Improving TNFα's Epitope Identification with Residue Level Hydroxyl Radical Protein Footprinting using the ZenoTOF 7600 system

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HRPF Introduction

The Higher Order Structure (HOS) of a protein plays a critical role in drug stability, safety and biological function. Incorrect HOS or incorrect protein interactions are linked to adverse drug reactions which can result in further sickness or death. Advanced techniques are required to address the HOS of proteins robustly. One such method is hydroxyl radical protein footprinting (HRPF). The Fox® Protein Footprinting System is a novel Hydroxyl Radical Protein Footprinting (HRPF) method that uses a proprietary flash oxidation lamp to generate hydroxyl radicals (•OH) that irreversibly modify solvent exposed amino acid side chains. As solvent accessibility changes, the •OH modification concordantly changes.

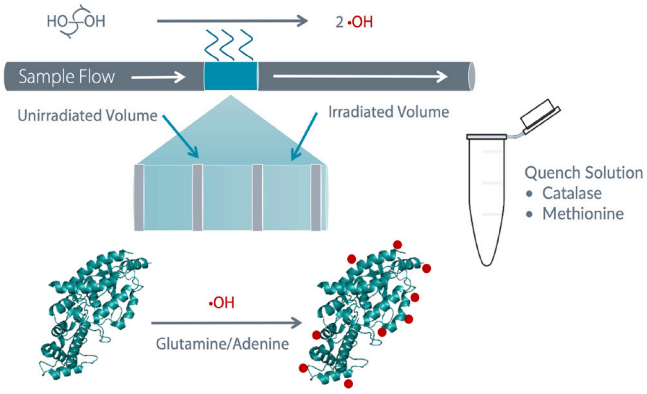


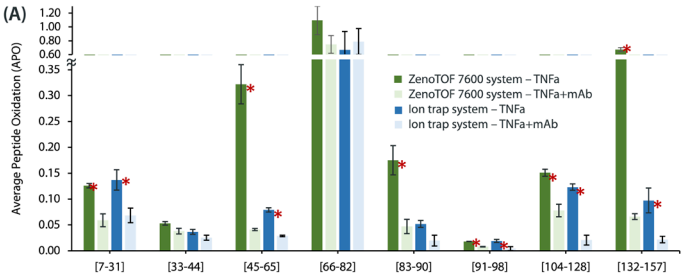
Figure 1: Schematic of an HRPF method, fast photochemical oxidation of proteins (FPOP). With FPOP, protein is mixed with hydrogen peroxide and flown past a pulsing light source which photolyzes the hydrogen peroxide into two •OH and modifies solvent exposed amino acids. Following labeling, the sample is deposited into a quench solution of catalase and methionine.

TNF-α Epitope Mapping Method

TNF-α is a pro-inflammatory cytokine and Adalimumab is a monoclonal antibody prescribed to treat inflammatory diseases. Using the Fox System coupled to a mass spectrometer (MS) with either Zeno trap or ion trapping, TNFα's epitope against Adalimumab was mapped. First, TNFα was labeled with and without Adalimumab in triplicates. All samples underwent trypsin digestion and split in half for analysis on the Zeno trap or ion trapping MS. Both systems used a reverse phase 300 µm x 150 mm C18 column with a flow rate of 5 µL/min and a 45-minute gradient. After LC-MS/MS, all samples were processed using FoxWare® Software to determine which amino acids were oxidized and to what extent. With the ZenoTOF 7600 system, the localization of oxidation was improved as well as the reproducibility.

Flash Oxidation (Fox) HRPF System

Peptide Level Analysis



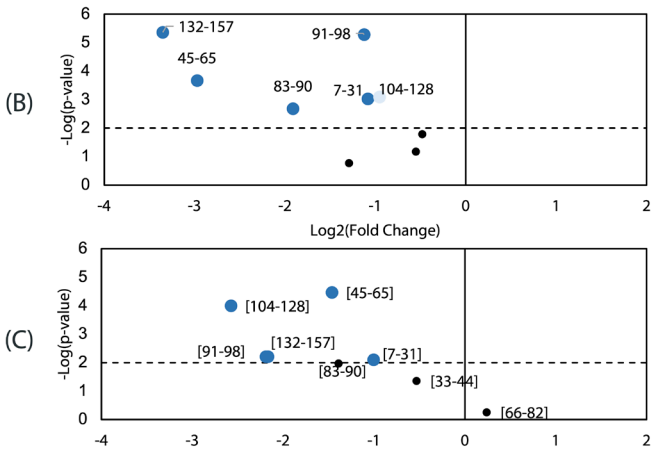
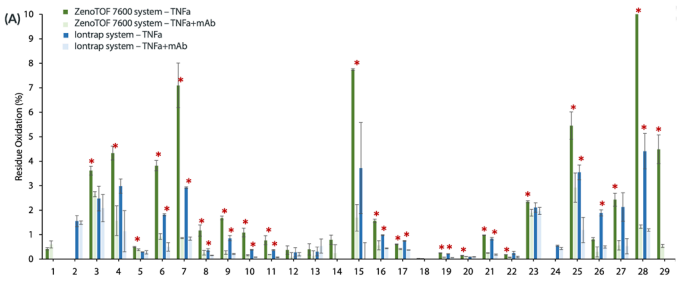
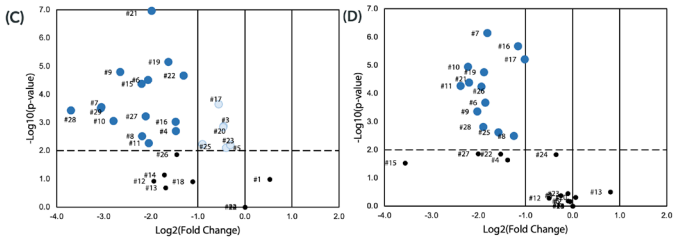


Figure 2: (A) Histogram of oxidized peptides from TNF-a. Peptides with a significant change in oxidation (p-value < 0.01) are marked with red asterisks.The relative standard deviation (RSD) of ZenoTOF 7600) was 3.4%-29% with an average RSD of 11.5%. The RSD range of the Ion trap system was 5%-92% with an average RSD of 26%. (B) Volcano plot of oxidized peptides from TNF-a. The average p-value for ZenoTOF 7600 's average p-value for all peptides was 0.029. The average p-value of Iontrap system was 0.079.

Residue Level Analysis





Decreased <2 Fold

Decreased >2 Fold

(B)

PEAK #	ZenoTOF 7600	IONTRAP
1	18-ANPQA-22	NA
2	NA	20-PQ-21
3	20-PQAEGQ-25	20-PQAEGQ-25
4	W28	W28
5	L29	L29
6	50-VPSEGLY-56	46-NQLVVPSEGLY-56
7	51-PSEGL-55	52-SEGLYLIYSQV-62
8	52-SEGLYLIY-59	58-IYSQV-62
9	Y59	58-IYSQVLF-64
10	59-YSQVLFK-65	62-VL-63
11	64-FK-65	F64
12	I83	I83
13	V85	V85
14	83-IAV-85	NA
15	Y87	Y87
16	88-QTK-90	Q88
17	V91	V91
18	N92	NA
19	L93	93-LL-94
20	97-IK-98	93-LLSAIK-98
21	96-AI-97	93-LLSAI-97
22	94-LSAIK-98	L94
23	104-ET-105	106-PEG-108
24	NA	107-EGAEAKPWYEPIYLGGVFQLEK-128
25	113-PW-114	113-PW-114
26	114-WY-115	114-WYEPI-118
27	141-YL-142	Y141
28	F144	144-FA-145
29	152-FGI-154	NA

Figure 3: (A) Histogram of oxidized residues from TNF-a with and without Adalimumab. Peptides with a significant change in oxidation (p-value < 0.01) is marked with red asterisks. ZenoTOF 7600's relative standard deviation (RSD) for the common peaks range 0.4%-173% with an average RSD of 21%. Iontrapsystem 's RSD range 0.9%-314% with an average RSD of 25%. (B) Peak table with the ZenoTOF 7600's and Iontrap system's residue/sub-peptide localization. Cells colored in blue show a significant change in oxidation after the addition of Adalimumab. The ZenoTOF 7600's average localization is within 2.8 residues while the Iontrap system is within 4. (C) Volcano plot of oxidized residues from TNF-a. ZenoTOF 7600's average p-value for all peptides is 0.025. Iontrap's average p-value is 0.142.

Residue Oxidation Localization (MS/MS Examples)

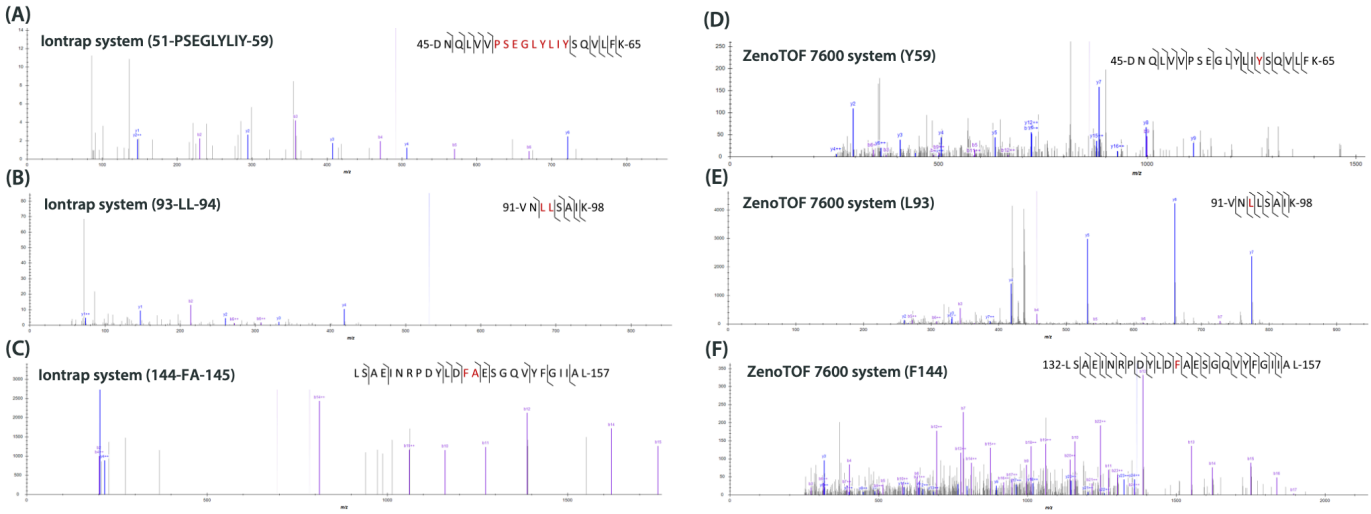


Figure 4: MS/MS spectra examples of labeled peptides. The residue(s) with the localized oxidation is colored red. (A-C) Three peptide examples from the Iontrap system system. (D-F) The same peptide examples from the ZenoTOF 7600 system. The ZenoTOF 7600 system improves ion coverage and thus improves the oxidation localization. An ambiguity score is calculated for each PSM. A higher score coordinates with higher confidence. The ZenoTOF 7600 's average Ambiguity score is 8.8 with 61.6% of the PSMs above 0. The Iontrapsystem's average Ambiguity score is 7.1 with 56.3% of the PSMs above 0. Furthermore, the %PSMs from the ZenoTOF 7600 system with a p-score >10 is 92.2% while the Iontrap system is 85.9%.

HRPF (Peptide and Residue Level) Mapping on Crystal Structure

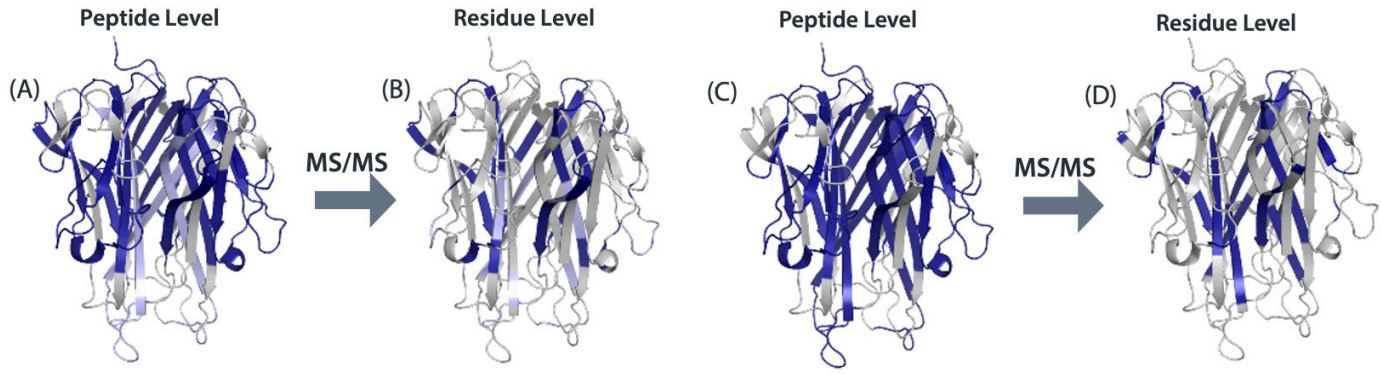


Figure 5: Regions with a significant change of oxidation are decorated on an available crystal structure. Dark blue indicates a decreased fold change >2 after adding Adalimumab and light blue is <2. (A,B) Are the peptides or residues, respectively, that were identified from the ZenoTOF 7600 system with a significant change in oxidation. (C,D) Are the peptides or residues, respectively, that were identified from the Iontrap system with a significant change in oxidation.

Conclusions

- Compared to the Iontrap system, the ZenoTOF 7600 system produces lower RSDs for peptide and residue oxidation with lower p-value when determining regions changing in solvent accessibility.
- The ZenoTOF 7600 system produces PSMs with better p-scores and improved oxidation localization across a chromatographic peak as well as individual PSMs.

GenNext has pioneered a superior, compact, cost-effective, and safe means of performing advanced HRPF analysis.

By replacing expensive, complicated, and hazardous lasers with our proprietary Flash Oxidation System, you can easily perform HRPF with a convenient benchtop instrument.

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