Mass spectrometry based peptide analysis and proteomics

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Central tasks

The Core Unit Mass Spectrometry and Proteomics (CUMP) is one of the Core Facilities established at the Medical Faculty. The facility offers state-of-the-art proteomic research to all members of the University Medical Center and Ulm University as well as to external industrial and academic partners.

As part of the Z01 project within the propose CRC1279, the CUMP will provide means for identification, detection and quantification of biological active peptides. In close cooperation with the group of Dr. Ständker, CUMP will further support the CRC in the identification and purification of bioactive peptides from complex peptide fractions.

Available instruments and services

Figure 1. As a core facility of the Medical Faculty, CUMP offers its services to members of Ulm University at all steps of a proteomic experiment. From conceptional advice and sample preparation to protein fractionation, the CUMP offers methods to tackle samples with varying complexity. Furthermore, tailored LC-MS/methods are available to accommodate for the needs of the various projects. Using bioinformatics, comprehensive qualitative and quantitative data analysis followed by data interpretation allows for the identification and quantification of thousands of proteins.

Figure 2. Selected equipment available at CUMP. (left) Expediton GELFREE 8100 fractionation station for size-based separation of medium length peptide entities. (middle two) Thermo Scientific Ultimate3000 nanoLC-ESI-MS/MS system coupled to a Thermo Scientific Orbitrap Velos Pro MS-system for high resolution proteome and peptide analyses. (right) Thermo Scientific BioLC uPLC for additional peptide separation prior LCMS-analysis.

Analysis strategies in use and development at CUMP

Figure 3. PTM-analysis platform. Due to their importance in cellular systems in general and their significant role under disease conditions, the ability to characterize a wide range of different PTMs is essential. CUMP currently establishes and improves protocols for multiple modifications, subsequently allowing use projects to employ these methods to facilitate their own research. Peptides harboring a given PTM will be enriched and/or fractionated using either uPLC (Dysglycosylation and Phosphorylation, left side) or bead-coupled antibodies specific for the respective PTM (Acetylation, Ubiquitylation and SUMOylation, right side).

Figure 4. A) Protein-derived endogenous peptides undergo multiple PTMs, cleavages on N- and C-termini being the obvious, resulting in differently modified entities, referred to as postproteins. Upon proteolytic digestion, as is common in bottom-up proteomics, information on the exact proteolysis composition is either lost or fragmented in close proximity. This necessitates the analysis of intact proteins in order to gain information on PTM synergies and dependencies. B) To this end, peptides will be fractionated according to their size using a Gelfree system. Following sample clean-up, proteins will be subjected to MS analysis. Information obtained from MS and MS/MS spectra will be used to identify peptides and locate PTMs.

Contact CUMP

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Aims

Z01 will provide members of this CRC with resources and methodologies required for peptide discovery and characterization. The specific aims of the Core Unit Mass Spectrometry and Proteomics within this research consortium are:

Aim 1: In close cooperation with Dr. Ständker, identify bioactive peptides in fractions of peptide libraries using mass spectrometry based techniques.

Aim 2: To apply MS-based methods for peptide quantification in body fluids and tissues.

Role of the CUMP within CRC1279

Identification and quantification of bioactive peptides. Within CRC1279, the Core Unit will provide expertise in MS-based peptide identification and quantification including stable-isotope labelling of amino acids in cell culture (SILAC) (Wrobel et al., 2015) and label-free proteomics (Noworol et al., 2011; Gronemeyer et al., 2013). In the past, CUMP was involved in the detection and quantification of bioactive peptides (Mohr et al., 2015; Zirilli et al., 2015) and cooperated with T. Well (A05, C01) to develop novel techniques for dual specific peptide labelling (Wang et al., 2016).

Figure 5. Schematic outline of the identification and quantification of bioactive peptides. Spectral data acquired on an Orbitrap Velos Pro will be subjected to de Novo sequencing, spectral networking and database searching in order to achieve peptide identification. Quantitative data will be used to profile peptides, abundances across different samples/fractions and to quantify peptides in various biological samples.

Identification of peptides in active fractions. Due to the unpredictable nature of endogenous protease generation, a traditional database search using existing approaches fails in reliably identifying the active components in fractions from peptide libraries. To overcome this limitation, de Novo sequencing for peptide identification and spectral networks (Bandera et al., 2007) will be applied. In the project, a profiling approach (Wiese et al., 2007) across different active fractions of a peptide library will be used to decrease the number of potential targets.

Quantitation of known peptides in tissue or body fluid samples. Projects within CRC1279 aim to optimize active peptides. CUMP will assist by quantifying peptides in different environments such as murine and human plasma. Since these experiments rely on accurate knowledge about peptide abundance, they are expected to be used to determine accurate peptide concentrations employing the proposed uPLC system.

Figure 6. De Novo sequencing (left) and spectral networking (right). Left: Peptide spectrum acquired on an Orbitrap Velos Pro was de Novo Sequenced using the PEAKS (Bioinformatics Solutions Inc.) software. Colored characters and peaks represent signals explained by the derived sequence, while black characters are amino acids unaccounted for by spectral data. (right) Spectral networking harboring the sequenced peptide. Nodes represent peptide entities and edges indicate similarities between spectra. Labelled edges indicate PTMs explaining the spectral differences between two peptides.

Figure 7. Plasma stability of EPI-X4 in murine plasma. Plasma was collected at different time points and subjected to LCMS-analyses. (Upper panel) Extracted ion chromatograms of EPI-X4 samples. (Lower panel) Peak areas plotted against incubation time. For EPI-X4 degradation is a first-order reaction, log transformation of these areas allows linear regression and subsequent half-life calculation.

Cooperations

The Core Unit Mass Spectrometry and Proteomics will provide means for identification, detection and quantification of biological active peptides. For peptide identification, CUMP will be involved in projects A02 (Münchner, Spillerbot), A05 (Kirchoff, Wost, B01 (Buske), B02 (Geiger) and B03 (Jumaa, Uehleb)). CUMP will cooperate with project A06 (Klein, Münch, Schanche) to determine the stability of EPI-X4 derivatives in human and murine plasma. With our efforts to enhancing the ability to identify active peptides, we expect further interest in our techniques and expertise.

References