

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*02:01, B*07:02, C*07:02

Data Summary

Dataset	Peptides
Background (unmodified)	77,100
Total Modified	13,904
Artifact Oxidation	6,382
Cysteinylation	2,494
Deamidation	1,481
Biological Oxidation	955
Phosphorylation	911
Ubiquitination	699 (GG: 464, G: 235)
Acetylation	341
Methylation	233
Citrullination	138
Dimethylation	137
N-Glycosylation	51
Carbamidomethylation	48
SUMOylation	34

PTM Categories

PTM Type	Target Residues
Phosphorylation	S, T, Y
Acetylation	K, N-terminus
Cysteinylation	C
Methylation	K, R, and others (C, H, N, Q, I, L, D, E, S, T, N-term)
Dimethylation	K, R, N, P, N-term
Deamidation	N, Q
Biological Oxidation	P, I, L, Q, S, T, V, C, D, E, N, Y, G, K, R
Artifact Oxidation	M, W, H, F
Carbamidomethylation	C
Citrullination	R
Ubiquitination	K, S, T, C
N-Glycosylation	N
SUMOylation	K

HLA Binding Classification

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

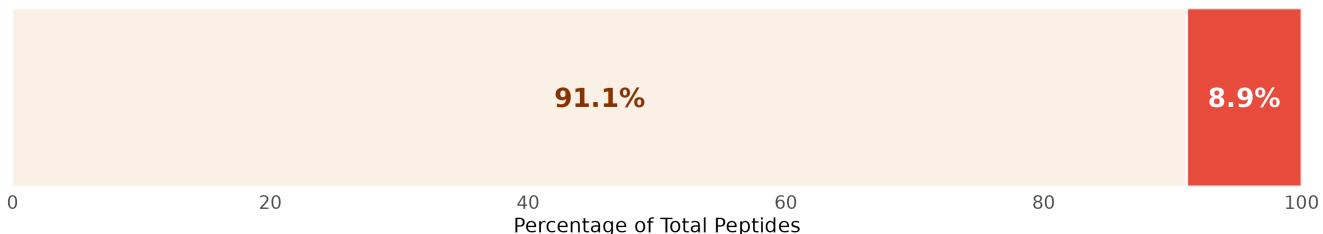
Figure 1: PTM Composition

Figure 1A: PTM Distribution

HLA-I Immunopeptidome Composition (n = 84,622 peptides)

Excludes artifact oxidation (n = 6,382)

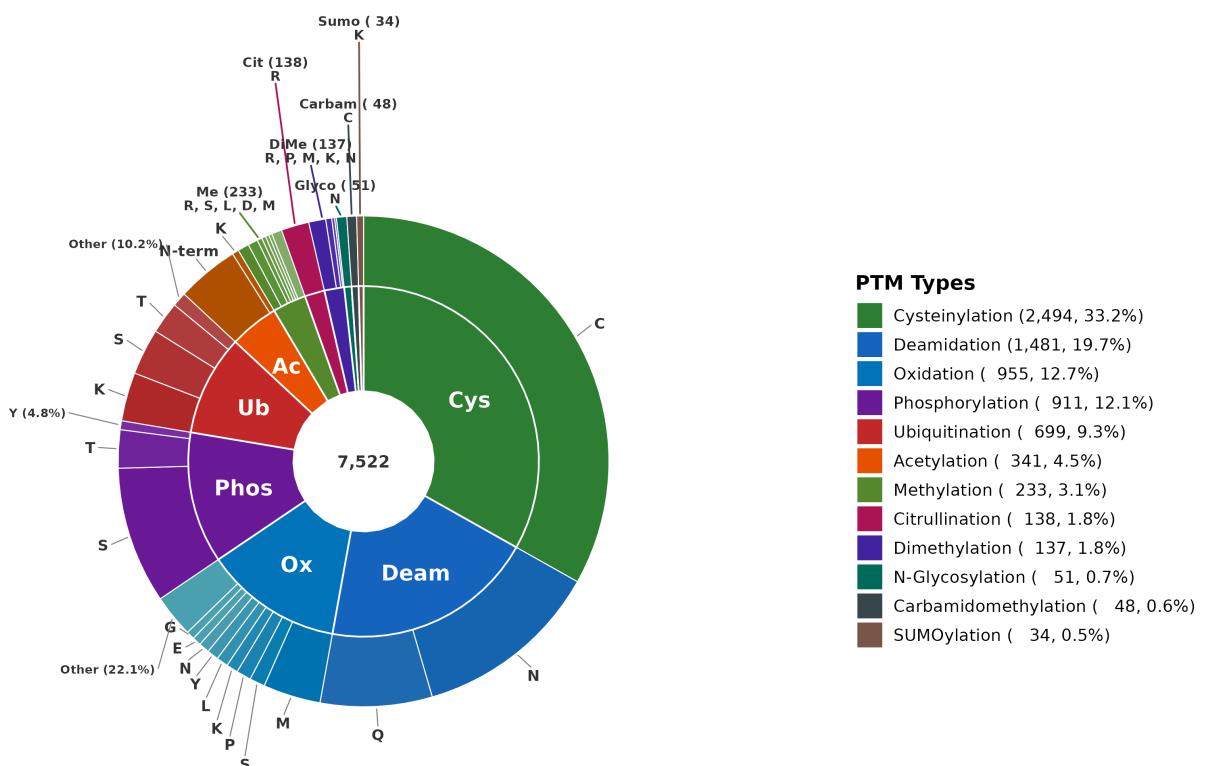
■ Modified (7,522) ■ Unmodified (77,100)



Distribution of unique modified peptides by PTM type (length 8-14 amino acids). Artifact oxidation (n=6,382) is excluded from the modified count to preserve visual clarity and is shown separately in Figure 1C.

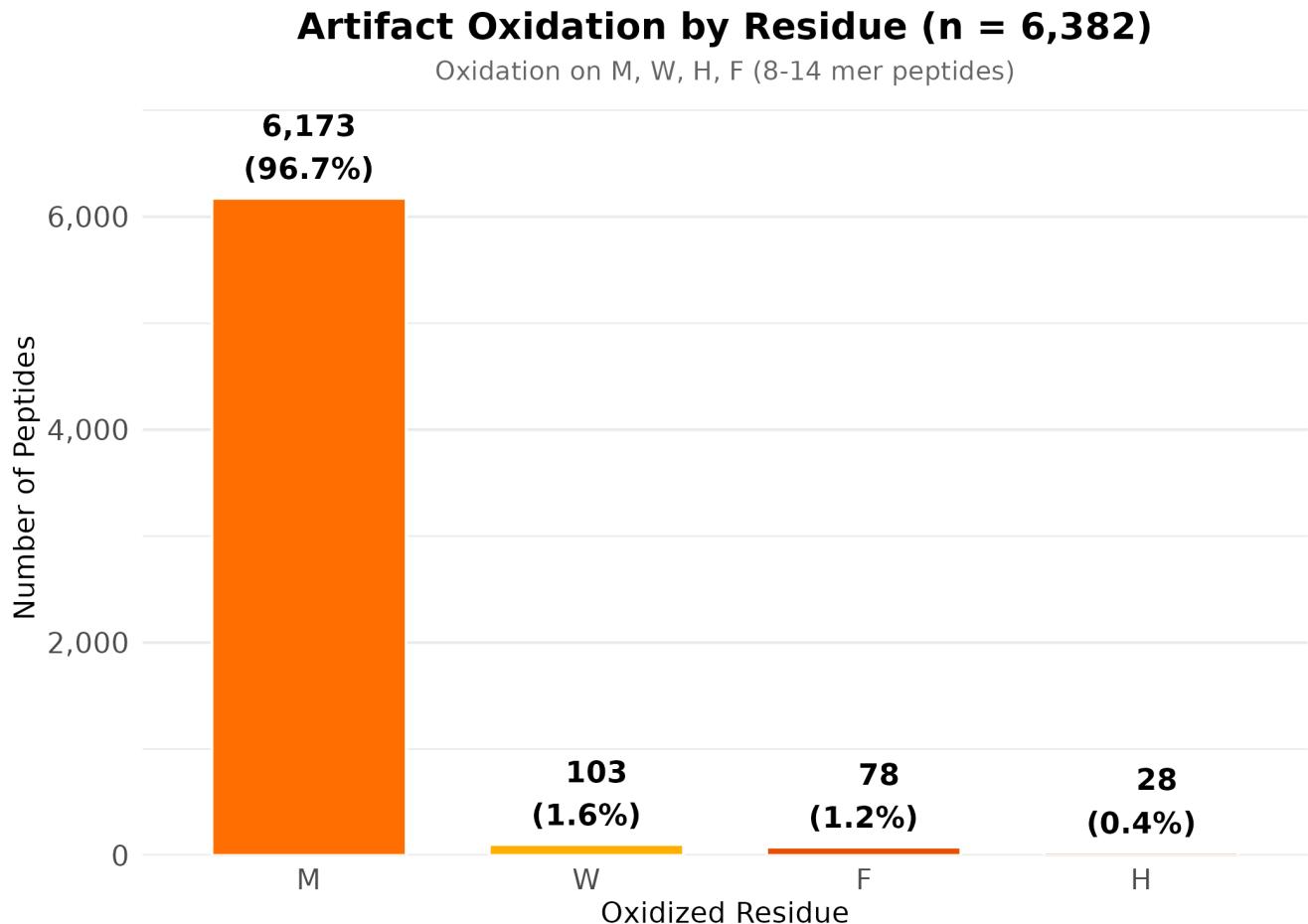
Figure 1B: PTM Proportions

HLA-I PTM Distribution by Modification Type and Residue



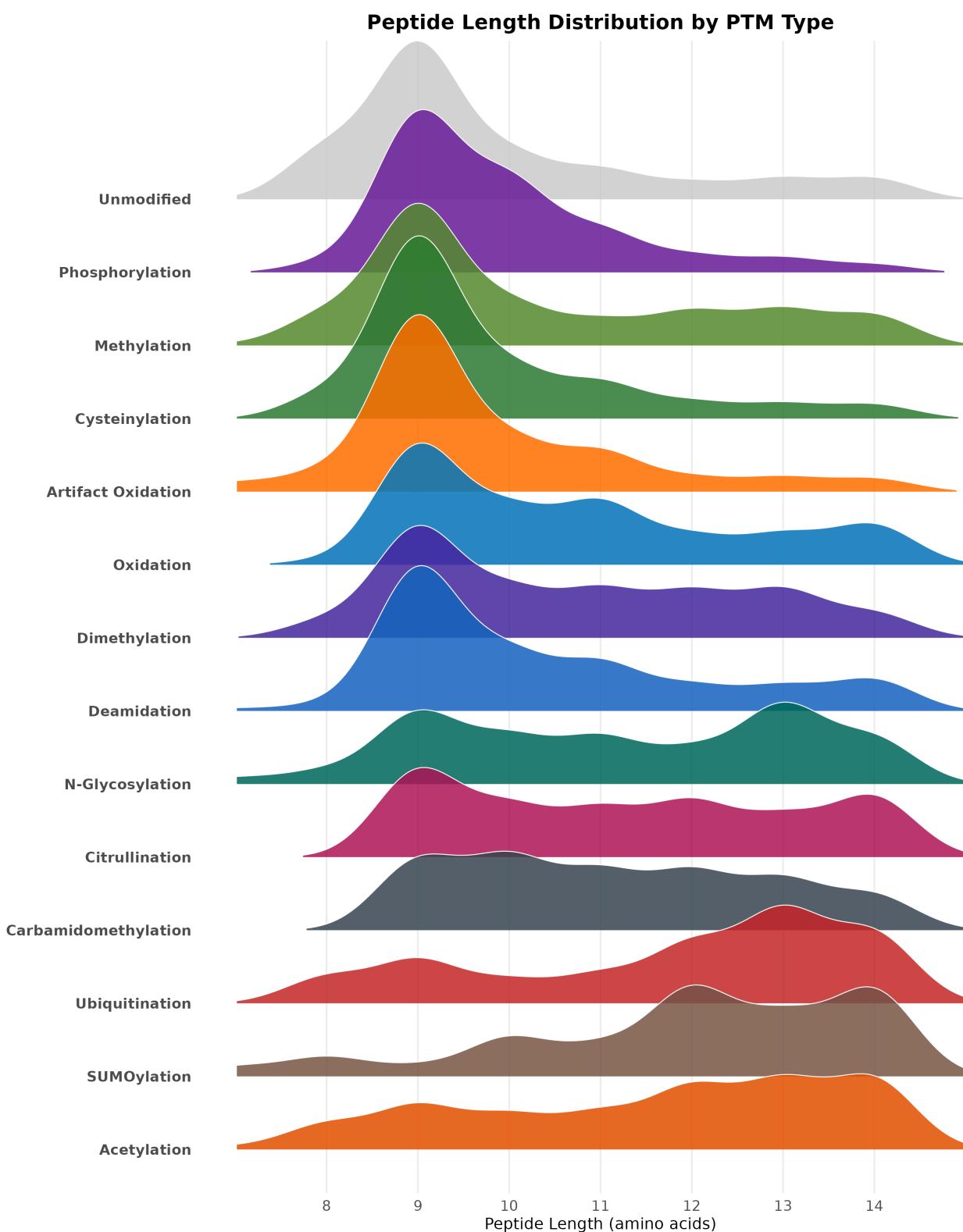
Proportional representation of each PTM class within the biologically modified peptidome. Artifact oxidation is excluded and shown separately in Figure 1C.

Figure 1C: Artifact Oxidation



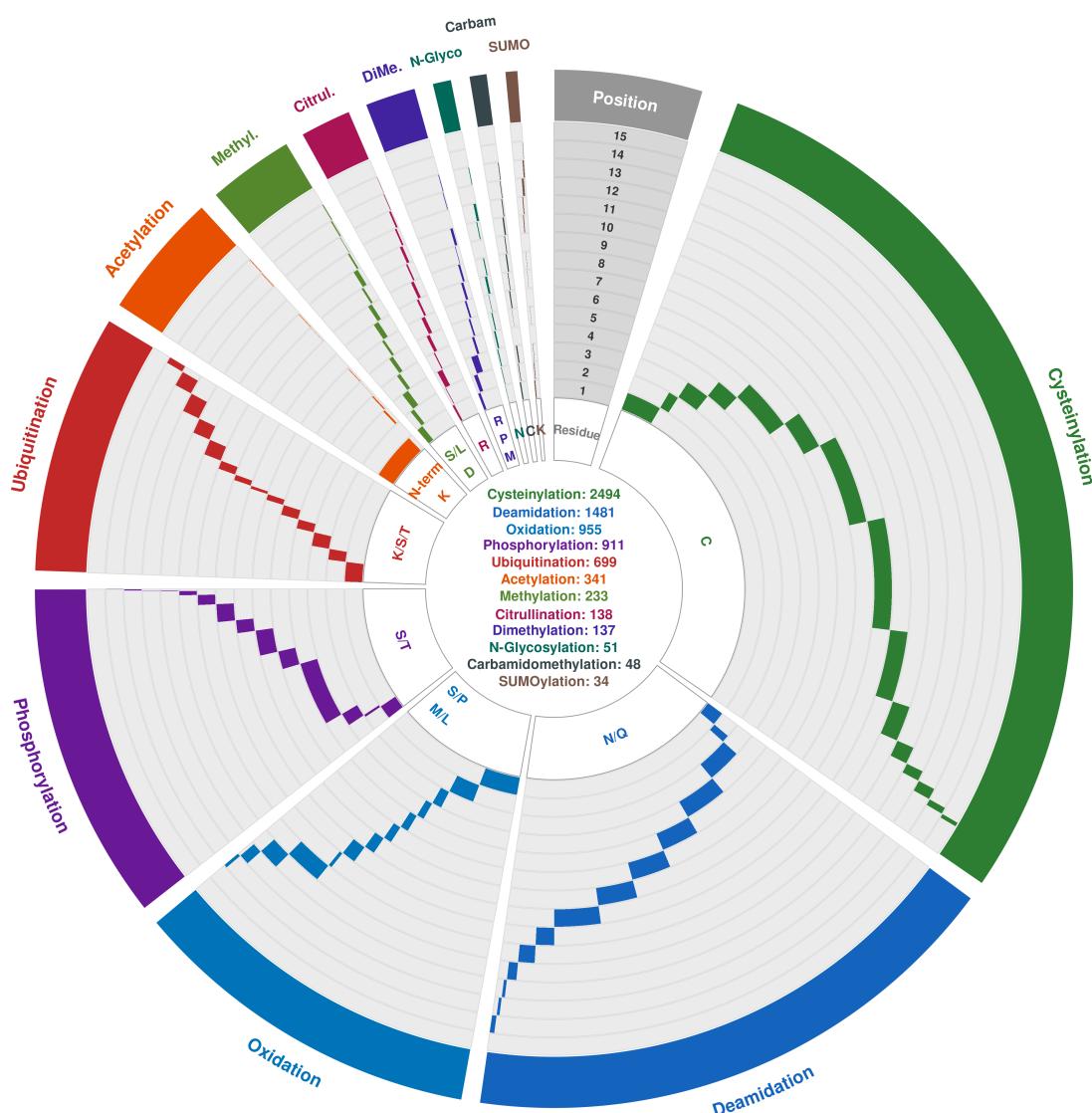
Artifact oxidation peptide counts by modified residue (M, W, H, F). Total: 6,382 peptides.

Figure 2: Peptide Length Distribution

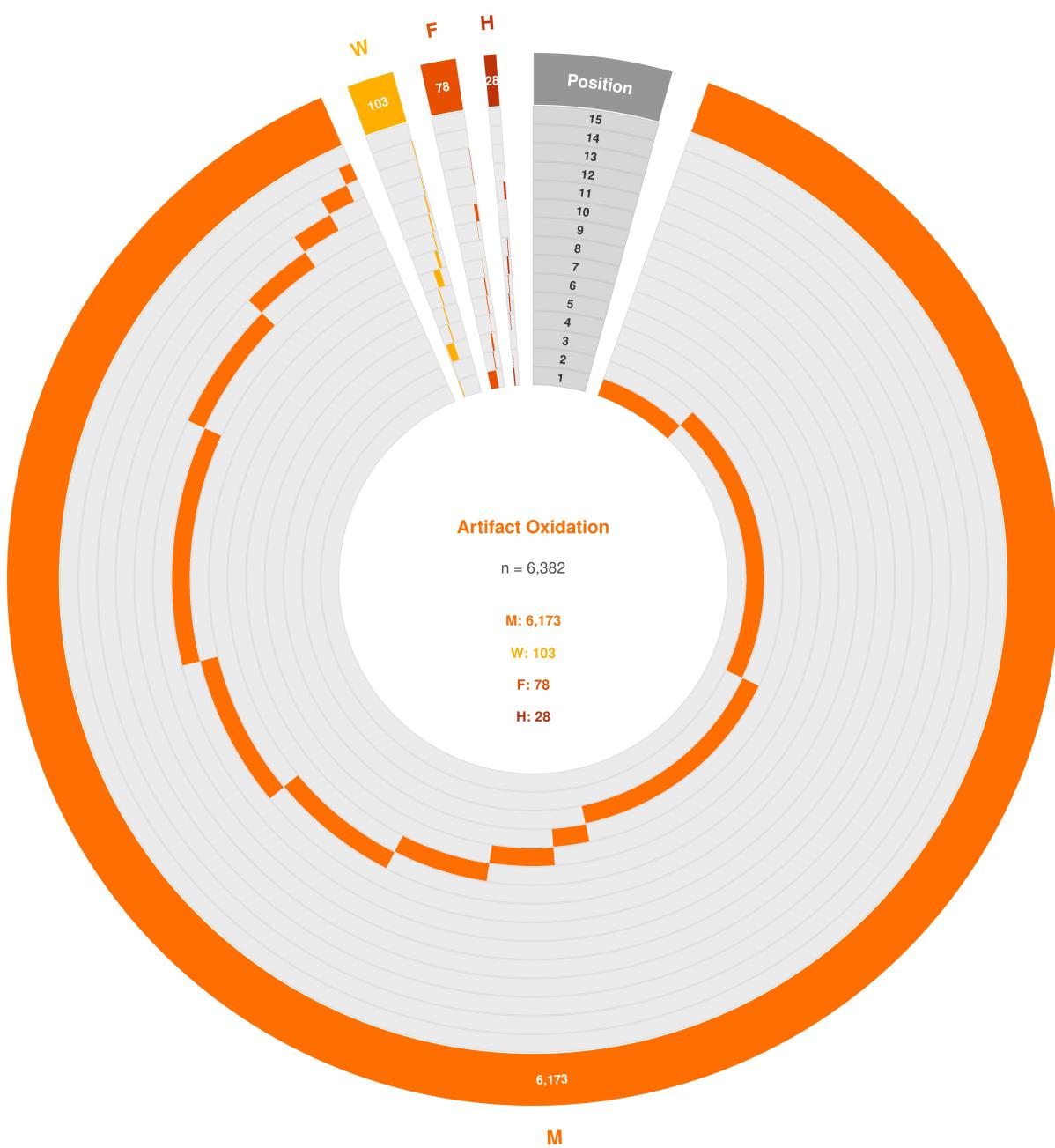


Ridgeline density plot comparing peptide length distributions across PTM types. Gray distribution represents unmodified background peptides.

Figure 4A: PTM Landscape



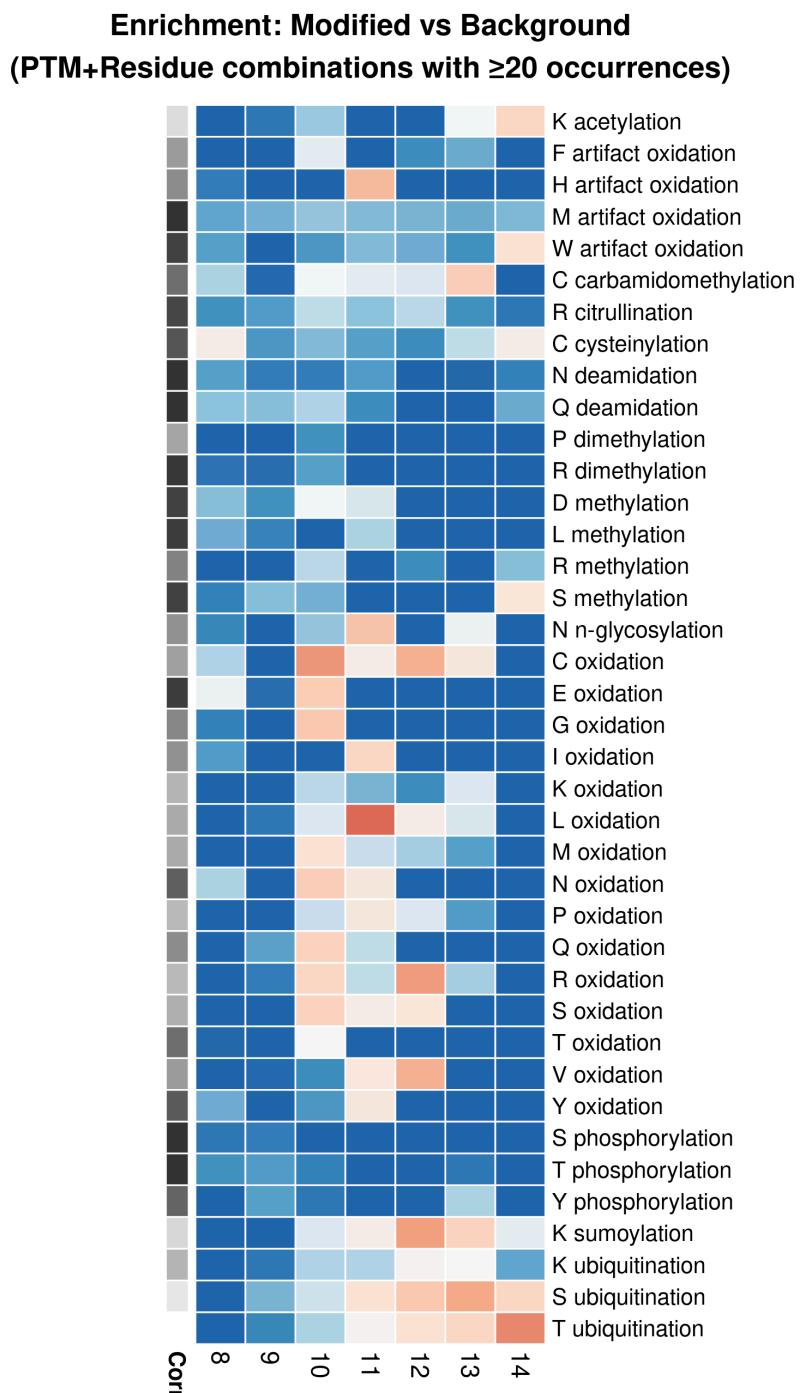
Circos diagram illustrating relationships between PTM types (outer sectors), modified residues (links), and modification site positions (inner elements). Sector width is proportional to peptide count. Artifact oxidation is excluded to preserve visual balance and is shown separately in Figure 4A2.

Figure 4A2: Artifact Oxidation Landscape

Circos diagram for artifact oxidation ($n=6,382$) showing modification distribution across residues M, W, H, and F with positional information.

Figure 4B: Positional Enrichment

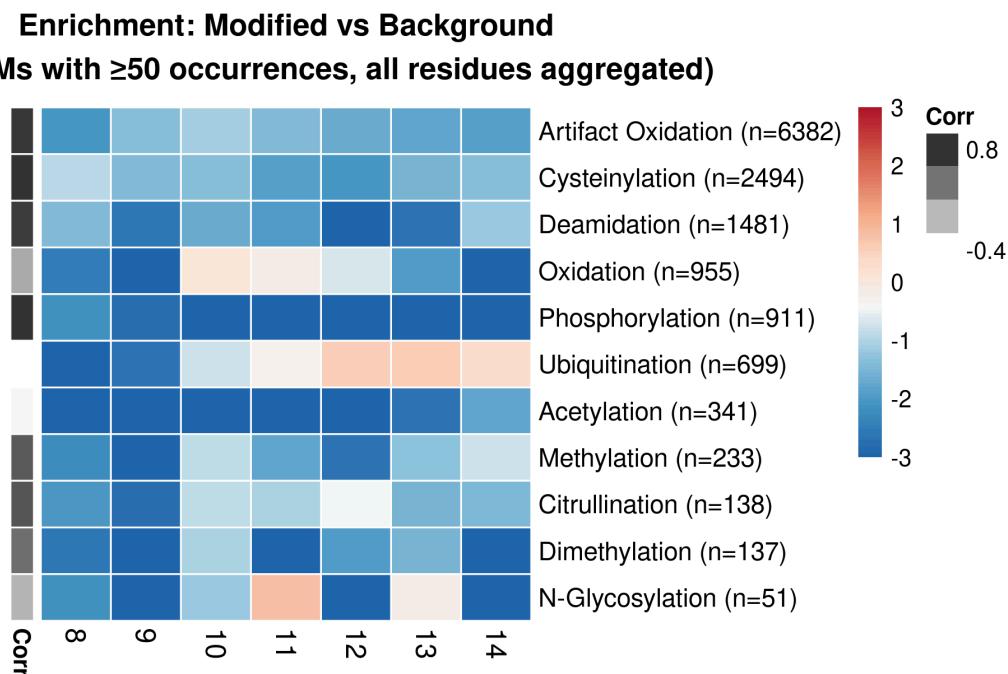
By PTM and Residue



Log₂ enrichment of PTM occurrence at peptide positions 8-14 relative to background amino acid positional distribution. PTM+residue combinations with ≥ 20 occurrences. Color scale: blue (depletion)

to red (enrichment), capped at ± 3 . Correlation column indicates similarity to background distribution.

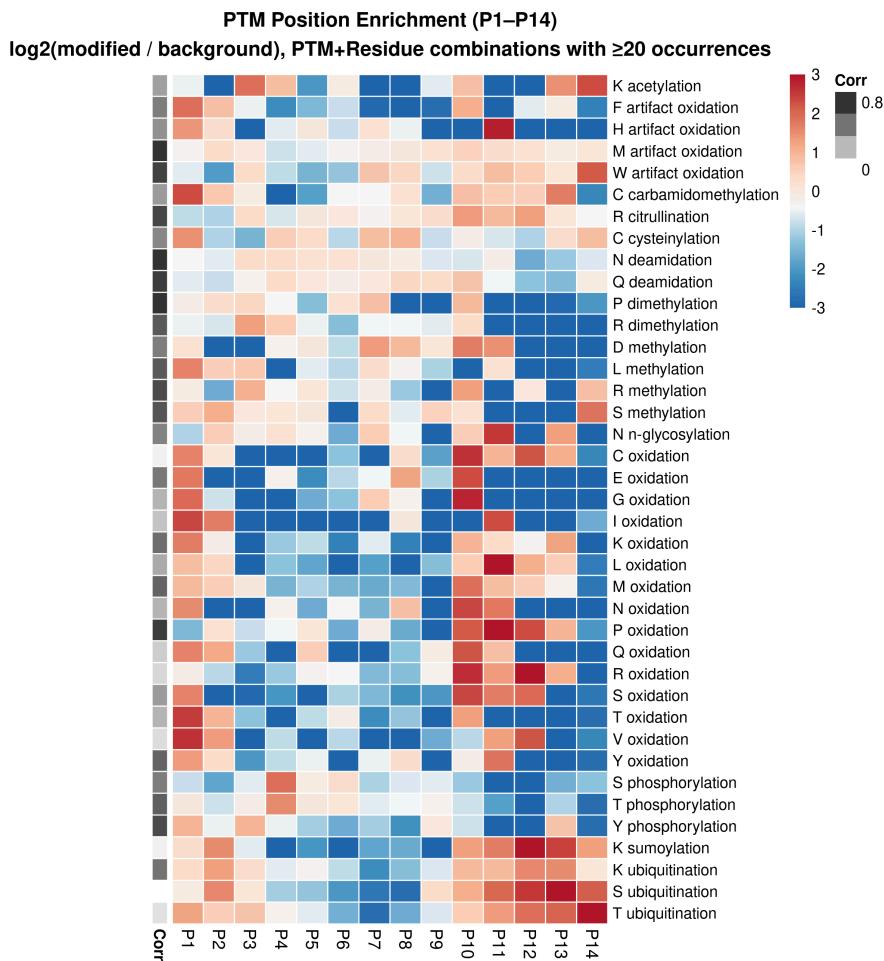
By PTM Type (Aggregated)



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

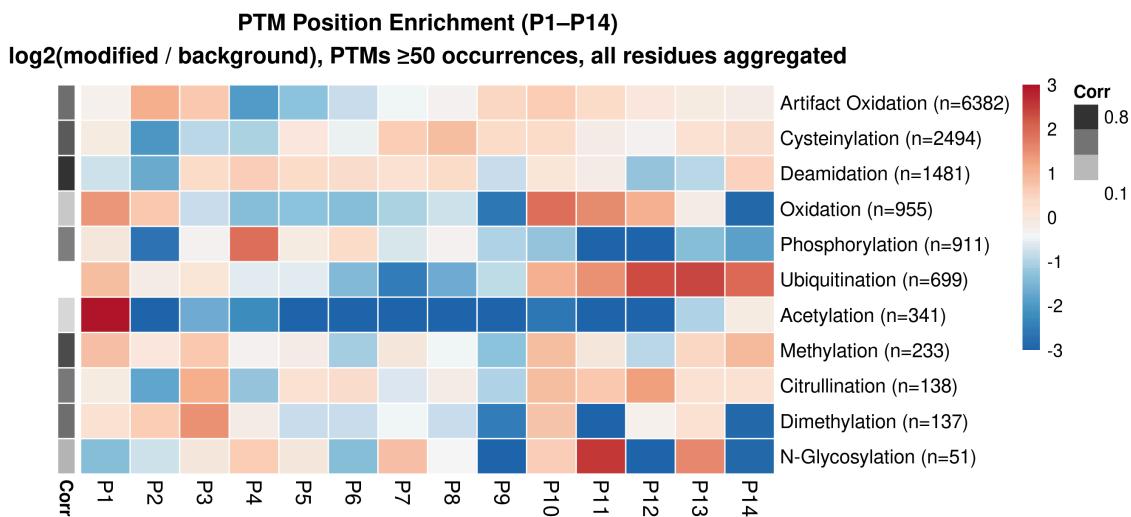
Figure 4X: Full Positional Enrichment (P1–P14)

By PTM and Residue



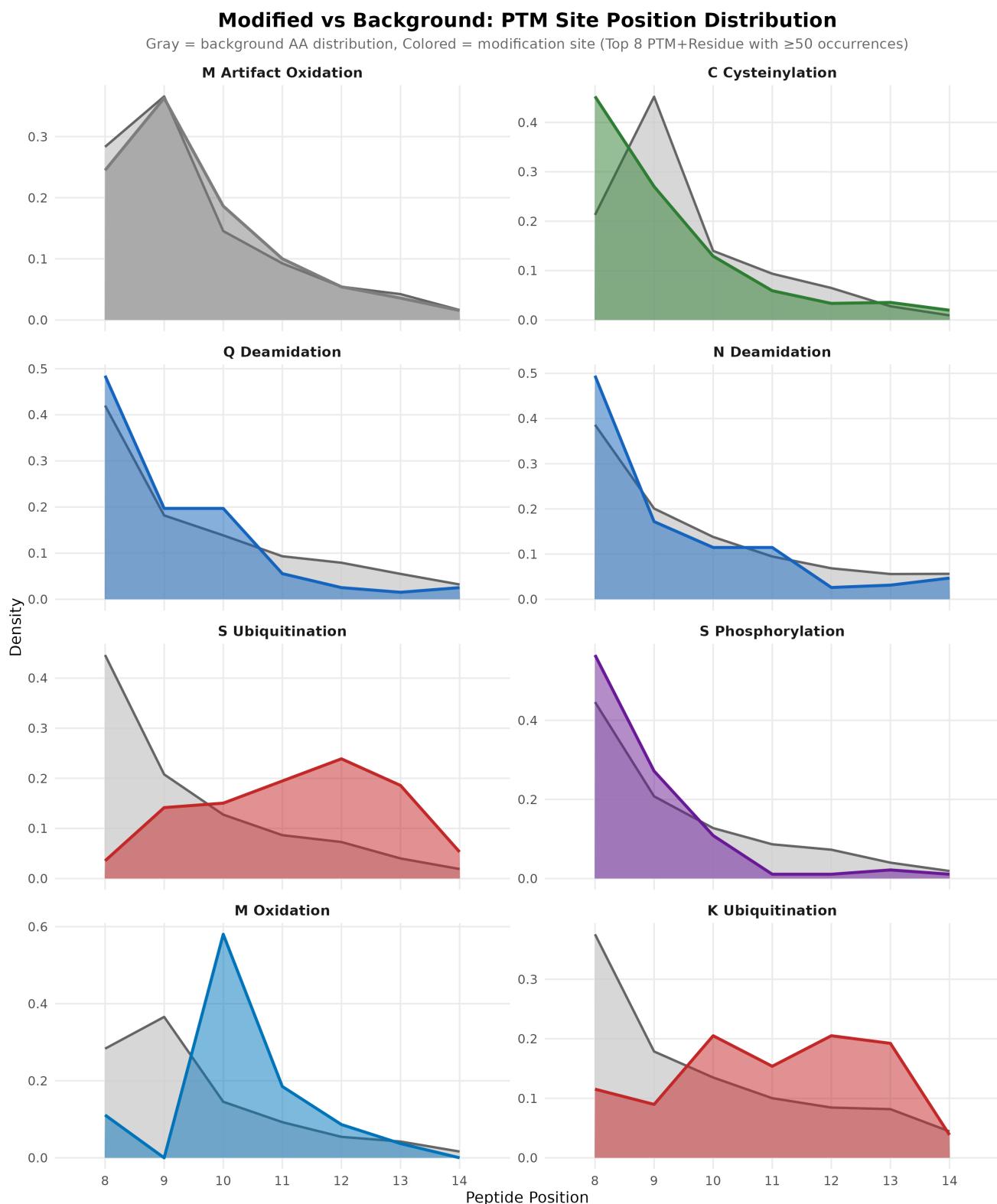
Log₂ enrichment of PTM occurrence across all peptide positions P1–P14, relative to background amino acid positional distribution. PTM+residue combinations with ≥ 20 occurrences. Unlike Figures 4B/4B2, which focus on the tail positions 8–14, this panel captures positional preferences across the full peptide length.

By PTM Type (Aggregated)



Aggregated positional enrichment by PTM type (≥ 50 occurrences) across P1–P14, combining all modified residues.

Figure 4C: PTM Position Density

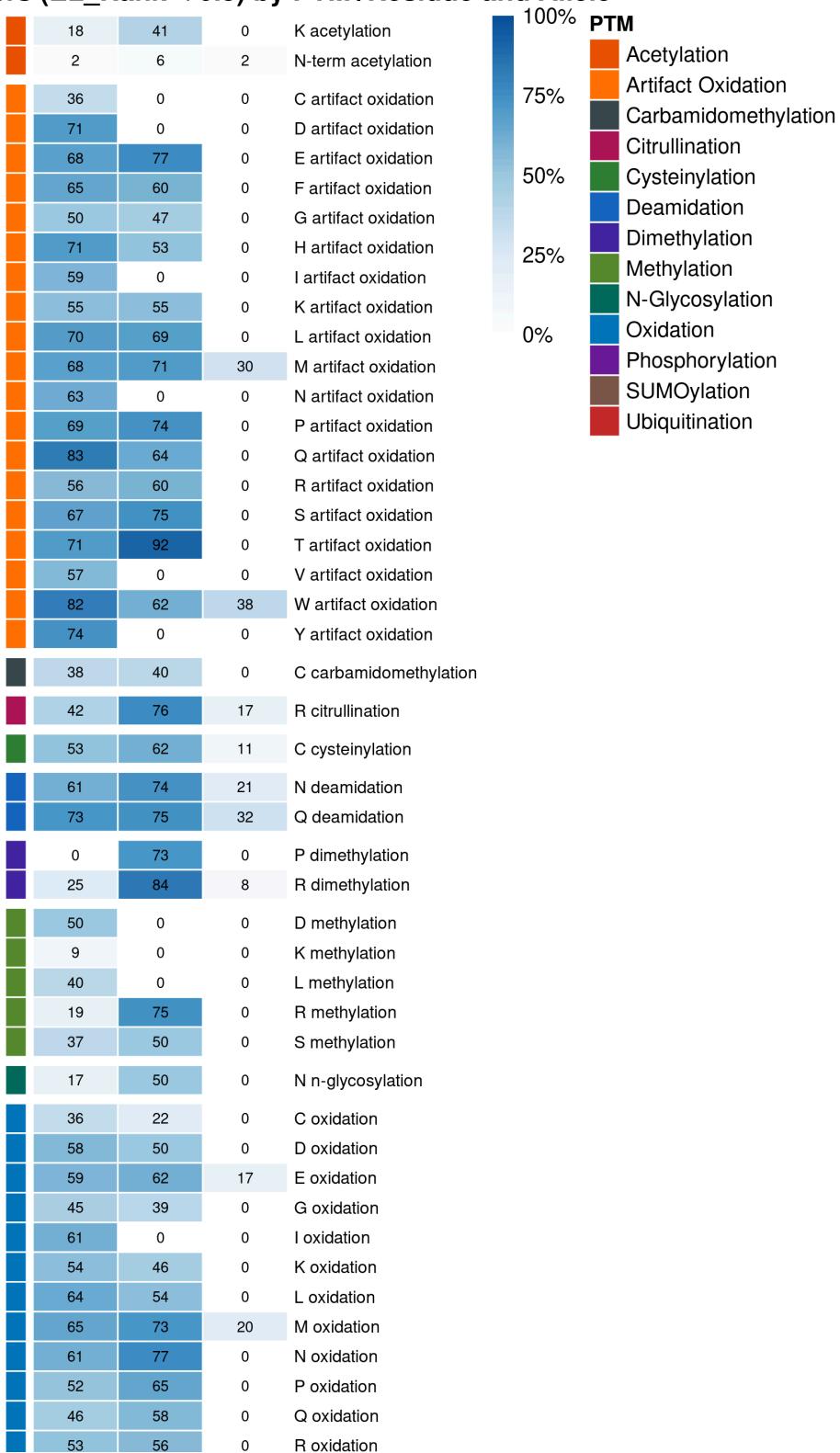


Density curves showing modification site distribution across peptide positions 8-14. PTM+residue combinations with ≥ 50 modifications.

Figure 5A: HLA Binding Affinity

Strong Binders by PTM and Residue

Strong Binders (EL_Rank < 0.5) by PTM+Residue and Allele

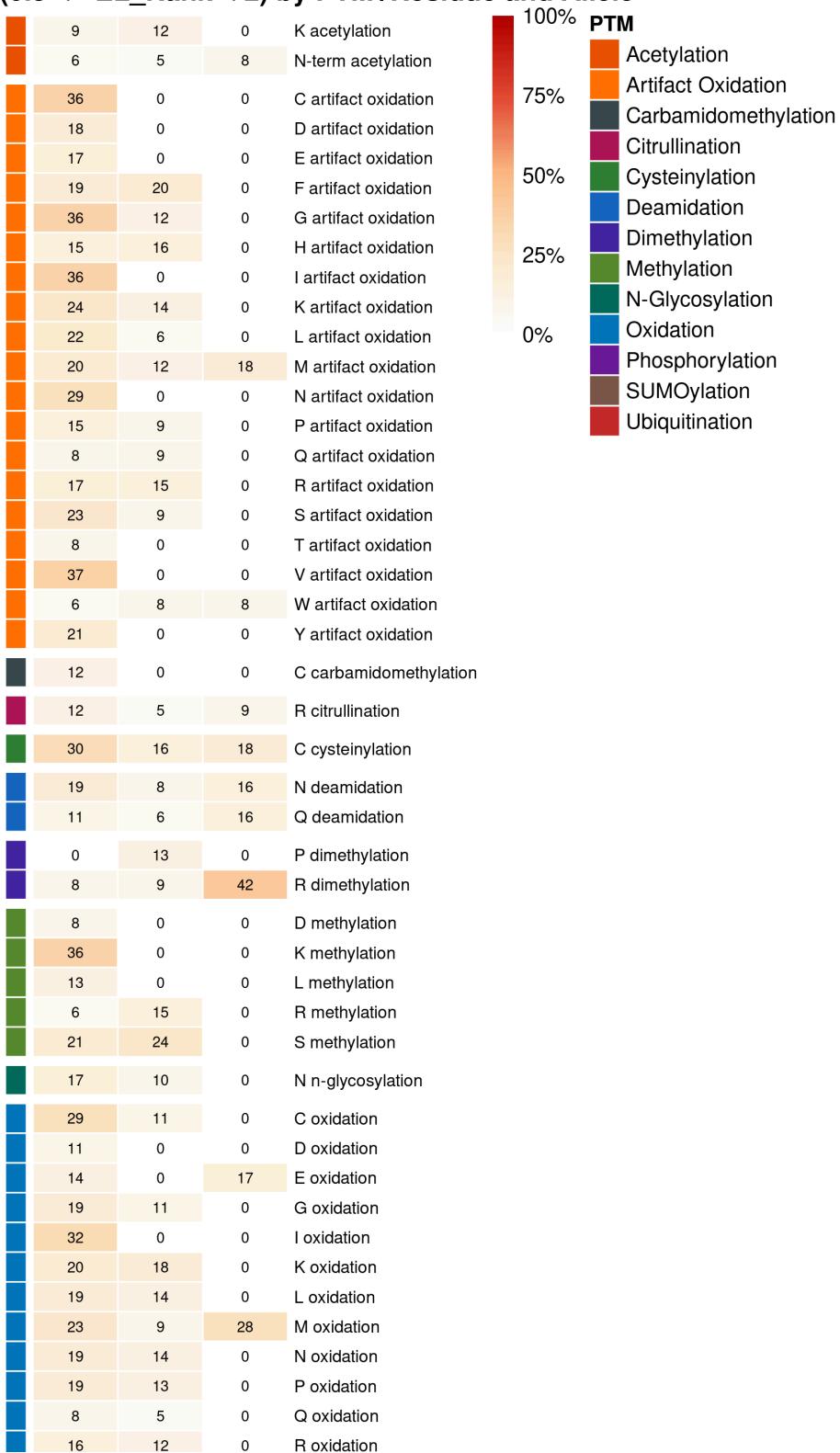


	47	72	8	S oxidation
	52	74	0	T oxidation
	44	64	0	V oxidation
	42	0	0	W oxidation
	62	0	35	Y oxidation
	63	92	64	S phosphorylation
	66	90	50	T phosphorylation
	68	0	0	Y phosphorylation
	10	18	0	K sumoylation
	37	35	5	K ubiquitination
	6	14	10	S ubiquitination
	7	21	4	T ubiquitination
PTM	A0201	B0702	C0702	

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

Weak Binders by PTM and Residue

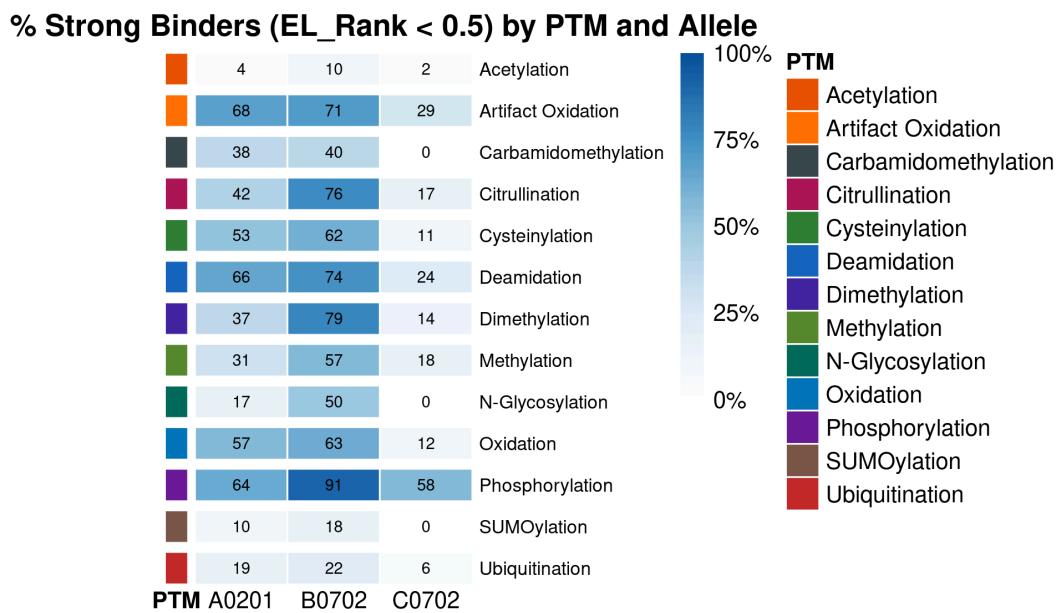
Weak Binders ($0.5 \leq EL_Rank < 2$) by PTM+Residue and Allele



	14	7	15	S oxidation
	5	0	0	T oxidation
	24	9	0	V oxidation
	17	0	0	W oxidation
	25	0	12	Y oxidation
	26	5	25	S phosphorylation
	14	6	25	T phosphorylation
	27	0	0	Y phosphorylation
	0	0	0	K sumoylation
	4	10	3	K ubiquitination
	4	10	4	S ubiquitination
	9	7	0	T ubiquitination
PTM	A0201	B0702	C0702	

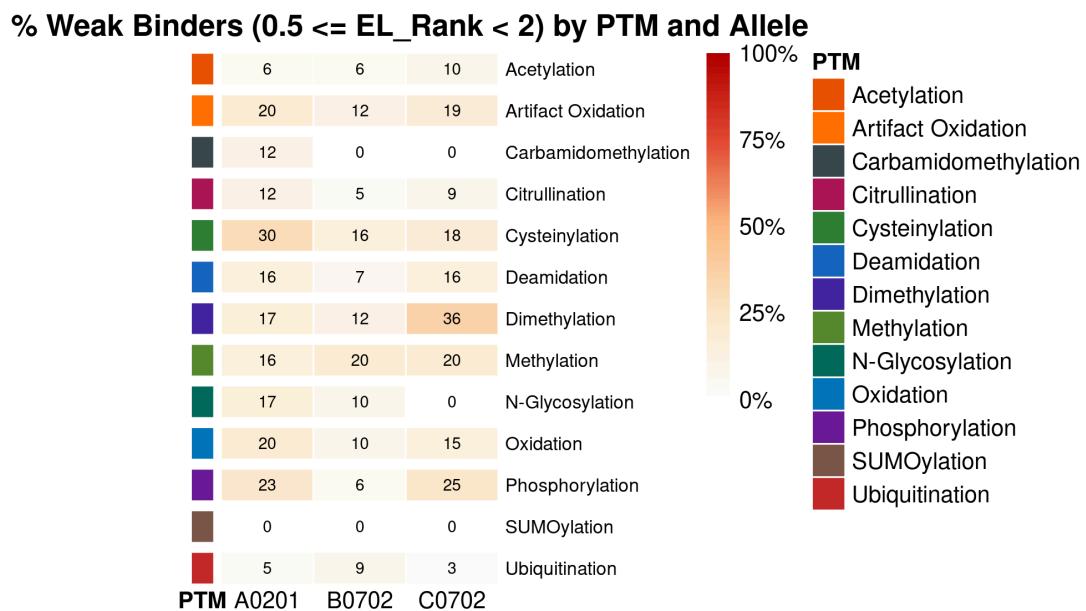
Percentage of weak HLA binders ($0.5\% \leq \text{EL Rank} < 2\%$) for each PTM+residue combination.

Strong Binders by PTM Type



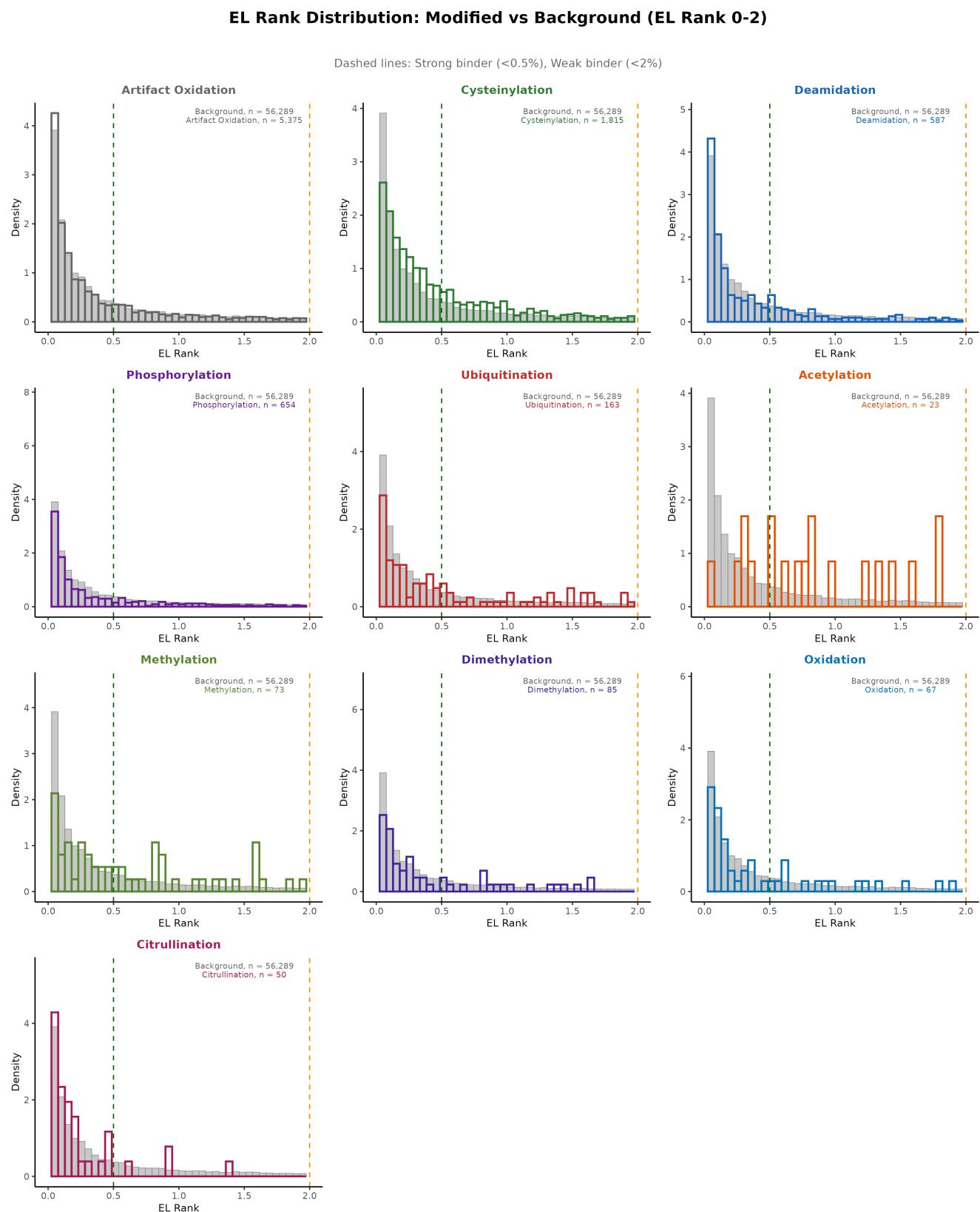
Strong binder percentages aggregated by PTM type across alleles.

Weak Binders by PTM Type



Weak binder percentages aggregated by PTM type.

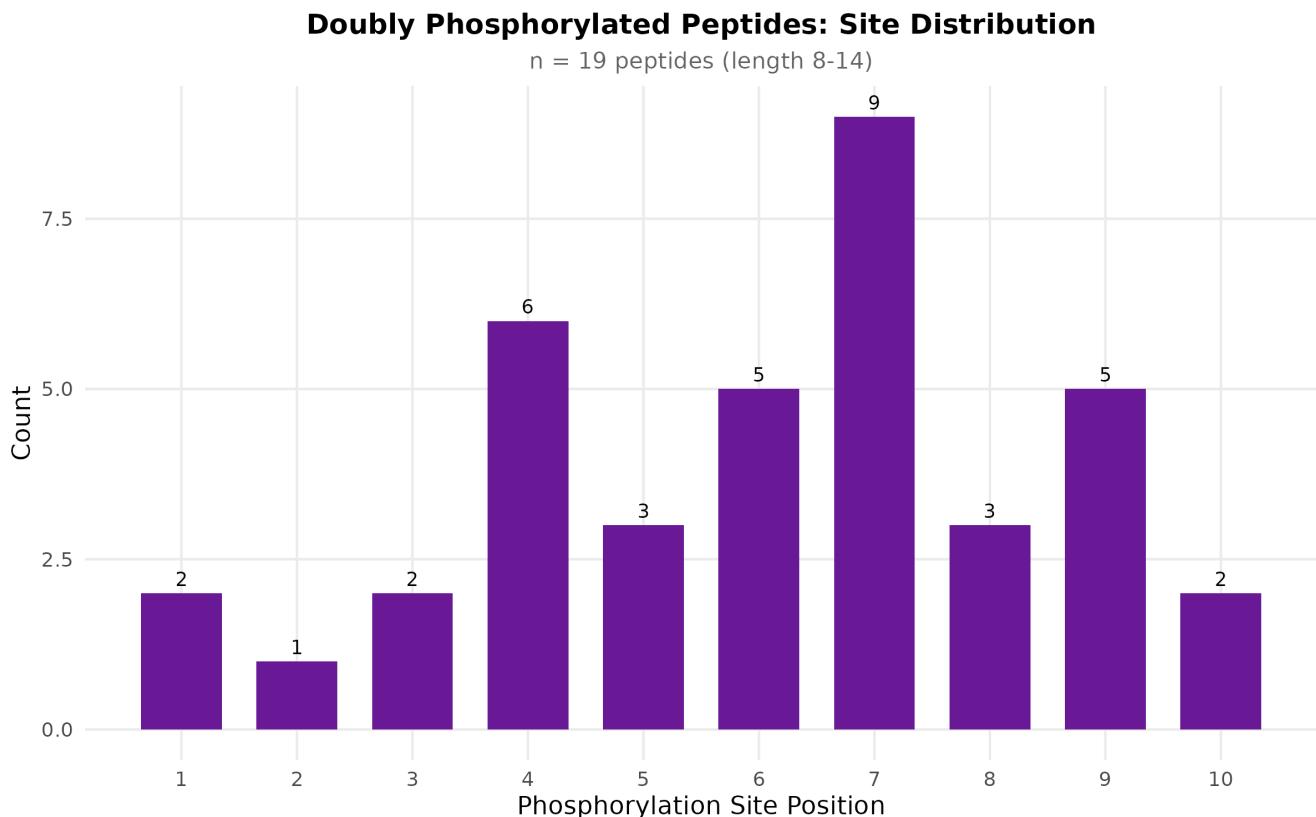
Figure 5B: EL Rank Distribution



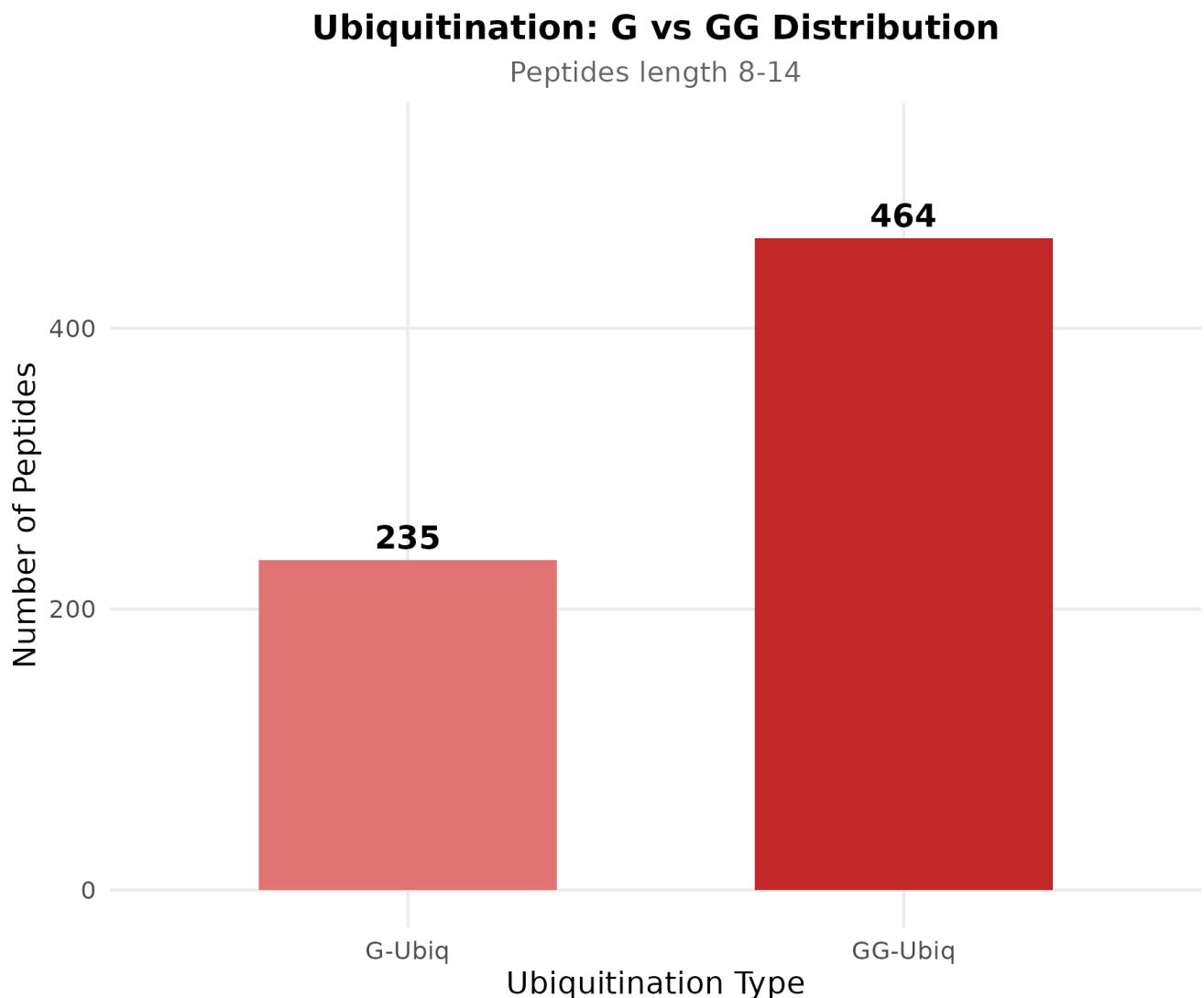
EL Rank distribution comparison between modified peptides (colored outlines) and background unmodified peptides (gray) within the binder range (EL Rank 0-2). Dashed lines indicate strong binder (0.5%) and weak binder (2%) thresholds.

Figure 6: Specific PTM Analyses

Figure 6A: Doubly Phosphorylated Peptides



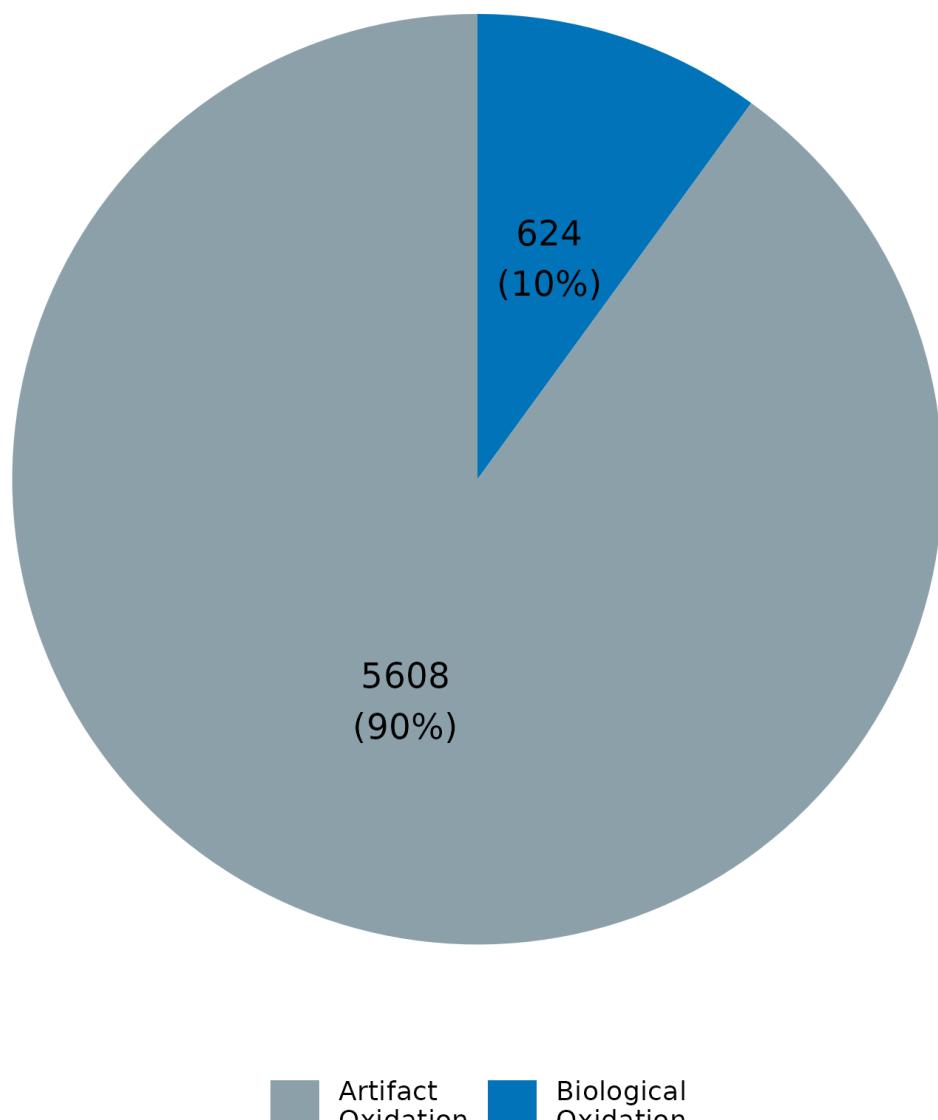
Site distribution for peptides carrying two or more phosphorylation sites.

Figure 6B: Ubiquitination Remnants

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

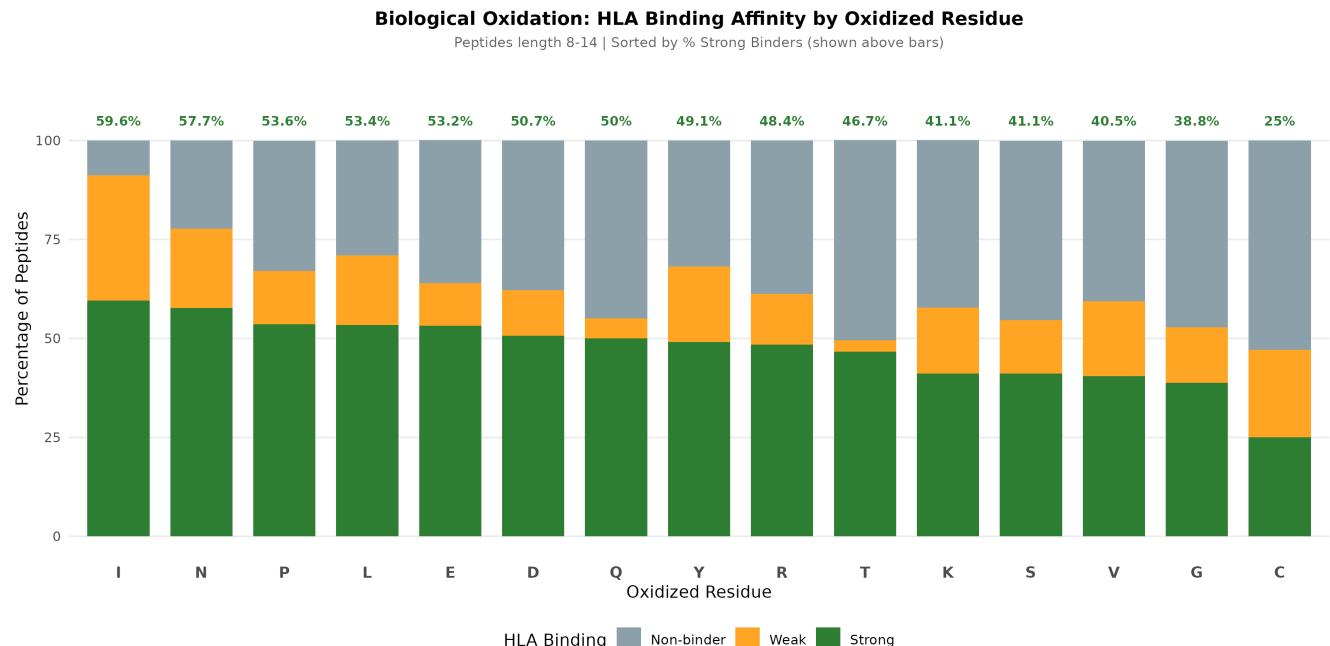
Figure 6C: Oxidation Classification**Oxidation: Biological vs Artifact**

Unique peptides (length 8-14)

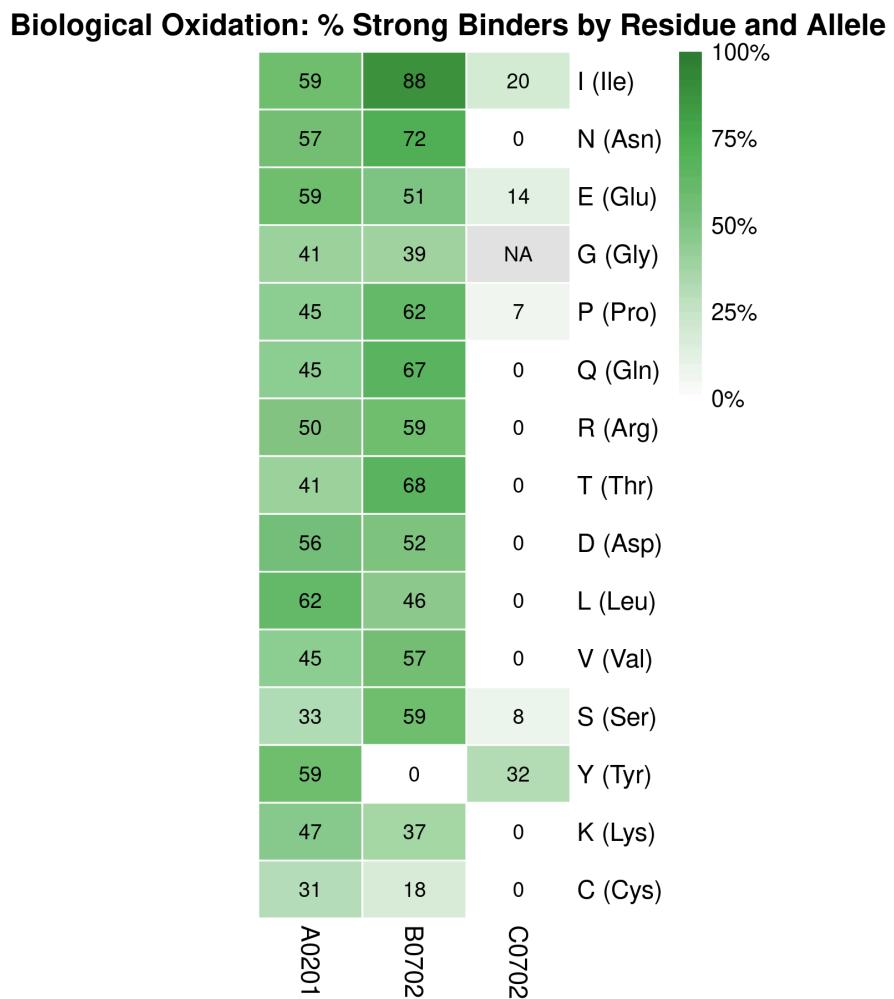


Classification of oxidized peptides: biological oxidation (P, I, L, Q, S, T, V, C, D, E, N, Y, G, K, R) versus artifact oxidation (M, W, H, F).

Figure 6D: Biological Oxidation by Residue



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

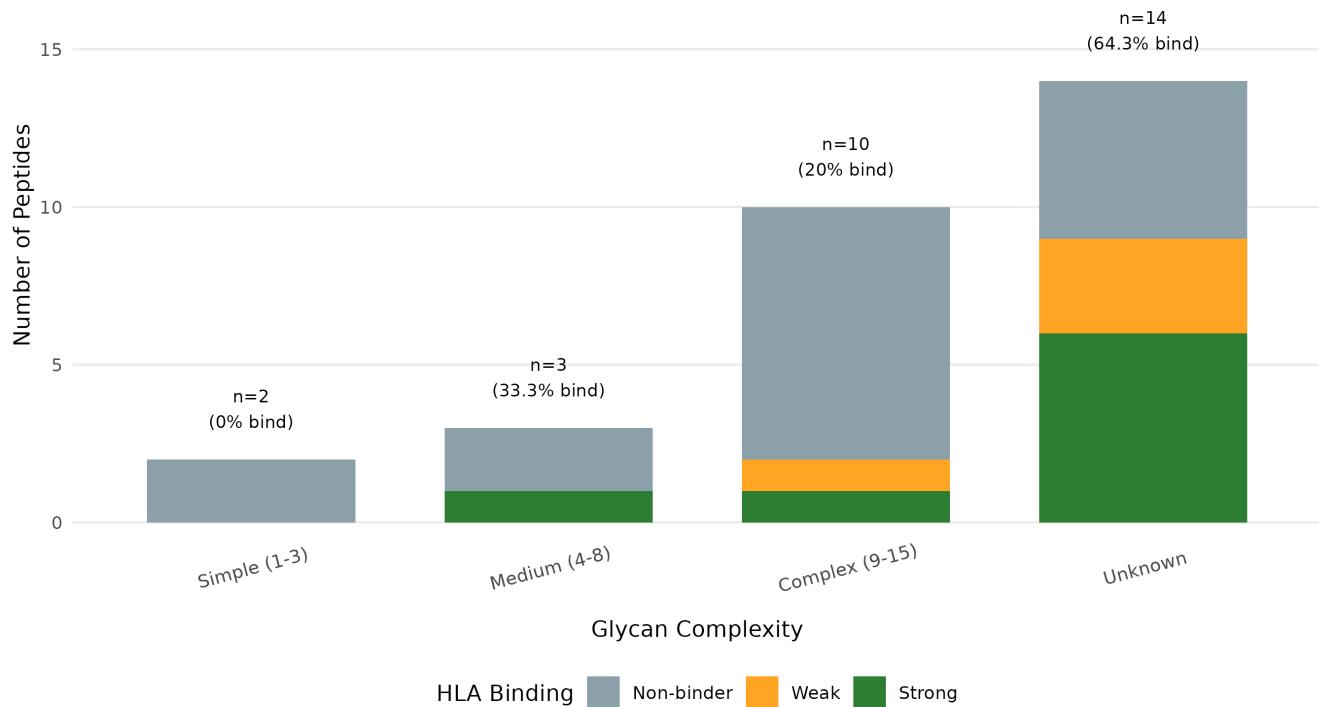
Figure 6D2: Biological Oxidation Heatmap

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

Figure 6E: Glycan Complexity

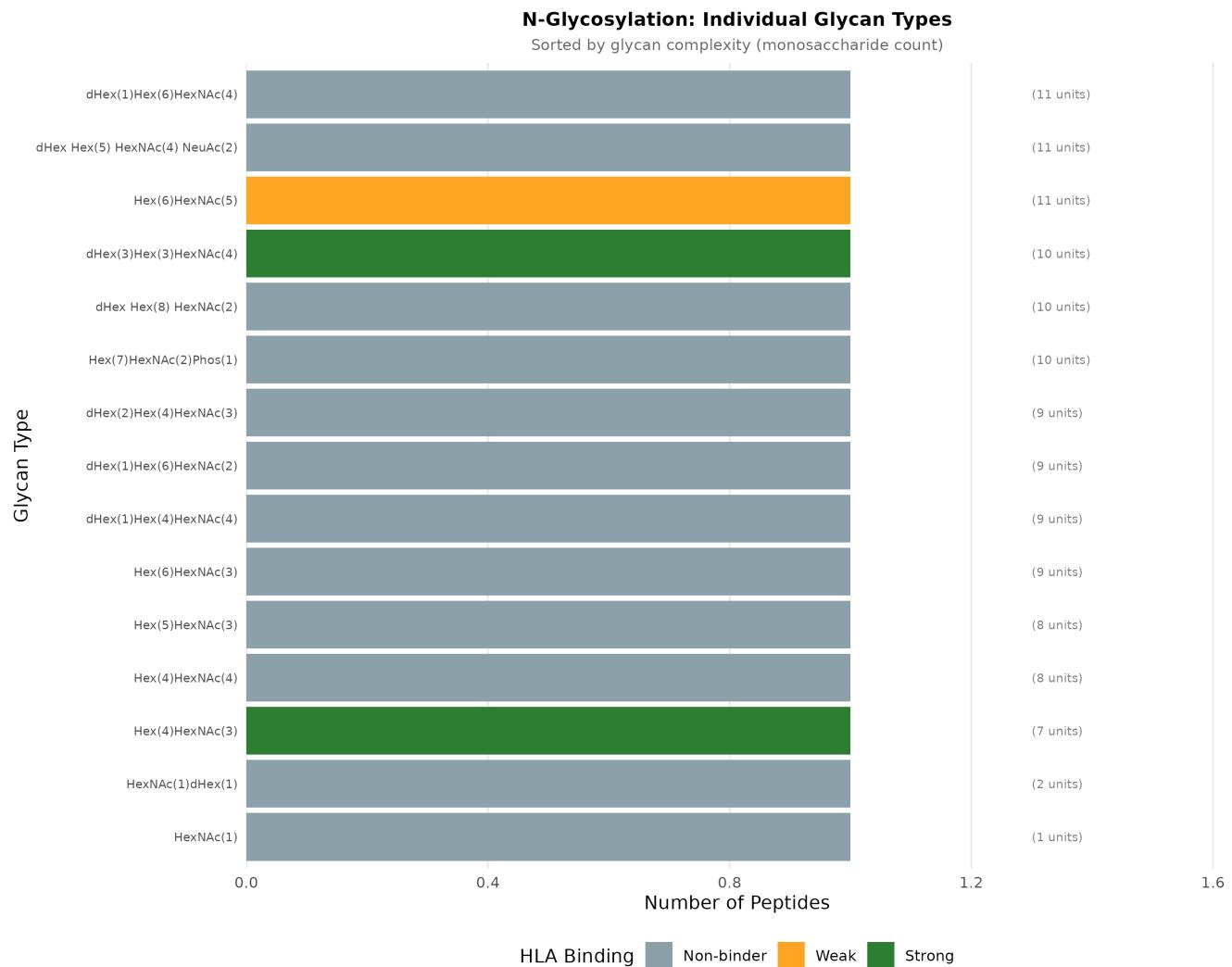
N-Glycosylation: Glycan Complexity vs HLA Binding

Complexity = sum of monosaccharide units | Peptides length 8-14



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



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

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