



GenNext® TECHNOLOGIES

## AutoFox® Footprinting System

# The Next-Generation of Protein Structural Analysis

**The AutoFox Footprinting System, powered by GenNext's patented Flash Oxidation (Fox®) Technology, performs Radical Protein Footprinting (RPF) in a simplified, automated workflow to study protein higher order structure (HOS), dynamics, and interactions under native conditions.**

**With minimal sample requirements, unmatched throughput, and real-time radical generation control, GenNext's platform offers biopharmaceutical researchers the optimal blend of precision, speed, and practicality.**

**The system delivers accurate, high-resolution structural insights within days—eliminating the complexity and high costs of traditional methods—giving researchers a cutting-edge yet accessible solution for faster, safer, and smarter biotherapeutic development.**

# Flash Oxidation (Fox®) Protein Footprinting Workflow

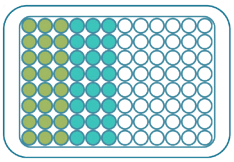
## *From Sample to Results in Days*

**GenNext delivers a fully automated means of performing Radical Protein Footprinting (RPF) via our proprietary Flash Oxidation (Fox) technology. The method uses hydroxyl radicals (•OH) to examine the solvent accessibility of amino acid side chains within a protein. By selectively abstracting hydrogen atoms from exposed amino acids, •OH radicals form oxidation products that can be analyzed for vital insights into protein structure and dynamics.**

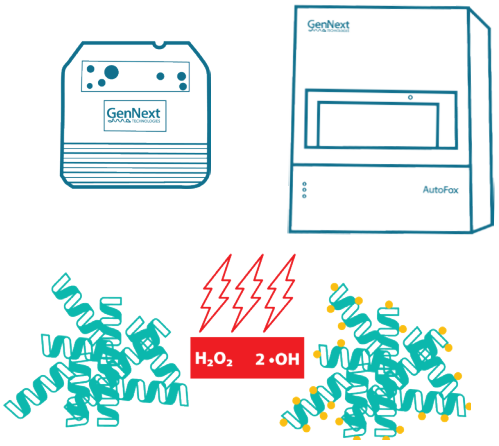
**Our approach to Higher Order Structure (HOS) studies enables in-solution structural and interaction analysis on a wide range of protein sizes, conformational states, and concentrations. The AutoFox System generates actionable data related to a protein's structure, stability, and interaction for insights into biological function and therapeutic efficacy/safety. Only AutoFox Footprinting can validate AI-driven drug discovery models in just days or weeks—accelerating the path to breakthrough therapies.**

### *1. Sample Introduction*

For comparative structural studies, researchers load two different protein samples (e.g., an antigen and its antibody complex) into a 96-well plate, which is then placed on the AutoFox System's sample deck.



### *2. Radical Generation, Labeling, and Quenching*

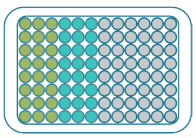



The AutoFox System automatically transfers each sample to the Optofluidic chip where the protein sample is mixed with labeling reagents, including adenine.

The mixture of proteins and labeling reagents is exposed to intense light pulses from a high-pressure flash lamp, which photolyzes the labeling reagent, generating reactive radicals capable of modifying proteins.

GenNext's proprietary inline radical dosimeter monitors and adjusts the flash lamp's intensity in real time to ensure optimal and reproducible labeling conditions. This adjustment accounts for sample variability—such as free proteins versus complexes, protein concentration, ligand presence, or changes in buffer components.

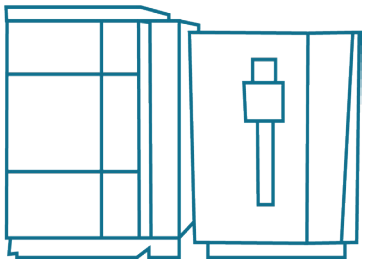
The dosimeter measures changes in adenine absorbance to determine the effective radical concentration. Within microseconds, these radicals covalently modify the protein solvent-accessible amino acid side chains. This irreversible labeling leads to a measurable mass shift in the modified peptides, reflecting changes in protein conformation and solvent accessibility.



Immediately after labeling, the modified protein sample is deposited into a 96-well plate containing a quench solution (typically methionine amide and N,N′-Dimethylthiourea) to arrest any further radical activity, preserving nascent structural information.

### *3. Proteolytic Digestion & LC-MS/MS*

The labeled proteins are proteolytically digested into peptides, which are then analyzed via liquid chromatography-tandem mass spectrometry (LC-MS/MS). This analysis identifies and quantifies site-specific modifications for comparison of solvent accessibility and conformational changes between the protein samples.



### *4. Analysis*

The data generated from this workflow is examined using LC-MS/MS software along with FoxWare® Software, developed by GenNext for evaluation of protein HOS studies. Results from this streamlined, yet advanced structural biology workflow enables researchers to elucidate structural change, mechanisms of action, ligand binding, and conformational dynamics—insights that are essential for optimizing the stability, efficacy, and safety of biologics.

## *Key Advantages of the AutoFox® System*

### *Reusable Optofluidic Chip*

- Performs on-board reagent mixing, photolysis, and dosimetry measurements.
- Labels up to 30 µL of samples in ten seconds.
- Integrated dosimetry cell measures real-time effective radical load for robust and reproducible results even with varying background scavenging.

### *Automated Robotics using 96-Well Microplate*

- AutoFox sample deck is driven by a custom robot that moves solutions through the optofluidic chip using a 96-well plate.
- Automated, high-throughput capacity labels up to 48 different samples in one run.

### *On-Chip, Real-Time Dosimetry*

- Determines effective radical concentration in real-time.
- Automatically adjusts to varying protein load, introduction of protein ligands, and variation in buffer composition/excipients.

### *FoxWare & Control Software*

- Easy-to-use control software for simple and reliable system programming and operation.
- FoxWare Software's user-friendly interface and built-in analysis tools address RPF workflow, chemical labeling/artifacts, and comparative study requirements.
- Intuitive algorithms analyze and report on qualitative and quantitative comparative studies.

# From Uncertainty to Insights in Days

## *For Advanced & Accelerated Biopharmaceutical Development*

### *Excellent Structural Resolution*

The AutoFox System enables high-resolution structural characterization by quantifying site-specific changes in oxidative labeling, which directly correlate with alterations in solvent accessibility at the amino acid level. This residue-level resolution facilitates detailed mapping of protein-protein and protein-ligand interaction interfaces, as well as conformational dynamics critical to functional analysis.

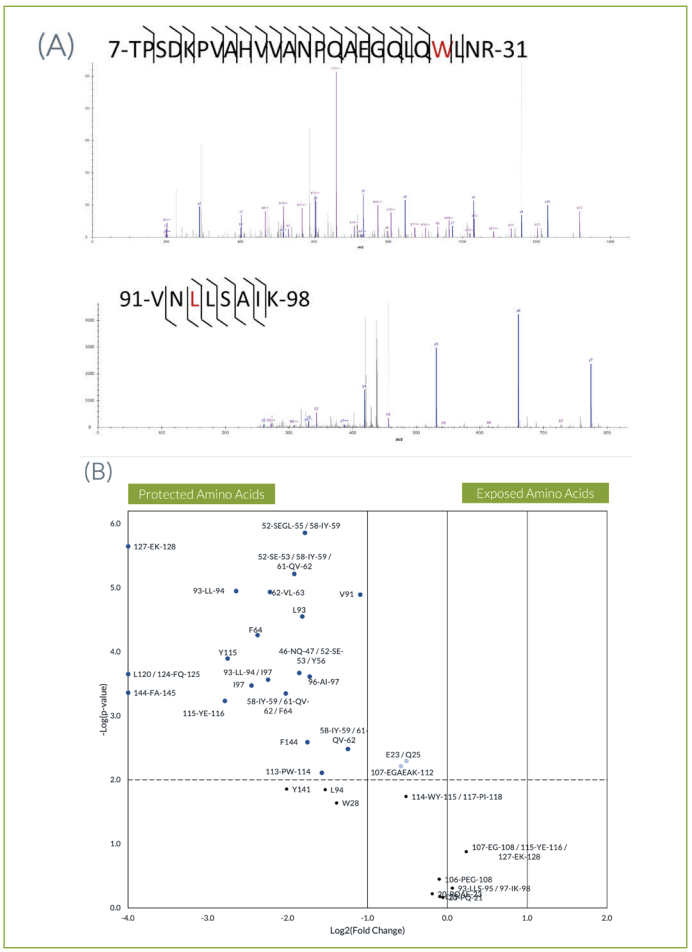



**FIGURE LEGEND.** *(A) MS/MS analysis detects fragment ions with a +16 Da mass shift, allowing FoxWare® Software to localize the site of oxidation at the amino acid level. (B) By quantifying the chromatographic peak areas of oxidized and unoxidized species, FoxWare® Software calculates average residue-level oxidation and generates volcano plots to highlight regions with statistically significant changes—revealing structural perturbations and changes in solvent accessibility.*

### *Highly Reproducible*

Consistent, reliable structural proteomics data is essential for confident decision-making in biopharmaceutical drug discovery and development. The AutoFox System delivers highly reproducible and accurate radical protein footprinting data across time, users, experimental conditions, optofluidic chips, and setups.

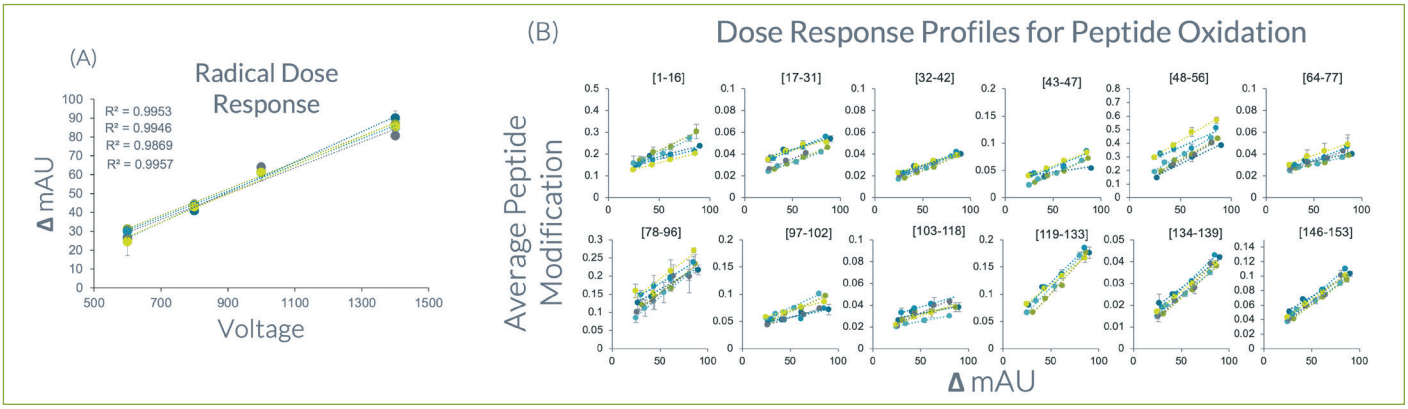
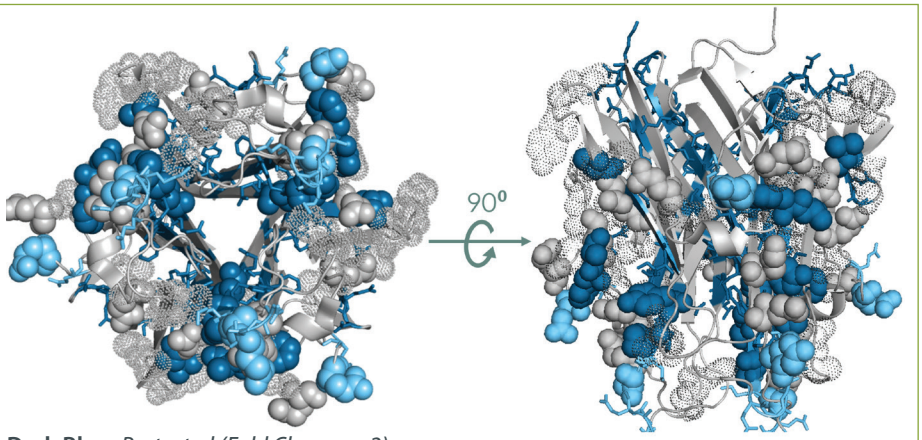



**FIGURE LEGEND.** *(A) Dose-response curves generated by the AutoFox System show strong linear correlation ($R^2 > 0.98$) between hydroxyl radical dose (ΔmAU) and applied voltage, with excellent relative standard deviations (~1–11%) across three technical replicates. Four independent biological replicates—conducted on different days using separate chips and operators—demonstrate the system's robust day-to-day reproducibility. (B) Peptide-level analysis further confirms the consistency of HRPF labeling. Average peptide oxidation (APO) values increase linearly with radical dose across all replicates, with no outliers observed.*

## *High Spatial Resolution*

By delivering precise spatial information on protein structures, the AutoFox® System enables researchers to accurately map protein surfaces and pinpoint regions involved in protein-protein or protein-ligand interactions. Its ability to resolve fine structural details and localize epitopes with high accuracy makes the AutoFox System a powerful and novel tool for biopharmaceutical characterization.




**Dark Blue:** *Protected (Fold Change >2)*
**Light Blue***: Protected (Fold Change <2)*
**Spheres:** *Substituted residues that resulted in >10-fold decrease in Adalimumab binding*
**Dots:** *Other residues IDed but did not decrease Adalimumab binding*

**FIGURE LEGEND.** *AutoFox footprinting results are mapped onto the crystal structure of TNFa, highlighting residues with significant protection upon adalimumab binding. Residues are shaded by fold change in oxidation: darker blue indicates >2-fold protection, lighter blue <2-fold. Known adalimumab-binding residues are shown as spheres (mutations that reduce binding >10-fold) or dots (mutations with minimal binding effect). The majority of protected residues identified correspond to those with the greatest impact on adalimumab binding. Additional protection observed at the trimer core suggests structural condensation upon antibody engagement.*

## *Versatility Across Protein Classes*

Free from limitations related to protein size, type, or structure, the AutoFox System can analyze a wide variety of systems—including protein complexes, cell lysates, and membrane targets—without requiring crystallization, labeling tags, or detergents. Its ability to provide residue-level insights into the higher order structures of diverse biopharmaceutical targets demonstrates the system's utility and versatility in addressing structural proteomics challenges.

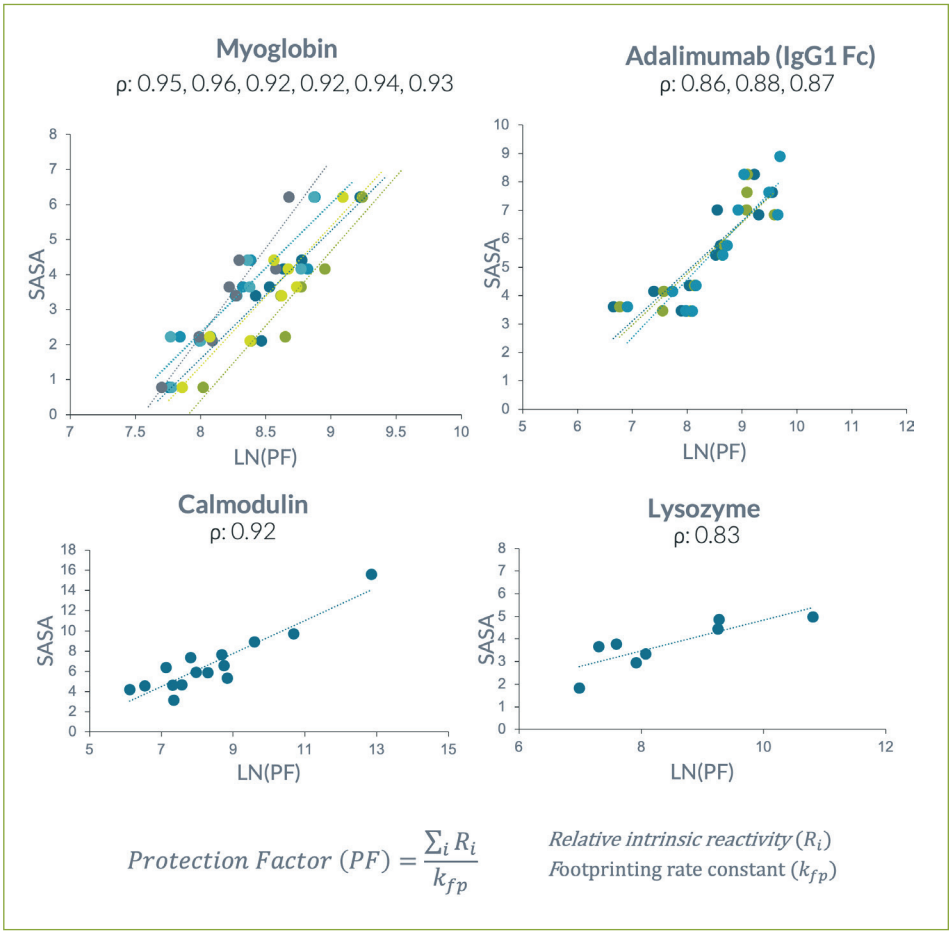



**FIGURE LEGEND.** *Hydroxyl Radical Protein Footprinting rate constants are converted into protection factors (PFs), which show strong correlation with solvent-accessible surface area (SASA) values derived from known protein structures. Across multiple replicates and protein classes, the AutoFox System consistently demonstrates high reproducibility and quantitative agreement with structural solvent accessibility. These results validate its utility for higher order structure confirmation and underscore its versatility in analyzing diverse biopharmaceutical targets.*

# Insights from Biosimilars to AI Model Validation

## *Powerful Tool to Accelerate Drug Discovery & Development*

### *Biotherapeutics & Monoclonal Antibodies (mAbs)*

**First-in-Class Drugs & Biosimilars**

***Interaction & Aggregation Analysis***

The AutoFox® System produces precise epitope and paratope mapping for monoclonal antibodies, providing high-resolution insight into protein-protein interactions which are crucial for optimizing mAb therapeutics. In addition, Fox® Footprinting identifies aggregation-prone regions in mAb formulations, providing data for formulation optimization strategies to mitigate risks and improve stability, efficacy, and immunogenicity.

***Biosimilarity Assessment & Stability Optimization***

This powerful method also provides a relative comparison of reference biologics and biosimilar candidates, detecting structural differences due to manufacturing or formulation variances to ensure comparability and regulatory compliance. Fox Footprinting helps researchers predict degradation pathways, optimize storage conditions, and enhance shelf-life stability by assessing the impact of formulation conditions, temperature, and storage on mAb structural integrity.

***Formulation & Delivery***

Fox Footprinting aids in formulation development of mAb therapeutics by comparing structural changes due to pH shifts, excipients, stabilizers, or process modifications, ensuring batch-to-batch consistency and product quality. Finally, the AutoFox System can monitor mAb stability under various delivery conditions to optimize drug delivery formulations, enabling the development of effective intravenous, subcutaneous, and novel administration strategies without compromising therapeutic integrity.

### *Small Molecule Drug Discovery*

**Allosteric & Orthosteric Therapeutics**

***Target Engagement & Response***

High-throughput primary screening aids in identifying initial hits for allosteric and orthosteric small-molecule therapeutics, while distinguishing high-affinity binders from non-productive interactions remains a significant challenge. The AutoFox Protein Footprinting platform enhances secondary screening by identifying therapeutic binding sites and characterizing target response, ensuring only promising leads advance and eliminating compounds that fail to bind or induce desired effects.

***Lead Optimization***

The chemical modifications that are introduced during lead optimization to enhance specificity and binding affinity can be analyzed by the AutoFox System to confirm the retention of productive target engagement and response.

## *Artificial Intelligence & Computational Biology*

### Bridging the Gap Between AI Models & Experimental Validation

***AI Drug Design Validation***

Although AI generates structural predictions at an unprecedented scale, these models fail to accurately predict protein binding sites, target response, allosteric regulation, and protein flexibility. AI predictions offer powerful hypotheses, but empirical validation remains essential to refine and guide machine learning models.

***Conventional Validation Methods***

Traditional structural biology methods, like multidimensional NMR, X-ray crystallography, and cryo-EM, require months or even years to confirm an AI-generated hypotheses. Despite their precision, today's ultra high-resolution structural biology techniques are costly, difficult, time-intensive, and require large amounts of purified material.

***High-Speed, Empirical Validation***

Only the AutoFox® Footprinting platform is capable of rapidly adjudicating AI-generated drug discovery models in just days, bridging a critical gap in computational biology. With its unique ability to map solvent accessibility and detect structural shifts, the AutoFox System quickly validates AI-predicted protein-drug interactions—making it a powerful asset for drug binding confirmation, protein conformation analysis, and structural biology research.

*In just one week, Fox Technology validated an AI-predicted drug binding and target response—results that were confirmed six to eight months later by cryo-EM and X-ray crystallography, proving the unmatched speed and reliability of the AutoFox® System.*

## *Applications of Fox® Technology*

- **Epitope & Paratope Mapping** to precisely define antigen-antibody interactions in mAbs.
- **Computational Biology Validation** to rapidly evaluate AI-generated drug discovery models in days.
- **Aggregation & Stability Studies** to detect conformational changes and optimize formulations.
- **Target Engagement & Drug Binding** to discover binding sites and track allosteric responses.
- **Biosimilarity & Comparability** to confidently assess structural equivalence of biosimilars.

# AutoFox Versus other Modalities

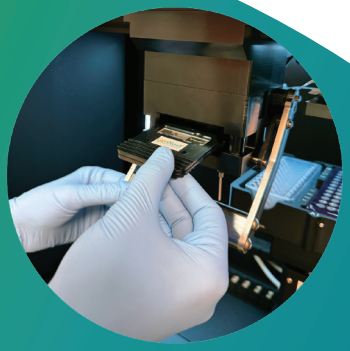
## *Finding the Optimal Balance for Fast, Effective Structural Analysis*

**Biopharmaceutical researchers often face difficult trade-offs between speed, resolution, and cost when selecting structural biology methods.**

**Fox® Footprinting provides the optimal balance—delivering actionable, high-quality structural data quickly and affordably.**

| | AutoFox® System | HDX | Cryo-Em | NMR | X-Ray Crystallography |
|---|---|---|---|---|---|
| Easy to Perform | ✓ | ✗ | ✗ | ✗ | ✗ |
| Rapid Results | ✓ | ✗ | ✗ | ✗ | ✗ |
| Cost-Effective | ✓ | ✓ | ✗ | ✗ | ✗ |
| Flexible/HT Workflow | ✓ | ✗ | ✗ | ✗ | ✗ |
| Residue Level Resolution | ✓ | ✗ | ✓ | ✓ | ✓ |
| Small Sample Quantities | ✓ | ✗ | ✗ | ✗ | ✗ |
| In-Solution Native Conditions | ✓ | ✓ | ✗ | ✗ | ✗ |
| No Protein Size Limitation | ✓ | ✓ | ✗ | ✗ | ✗ |
| Intrinsically Disordered Domains Compatibility | ✓ | ✓ | ✗ | ✗ | ✗ |
| Cell Lysates Compatibility | ✓ | ✗ | ✗ | ✗ | ✗ |
| Glycoprotein Compatibility | ✓ | ✗ | ✗ | ✗ | ✗ |





# Accelerate Discovery, Empower Precision

**Join the next generation of biopharmaceutical discovery and development with the unmatched speed, resolution, and reproducibility of the AutoFox® System for protein structure and interaction analysis.**

### *Instrument Purchase*

Harness the power of fully automated, chip-based RPF with the AutoFox System. Save time and money, experience a simplified and faster workflow, and produce robust and reproducible HOS data.

### *Service Contract*

Our Research Services offer a way to easily, quickly, and cost-effectively take advantage of Fox® Technology without investing in capital equipment, or as a proof of principle before a system purchase.

**Contact us to learn how our technology can easily fit into your lab's workflow or test-drive the AutoFox System on an outsourced project basis.**

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