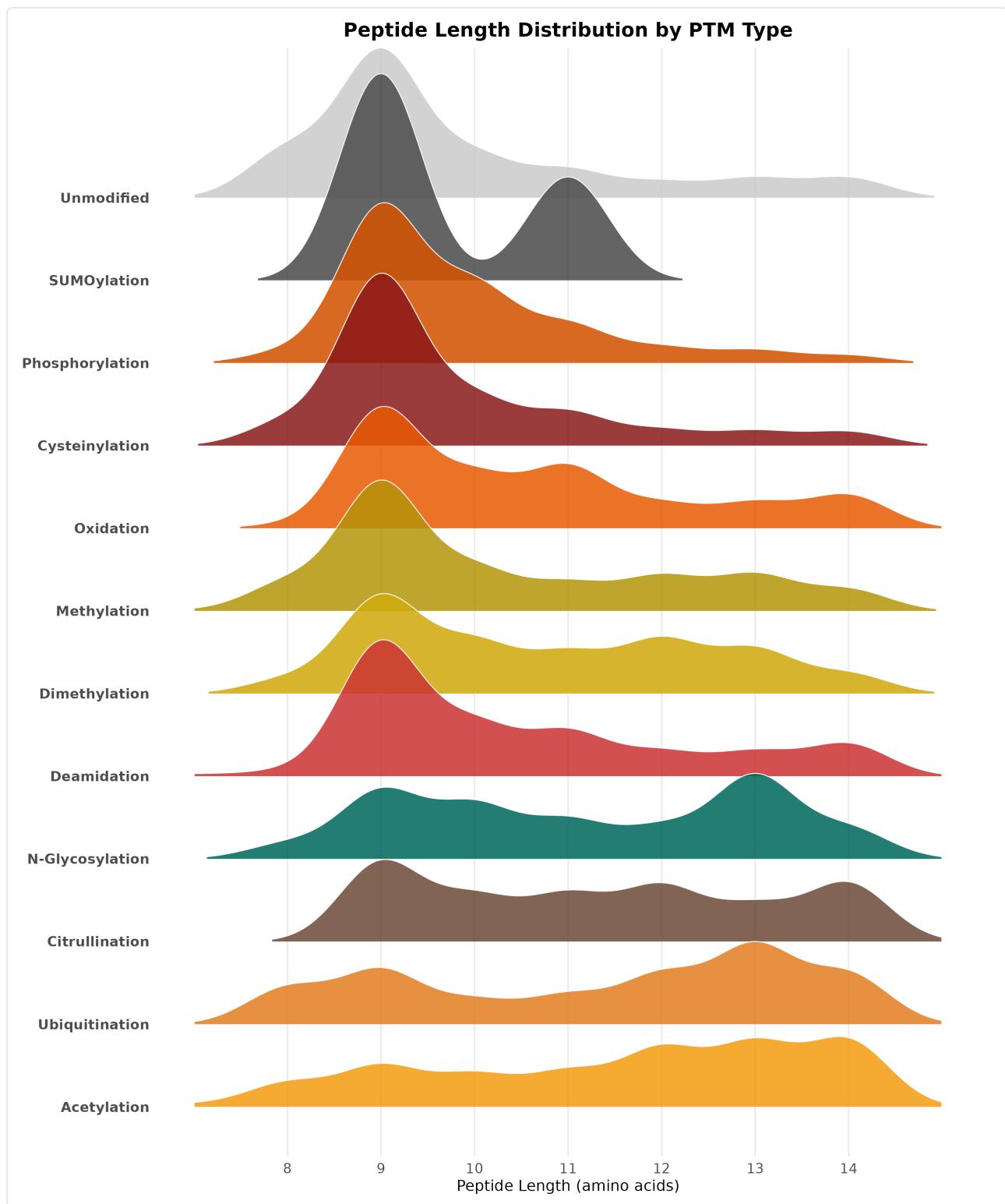
## Figure 1B: PTM Proportions



!/[Figure 1B](figure\_panels/Figure1B\_Donut.png)

Proportional representation of each PTM class within the modified peptidome.

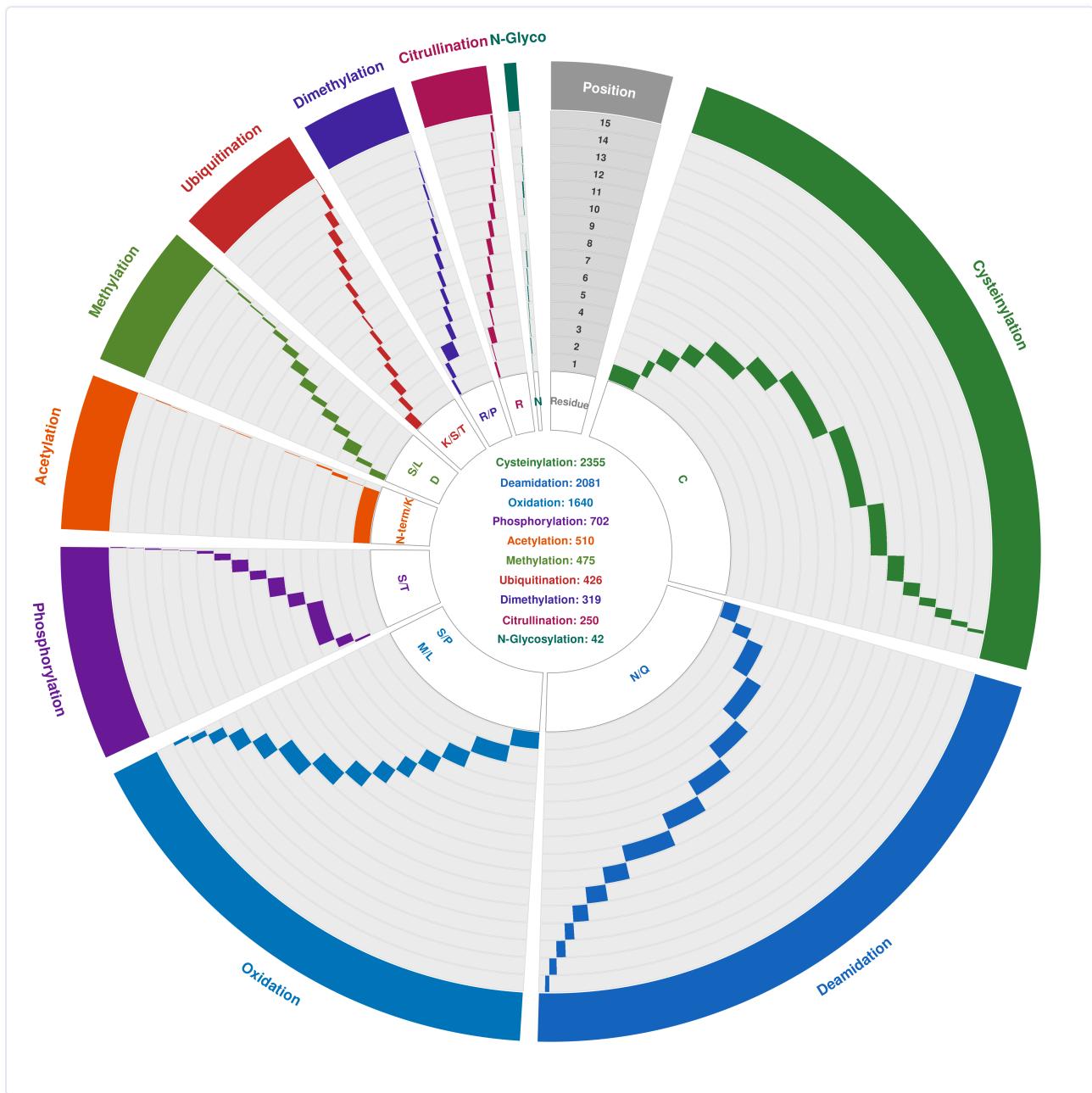
## Figure 2: Peptide Length Distribution



![Figure 2](figure\_panels/Figure2\_Length\_Distribution.png)

Ridgeline density plot comparing peptide length distributions across PTM types. Gray distribution represents unmodified background peptides.

## Figure 4A: PTM Landscape



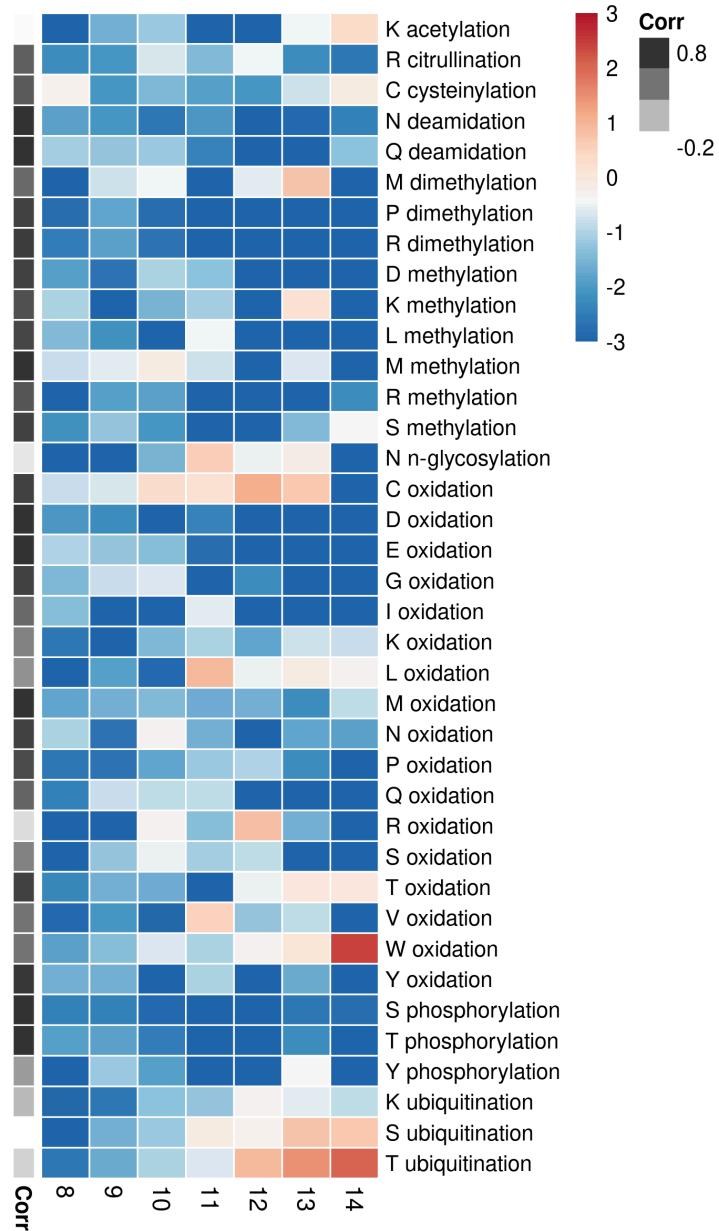
!/[Figure 4A](figure\_panels/Figure4A\_Circos\_PTMs\_Landscape.png)

Circos diagram illustrating relationships between PTM types (outer sectors), modified residues (links), and HLA binding alleles (inner elements). Link density reflects relative abundance of each PTM-residue combination.

## Figure 4B: Positional Enrichment

### By PTM and Residue

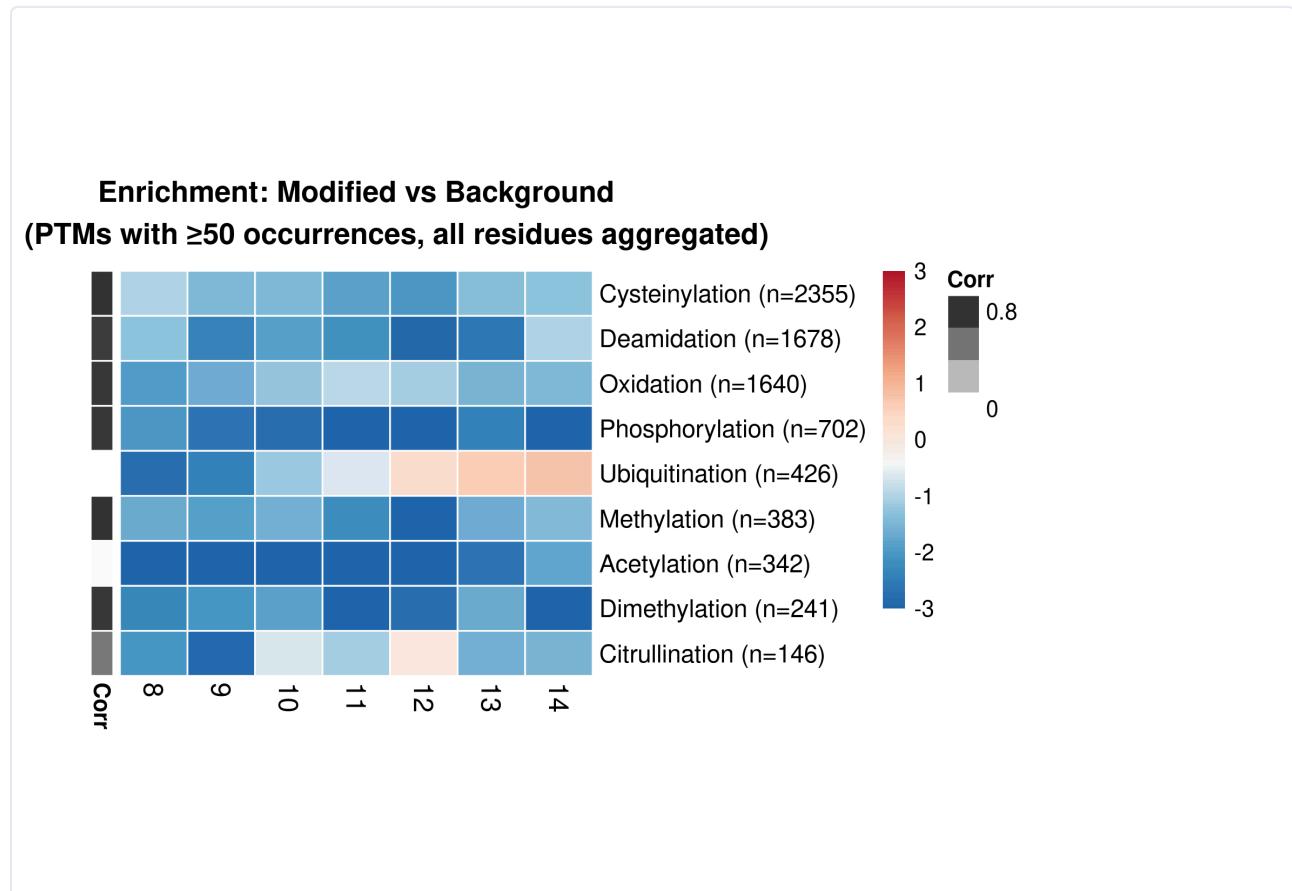
**Enrichment: Modified vs Background  
(PTM+Residue combinations with  $\geq 20$  occurrences)**



!/[Figure 4B](figure\_panels/Figure4B\_Position\_Enrichment.png)

$\log_2$  enrichment of PTM occurrence at peptide positions 8-14 relative to background amino acid positional distribution. PTM+residue combinations with  $\geq 20$  occurrences. Color scale: blue (depletion) to red (enrichment), capped at  $\pm 3$ . Correlation column indicates similarity to background distribution.

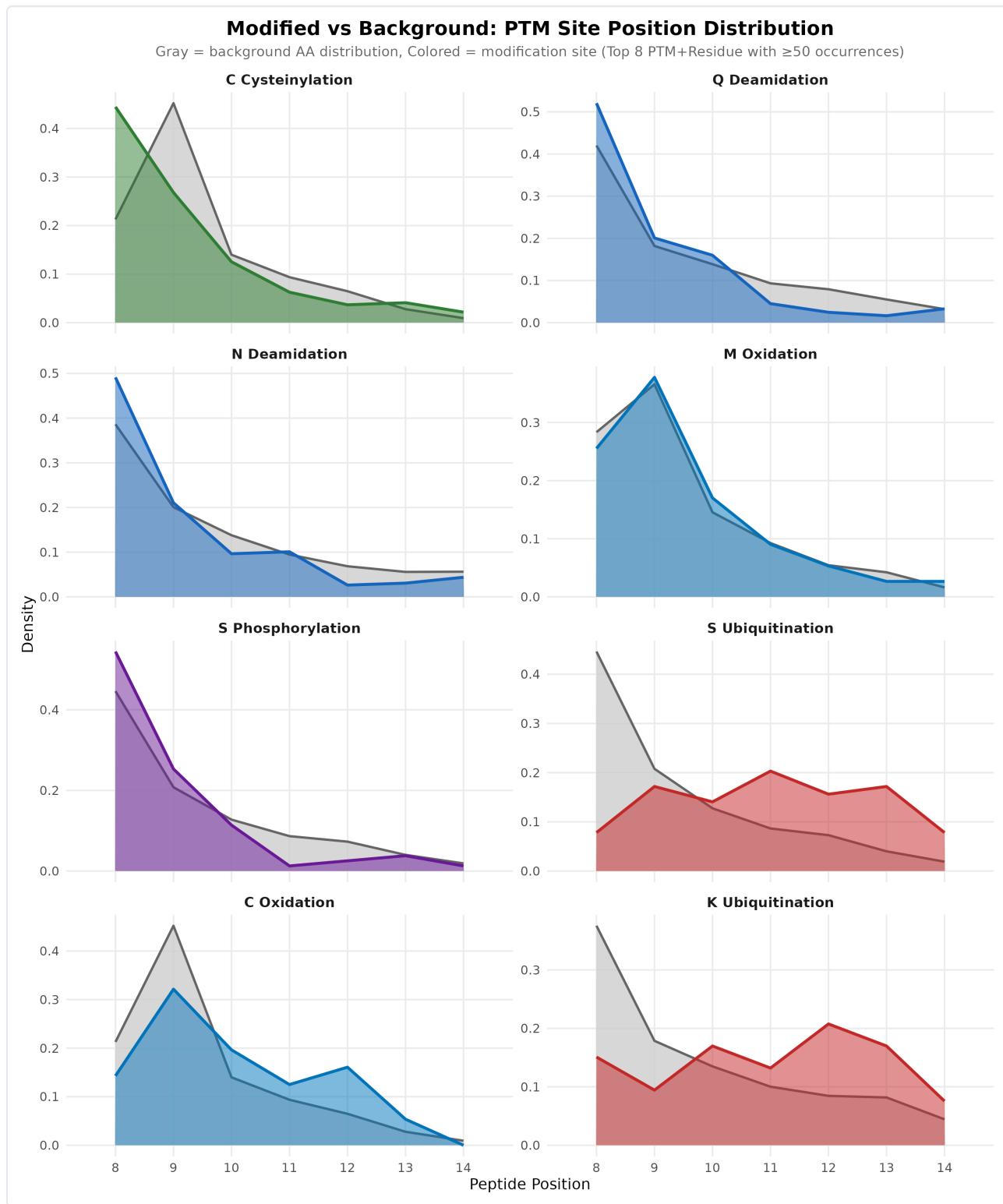
## By PTM Type (Aggregated)



!/[Figure 4B2](figure\_panels/Figure4B2\_Position\_Enrichment\_ByPTM.png)

Aggregated positional enrichment by PTM type ( $\geq 50$  occurrences), combining all modified residues.

## Figure 4C: PTM Position Density

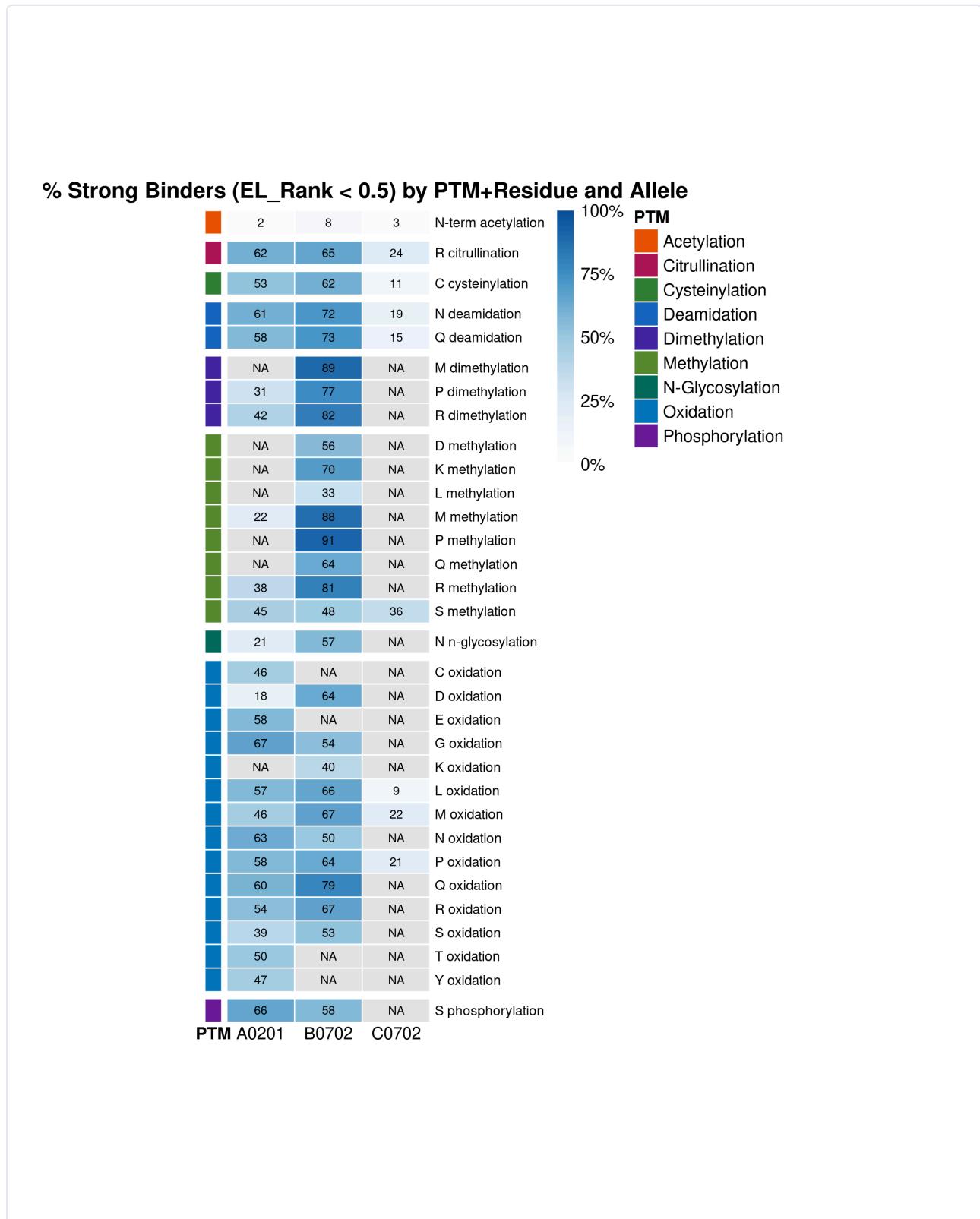


!/[Figure 4C](figure\_panels/Figure4C\_Position\_Density.png)

Density curves showing modification site distribution across peptide positions 8-14. PTM+residue combinations with  $\geq 50$  modifications.

## Figure 5A: HLA Binding Affinity

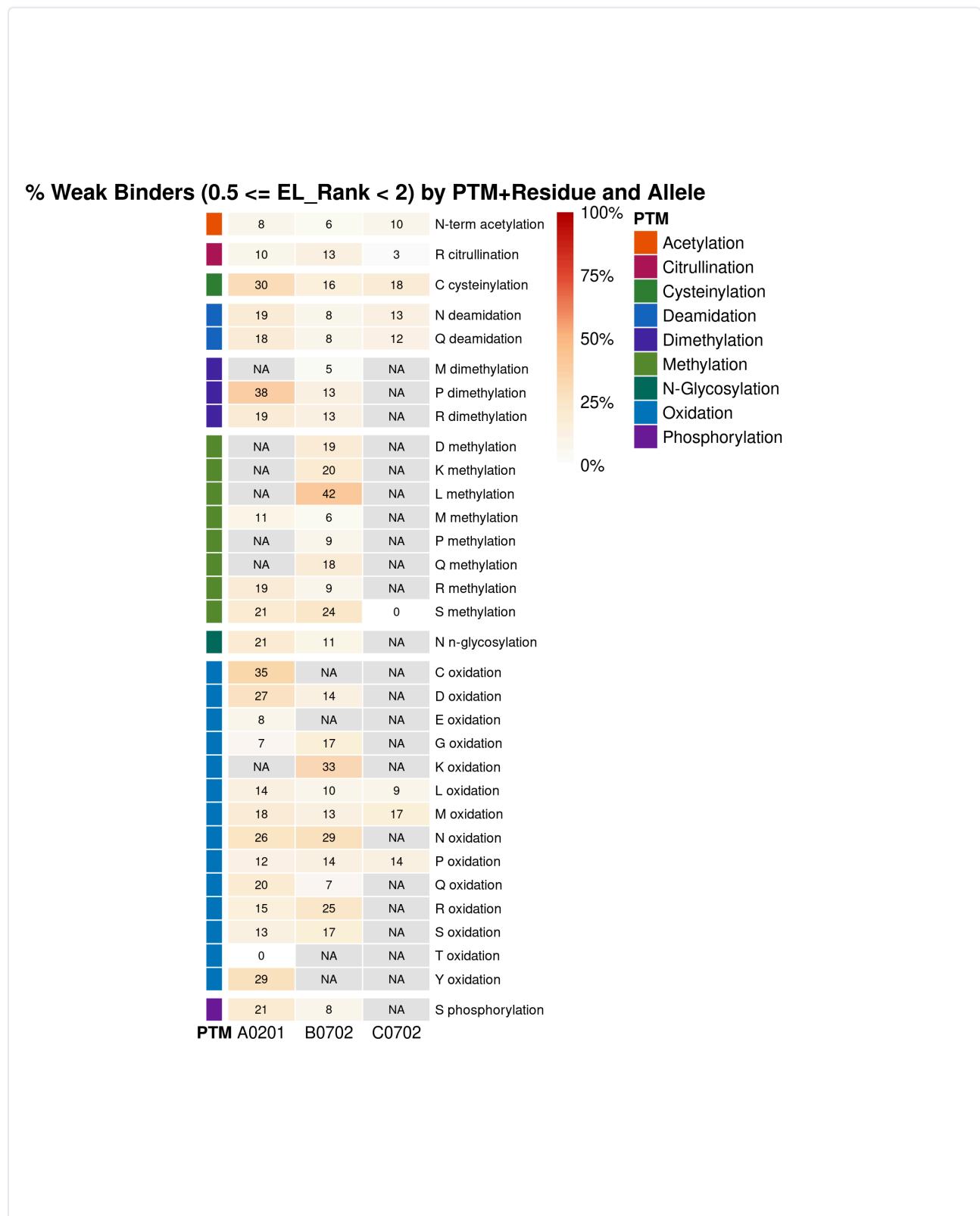
### Strong Binders by PTM and Residue



![Figure 5A Strong by Residue](figure\_panels/Figure5A\_StrongBinders\_ByResidue.png)

Percentage of strong HLA binders (EL Rank < 0.5%) for each PTM+residue combination across alleles A0201, B0702, and C0702. Combinations with  $\geq 10$  peptides.

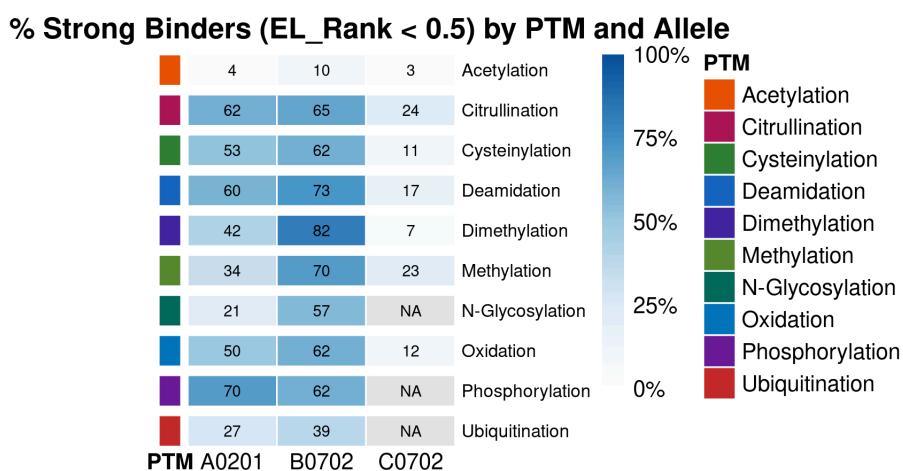
## Weak Binders by PTM and Residue



![Figure 5A Weak by Residue](figure\_panels/Figure5A\_WeakBinders\_ByResidue.png)

Percentage of weak HLA binders ( $0.5\% \leq \text{EL Rank} < 2\%$ ) for each PTM+residue combination.

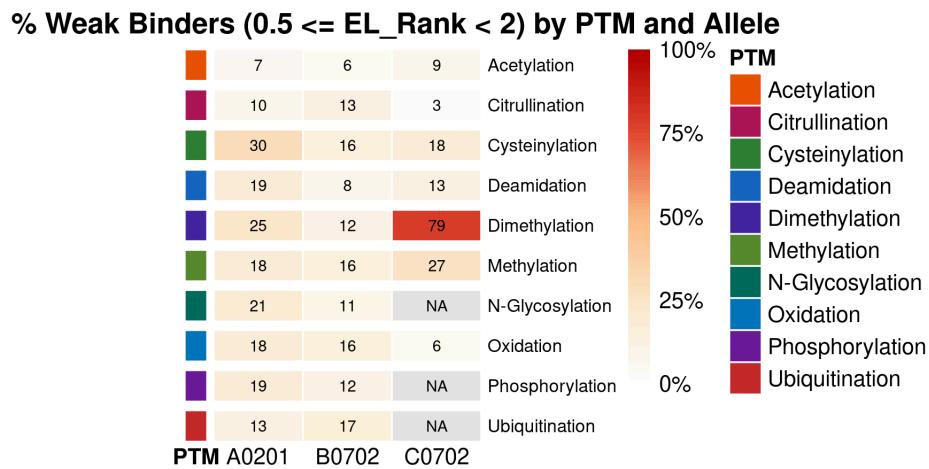
## Strong Binders by PTM Type



!/[Figure 5A Strong by PTM](figure\_panels/Figure5A\_StrongBinders\_ByPTM.png)

Strong binder percentages aggregated by PTM type across alleles.

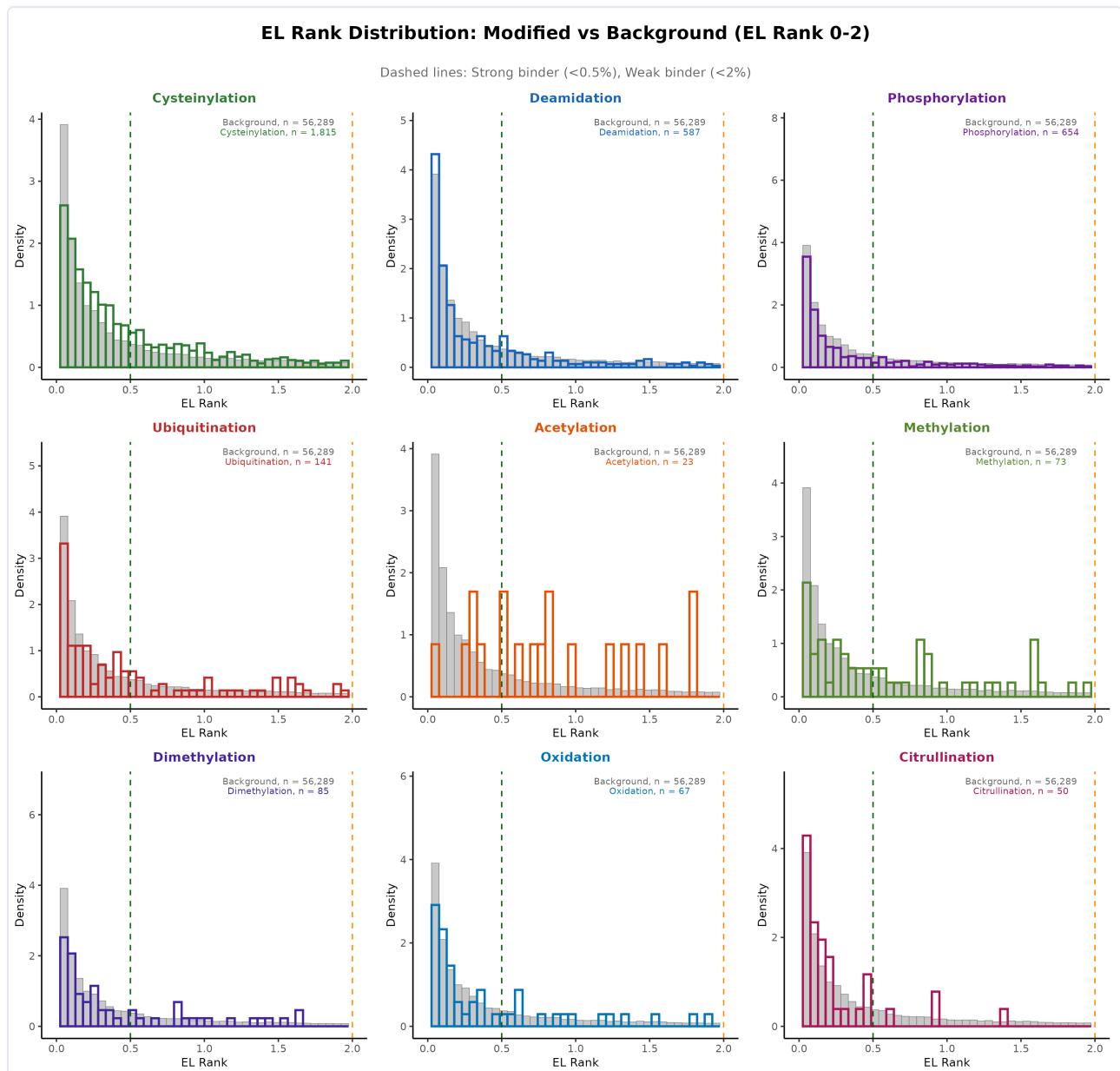
## Weak Binders by PTM Type



![Figure 5A Weak by PTM](figure\_panels/Figure5A\_WeakBinders\_ByPTM.png)

Weak binder percentages aggregated by PTM type.

## Figure 5B: EL Rank Distribution

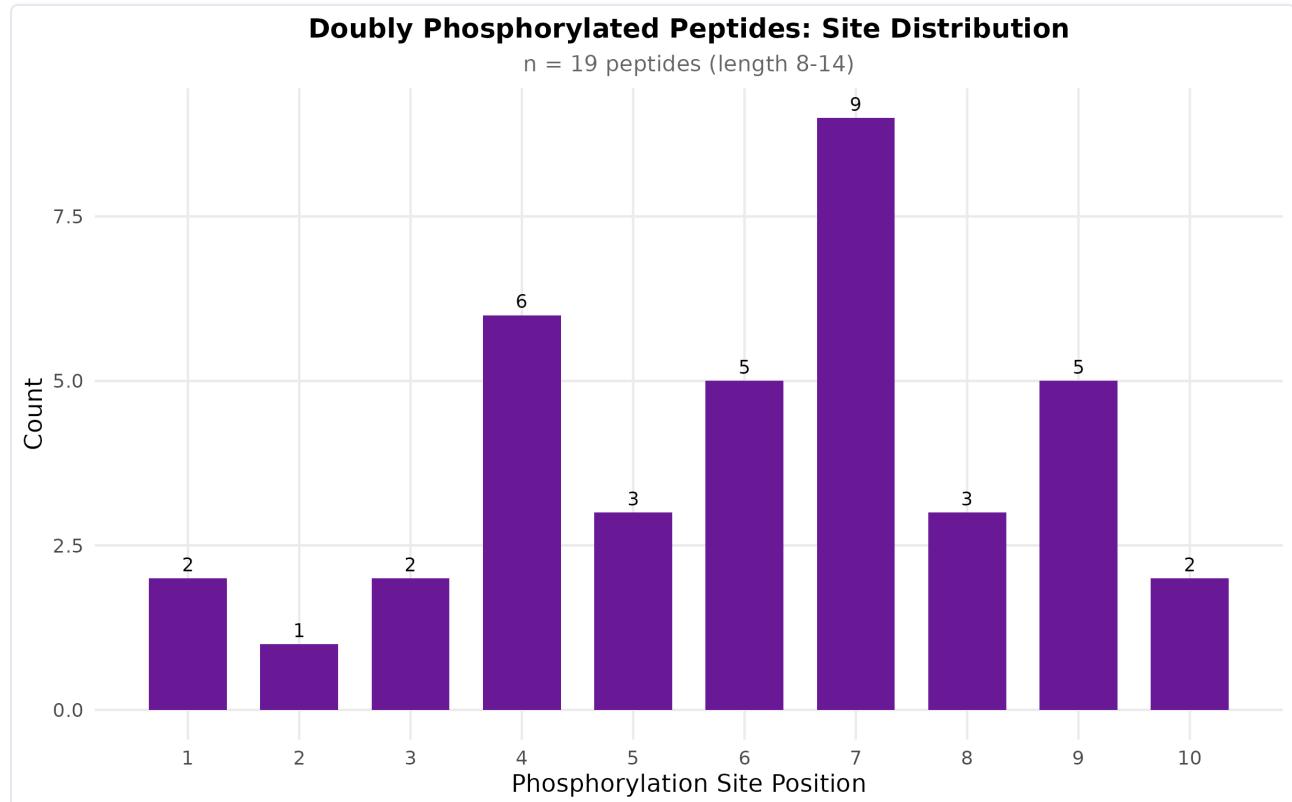


![Figure 5B](figure\_panels/Figure5B\_ELRank\_Distribution.png)

EL Rank distribution comparison between modified peptides (colored outlines) and background unmodified peptides (gray) within the binder range (EL Rank 0-2). Dashed lines indicate strong binder (0.5%) and weak binder (2%) thresholds.

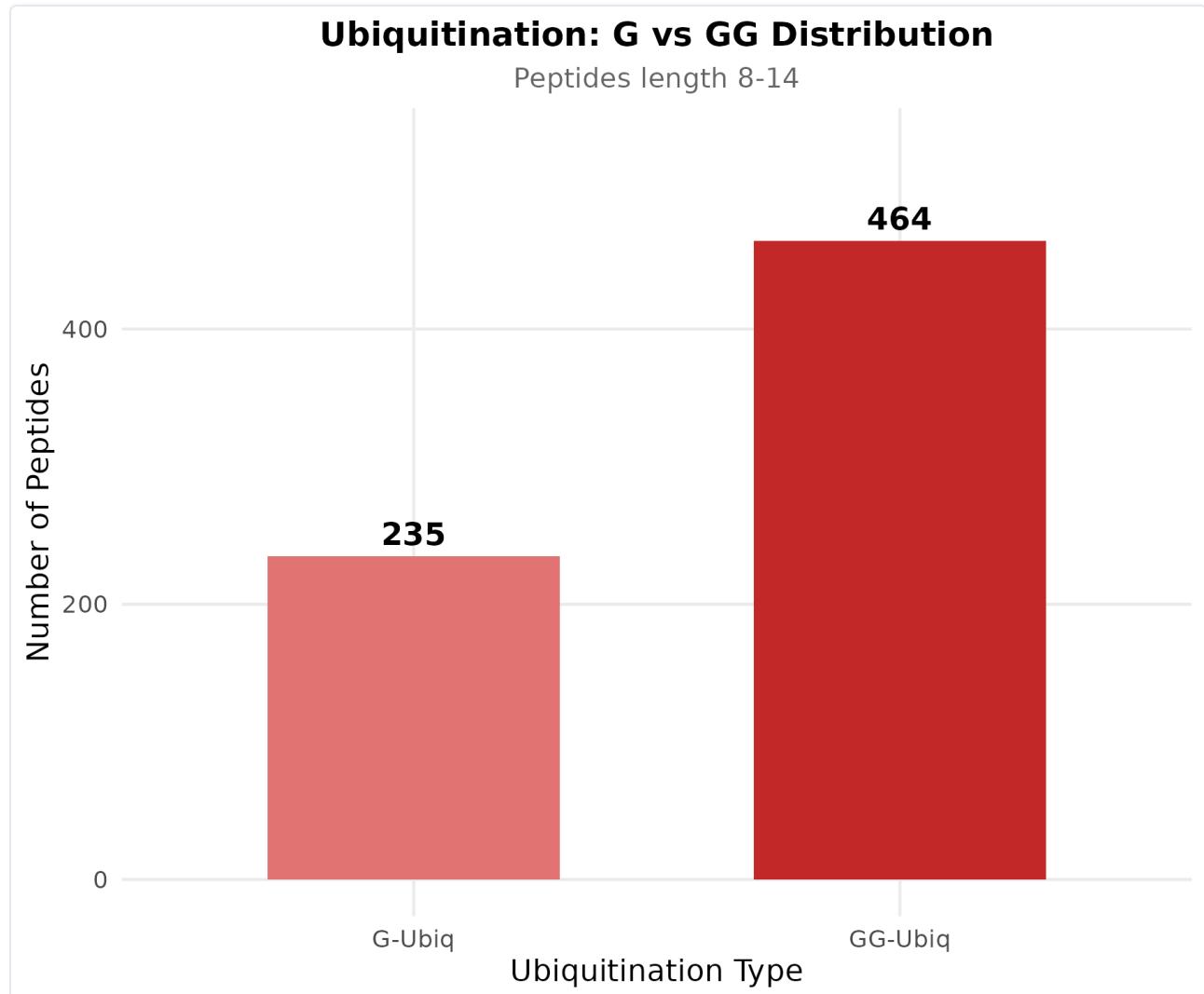
## Figure 6: Specific PTM Analyses

### Figure 6A: Doubly Phosphorylated Peptides



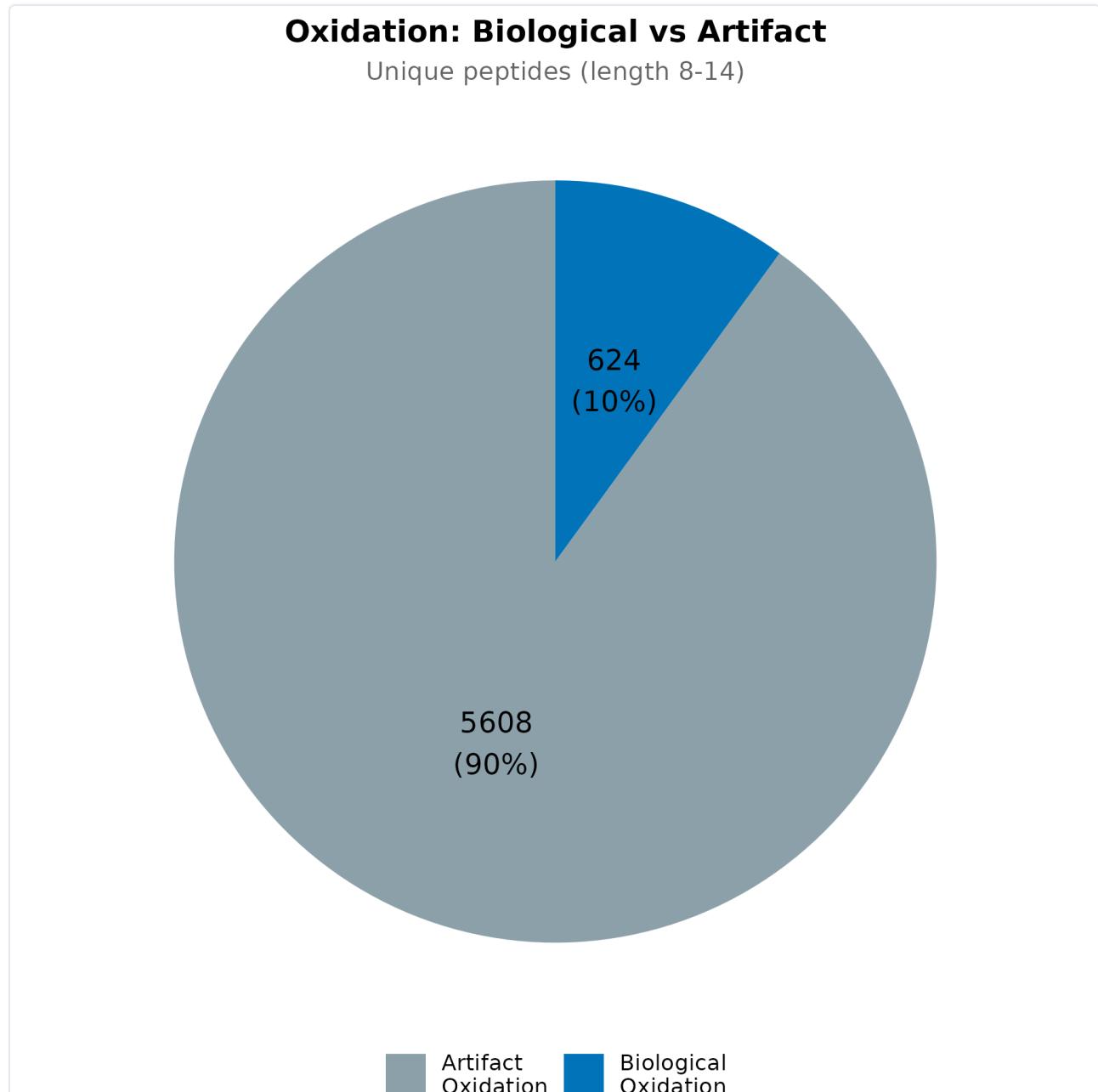
!/[Figure 6A](figure\_panels/Figure6A\_DoublyPhospho\_Sites.png)

Site distribution for peptides carrying two or more phosphorylation sites.

**Figure 6B: Ubiquitination Remnants**

*![Figure 6B](figure\_panels/Figure6B\_Ubiq\_G\_vs\_GG.png)*

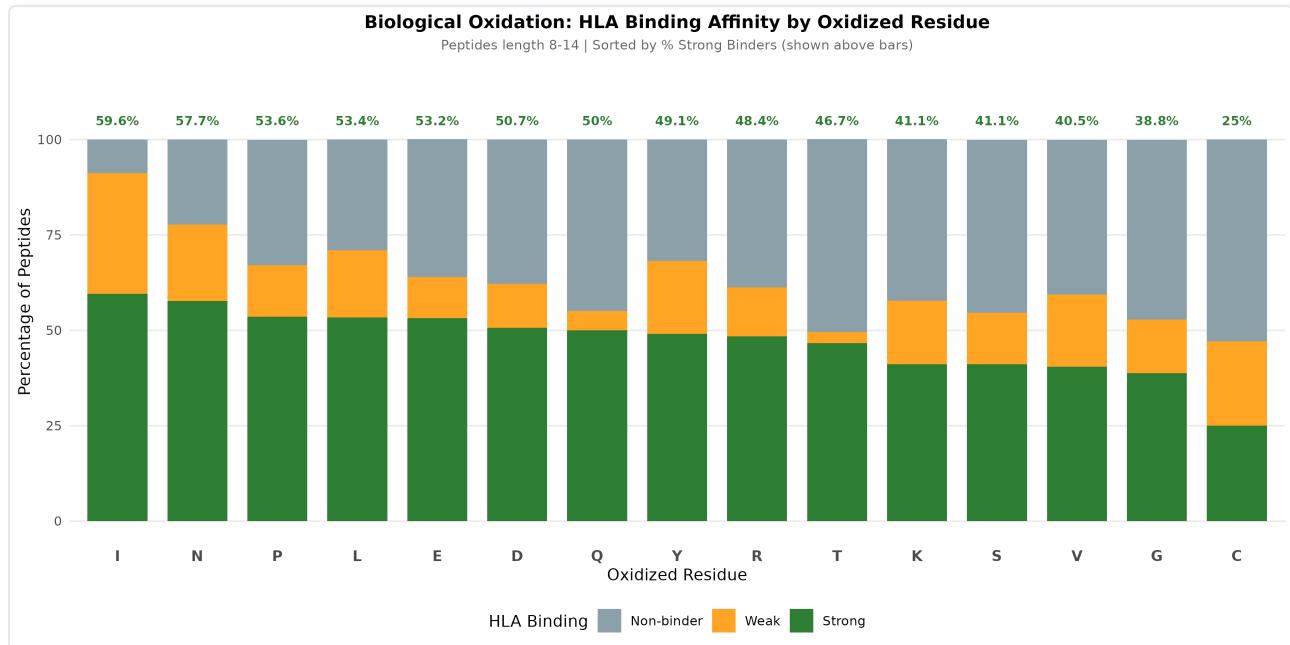
Comparison of ubiquitin remnant types following tryptic digestion: single glycine (G, +57 Da) versus diglycine (GG, +114 Da).

**Figure 6C: Oxidation Classification**

![Figure 6C](figure\_panels/Figure6C\_Oxidation\_Bio\_vs\_Art.png)

Classification of oxidized peptides: biological oxidation (P, I, L, Q, S, T, V, C, D, E, N, Y, G, K, R) versus artifact oxidation (M, W, H).

## Figure 6D: Biological Oxidation by Residue

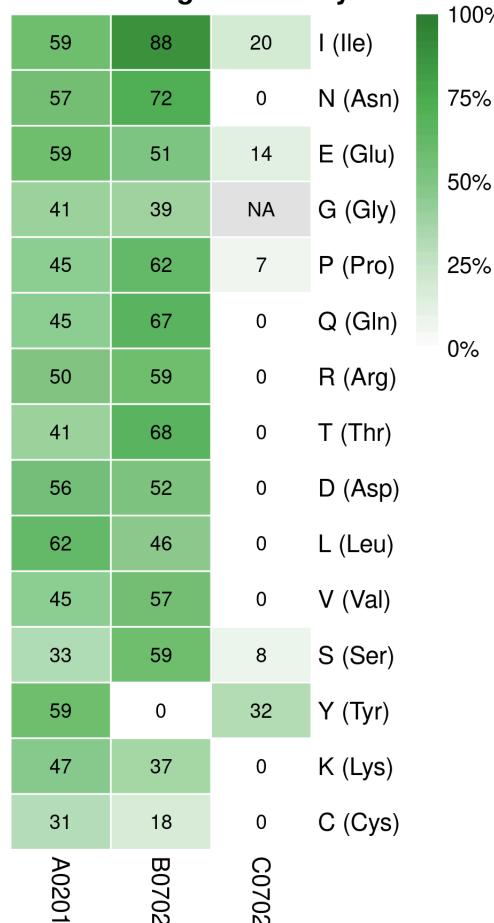


!/[Figure 6D](figure\_panels/Figure6D\_BioOxid\_BindingByResidue.png)

HLA binding classification for biologically oxidized peptides stratified by oxidized residue. Sorted by percentage of strong binders.

## Figure 6D2: Biological Oxidation Heatmap

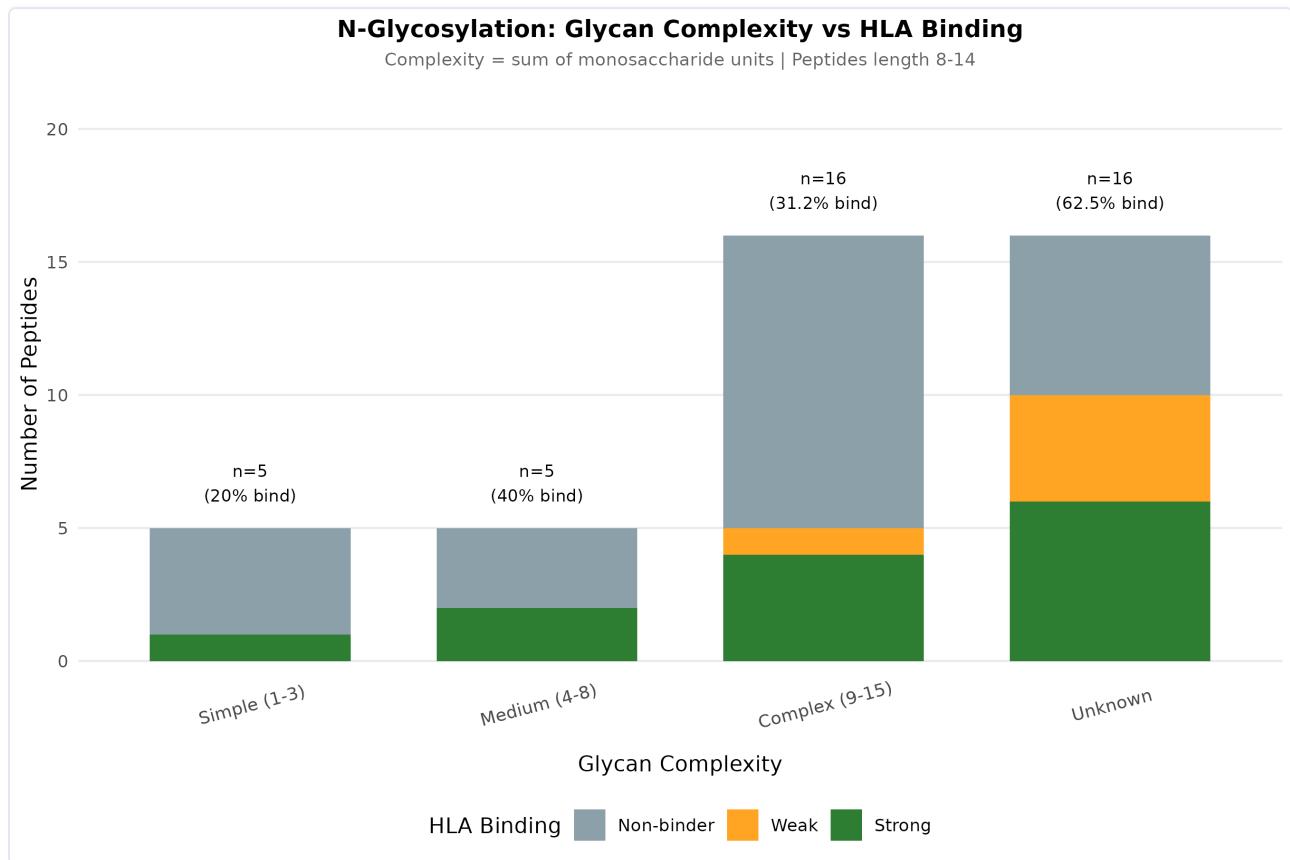
**Biological Oxidation: % Strong Binders by Residue and Allele**



![[Figure 6D2](figure\_panels/Figure6D2\_BioOxid\_Heatmap.png)]

Strong binder percentages for biological oxidation cross-tabulated by residue and HLA allele.

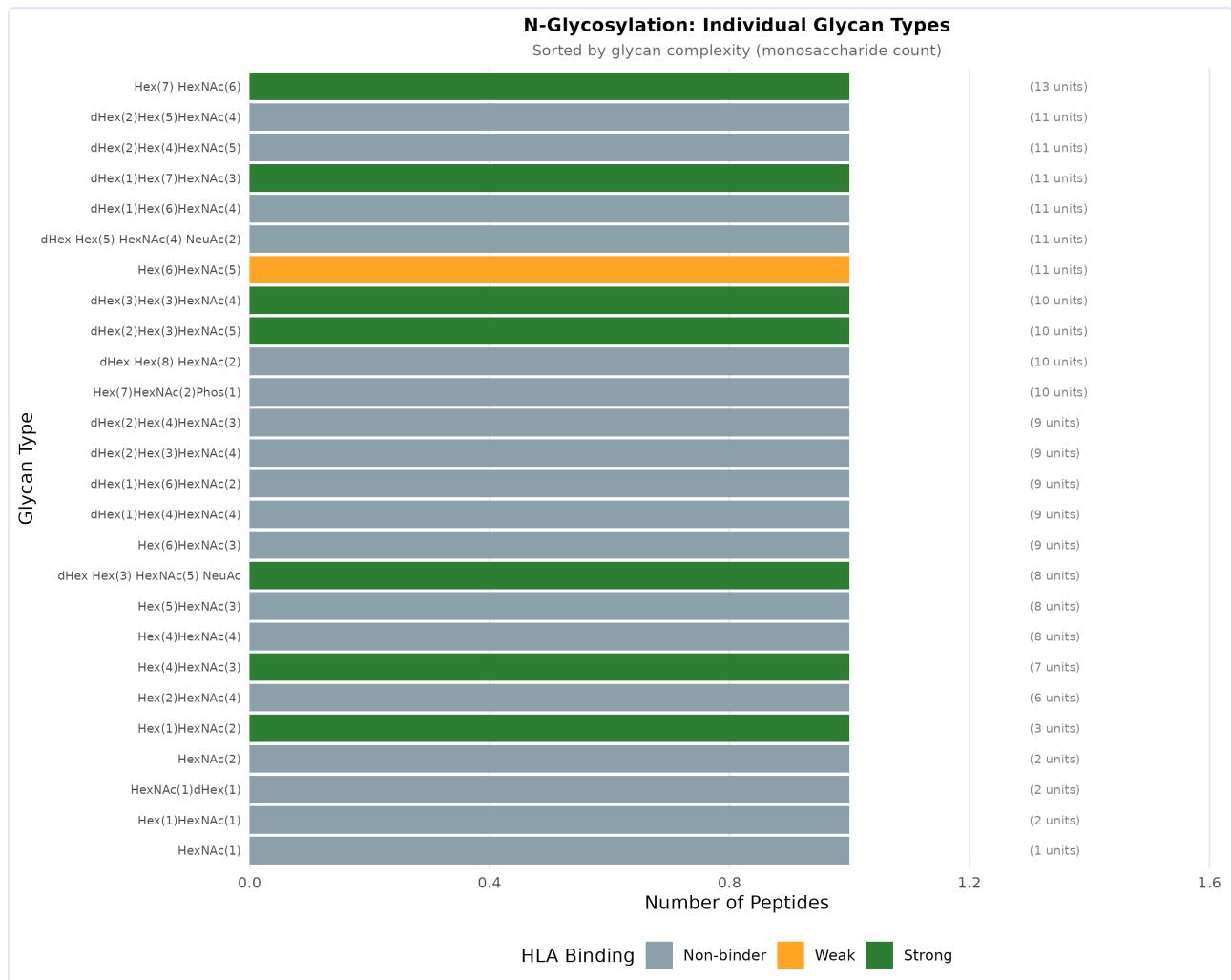
## Figure 6E: Glycan Complexity



!/[Figure 6E](figure\_panels/Figure6E\_Glycan\_Complexity.png)

N-glycosylation analysis stratified by glycan complexity (sum of monosaccharide units). Categories: Simple (1-3), Medium (4-8), Complex (9-15).

## Figure 6E2: Glycan Types



!Figure 6E2](figure\_panels/Figure6E2\_Glycan\_Types\_Detail.png)

Individual glycan structures with HLA binding classification, sorted by monosaccharide count.

**Analysis Date:** January 2026 | **Made by:** Peter Kubiniok