

# FoxWare<sup>®</sup> Protein Footprinting Software Provides Unique Features to Accurately Track Changes in Amino Acid Solvent Accessibility

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## Introduction

Hydroxyl Radical Protein Footprinting (HRPF) can be analyzed using FoxWare Data Processing Software to map binding interactions between proteins and antibodies. Changes in residue-level oxidation of TNF $\alpha$  between bound (to Adalimumab) and unbound states are calculated based on XIC-level differences validated via 100–400 PSMs per individual peptide. New innovations in the software tool allow automation of residue-level changes with confidence metrics that inform upon spectral quality.

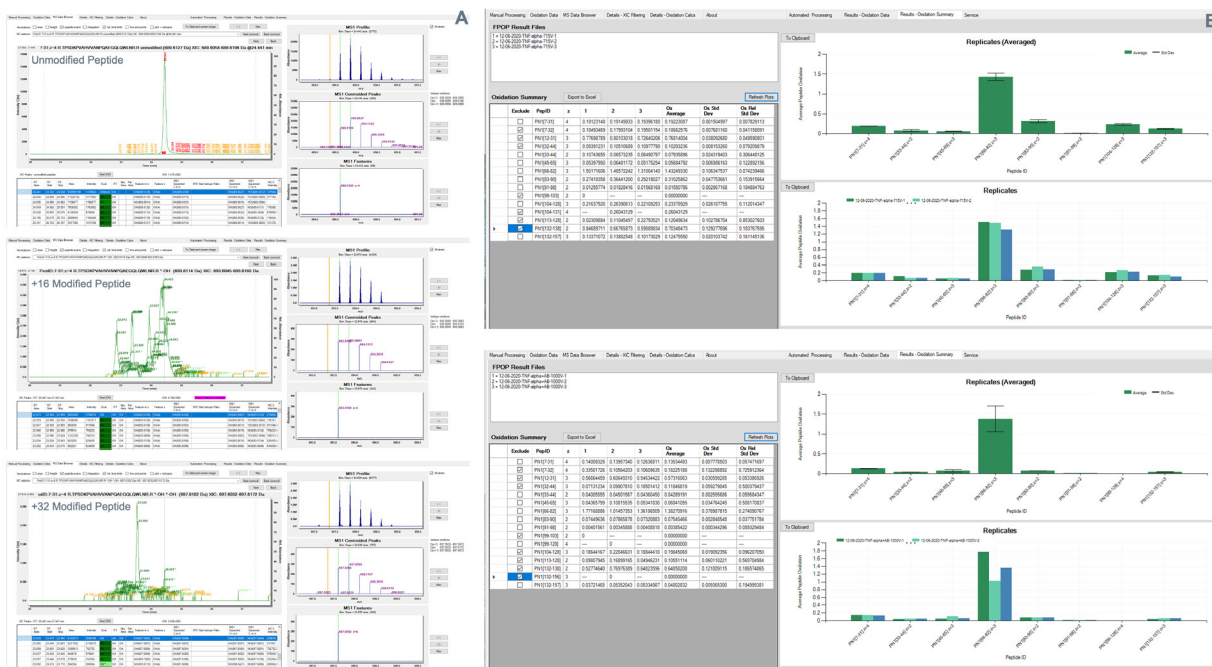
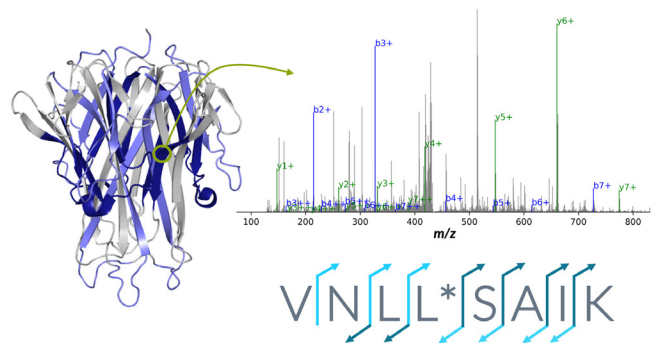
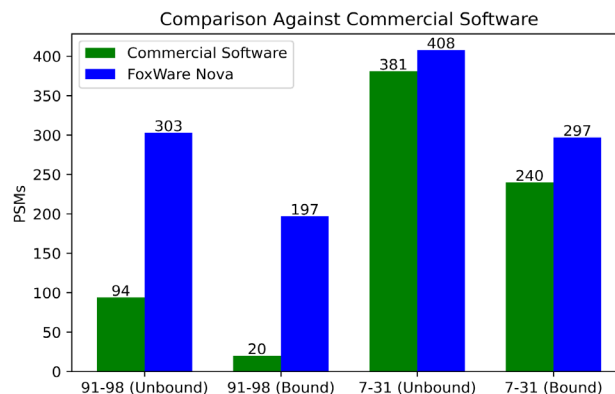


Figure 1: (A) FoxWare Data Processing Software provides an interface in which users can view and validate both unoxidized PSMs as well as PSMs with one or more modifications. (B) Software can automate relative quantitation among any number of replicates, providing average peptide oxidation as well as individual replicate comparisons.

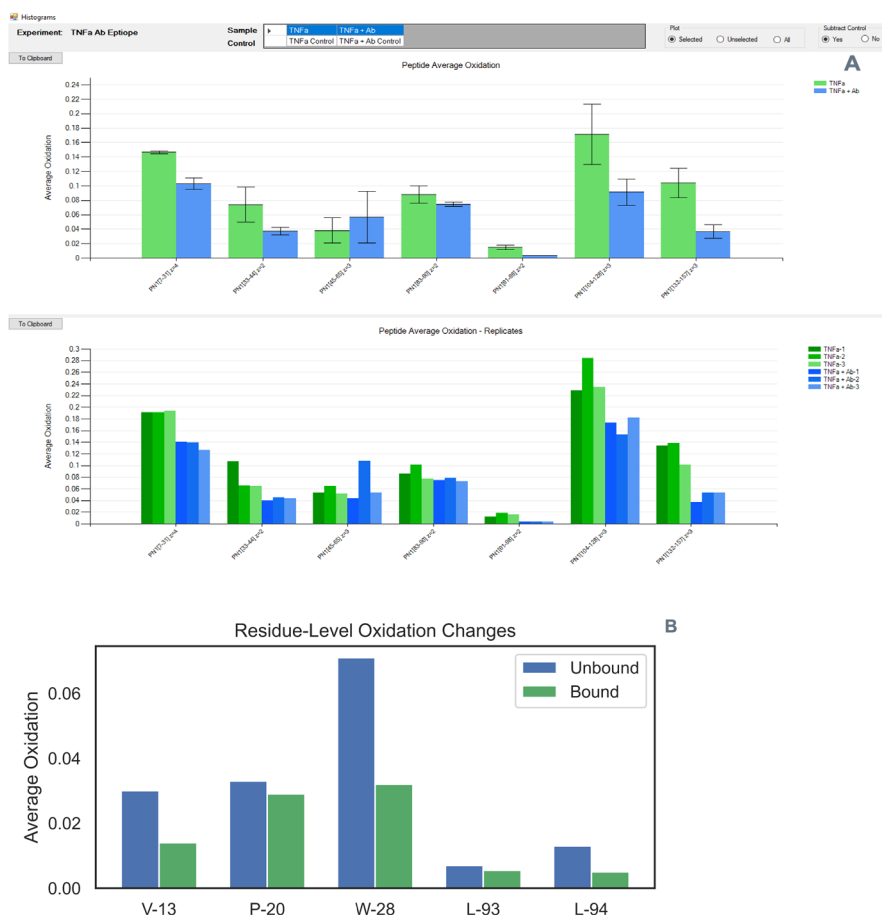
## FoxWare Software Performance for Residue-Level PSMs

The recently developed FoxWare Nova<sup>™</sup> Software utilizes a novel algorithm specifically targeting residue-level oxidation indicators in tandem mass spectra, resulting in substantially greater number of residue-level PSMs compared to commercially available software with increased confidence.

*Figure 2: FoxWare Software is designed to improve residue-level oxidation discovery in tandem mass spectra without any additional post-processing of raw data files. Compared to commercially available software, PSMs discovery improves an average of 20% for larger peptides (>10 amino acids) and >300% for shorter sequence peptides (5-8 amino acids).*



## Oxidation Shifts in Bound Versus Unbound TNFa



*Figure 3: PSMs detected by the FoxWare Software are reported for individual proteins and their respective peptides. FoxWare Software allows for comparison of oxidation states between bound and unbound TNFa directly. (A) Peptide-level oxidation changes are reported in the FoxWare Software user interface at the individual replicate level and overall average oxidation. (B) When data permits (such as when utilizing a targeted inclusion list), residue-level changes are also able to be determined using the FoxWare Nova. Findings on site-specific changes in oxidation are supported from previous X-ray crystallography results.*

## Automated Discovery of Residue-Level Oxidation Changes

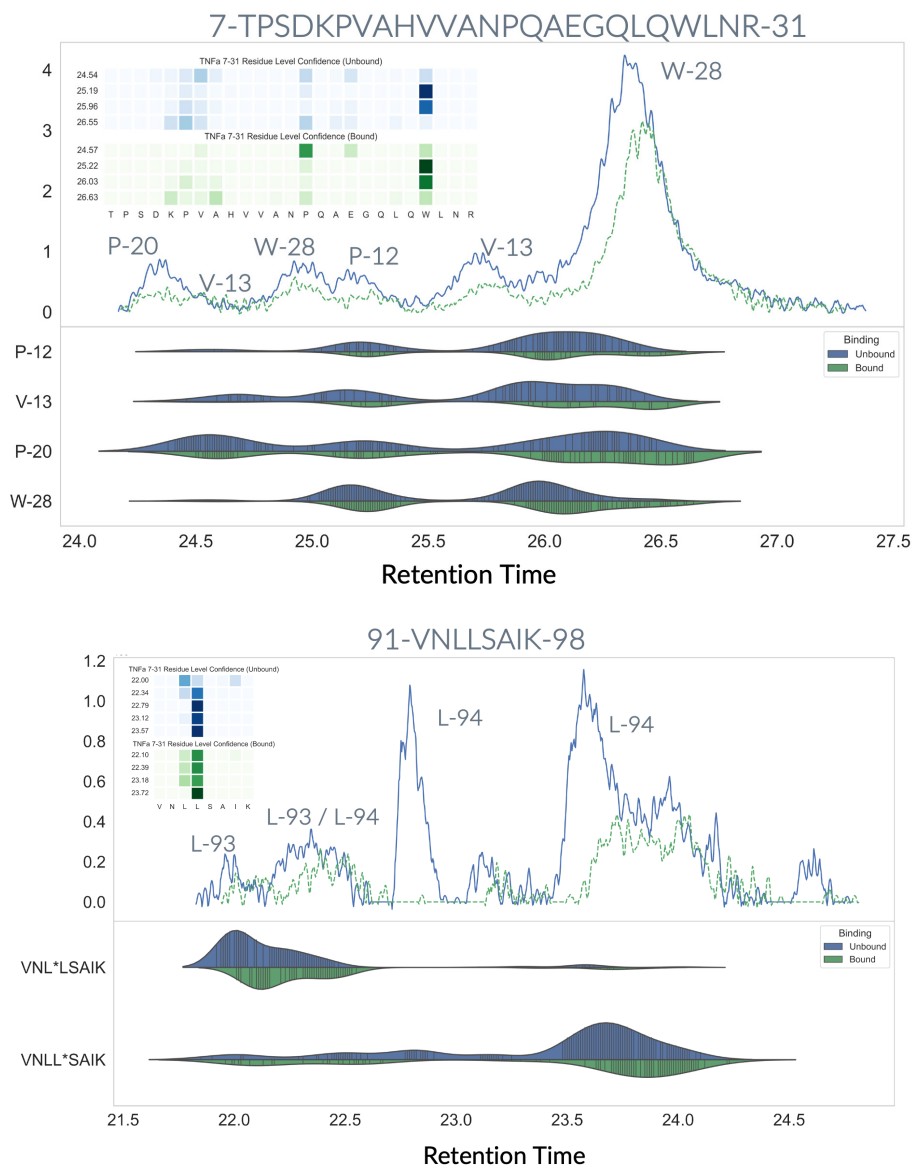


Figure 4: Comparative XICs and respective violin histograms showing changes in residue-level oxidation between bound and unbound TNFα peptides (AA7-31 and AA91-98). Only the highest confidence-level results are shown. (Insets) Heatmaps of confidence estimates of all potential oxidation sites based on the tandem mass spectra. Spectral assignment and validation of PSMs is automated in FoxWare Nova Software.

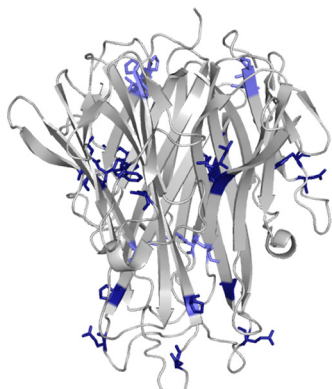


Figure 5: TNFα 3D structure highlighting major sites of oxidation that decreased significantly upon binding to Adalimumab according to analysis using FoxWare Nova Software. All residues fall within the expected binding domain of the TNFα epitope.

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## Conclusions

- FoxWare Software offers automated assignment and relative quantitation of residue-level changes in protein-binding interactions.
- Use of an inclusion list further enhances the ability to elucidate residue-level changes in response to protein-binding activity.



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FoxWare Software accurately interprets results from Hydroxyl Radical Protein Footprinting (HRPF) experiments for qualitative and quantitative comparative studies of Higher Order Structure (HOS).

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