



Fox[®] Footprinting: The Next Generation of Protein HOS Analysis

Reinventing the study of protein Higher Order Structure (HOS) through automated Hydroxyl Radical Protein Footprinting (HRPF)

Advantages

- ✓ Direct Assessment of Solvent Accessibility
- ✓ Higher-Order Structure Validation
- ✓ High Spatial Resolution
- ✓ Versatility Across Protein Classes
- ✓ Complementary to Other Techniques

Insights

- ✓ Protein-Protein Interactions
- ✓ Epitope Mapping
- ✓ Protein-Ligand Interactions
- ✓ Protein Folding & Conformational Changes
- ✓ Identifying Regions Impacted by Allostery
- ✓ Glycoprotein Analysis

Fox Protein Footprinting Instrumentation & Software

The Flash Oxidation (Fox®) Protein Footprinting System employs hydroxyl radicals ($\bullet\text{OH}$) to examine the solvent accessibility of amino acid side chains within a protein. By selectively abstracting hydrogen atoms from exposed amino acids, $\bullet\text{OH}$ radicals induce the formation of oxidation products, enabling the detection and analysis of vital information concerning protein structure and dynamics.

Comprised of a user-friendly benchtop instrument coupled with intuitive data analysis FoxWare® Software, the Fox System makes it possible for biopharmaceutical researchers to easily and efficiently unravel the mysteries of protein HOS.

This powerful tool opens new avenues for studying and understanding proteins, ultimately accelerating advancements in biopharmaceutical research and development.



Why Fox Protein Footprinting: *What can you investigate & discover with HRPFP?*

The insights gained from studying a diverse range of protein classes and systems with Fox Footprinting have the potential to greatly influence decision-making at every stage of the drug discovery and development journey. Leading to the design and production of safer, more effective, and stable biologic therapeutics.



Unique Advantages of the Fox System

Direct Assessment of Solvent Accessibility

The Fox[®] System directly measures the solvent accessibility of many amino acid side chains within a protein. By reacting hydroxyl radicals ($\cdot\text{OH}$) with amino acid side chains, the Fox System can identify residues that are exposed to the solvent and those that are shielded within the protein structure. This information is critical for understanding protein folding, dynamics, and interactions.

Higher-Order Structure Validation

The Fox System validates the higher-order structure of complex biologics, such as mAbs, by confirming the correct folding and assembly of the protein. By comparing the experimental Fox data with the expected solvent accessibility based on the known protein structure, researchers can assess the structural integrity and accuracy of the higher-order structure of the biologic.

High Spatial Resolution

The Fox System delivers high spatial resolution information about protein structure. It can pinpoint specific amino acids and regions within a protein that are solvent-accessible or buried. This level of detail allows researchers to map protein surfaces and identify regions involved in protein-protein or protein-ligand interactions accurately.



Versatility Across Protein Classes

The Fox System handles a wide range of protein classes, including soluble proteins, membrane proteins, and protein complexes. It is not limited by protein size, type, or structure, allowing researchers to study diverse protein systems using this technique. Able to handle very large and heterogeneous systems, insights generated by the Fox System contribute to a better understanding of their function and communication.

Complementary to Other Techniques

The Fox System complements other structural biology techniques, such as X-ray crystallography, nuclear magnetic resonance (NMR) spectroscopy, Hydrogen/Deuterium eXchange (HDX), and cryo-electron microscopy (cryo-EM). It provides information about solvent accessibility and dynamics in native solution state conditions that may not be readily available from other methods. By combining the Fox HRP method with other techniques, researchers can obtain a more comprehensive understanding of protein structure and function.

Novel Insights Via the Fox System

Protein-Protein Interactions

The Fox[®] System identifies protein-protein interaction sites and interfaces. By measuring how protein-protein interactions change the solvent accessibility of residues at the interaction interface, the Fox System can pinpoint the regions or domains responsible for binding. This information helps researchers understand the structural basis of protein interactions, including those involving mAbs and their target antigens or other interacting proteins.

Epitope Mapping

The Fox System generates valuable information about the solvent accessibility of residues involved in antibody-antigen interactions, and can rapidly and confidently identify changes that occur upon binding. This approach allows for the identification and mapping of epitopes on mAbs, providing insights into the binding interfaces and the regions responsible for antigen recognition. This knowledge is crucial for the design and optimization of therapeutic antibodies and diagnostic reagents.

Protein-Ligand Interaction Characterization

The Fox System uncovers valuable information about protein-ligand interactions. By assessing changes in solvent accessibility upon ligand binding, the Fox System can identify residues involved in the binding site and reveal the conformational changes induced by the ligand. This information aids in understanding the binding mechanism and guiding drug discovery efforts.



Protein Folding & Conformational Changes

The Fox System detects conformational changes and dynamics within a protein. By comparing Fox data across different conditions or time points, researchers can identify regions or domains that undergo structural rearrangements, exposing or shielding certain residues. This capability allows for the study of conformational dynamics, such as ligand-induced conformational changes or structural responses to environmental factors.

Identifying Regions Impacted by Allosteric

The Fox System identifies regions influenced by allostery which are critical for understanding how proteins transmit signals and undergo functional changes. By uncovering the regions impacted by allostery, the Fox-based HRP method helps elucidate the underlying mechanisms of protein function and assists in drug discovery efforts targeting allosteric sites.

Glycoprotein Analysis

The Fox System reveals the solvent accessibility of glycoproteins. Glycans shield certain amino acids from oxidation, allowing researchers to understand glycan-protein interactions. Additionally, the approach helps analyze glycoprotein conformational changes and glycoprotein-protein interactions under various conditions, providing a more comprehensive understanding of glycoprotein structure and function.

Quickly and easily providing invaluable insights into protein structure and interactions, the Fox Footprinting System ultimately supports researchers in the development and optimization of more advanced and effective biopharmaceutical compounds.

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