

Agilent & GenNext Technology Integration

Accelerating High-Value Decision Making in Biopharmaceutical Research

Advancing structural biology research via protein footprinting combined with ion mobility LC/Q-TOF

The integration of GenNext's AutoFox[®] Protein Footprinting System with Agilent's 6560 Ion Mobility Quadrupole Time-of-Flight (IM-QTOF) LC/MS System presents an unparalleled approach to detailed protein structure analysis and biomolecular interaction studies. This fusion of technology platforms enables biopharmaceutical researchers to achieve deeper insights with greater ease, facilitating high-value decisions faster than ever before.

GenNext AutoFox[®] Protein Footprinting System



Agilent 6560 Ion Mobility LC/Q-TOF



Cutting-Edge Technology Synergy

The advanced workflow starts with Hydroxyl Radical Protein Footprinting (HRPF) by the GenNext AutoFox System, followed by detection and quantification on the Agilent IM-QTOF System, and then finishes with data analysis using GenNext's FoxWare[®] and Agilent's MassHunter IM-MS software.

GenNext's AutoFox Protein Footprinting System with FoxWare Software is trusted for delivering high-resolution protein footprinting data, enabling researchers to gain profound insights into protein structures and conformational changes. This system is highly regarded for its precision, reproducibility, and efficiency in mapping protein-ligand interactions, therapeutic-target interactions, and protein conformations in their native environments.

Agilent's 6560 IM-QTOF LC/MS system, known for its advanced ion mobility separation coupled with high-resolution mass spectrometry, allows for the precise analysis of complex biological samples. Its capability to separate ions based on their shape and charge provides an added dimension of structural information crucial for comprehensive protein analysis.

Ion Mobility and AutoFox Enhance Protein Footprinting

Historically, protein footprinting faced complications from co-eluting peptidomorphs that resulted in chimeric tandem mass spectrometry measurements. The combination of ion mobility-tandem mass spectrometry with AutoFox protein footprinting resolves this issue, as ion mobility deconvolves complex tandem mass spectrometry measurements, providing confident amino acid-level structural resolution.

This technology combination facilitates the collection of more comprehensive and detailed data, deepening the understanding of protein functions and mechanisms through enhanced protein structural resolution.

TECHNOLOGY WORKFLOW

Phase One:

Protein Footprinting using the GenNext AutoFox[®] System and FoxWare Software

In the AutoFox System, the protein of interest is mixed with hydrogen peroxide and then flowed pass 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 DMTU and methionine. After quench, oxidation is detected and then quantified using bottom-up proteomics.

Phase Two:

Detection & Quantification on the Agilent 6560 IM-QTOF System

The Agilent 6560 ion mobility Q-TOF LC/MS system combines chromatography, ion mobility, and mass spectrometry to deliver unmatched separation and selectivity. With its electrodynamic ion funnel technology, the system improves sensitivity while maintaining a low field drift tube design, allowing for precise measurement of collision cross sections (CCS) and the preservation of labile targets. Additionally, the 6560 can uncover structural information, including the separation of in-class isomers, offering insights that traditional LC/MS systems cannot provide.

Phase Three:

Data Analysis using GenNext FoxWare Software and Agilent MassHunter IM-MS Software

The researcher can use FoxWare Software's intuitive algorithms to generate qualitative and quantitative comparative studies of Higher Order Structure (HOS). MassHunter IM-MS software provides a platform for interactive browsing and visualization of data from single LC-IM-MS data files.

TECHNOLOGY ADVANTAGES

The fusion of these two state-of-the-art technologies allows researchers to:

- Achieve Greater Structural Insights**
 The integrated system offers unparalleled resolution in mapping protein structures and interactions, providing deeper insights that are essential for understanding complex biological mechanisms.
- Make Faster, High-Value Decisions**
 By significantly reducing the time required for protein structure and drug-target interaction analysis, researchers can obtain rapid and accurate results, enabling faster decision making in drug discovery and development.
- Gather Comprehensive Data**
 The combination of protein footprinting and ion mobility mass spectrometry delivers a wealth of detailed data, enhancing the ability to make informed decisions.
- Enhance Drug Design**
 Detailed structural insights facilitate the design of more effective and targeted therapeutics, accelerating development pipelines and improving drug efficacy.



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