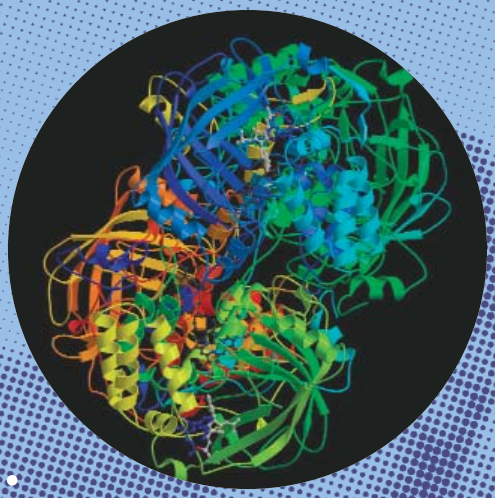


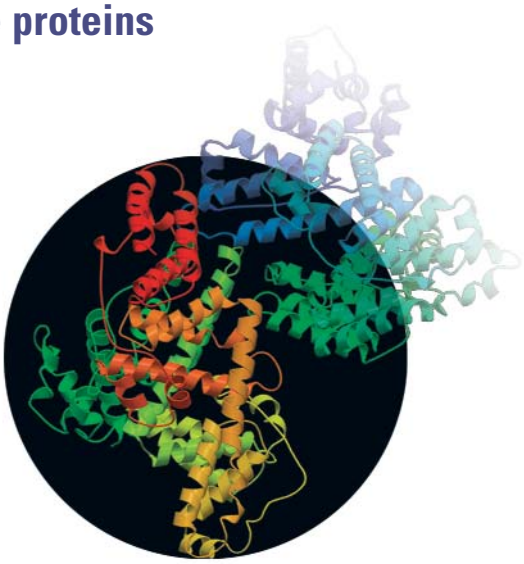
Agilent Nanoflow Proteomics Solution

High-throughput identification of low-abundance proteins

Agilent Technologies

High-throughput identification of low-abundance proteins

LC/MS^n analysis offers one of the most effective approaches to the identification of proteins in complex mixtures. The Agilent Nanoflow Proteomics Solution carefully integrates state-of-the-art nanoflow HPLC and ion trap MS^n to create a high-throughput solution to the separation and identification of low-abundance proteins.



Superior separations...

The nanoflow LC system is supplied with high-efficiency nanocolumns for sample cleanup, concentration, and separation. It can be configured for one-dimensional separation, one-dimensional separation with sample cleanup and concentration, or full two-dimensional separation—whatever is appropriate for your samples.

If analyses include human plasma, serum, or cerebrospinal fluid, the Agilent multiple affinity removal system can be added to the Nanoflow Proteomics Solution. These proprietary columns and buffers can remove 98-99% of albumin, IgG, IgA, haptoglobin, transferrin, and antitrypsin from your samples.

The Nanoflow Proteomics Solution features cutting-edge LC technology. Its revolutionary electronic flow control (EFC) system has active feedback for unprecedented stability and reliability at flow rates from 100 nl/min to 1µl/min. The entire flow path is designed to maximize and maintain separation efficiency.

...ultrasensitive analysis...

Central to the Nanoflow Proteomics Solution is Agilent's newest, most sensitive ion trap mass spectrometer equipped with an improved, second-generation nanospray ion source. The mass spectrometer provides attomole-level sensitivity and a superior combination of scan speed and resolution. Its specialized peptide scan mode enhances analysis of low-abundance proteins.

...and positive identification

The available Spectrum Mill MS proteomics workbench provides multiple options for protein identification, including MS and MS/MS database searching and *de novo* sequencing. Through intelligent spectral extraction and processing, the Spectrum Mill workbench speeds searching and reduces the number of false positive matches. Match scoring based on information content instead of probabilities is independent of the database being searched, making cross-database comparisons possible.

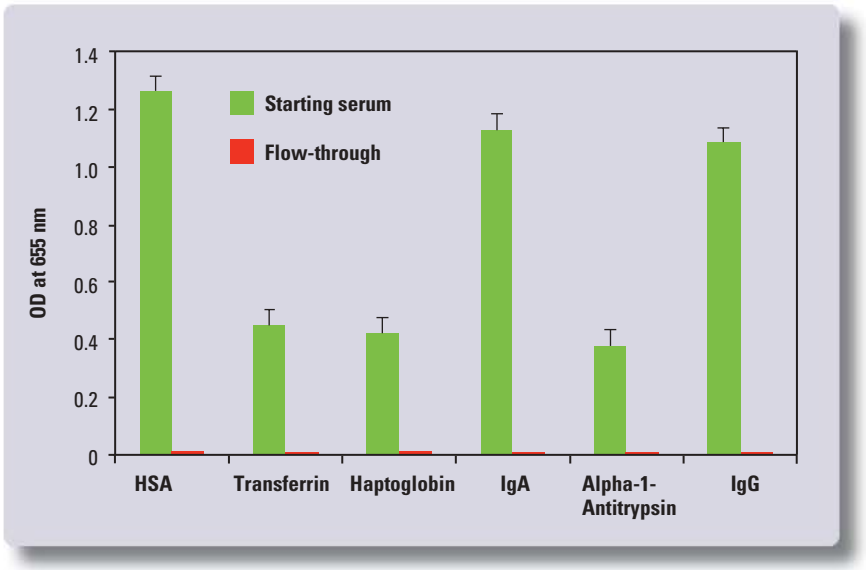
Superior separation technology

Identification of low-abundance proteins requires careful sample preparation and good separations. Agilent has a wide selection of innovative sample preparation and separation technologies carefully matched to the Nanoflow Proteomics Solution.

Immunoaffinity columns remove high-abundance proteins

In biological samples, common high-abundance proteins often obscure the low-abundance proteins of real analytical interest. The Agilent multiple affinity removal system uses immunoaffinity-based LC columns and proprietary, optimized buffers to simultaneously remove 98-99% of six high-abundance proteins (albumin, IgG, IgA, haptoglobin, transferrin, and antitrypsin) from human plasma, serum, or cerebrospinal fluid (CSF), leaving samples almost free of these interferences and ready for further study by LC/MS or gel electrophoresis. The system features:

- High efficiency
- Low non-specific protein binding to minimize loss of important proteins
- Long column life (at least 200 injections under proper conditions)
- Compatibility with almost all LC or HPLC systems



ELISA analysis of a serum sample before and after a single pass through Agilent's immunoaffinity column demonstrates removal of 98–99+% of targeted high-abundance proteins

High-efficiency columns provide optimum separations

Agilent offers analytical and enrichment columns designed specifically for use with the Nanoflow Proteomics Solution. They are ideally suited to high-sensitivity, high-throughput separations of peptide digests.

Analytical columns

The Nanoflow Proteomics Solution includes 75 µm id, 50 or 150 mm, C18 or C8 wide-pore nanocolumns featuring Agilent's famous ZORBAX StableBond (SB) technology. These reversed-phase HPLC columns are excellent for peptide separations and are extremely stable and durable. Features include:

- PEEK-coated fused silica
- Standard 1/16-inch fittings
- Flexibility for direct connection to an MS
- 100 nl/min – 1 µl/min flow rates

The same column is also available in a 100 µm id for applications with slightly higher flow rates.

Enrichment columns

The Nanoflow Proteomics Solution also includes ZORBAX 300SB C18 and C8 enrichment columns (0.3 x 5 mm). The enrichment columns do an excellent job of concentrating and desalting low-concentration peptide mixtures. Also available are ion-exchange columns that can be incorporated into full two-dimensional LC separations.

Unsurpassed separations at sensitivity-enhancing ultralow flows

Stable, ultralow-flow-rate separations are essential for high-sensitivity, high-throughput analyses of complex protein mixtures. The Nanoflow Proteomics Solution includes cutting-edge LC technology that provides ultrastable, nanoliter-per-minute flow rates and outstanding sample cleanup, concentration, and separation.

Active-feedback flow control provides stable nanoliter flows

Ultrastable flows

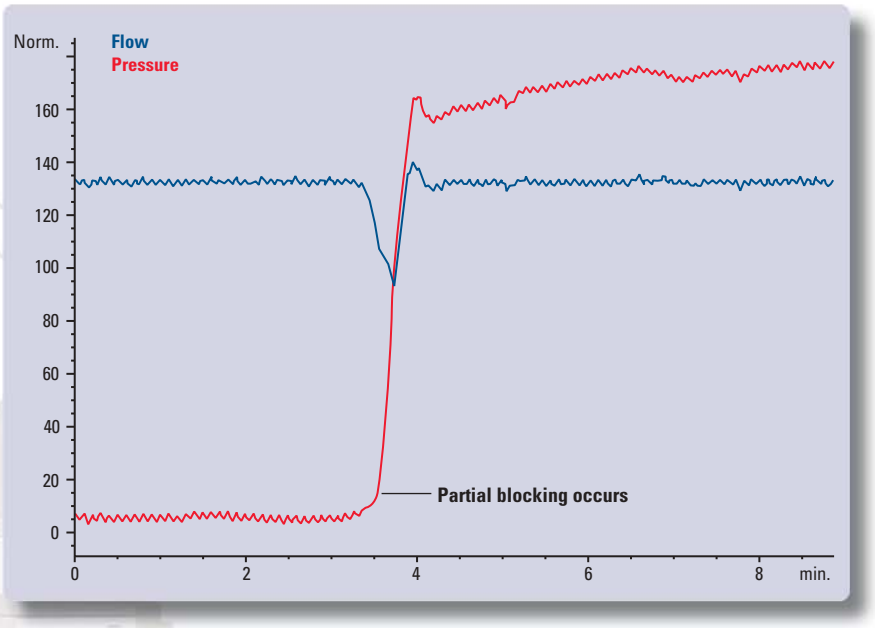
The heart of the Nanoflow Proteomics Solution is an Agilent 1100 Series Nanoflow LC System for MS. The nanoflow LC system is specifically designed to provide stable flows that enhance MS sensitivity. It features a revolutionary electronic flow control (EFC) system with active feedback for unprecedented stability at flow rates from 100 nl/min to 1 µl/min.

Maintain separation efficiency

The entire flow path has been designed to maximize and maintain separation efficiency. The switching valves, fittings, connectors, and PEEK-coated fused silica tubing have all been carefully selected to minimize dead volumes.

Multiple separation options

The nanoflow LC system is supplied with high-efficiency nanocolumns for sample cleanup, concentration, and separation. It can be configured for one-dimensional separation, one-dimensional separation with sample cleanup and concentration, or full two-dimensional separation — whatever is appropriate for your samples.



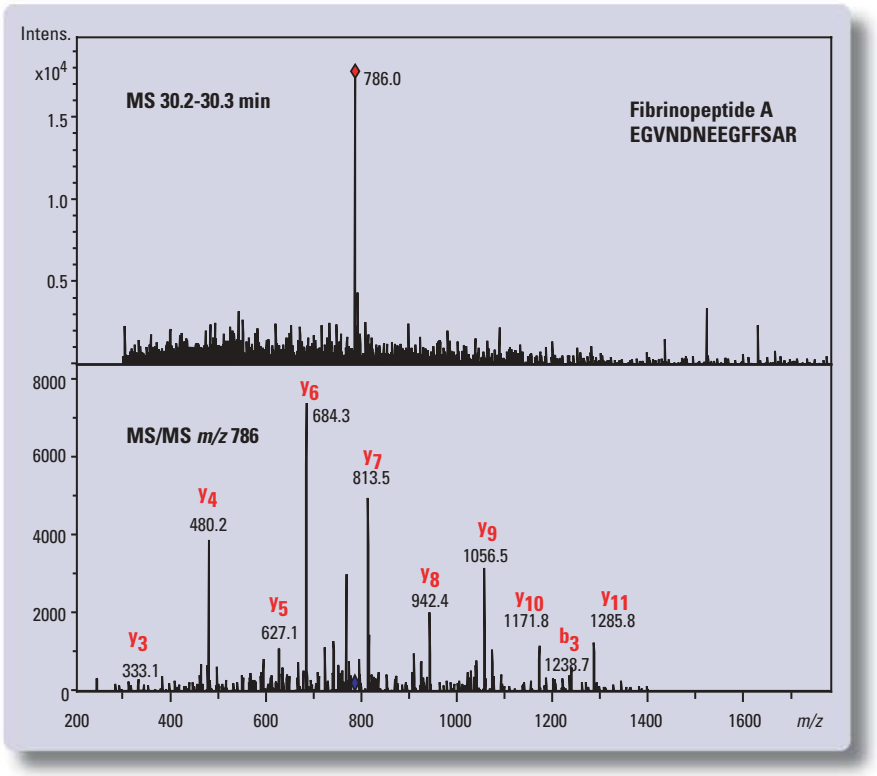
Unlike passive splitters, electronic flow control with active feedback compensates for partial plugging in the flow path, saving precious time and samples. Real-time display of both flow and pressure also makes diagnosis of leaks and blockages dramatically easier.

Superb MS/MS performance

Identification of low-abundance proteins requires exceptional sensitivity and specificity. The Nanoflow Proteomics Solution includes Agilent's most sensitive ion trap mass spectrometer equipped with a second-generation nanospray ion source. Together, they provide attomole-level sensitivity and the specificity of MS^n data.

Sensitivity, resolution, and scan speed simplify protein identification

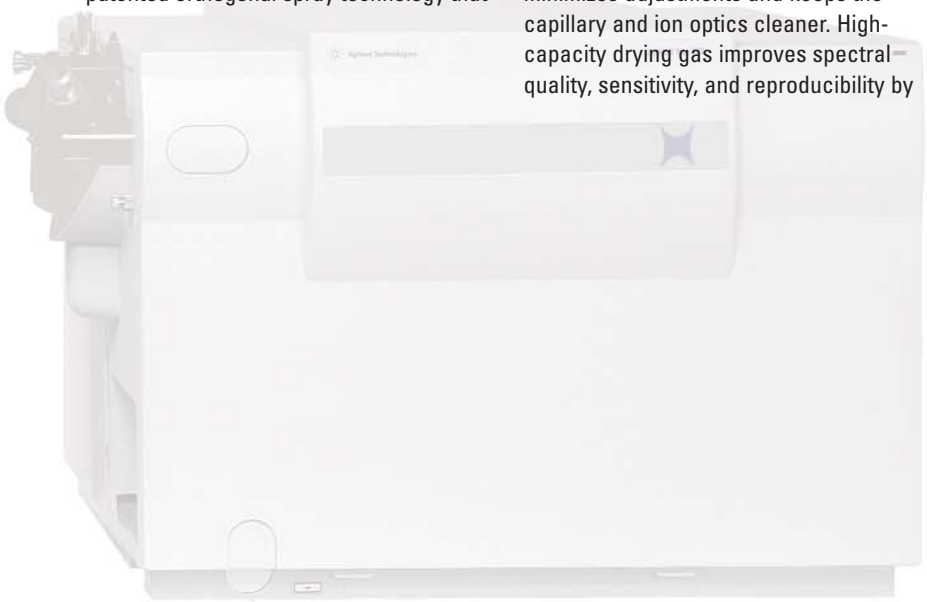
The Nanoflow Proteomics Solution includes the Agilent 1100 Series LC/MSD Trap XCT; Agilent's most sensitive solution for MS^n analyses. The LC/MSD Trap XCT feature a high-capacity ion trap for heightened sensitivity. At the same time, it retains the resolution and scan speed advantages inherent in a true three-dimensional ion trap. The LC/MSD Trap XCT offers an outstanding combination of resolution and scan speed including a specialized peptide scan mode for enhanced analysis of low-abundance proteins. SmartFrag collision-energy ramping simplifies method development and ensures that every peptide receives the correct collision energy for dissociation.



LC/MS/MS analysis of 100 attomoles of fibrinopeptide A on column

Nanospray for ultimate online analyses

The Agilent nanospray ion source is a second-generation source that provides attomole-level sensitivity for online LC separations such as the separation of proteolytically digested proteins. It is the only nanospray source to incorporate Agilent's patented orthogonal spray technology that minimizes adjustments and keeps the capillary and ion optics cleaner. High-capacity drying gas improves spectral quality, sensitivity, and reproducibility by reducing solvent clusters and mobile-phase adducts. The nanospray source is sealed for increased safety when working with potentially hazardous biological samples.



Better results through intelligent data acquisition

The Nanoflow Proteomics Solution software includes advanced, second-generation data-dependent acquisition capabilities; capabilities that can increase both the amount and quality of unique peptide data, and with it your chances for success.

Increase the amount of unique data acquired

When searching for low-abundance proteins, the most abundant component is frequently not be the component of greatest interest. The Nanoflow Proteomics Solution software features a wide array of intelligent, data-dependent acquisition features to help ensure that more unique data is automatically acquired from every scan.

Active exclusion

Active exclusion brings intelligence and automation to ion exclusion. The repeat-count feature increases the amount of unique data acquired by preventing acquisition of MS/MS data from the same precursor ion more than a user-specified number of times.

To ensure that ions of the same *m/z* value present in more than one chromatographic peak are always acquired, the exclude-time feature removes ions from the active-exclusion list after a user-specified time.

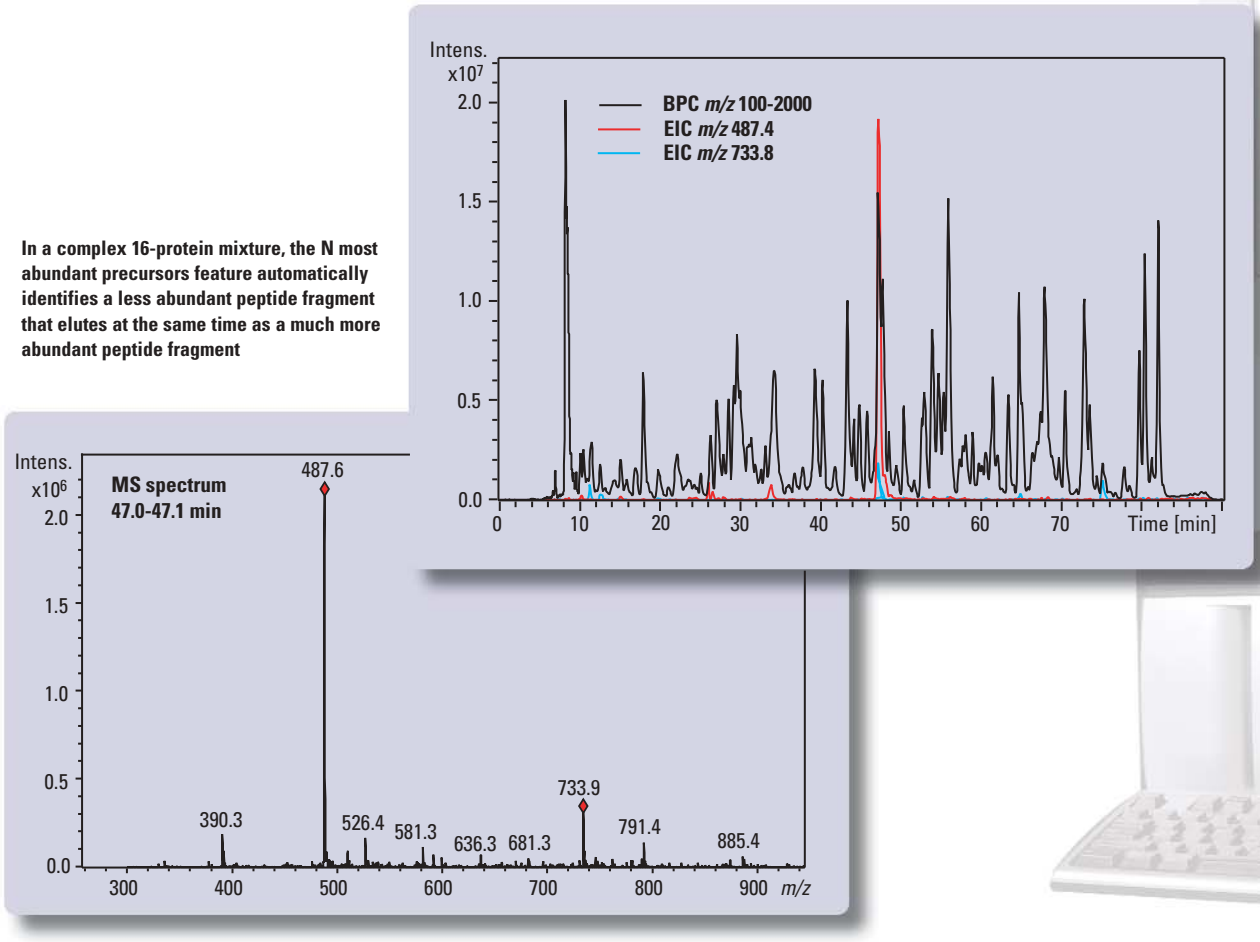
Static and preferred ion exclusion lists can also be applied, along with absolute and relative abundance thresholds.

Isotopic exclusion

Isotopes can result in the acquisition of unnecessary mass spectral data. Isotopic exclusion prevents acquisition of MS/MS data from isotopes.

N most abundant precursors

The N most abundant precursors feature determines the number of unique precursor ions from which data are acquired. It is especially helpful in acquiring data from less abundant precursor ions in coeluting peaks.



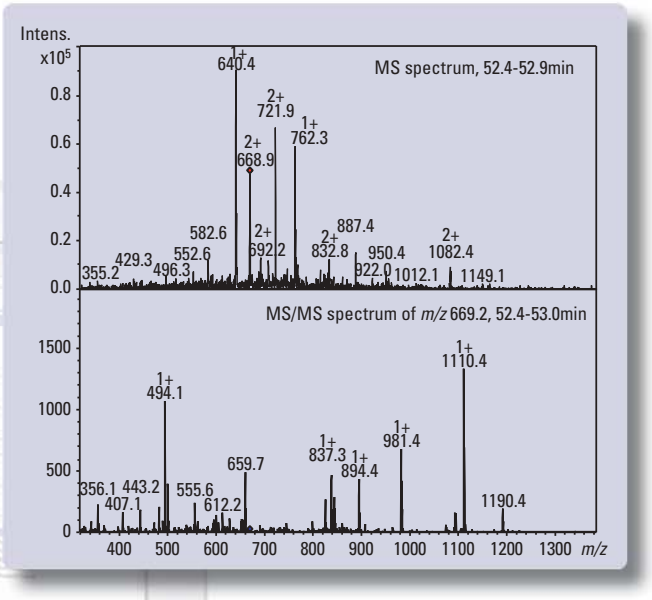
In a complex 16-protein mixture, the N most abundant precursors feature automatically identifies a less abundant peptide fragment that elutes at the same time as a much more abundant peptide fragment

Ensure better quality data for better matches

You need the highest quality data possible to increase your chances of a good match. The Nanoflow Proteomics Solution software includes essential features for improving data quality.

Preferred charge-state selection

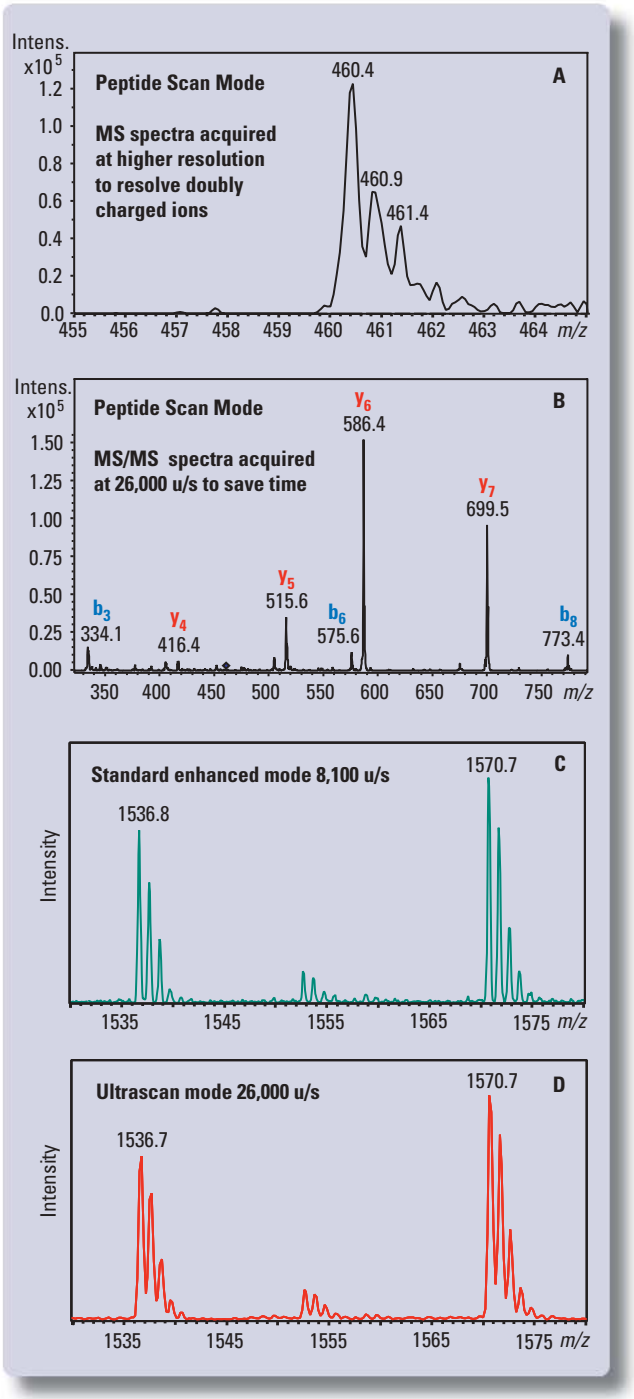
Generally, doubly charged ions yield the best collision-induced dissociation spectral data for protein database searching and matching. Preferred charge-state selection ensures preferential selection of these doubly charged ions.



Preferred charge-state selection (+2) and N most abundant ions (2) result in acquisition of MS/MS data from a less-abundant peptide fragment that would otherwise not have been selected for MS/MS

Peptide scan mode for better protein ID

Peptide scan mode combines the LC/MSD Trap XCT's enhanced and ultrascan modes to achieve better MS/MS and MS^n. It uses the higher-resolution enhanced mode during MS^1 for more accurate determination of charge states for +1, +2, and +3 charged peptides. It switches to the faster ultrascan mode in MS^2 and beyond to maintain shorter overall experiment cycle times. The net result is more proteins identified from complex peptide digest mixtures.



Peptide scan mode (A, B) enhances peptide analyses by using separate combinations of scan speed and resolution for MS and MS/MS. Data from singly charged AP-MALDI ions can be acquired successfully using either enhanced or ultrascan mode (C, D).

Unprecedented capabilities for high-throughput MS proteomics data analysis

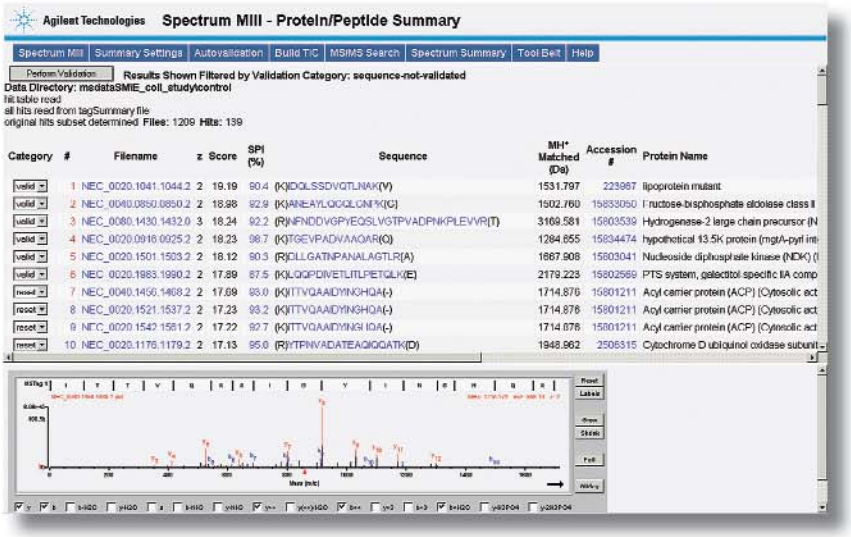
Agilent's Spectrum Mill MS proteomics workbench is a comprehensive suite of software tools that facilitate high-throughput proteomics experiments using mass spectrometry. It is highly recommended for use with the Nanoflow Proteomics Solution.

A wide range of capabilities

Agilent's Spectrum Mill MS proteomics workbench provides:

- Intelligent spectral extraction and quality assessment
- Database searching and *de novo* sequencing
- Automated and interactive results validation
- Quantitative and semiquantitative analyses
- Cross-experiment result summaries

This open-platform software can process mass spectral data from multiple instrument types and vendors.



Interactive validation facilitates comparison of the proposed match to the actual MS/MS spectra

Intelligent spectral extraction speeds protein identification

The extreme speed and efficiency with which Spectrum Mill workbench searches protein databases is due in large part to its intelligent extraction and processing of mass spectra. The Spectrum Mill extraction software assesses MS/MS spectral quality based on sequence tag length and signal-to-noise criteria. By identifying and excluding noise spectra and poor quality spectra before searching, search speed is greatly increased. This also reduces the number of false positive matches.

Multiple options for protein identification add flexibility

Spectrum Mill MS proteomics workbench provides multiple options for protein identification and characterization. It can search MS/MS spectra from ion trap and hyphenated mass spectrometers. It can also use peptide mass fingerprinting (PMF) to process MS spectra from MALDI-TOF and similar mass spectrometers.

The innovative MS/MS database search algorithm uses intelligent parallelization to provide rapid processing—without the creation of gigantic index files. It can operate in *identity mode* to find unmodified peptides or in *homology* mode to look for mutations and modifications.

Scoring is based on the information content (scored peak intensities, fragment ion types, etc.) of the spectra, not on probabilities. This produces a score that is independent of the database being searched and makes cross-database comparisons possible.

Automatic and manual match validation

Database matches can be validated automatically based on overall score and percent-scored peak intensity. Spectra associated with matches that are clearly correct are isolated from spectra associated with unvalidated matches. Unvalidated spectra can then be examined and validated interactively. Numerous features are included to make interactive validation fast and easy. Spectra from remaining unvalidated matches can be re-searched using alternate parameters or databases.

De novo spectral interpretation for peptides not found in any database

The *de novo* sequencing algorithm uses advanced graph theory to generate a ranked list of potential peptide sequences. It discards unrealistic solutions and compensates for common spectral difficulties such as noise and incomplete fragmentation. Result review enables simultaneous viewing of both database search results and *de novo* results.

Quantitative as well as qualitative information

The Spectrum Mill workbench includes a unique feature for comparing relative protein abundances.The comparisons are based on mean peak intensities of all the component peptides from a protein. Although semiquantitative, these comparisons are sufficient to reveal two- to five-fold changes in relative abundances. For researchers who need more precise quantification, the Spectrum Mill workbench supports ICAT analyses. Cys modifications include D_0/D_8 and $^{12}C/^{13}C$ ICAT labeling.

Complex data made accessible

The Spectrum Mill workbench can summarize and correlate results in ways that make the information accessible to biologists and biochemists as well as mass spectrometrists. A mass spectrometrist can review peptides in order of match quality; viewing peptide masses, mass spectra, and molecular weights. Biologists or biochemists can review the same data and examine protein names, match scores, percent coverages, and relative protein abundances.

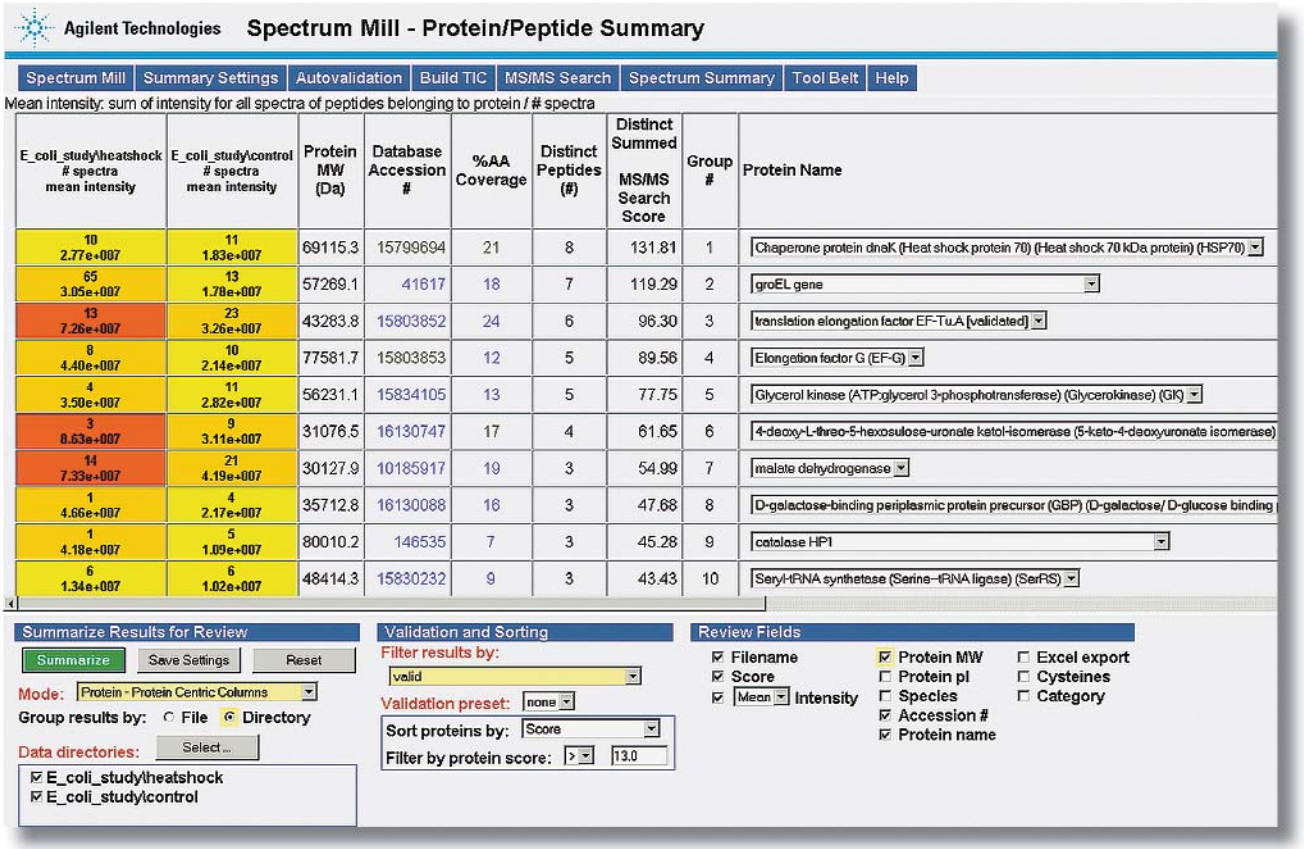
Users can review multiple samples, comparing information such as expression levels. Large data sets can be compared across multiple experiments and the results can be summarized at the protein level.

Compatibility with data from multiple vendors

Add-on modules are available that allow the Spectrum Mill MS proteomics workbench to process non-Agilent data formats:

- .RAW (Thermo Finnigan)
- .wiff (SCIEX LC/MS)
- .pkl (peak list) and multispectral appended .pkl (Waters/Micromass and others)

The extractor modules are optimized for the unique mass spectral characteristics of the type of mass spectrometer that generated the data.



Protein-protein mode allows comparison of data, including color-coded relative abundance information, from multiple samples

AP-MALDI — a complementary approach to protein ID

When sophisticated LC separations are not required, the LC/MSD Trap XCT equipped with Agilent's revolutionary atmospheric pressure matrix-assisted laser desorption/ionization (AP-MALDI) ion source provides a very fast, very easy, and extremely sensitive alternative to nanospray ionization.

Atmospheric pressure ionization makes it fast and easy to load and unload sample plates

Easy operation for fast analyses

When working with samples such as tryptic digests of two-dimensional gel spots, the LC/MSD Trap and AP-MALDI source offer a very fast way to analyze large numbers of samples. You can target sample spots manually, or, after initial positioning, the system will automatically move the sample plate to ensure uniform sampling. The 96-well sample plates are compatible with robotic deposition.

Attomole-level sensitivity

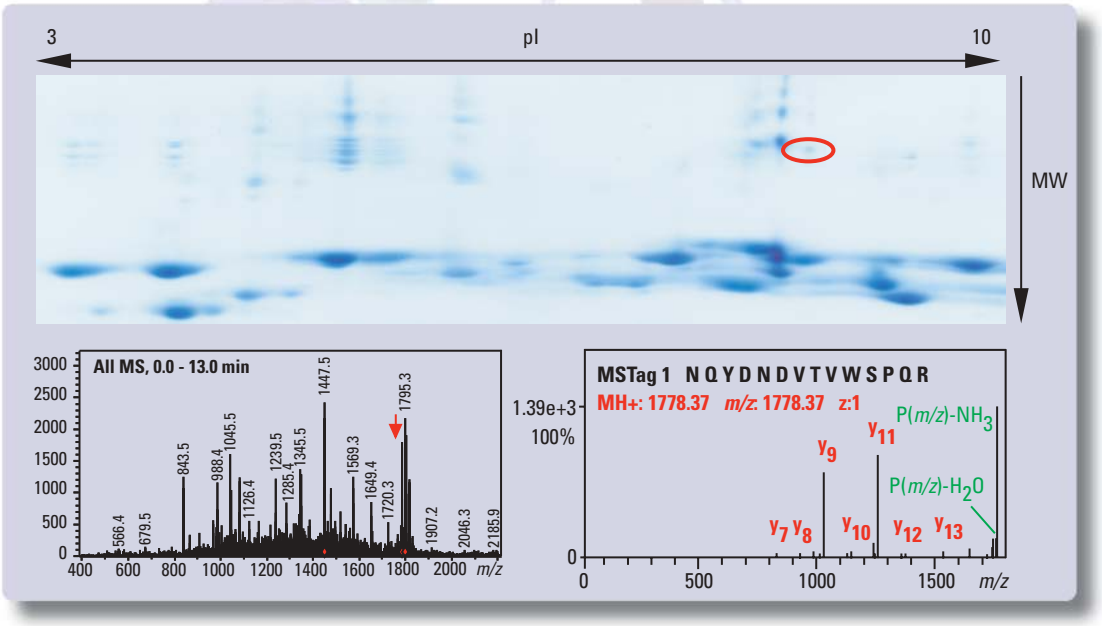
Fast analyses do not require you to sacrifice sensitivity. The LC/MSD Trap XCT with AP-MALDI demonstrates attomole-level sensitivity—equivalent to, or in some cases even better than, that provided by the standard Nanoflow Proteomics Solution.

Searchable MS/MS spectra

Unlike MALDI-TOF instruments, which require a difficult-to-use post-source decay process to generate additional spectral information, the LC/MSD Trap XCT with an AP-MALDI source easily produces true MS/MS spectra that are clean and searchable. The added specificity of MS/MS data reduces the need for high coverage.

Easy conversion between operating modes

AP-MALDI provides a useful complement to nanospray without the expense of another mass spectrometer. It uses Agilent's standard ion source mounting system so you can convert back and forth between nanospray and AP-MALDI, or any of Agilent's other LC/MS ion sources, in a matter of minutes.



AP-MALDI MS analysis of the proteolytic digest of a light spot from a 2D PAGE separation of 50 µg of rat proteosomes. Spectrum Mill workbench search yielded positive identification.

More proteomics challenges, more Agilent solutions

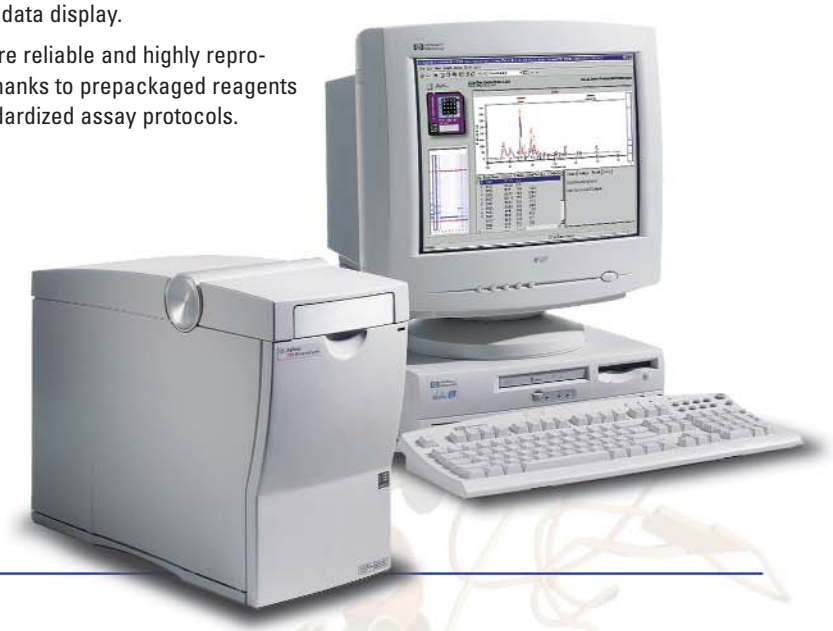
In addition to the Nanoflow Proteomics Solution, Agilent offers solutions for many other proteomics challenges.

Bioanalyzer and protein assays do more with less

Checking cell lysates for recombinant protein expression, identifying the protein of interest in column fractions, or assessing protein purity? The Agilent 2100 bioanalyzer equipped with our enhanced Protein 200 Plus LabChip® kit helps you collect more information in less time. Each chip, featuring Caliper Technologies Corporation's LabChip approach, is capable of producing size, purity, and concentration information for ten different protein samples in less than 30 minutes.

The bioanalyzer and LabChip kit make these analyses far easier than traditional techniques.

- No more gels and buffers, tedious staining, or imaging; simply add your proteins to the chip, start the run, and watch the real-time data display.
- Results are reliable and highly reproducible thanks to prepackaged reagents and standardized assay protocols.
- Alternative data display options show results in gel-like image, electropherogram, and tabular formats.
- The data is digital, making it easy to share with colleagues, export for publication, or archive.



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Simplified protein purification

Based on Agilent's industry-leading 1100 Series HPLC system, the 1100 Series purification platform provides unified, high-throughput analytical-scale to preparative-scale purification of proteins. It can be configured based on the amount of sample to be isolated, required purity, desired throughput, and analytical costs. Features include:

- A choice of pumps, autosamplers, fraction collectors, detectors, and flow cells that can be matched to throughput demands and sample amounts
- Optional MS detection allows fraction collection based on time, peak, and/or mass – a unique combination
- Application-specific purification software and optional easy-access software that provides walk-up access for non-experts
- Single-vendor solution for your equipment, supplies, and support needs

Service and support—global reach, local response

Agilent's reputation as a premier supplier of instrument service and support is worldwide. Our sophisticated communications technology and infrastructure enable us to operate seamlessly in more than 40 countries; so we can serve you wherever you are. And our global perspective doesn't stop us from being responsive down to the last local detail.

Agilent's worldwide network of support services can simplify lab management and eliminate maintenance and repair worries. Our wide range of options lets you choose a package that's exactly right for your organization and your budget, with predictable costs that facilitate fiscal planning. Your support package can include enhanced warranty protection, preventive maintenance, troubleshooting and repair services, and regulatory compliance assistance. Support services can be purchased with your instrument, with an annual contract, or as you need them.

A service partnership with Agilent puts our responsive, highly skilled professionals—trained in an ISO 9001 factory program—to work for you. These expert professionals use only genuine Agilent replacement parts, and procedures and specifications designed to maximize the productivity, maintain the integrity, and extend the life of your instruments.

Our contractual support services are backed by an escalation process that focuses a network of elite repair experts, application specialists, and even the instrument's design engineers for rapid resolution of difficult problems.

For more information

For more information about the Agilent Nanoflow Proteomics Solution, Agilent's other solutions for proteomics, or for information about individual products for liquid chromatography and mass spectrometry, call toll free:

1-800-227-9770 (U.S. and Canada)

In other countries, please call your local Agilent Technologies life sciences sales office or authorized Agilent Technologies distributor.

You can also visit our site on the World Wide Web at:

www.agilent.com/chem/proteomics

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