Streamline Mass Spec-Based Structural Biology Experiments with the High-Throughput AutoFox® Protein Footprinting System

Emily E. Chea[1], Zhi Cheng[1], Scot R. Weinberger[1]

1. *GenNext Technologies, Inc., Half Moon Bay, CA*

Fully Automated HRPF Labeling using the AutoFox® System

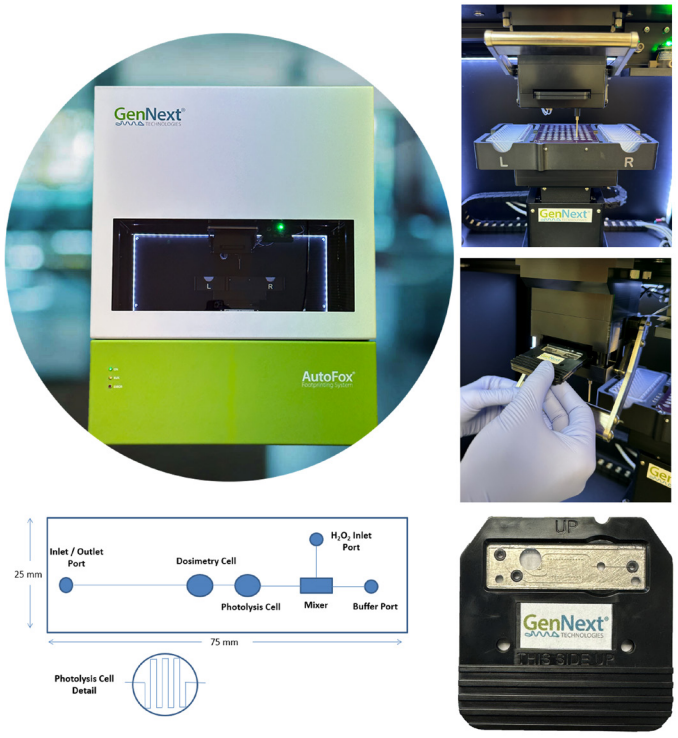


Figure 1: *HRPF labeling is performed in an automated fashion using a 96-well plate in the AutoFox System. Sample delivery, reagent mixing, and flashing are performed through a microfluidic chip designed to maximize irradiated volume. Samples are quenched in an adjacent well prior to LC-MS/MS analysis.*

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 lamp to generate hydroxyl radicals (•OH) that irreversibly modify solvent exposed amino acid side chains. As solvent accessibility changes, the •OH modification concordantly changes.

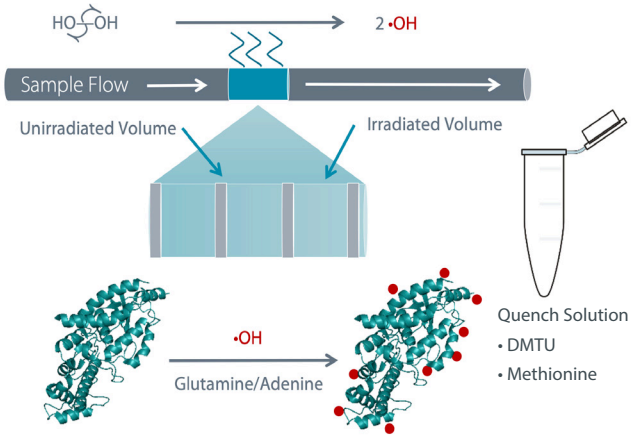


Figure 2: *Schematic of an HRPF method, fast Photochemical Oxidation of Proteins (FPOP). With FPOP, protein is mixed with hydrogen peroxide and flowed passed a pulsing light source which photolyzes the hydrogen peroxide into two •OH and modifies solvent exposed amino acids. Following labeling, the sample is deposited into a quench solution of catalase and methionine.*

Unparalleled Labeling Reproducibility: •OH Dosimetry

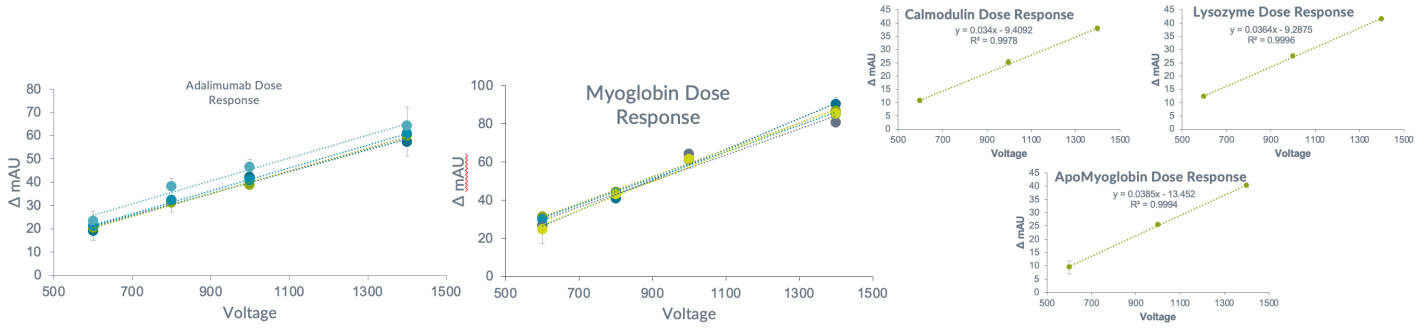
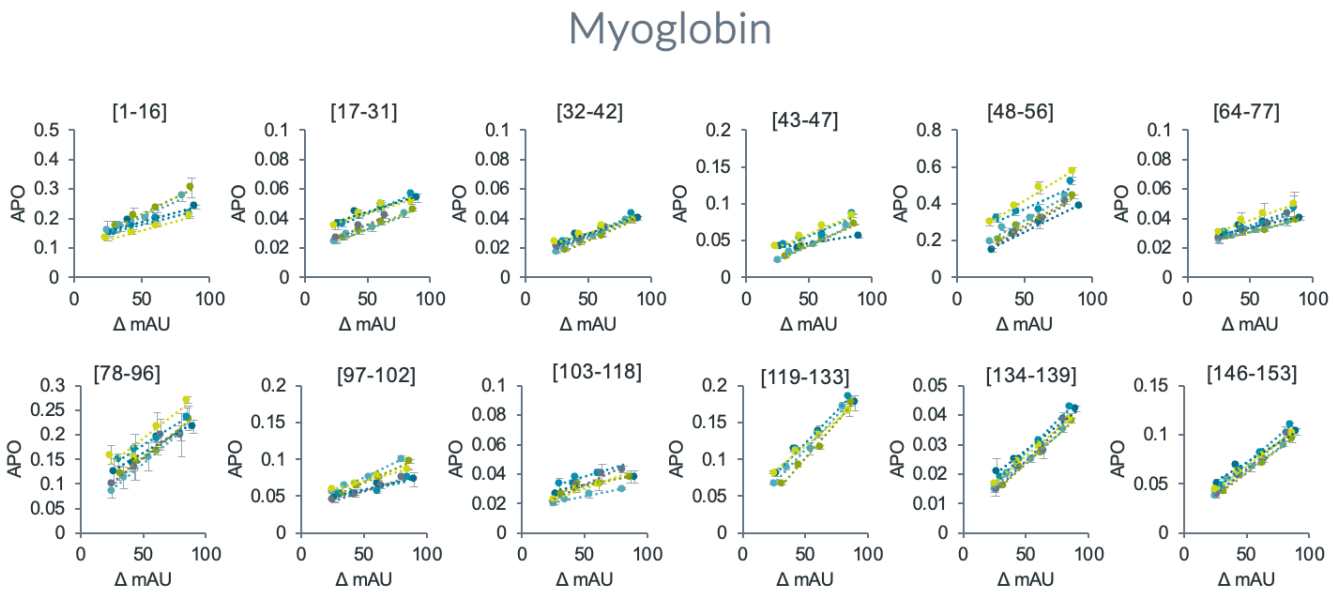


Figure 3: *AutoFox® System dose response curves using adenine and target proteins of variable size (adalimumab: 157kDa, myoglin: 17kDa, Lysozyme: 14kDa, and Calmodulin: 17kDa). Dose responses demonstrated robust correlation (R2 > 0.95) across a series of 4 biological replicates performed in sets of 3 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%.*

Reproducible Protein Dose Response Oxidation Experiments



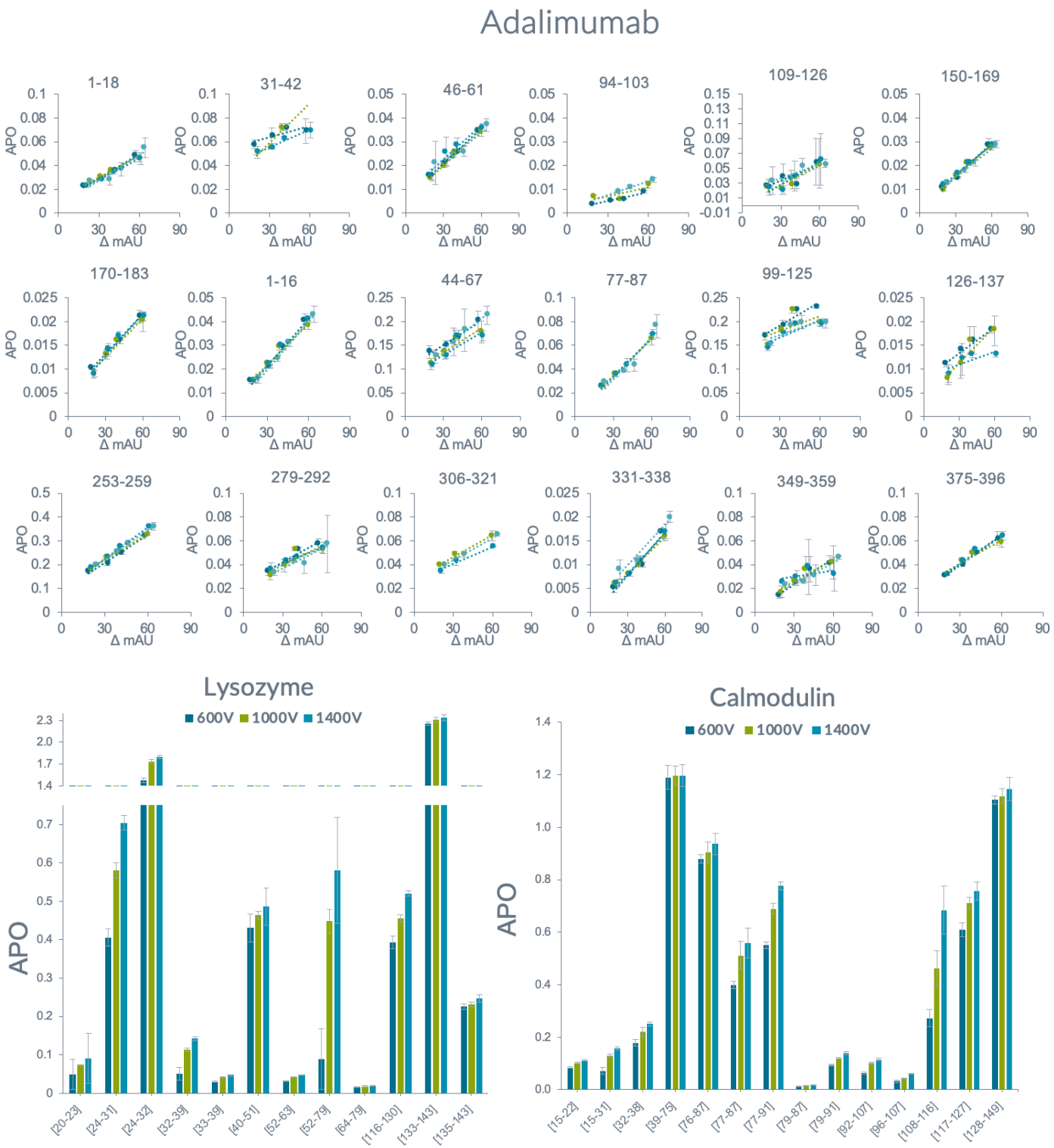


Figure 4: *AutoFox® System protein dose response results demonstrate high reproducibility. Following HRPF labeling by the AutoFox System, samples underwent bottom-up proteomics to detect and quantify the extent of oxidation. All protein samples were exposed to varying OH radical concentrations. As radical concentration increased, the average peptide oxidation (APO) also increased linearly. Myoglobin and adalimumab samples were labeled across four biological replicates, with each set including three technical replicates. No replicate was identified as an outlier, illustrating the robustness and reliability of the AutoFox System.*

Accurate Measurement of Solvent Accessibility: SASA vs LN(PF)

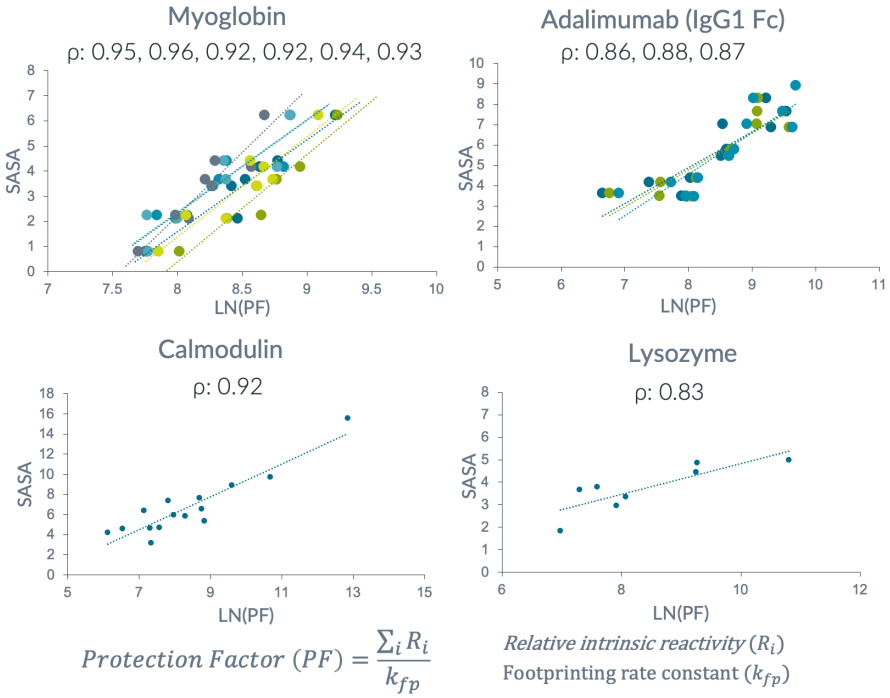


Figure 5: *The AutoFox® System accurately and reliably measures proteins' solvent accessibility. An algorithm developed by Huang et al. (2015)1 converts the measured footprinting rate constant into a protection factor (PF) by considering the intrinsic reactivity of amino acid side chains. The calculated PFs correlate well with known crystal structure solvent accessibility (SASA) data. For each biological replicate across all proteins the SASA and the LN (PF) are compared, and a Pearson correlation coefficient was determined. As the SASA increases, the extent of oxidation (i.e. PF) also increases. In the absence of a crystal structure for adalimumab, the Fc region of an IgG1 was used for comparison. Only peptides that aligned with those from the crystal structure were used in the analysis. Across all proteins and biological replicates, the solvent accessibility data from the AutoFox System show strong quantitative agreement with crystal structure solvent accessibility.*

1. Quantitative Mapping of Protein Structure by Hydroxyl Radical Footprinting-Mediated Structural Mass Spectrometry: A Protection Factor Analysis. Huang, Wei et al. Biophysical Journal, Volume 108, Issue 1, 107 - 115

Conclusions

The AutoFox System represents a major innovation in protein footprinting, providing a significant advancement in mass spectrometry-based structural biology by enabling high-throughput and precise protein footprinting.

The AutoFox System demonstrates versatility, effectively probing solvent accessibility across a wide range of proteins.

The AutoFox System shows high reproducibility and robustness in protein labeling, delivering consistent results across multiple biological replicates and technical triplicates.

The AutoFox System provides accurate measurements of solvent accessibility, as indicated by strong correlations between the calculated protection factors (PF) and known crystal structure solvent accessibility (SASA).