Agilent Proteomics

ENHANCE YOUR PROTEOMICS RESEARCH

Agilent Technologies
PROTEOMICS SOLUTIONS FOR YOUR APPLICATION

Proteomics is a key tool that can be applied to biological questions across a wide range of disciplines and areas of study. Wherever proteins play a role, proteomics offers the potential for insight that often can be gained no other way.

**Basic and clinical research**
Identify and verify peptide/protein biomarkers that provide fundamental insights into biology.

**Agriculture and crop optimization**
Identify and understand protein pathways to optimize crop development, increase yield, and improve pesticide/herbicide resistance.

**Food analysis**
Identify proteins that correlate with major traits such as food quality, authenticity, immunogenicity, taste, and nutritional value.

**Pharmaceutical discovery**
Identify proteins as markers of drug-induced toxicity during drug discovery and development.

**Biofuels and synthetic biology**
Verify protein synthesis to maximize output of the desired end product.
The field of proteomics has rapidly expanded to cover just about every aspect of protein research from identification and characterization of post-translational modifications, to biomarker discovery and quantitation. Because proteomics analysis presents many distinct challenges, your research goals need to be met by comprehensive, optimized, and practical workflows for accurate and reproducible results.

Proteomics studies can be broadly categorized as:

**Discovery Proteomics**

Identification of proteins without any prior knowledge of what proteins may be present. This is accomplished by searching MS/MS spectra against a protein database. Discovery proteomics can also be used to find differences between sample types, such as control versus treated.

**Targeted Proteomics**

Confirmation of proteins based on prior knowledge, such as literature, genomic studies, or pathway analysis. Targeted proteomics may also involve accurately quantitating proteins of interest. Agilent provides the tools you need for both discovery and targeted proteomics research, with a product portfolio that supports nanoflow and standard-flow HPLC and mass spectrometers that deliver accurate protein identification and quantitation.
The abundance of proteins in a sample of interest can vary by orders of magnitude, and often the most interesting proteins are present at very low levels. Discovery proteomics places particularly high demands on separation, analytical sensitivity, and informatics.

Protein samples are typically analyzed by LC/MS/MS in a data-dependent mode; therefore, a well-designed data-dependent engine (DDE) is an essential feature of the MS instrument. Done well, this approach delivers more high-quality peptide MS/MS spectra, leading to the identification of more peptides and a more complete picture of the proteins present in the sample.

The effectiveness of the data-dependent engine depends on the ion selection algorithm to choose the precursor ions most likely to be peptides and to yield high-quality MS/MS spectra. The challenges for effective peptide ion selection are highlighted in the figure below.

Agilent’s 6550 iFunnel Q-TOF LC/MS System dramatically boosts analytical sensitivity, enhancing the detection of low-level peptides. It also includes an advanced DDE algorithm incorporating all the elements for peptide ion selection, resulting in more confident protein identifications.

The ability of an HPLC to separate peptides can also significantly impact discovery proteomics results. Better separation yields sharper peaks, which simplifies the task of the DDE, leading to more identified proteins.

### Ion selection
- Select ions most likely to produce high quality spectra
- Select monoisotopic ion with peptide-like isotope pattern

### Charge state determination
- Accurately identify charge state
- Preferentially select multiply charged ions

### Precursor purity
- Select primary ions within quadrupole isolation window
- Avoid mixed spectra

### MS/MS time management
- Optimize time spent fragmenting ions

Data-dependent logic used to maximize protein identification.
One way that targeted proteomics differs from discovery proteomics is that it screens for peptides from proteins expected to be present based on prior knowledge, such as literature, genomic studies, or pathway analysis.

Accurate mass quadrupole time-of-flight (Q-TOF) instruments are the tools of choice for screening large numbers of proteins simultaneously. Targeted Q-TOF proteomics acquires peptide MS/MS spectra in a data-independent acquisition (DIA) mode. Peptides from specific proteins of interest are identified by screening acquired peptide MS/MS spectra against a peptide MS/MS library.

Agilent’s data-independent analysis can either use All Ions MS/MS methodology or wide band isolation. All Ions MS/MS does not use quadrupole isolation, and fragments ions in the collision cell with two or more collision energies. Wide band isolation reduces data complexity through isolating and fragmenting a series of narrow mass ranges (~10 Da). These data-independent analysis approaches are only feasible because of the high mass accuracy measurements of the Agilent 6550 iFunnel Q-TOF. Accurate mass peptide MS and MS/MS spectra dramatically reduce incidental false matches when screening a peptide spectral library.

The other approach for targeted proteomics is quantitative analysis of known proteins. The Q-TOF is a very flexible and versatile instrument that can also be used for quantitative proteomics. However, most scientists regularly use triple quadrupole mass spectrometry for routine quantitative analysis because of the superior analytical sensitivity and dynamic range that it delivers.

Regardless of your approach, data is only the beginning—sophisticated analysis software is key to extracting the maximum knowledge from your experimental results.

Data-independent workflows for targeted proteomics.
It is now feasible to use UHPLC for high-throughput, robust and reproducible LC/MS analysis of complex proteomics samples for both discovery and targeted workflows. Agilent’s unique Jet Stream source technology is at the center of this option.

Historically, proteomics researchers struggled to detect low level proteins using conventional flow chromatography. Due to its enhanced analytical sensitivity, nanoflow LC/MS became widely accepted and routinely used; however, the increased analytical sensitivity of this method comes at the cost of reduced robustness, reproducibility, and ease-of-use.

Now, with the innovative Agilent iFunnel technology, which includes the Agilent Jet Stream ion source, researchers can achieve near-nanoflow HPLC analytical sensitivity using conventional-flow HPLC. The Jet Stream Proteomics solution results in higher speed, greater robustness, and better reproducibility for proteomics analysis.

When sample quantity is not an issue, Jet Stream Proteomics offers the best of both worlds—analytical sensitivity and data quality.

Agilent Jet Stream provides a 3–5X increase in signal for peptides compared to electrospray.
When ultimate analytical sensitivity is required, nanoflow LC/MS using very small bore columns and nanoelectrospray (nanoES) provides the best results. Agilent offers a flexible nanoESI source and the Nanodaptor kit to enable nanoflow chromatography using an Agilent 1290 Infinity system.

**Agilent nanoESI**

The flexible, easy-to-configure Agilent G1992A nanoESI source is designed to provide the ultimate flexibility for experienced users. The design easily accommodates columns of different lengths and inner diameters as well as multi-dimensional chromatographic configurations to facilitate optimal performance for a wide variety of samples. The enclosed source both enhances safety and reduces background contamination within the analytical laboratory. The Agilent nanoESI source can be used for offline or online nanoES, and provides easy connections for nanocolumns with integral (pulled tips) or separate emitters.

**Infinity UHPLC Nanodapter**

When sample amount is limited, nanoflow LC/MS is the preferred option. The Agilent Infinity UHPLC Nanodapter converts a 1290 Infinity binary pump to deliver flow rates optimized for nanoflow LC separations. The combination of the Nanodapter with a 1290 Infinity UHPLC pump precisely delivers optimal flow rates from 100 to 900 nL/min for separations using columns with internal diameters from 75 to 150 µm at pressures up to 1300 bar. This solution is compatible with a wide range of nanoLC columns coupled to Agilent’s nanoESI source giving users flexibility to switch between standard flow Agilent Jet Stream and nanoflow LC/MS depending on sample availability.
Agilent provides a comprehensive portfolio of software tools supporting both discovery and targeted proteomics workflows.

**Agilent Spectrum Mill**

Quickly identify proteins and peptides through fast database searches with automatic or manual match confirmation. Advanced algorithms minimize incorrect matches and calculate false discovery rate. It can also be used to create custom libraries for Skyline software (from the MacCoss group at the University of Washington) for targeted proteomics.

**Skyline**

Designed specifically for proteomics researchers, the Skyline (from the MacCoss Group at the University of Washington) interface takes you from protein to peptides to precursors and product ions. Tight integration between Agilent and Skyline delivers a seamless user experience. Skyline can be used for Q-TOF analysis in data-independent acquisition mode for targeted proteomics. Peptides are identified by mining acquired MS/MS spectra against a public or custom peptide MS/MS library.

**Agilent Mass Profiler Professional (MPP)**

A member of the GeneSpring Suite designed specifically for MS data, MPP effectively analyzes and compares proteins or peptides from multiple samples with simple or advanced statistical analysis and visualization tools. MPP facilitates integrated biology analysis by smoothly combining results from genomics, transcriptomics, proteomics, and metabolomics experiments.
The optional MPP Pathway Architect module offers a seamless platform to gain deeper insights into data. It allows users to take the results from single or multi-omics experiments and map them onto canonical biological pathways, concurrently analyzing, visualizing, and interpreting pathway information. This pathway-centric workflow speeds the route from discovery and insight to evaluation, and enables efficient planning and execution of the next series of experiments.

Rapidly extracts peptide or protein signatures from label-free MS-only data and enables automated two-pass batch feature extraction from large complex data sets. Profinder uses information obtained from a large batch of samples to yield reliable extracted features for high-quality statistical analyses. The results of Profinder data processing can be easily transferred to Agilent’s statistical analysis software, Mass Profiler Professional.

Uses output files from one or more MS/MS protein search engines (including Spectrum Mill) to evaluate, organize, and interpret mass spectrometry search results. Scaffold software (from Proteome Software) allows the user to more easily manage large amounts of data and compare samples or results from multiple search engines.
**Chromatographic separation**

**The Agilent 1290 Infinity II System**
Offers the industry’s best chromatographic performance and flexibility. Taking full advantage of modern sub-2 μm packing material, the 1290 enables outstanding chromatographic separation of complex protein samples. Together with Agilent Jet Stream technology, it delivers fast, reproducible, and sensitive protein results.

**Agilent Infinity UHPLC Nanodapter**
Switching between standard flow and nanoflow LC has never been easier using Agilent’s Infinity UHPLC Nanodapter to modify an Agilent 1290 Infinity binary pump to precisely deliver flow rates from 100 to 900 nL/min to provide a robust and easy-to-use nanoflow LC/MS solution for proteomics.

**Sample preparation**

**Agilent AssayMAP Bravo Platform**
Automates protein/peptide purification, protein digestion, and affinity purification workflows. It is designed for ease of use, and systematically increases reproducibility and throughput.

**Agilent Multiple Affinity Removal System**
Designed to effectively remove up to 99% of the top 1–14 high-abundance proteins from human or mouse serum samples. It is available in reusable HPLC and spin-filter formats.
LC/MS analysis

**Agilent 6400 Series Triple Quadrupole LC/MS**

The proven choice for quantitative applications requiring superior analytical sensitivity, reliability, and overall system robustness. The 6495 with iFunnel Technology incorporating the Agilent Jet Stream source dramatically increases ion sampling, which results in lower detection limits. When sample quantity is not limiting, Jet Stream Proteomics offers the best combination of analytical sensitivity, reproducibility and throughput for peptide quantitation.

**Agilent 6500 Series Quadrupole Time-of-Flight LC/MS**

A versatile choice for qualitative or quantitative proteomics. These instruments deliver accurate mass measurements with wide linear dynamic range, enabling simultaneous detection of both high- and low-level peptides in complex mixtures. With Agilent iFunnel technology, the 6550 Q-TOF delivers the lowest detection levels of any accurate mass LC/MS/MS instrument.

Ion sources for proteomics

**Agilent Jet Stream Technology**

Utilizes thermal gradient focusing technology with super-heated nitrogen sheath gas for greater peptide signal with reduced noise. Agilent Jet Stream technology is designed for standard flow UHPLC separation, and improves peptide sensitivity 3–6X when compared to conventional ESI sources.

**Agilent nanoESI Source**

A nanoflow ESI source that provides maximum flexibility for experienced users. The design easily accommodates columns of different lengths and inner diameters, facilitating optimal performance for a wide range of proteomics samples.

LC/MS standards

**MRM PeptiQuant Kits**

Developed by MRM Proteomics and available from Cambridge Isotope Laboratories, these kits are designed to evaluate instrument performance for quantitative plasma proteomics. Kits contain tryptic digested plasma samples spiked with isotope-labeled standard peptides of known concentrations; use them to standardize sample preparation workflow or to evaluate day-to-day instrument performance to ensure that you are obtaining consistent results across multi-day experiments. PeptiQuant kits are also available for quantifying large panels of proteins in experimental samples for biomarker discovery and evaluation.

**Agilent Complex Proteomics Standard Kit**

This kit provides an extract of proteins from Pfu (Pyrococcus furiosus). The standard is used for mass spectrometry based proteomics applications to benchmark or evaluate workflow performance and facilitate cross-experiment, cross-instrument, or cross-laboratory data comparisons.