Automated Hydroxyl Radical Protein Footprinting in a 96-Well Format

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HRPF Introduction

The higher order structure (HOS) of a protein plays a critical role in a drug's stability, safety and biological function. Incorrect HOS or protein interactions are linked to adverse drug reactions which can result in further sickness or death.

Advance techniques are required to robustly address the HOS of proteins. One such method is Hydroxyl Radical Protein Footprinting (HRPF). The Fox® Protein Footprinting System is a novel HRPF method that uses a proprietary flash oxidation system to generate hydroxyl radicals (•OH) that irreversibly modify solvent exposed amino acid side chains. As solvent accessibility changes, the •OH modification concordantly changes.

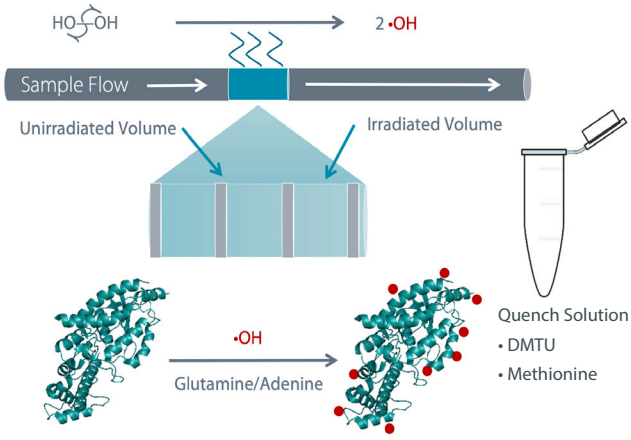


Figure 1: *Schematic of an HRPF method, fast Photochemical Oxidation of Proteins (FPOP). With FPOP, protein is mixed with hydrogen peroxide and flowed passed a pulsing light source which photolyzes the hydrogen peroxide into two •OH and modifies solvent exposed amino acids. Following labeling, the sample is deposited into a quench solution of DTMU and methionine.*

Fully Automated HRPF Labeling using the AutoFox® System

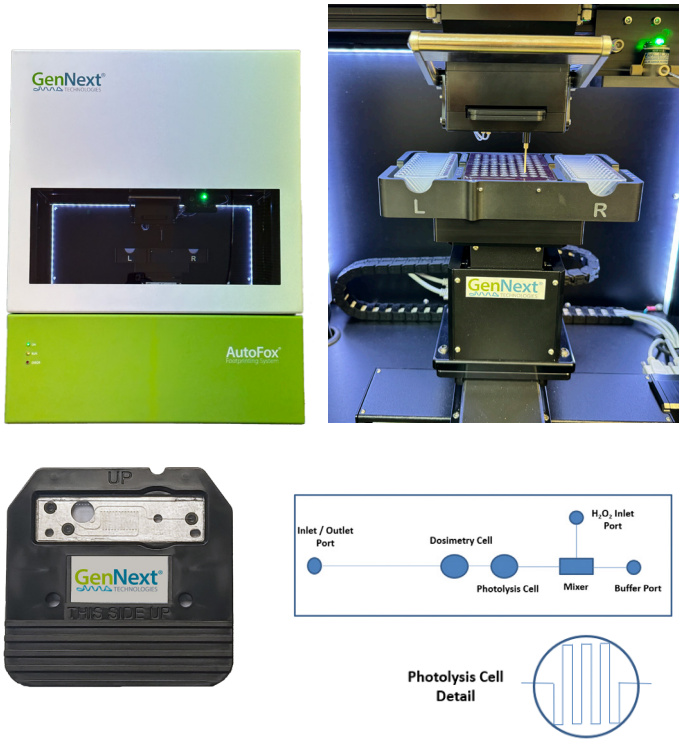


Figure 2: *HRPF labeling is performed in an automated fashion using a 96-well plate in the AutoFox System. Sample delivery, reagent mixing, and flashing are performed through a microfluidic chip designed to maximize irradiated volume. Samples are quenched in an adjacent well prior to LC-MS/MS analysis.*

Unparalleled Reproducibility for Labeling any Protein

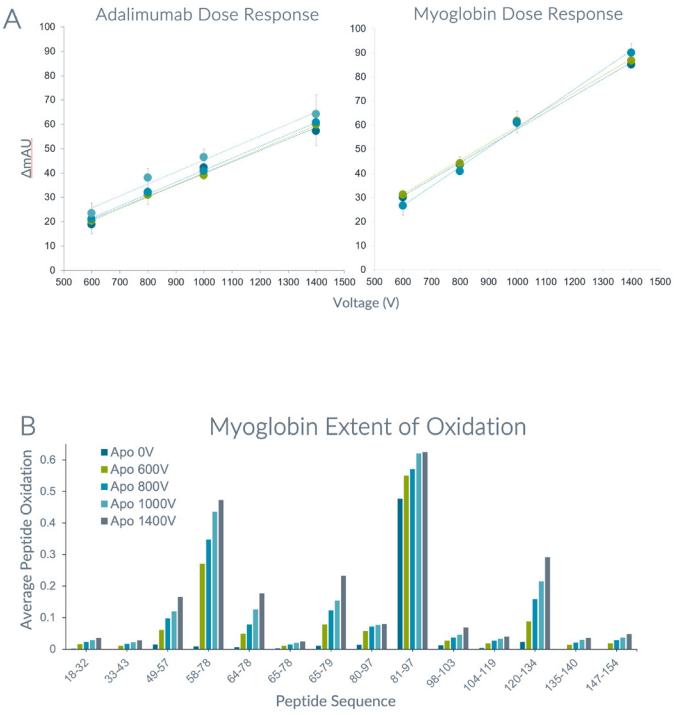


Figure 3: *(A) AutoFox System dose response curves using adenine and target proteins of variable size (adalimumab, 157kDa, and myoglobin, 17kDa). Dose responses demonstrated robust correlation (R2 > 0.95) across a series of 4 biological replicates performed in sets of 3 technical triplicate runs in LC-MS. Each biological replicate was performed on separate days with separate chips by different users. Relative standard deviations (RSDs) from replicates ranged from ~1-11%. (B) Peptide-level dose response of individual replicates for Apo-Myoglobin shows consistent linear response with respect to radical load.*

Conformational Change Analysis of Holo- and Apo-Myoglobin

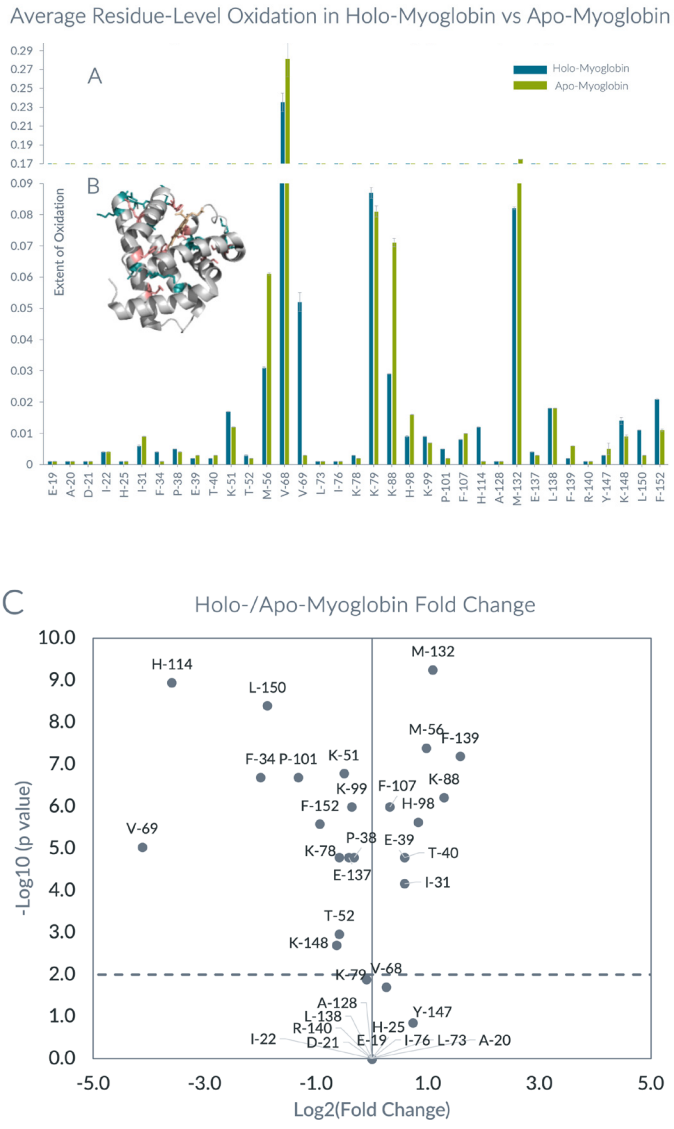


Figure 4: *The AutoFox System is capable of providing residue-level extent of oxidation (A) of a single protein in two different conformational states (e.g. apo- versus holo-myoglobin). The extent of oxidation at the residue level shows on average an ~6% RSD (ranging from 0.51-13%). Results can be mapped onto a 3D model (B). Major changes in solvent accessibility are determined based on fold change analysis (C) that exceed a p-value of 0.01. Results from the AutoFox System match previously published HRPF studies on the same protein and solvent systems.*

Automated TNFα – Adalimumab Epitope Mapping

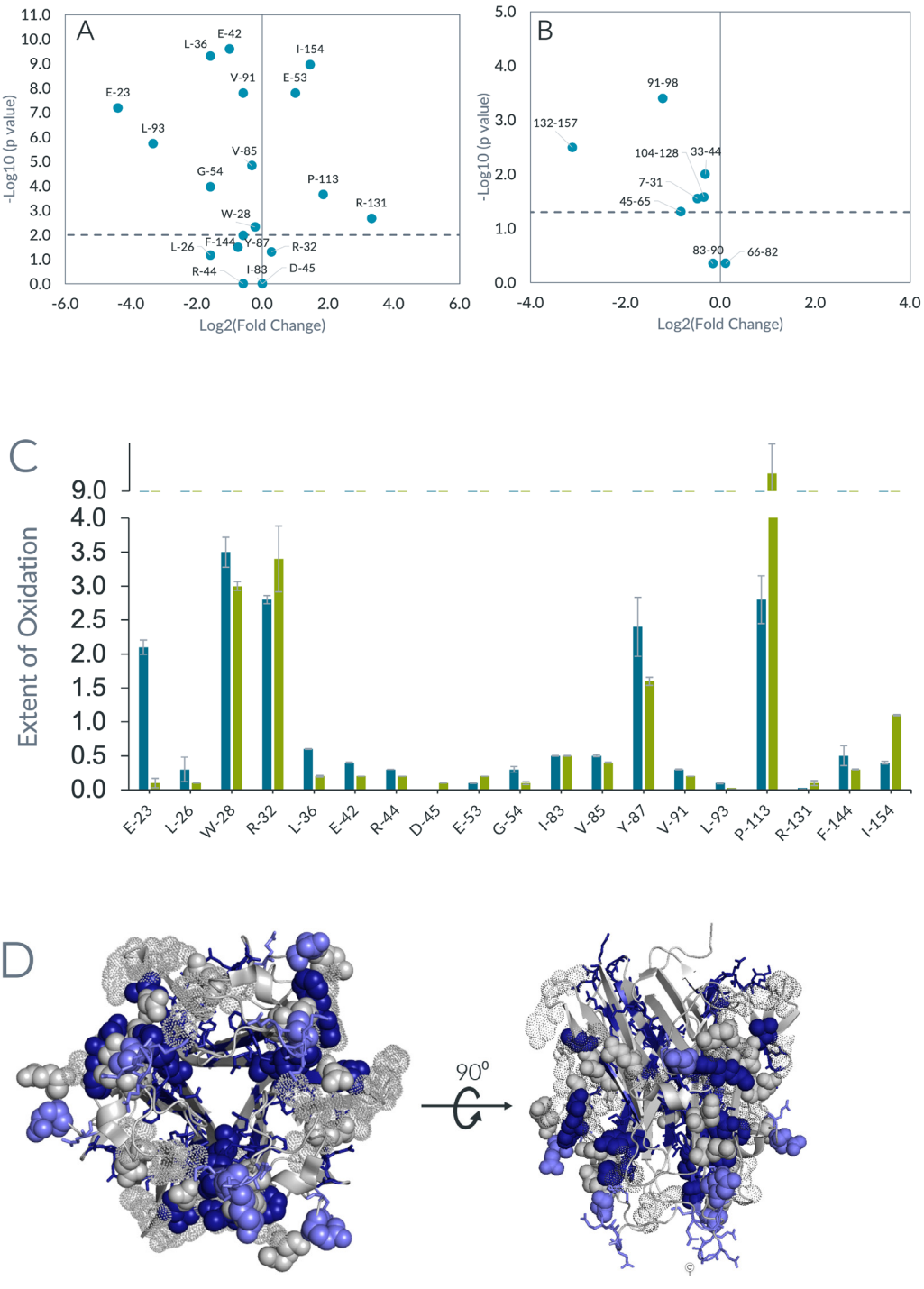


Figure 5:

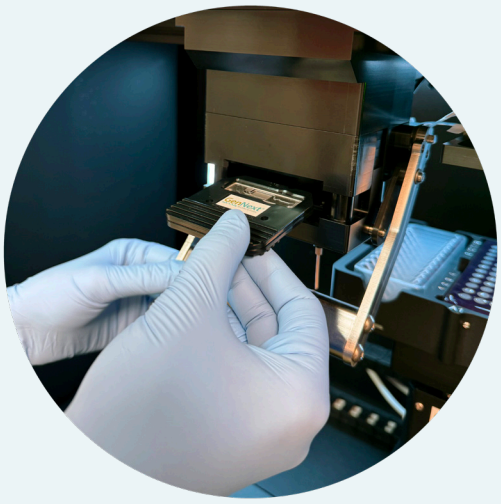
(A) Residue and (B) peptide level fold change analysis of tumor necrosis factor alpha (TNFα) when interacting with adalimumab. The left side of either plot represents residues that were protected upon formation of the complex.

(C) Average extent of oxidation at the residue level for TNFα. Results are determined using FoxWare® Protein Footprinting Software.

(D) 3-dimensional structure of TNFα. 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. Spheres represent substituted residues that resulted in >10-fold decrease in Adalimumab binding and dots indicate other residues involved in Adalimumab – TNFα binding.

Conclusions

- The AutoFox System is a first-in-class instrument designed for fully automated HRPF of proteins. Multiple applications such as conformational studies and paratope-epitope mapping can be done using an automated labeling and data processing workflow.
- Fox Technology provides highly precise and accurate quantification of HRPF results for numerous higher order structure (HOS) mapping experiments. Results are comparable to findings in literature from laser-based techniques (myoglobin) as well as crystal structure (TNFα.)



GenNext has pioneered a fully automated, high resolution 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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