Thermo Orbitrap Fusion

Proteomics mass spectrometer hyphenated to a nanoHPLC via an Advion NanoMate

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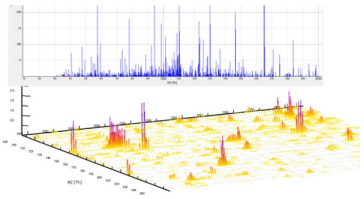
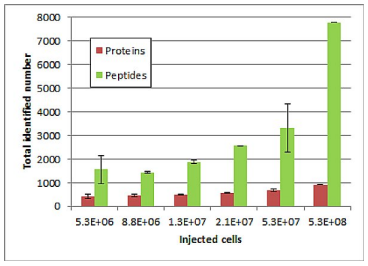
Instrument Description:

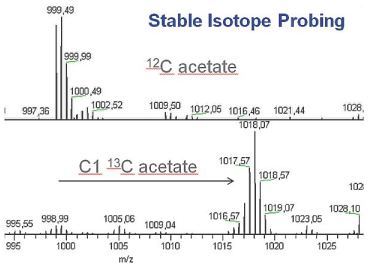
The whole setup comprises a nano-HPLC system, an Advion NanoMate and an Orbitrap Fusion mass spectrometer. The nanoHPLC provides precise separation of peptides, proteins or metabolites, the Advion couples reliably the liquid stream from the nanoHPLC to the mass spectrometer and ionizes via an electron spray, the Orbitrap analyses m/z values with very high speed, mass accuracy and resolution. The Orbitrap Fusion contains three mass analysers (Orbitrap, Ion trap, Quadrupole) which can be used for mass measurements and filtering of target ions. The Orbitrap is fast and very precise up to a resolution of 450,000 at $200\ m/z$. The instrument can fragment via CID, HCD or ETD. Internal lock masses guarantee high mass precision down to $1-2$ ppm. Through full control on each part of the instrument, we have high flexibility towards many different parameters. The whole instrument and our periphery are optimized for answering specific research question, rather than routine protein analysis.

Applications:

- Shotgun proteomics: identification of peptides from trypsin digests; comparison of expression profiles; concurrent identification of up to 1000 proteins per 2h gradient
- Protein identification from SDS-gel bands
- Protein Stable Isotope Probing (Protein-SIP): Tracing of stable isotopes into amino acids and proteins
- Top-down proteomics of intact proteins
- Analysis of cross-linked peptides
- Protein quantification
- Identification of cofactors and their modifications (e.g. B_{12}, THF, other tetrapyrroles)
- Analysis of complex analytes between 100 and $6000\ m/z$







Requirements for Samples:

Only very low amounts of protein between 100 ng and $1\ \mu$g are required per injection. For *E. coli* $1\ \mu$g represents about 6×10^6 cells. We inject only purified samples. Proteins need to be extracted, derivatized (to protect cysteine residues) and desalted. Mostly we require C_{18}-ZipTipped-samples ($ZipTip_{\mu-C_{18}}$, Millipore, $2\ \mu$g capacity). On request, we provide a detailed standardized operating procedure for sample preparation. For automatized identification of proteins, a protein sequence database in fasta-format is required. Metaproteome identifications are mostly limited by the metagenome database.

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Picture captions (from top):

- Instrument Setup
- Ion path
- Mass data 2D & 3D
- Peptide/Protein ID
- Protein SIP