



ABSTRACT

The Proteomics and Mass Spectrometry Facility of the Biotechnology Resource Center (BRC) provides state-of-the-art instruments and services, and expertise in their applications, to the university community and to outside investigators. Services include sample preparation, protein identification, protein characterization, quantitative proteomics and protein PTMs, and small molecule profiling and quantitation. The facility also provides consultation on project design and data analysis, and offers educational workshops, seminars and training.



OVERVIEW

Services provided: The facility provides proteomics and mass spectrometry based sample processing and data analysis both for individual samples and for large scale projects. Sample submission, tracking, data retrieval and billing are integrated into a web based laboratory information management system (LIMS). Training is available on sample preparation techniques, use of instruments, data analysis and interpretation.

Mass spectrometry: The facility has four electrospray ionization (ESI)-based mass spectrometers, including: (1) Orbitrap Fusion (Thermo Scientific); (2) Orbitrap Elite (Thermo Scientific); (3) hybrid triple quadrupole/linear ion trap 4000 Q Trap (ABSciex); and (4) hybrid quadrupole, ion mobility, orthogonal acceleration time of flight (Q-TOF) SYNAPT HDMS (Waters). All instruments have high sensitivity (low fmol for peptides) with high resolution and mass accuracy available in Orbitrap and Synapt.

High performance liquid chromatography (HPLC): Five HPLC systems: (1) UHPLC E3000 Proteomics MDLC (Thermo/Dionex); (2) 2 units of UHPLC3000 RSLCnano (Thermo/Dionex); (3) nanoACQUITY UPLC (Waters); and (4) 1100 HPLC (Agilent). All four nanoLC platforms are interfaced with mass spectrometers for on-line LC-MS/MS analysis of macromolecule and small molecules. The standalone Agilent 1100 HPLC system is used for off-line analytical-scale LC analysis.

Gel-based and other analysis: 2D gel electrophoresis systems Multiphor II IEF and Etan DALT Six/Twelve Vertical Gel Systems (GE Healthcare); Typhoon 9400 image system (GE Healthcare) for fluorescence imaging analysis; Spectra MAX plus spectrophotometer (Molecular Devices); GelFree 8100 Fractionation System (Expedeon) for size-based intact protein separation in solution.

Administration: The Proteomics and Mass Spectrometry Facility is part of the Biotechnology Resource Center of the Cornell University Institute of Biotechnology.

Open to all: The resources and services of the facility are open to all investigators at Cornell University and Cornell-affiliated institutions. The facility also provides services to external investigators at both academic institutions and commercial enterprises. In association with the NY State Center for Advanced Technology (CAT) in Life Science Enterprise, NY State companies receive a discount on facility services.



RESOURCES

Mass spectrometry: Orbitrap Fusion and Orbitrap Elite (Thermo Scientific), 4000 Q Trap (ABSciex), Q-TOF SYNAPT HDMS (Waters).

HPLC systems: UHPLC3000 Proteomics MDLC (Thermo/Dionex), 2xUHPLC3000 RSLCnano (Thermo/Dionex), nanoACQUITY UPLC (Waters), 1100 HPLC (Agilent).

Gel-based and other analysis: 2D gel electrophoresis systems (GE Healthcare), Typhoon 9400 image system (GE Healthcare) for fluorescence imaging analysis, Spectra MAX plus spectrophotometer (Molecular Devices), GelFree 8100 Fractionation System (Expedeon).

Bioinformatics tools: Database search engines: Mascot, Proteome Discoverer, ProteinPilot, ProteinTyr Global Server (PTGS), Scaffold, Biometric, MaxQuant. MS data analysis software: Xcalibur, SILV, Analyst, MultiQuant, MarkerView, MassLynx. Gel imaging analysis software: ImageQuant TL, ImageMaster, DeCyder, Same Spot.

Instrument Systems nanoLC Mass Spectrometry



UHIMate3000 RSLCnano/Orbitrap Fusion: Provides both nano and analytical scale of high mass accuracy, ultra high resolution and fast-scanning LC-MS/MS analyses for a broad range of applications, from in-depth discovery experiments to characterization of complex PTMs to comprehensive qualitative/quantitative workflows. The hybrid architecture (quadrupole, Orbitrap and ion trap mass analyzers) with the availability of multiple fragmentation techniques (CID, HCD, ETD and ETHCD) at any stage of MSⁿ is especially powerful when facing complex and low abundance samples in proteomics, glycomics, lipidomics and metabolomics.



UHIMate3000 RSLCnano/Orbitrap Elite: Provides both nano and analytical scale of high mass accuracy, high resolution and fast-scanning LC-MS/MS analyses for most discovery proteomics and protein characterization studies including protein IDs, of complex samples, high quality PTM analysis, quantitative proteomics, and small molecule profiling and identification.



UHIMate3000 2DLC/4000 Q Trap: Provides both 2D nano and analytical scale LC-MS/MS for data-dependent shotgun proteomics or data-independent acquisition analysis of complex samples. The system is also useful for intact protein analysis for the characterization of protein complexes (protein-protein non-covalent interaction) and protein-ligand interactions.



nanoACQUITY UPLC/SYNAPT HDMS: Provides 2D nano LC-MS/MS for data-dependent shotgun proteomics or data-independent acquisition analysis of complex samples. The system is also useful for intact protein analysis for the characterization of protein complexes (protein-protein non-covalent interaction) and protein-ligand interactions.

Protein/peptide separation and quantitation



Agilent 1100 HPLC: Provides routine analytical scale for HPLC separation of a variety of complex samples (protein/peptides and small molecules) through SCX, SAX, HILIC, SEC and RPLC.



GelFree 8100 (Expedeon): provides intact protein molecular weight-based fractionation (3.5 to 150 kD) with liquid phase recovery for complex protein mixtures.

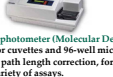


2D/1D gel-based separation systems: Multiphor II IEF (GE Healthcare), EPS 601 Vertical Gel System (GE Healthcare), Etan DALT Six/Twelve Vertical Gel Systems (GE Healthcare), and XCell SureLock Vertical Gel System (Invitrogen).

Gel imaging / Spectrophotometry



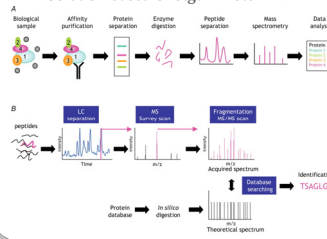
Typhoon 9400 Laser Scanner (GE Healthcare): Allows quantitative imaging analysis of Coomassie Blue and various fluorescence dye stained gels and blots.



Spectra MAX plus spectrophotometer (Molecular Devices): Provides fast, full spectral range detection for cuvettes and 96-well microplates, with temperature-independent path length correction, for quantitative measurement of a wide variety of assays.

Protein Identification

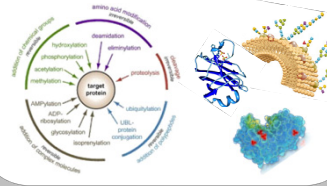
Gel-based Protein ID Solution-based Shotgun Protein ID



Protein Characterization

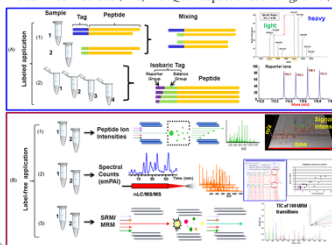
Detection and quantitation of post-translational modifications (PTMs) & other modifications in purified protein samples & complex sample mixtures

Acylation – ADP-ribosylation – Amino acid mutation – Chemical modifications –
Deamidation – Glycosylation – Methylation – Nitration – N- or C-terminal
detrimination – Hyposulfite – Phosphorylation – Ubiquitination, SUMOylation

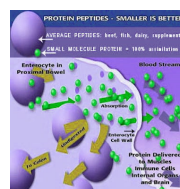


Quantitative Proteomics

Labeling Strategies (GTRAQ, TMT, SILAC, Dimethylation)
Label-Free Methods (XIC, MaxQuant spectral counting, SRM)



Small Molecule Profiling and Quantitation



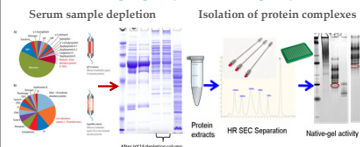
LC-MS/MS with SRM / MRM

Allows detection of ng/ml amounts of compound in complex biological matrices, including plasma, serum, urine, and cell extracts.

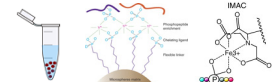
Sample Preparation

Sample preparation services include:

- 1D/2D gel
- 1D/2D HPLC (SCX, SAX, HILIC, SEC and RP)
- affinity enrichment/depletion
- in-gel and in-solution digestion
- sample desalting
- sample concentration
- sample quality assessment / quality control



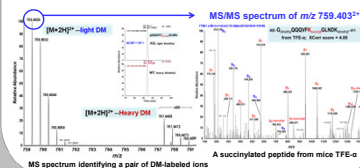
Phosphopeptides enrichment by TiO2 and IMAC beads



MS Data Analysis

Data analysis services include:

- de novo peptide sequencing
- database interrogation
- database searching
- search results reporting
- manual inspection of MS and MS/MS spectra data for validation of identification and quantitation analysis



Consultation, Workshops and Training

- Consultation on project design and data analysis.
- Software analysis tools and training.
- Training on core equipment that is open for user-access, including the Typhoon 9400, Agilent 1100 HPLC, and spectrophotometer.
- Educational workshops and hands-on training.
- Seminars on emerging proteomics and mass spec technologies.
- Coordinated project design consultation and data analysis support with other BRC facilities (e.g. genomics, bioinformatics, bio-IT, imaging and advanced technology assessment)

Contact Information



Proteomics & Mass Spectrometry Facility



Sheng Zhang, Director
BRC_protomics@cornell.edu
www.biotech.cornell.edu/brc/proteomics-and-mass-spectrometry

www.biotech.cornell.edu