

Mass Spectrometry Instrumentation

AutoflexIII MALDI-TOF-TOF

The AutoflexIII (Bruker Daltonics) is an orthogonal MALDI-TOF-TOF system that offers high mass accuracy (down to 5 ppm) and resolution (up to 25,000) in both positive and negative ionization modes. The instrument also offers MS/MS capabilities that can be used to obtain structural information by fragmentation of biomolecules. The AutoflexIII can be used for the analysis of low complexity samples including identification of proteins from 1D-, 2D- or 2D DIGE-gels (PMF, Peptide Mass Fingerprint), detection and identification of metabolites, peptides and proteins as well as Top-Down mass spectrometry of intact proteins enabling N-terminal sequencing of proteins.

MS Analysis of Proteins (Proteomics)

Exploris 480 and Q-Exactive HF

For a detailed characterization of proteins and post-translational modifications two high-resolution Orbitrap Fourier Transform (FT) electrospray mass spectrometers are available in the CFH. The Exploris 480 mass spectrometer (Thermo Fischer Scientific) is coupled to an Ultimate 3000 nano-RSLC UHPLC (Thermo Fischer Scientific) and offers high resolution (up to 480,000 at $m/z = 200$), high mass accuracy (≤ 1 ppm) and high scan rates (up to 40 Hz), making this instrument an ideal tool for the characterization of highly complex protein samples. In addition, the CFH operates a Q-Exactive HF mass spectrometer (Thermo Fischer Scientific) together with the group of Prof. Waltraud Schulze (Department of "Plant Systems Biology", 190d). The Q-Exactive HF is coupled to an EASY-nLC 1200 nano-UHPLC system (Thermo Fisher Scientific) and offers resolution up to 240,000 at $m/z = 200$), ≤ 1 ppm mass accuracy and up to 18 Hz scan rate. Besides non-targeted analysis of proteins and protein modifications both instruments can be also used for targeted protein quantification using SIM (Single Ion Monitoring) or PRM (Parallel Reaction Monitoring) experiments.

Applications for both high resolution nano-LC-ESI-MS/MS instruments:

- Accurate mass determination of peptides and proteins for quality control or identification
- Identification of proteins from highly complex samples
- Identification of posttranslational protein modifications
- Relative and absolute quantification of proteins and posttranslational protein modifications (with isotope labelling or label free) in highly complex samples
- Targeted quantification of proteins and peptides using SIM or PRM

MS Analysis of Metabolites/Small Organic Molecules (Metabolomics):

5500 QTRAP and 6500+ QTRAP ESI Mass Spectrometers (AB SCIEX)

The 5500 QTRAP and 6500+ QTRAP ESI-MS mass spectrometers (both AB SCIEX) of the CFH are used for targeted quantification of metabolites and proteins. Both instruments are coupled to 1290 Infinity UHPLC systems (Agilent). Metabolites or peptides with known m/z ratio can be detected and quantified with high sensitivity even in very complex mixtures by SRM (Selected Reaction Monitoring) or MRM (Multiple Reaction Monitoring) on Triple-Quadrupole or QTRAP mass spectrometers. In a SRM experiment combinations of a precursor ion and one or more

characteristic fragment ions of the metabolite/peptide of interest are analyzed. Since for both precursor ion and fragment ions only very narrow m/z ranges are selected by the quadrupoles (usually 1 Da or less) the chemical background is low and therefore the metabolite of interest can be detected with high sensitivity. Both QTRAP instruments have high scan rates in SRM mode (50 ms dwell time) and offer fast polarity switching (5 ms), which enables quantification of a large number of analytes in a single LC-MS/MS analysis. In addition, QTRAP mass spectrometers can also be operated in linear ion trap (LIT) mode allowing acquisition of LIT MS/MS or MS3 scans with high scan rates for confirmation of metabolite identity. Furthermore, the 5500 and 6500+ QTRAP mass spectrometers can be used for precursor ion or neutral loss scan experiments to screen for the presence of certain substance classes or modifications (for example lipids, glycosylation, phosphorylation, etc.) within a sample.

The 6500+ QTRAP instrument is equipped with a Selexion Differential Mobility Separation (DMS) device that can be used for the gas phase separation of isomeric or isobaric metabolites or to reduce background signals, which can in turn increase selectivity and sensitivity of a targeted quantification experiment.

Applications:

- Targeted quantification (relative and absolute) of metabolites and proteins using SRM/MRM experiments
- Precursor ion or neutral loss scans for the identification of certain substances classes or modifications

Q-Exactive Plus

For the identification of small molecules and metabolites a high resolution Q-Exactive Plus (Thermo Fisher Scientific) mass spectrometer is available at the CFH. The Q-Exactive Plus is coupled to a 1290 Infinity UHPLC system (Agilent) for chromatographic separation of metabolites. The Q-Exactive Plus can be operated at resolutions up to 140,000 (at $m/z = 200$) and offers high mass accuracy (≤ 1 ppm) and scan rates up to 10 Hz. The high mass accuracy of the instrument allows precise determination of the m/z ratio of an unknown metabolite, which is usually sufficient to calculate a sum formula. MS/MS spectra can be used for further characterization of a metabolite and can be compared to MS/MS spectral libraries (if available), which finally can lead to the identification of an unknown metabolite. The scan rates and the dynamic range of the instrument are sufficient to detect a high number of metabolites in positive and negative ion mode with sufficient data points for a precise quantification in a single analysis. This is particularly important for metabolite profiling or metabolomics experiments. The idea behind these experiments is to detect, identify and (relatively) quantify as many substances as possible. Comparison of metabolic profiles of a biological system under different environmental conditions leads to a better understanding of the regulatory processes within the biological system.

Applications:

- Identification of metabolites by determination of precise m/z ratio
- Fragmentation of metabolites for structural elucidation
- Metabolite Profiling/Metabolomics (relative quantification)

LTQ-Orbitrap XL

The LTQ-Orbitrap XL (Thermo Fisher Scientific) is a hybrid Fourier Transform (FT) electrospray mass spectrometer including two mass analyzers, an Orbitrap and a linear ion trap that work in parallel. The LIT has high scan speed, while the Orbitrap offers high mass accuracy (≤ 1 ppm) and high resolution (up to 100,000 at $m/z = 200$). Metabolites can be fragmented either in the linear ion trap (CID, including MS^n) or in the collision cell (HCD) which provides flexibility for MS/MS experiments. The instrument is operated in direct infusion mode using a syringe pump, but can also be coupled to an UHPLC system for the analysis of more complex samples.

Applications:

- Identification of small organic molecules/metabolites by determination of precise m/z ratio
- Fragmentation of small organic molecules/metabolites for structural elucidation

Gas Chromatography –Mass Spectrometry (GC-MS/MS)

7890B Gas chromatograph and 7000D Triple quadrupole mass spectrometer (Agilent)

This analytical system can be used versatilely for different analytical objectives. The instrument is equipped with two different sources: EI (Electron ionization) where the electrons are accelerated by a potential of 70 eV (standardized energy stable, instrument independent mass spectra for database searching) resulting in complex mass spectra, that are useful for structural identification and a CI (Chemical ionization) which is „softer“ (lower energy) than EI, the ionization takes place via proton transfer reactions. The advantage of latter is the reduced fragmentation which results in a peak in the mass spectrum indicative of the molecular weight of the analyte.

Substances can be analyzed in a mass range up to m/z 1050. This analytical technique can be used for volatile substances like terpenes or pesticides but also for non-volatile molecules. These have to be derivatized before analyzing.

Applications:

- Identification and/or quantification of small organic molecules/metabolites
- Fragmentation of small organic molecules/metabolites for structural elucidation