Info Sheet: **Protein identification**

Enzymatic digestion and mass spectrometry analysis

General description:

The identification of proteins in complex mixtures is performed using high performance liquid chromatography in combination with mass spectrometry (LC-MS/MS). Matrix-assisted laser desorption/ionization (MALDI)-MS/MS can be used in case of purified proteins.

The workflow consists of:

- Protein digestion (Trypsin) and MS analysis
  - In solution (incl. optional TCA precipitation) / on-beads / in-gel / others
  - LC-MS/MS analysis (or MALDI-MS/MS)
- Data analysis
  - Protein identification (Mascot / PEAKS)
  - Visualization of protein identities (Scaffold)
- Report
- Expected turnaround time (after arrival of samples):
  - 3-7 days

Requirements and considerations:

- Submit samples as Coomassie Blue stained gel bands (whenever possible avoid silver staining), in solution (free of protease inhibitors!) or on (possibly magnetic) beads.
- For pull-down analyses, consider including controls and replicates.

Analysis of post-translational modifications

General description:

The identification of post-translational modifications (PTMs) in complex protein mixtures requires enrichment strategies, which are different for each PTM. For this reason, only a few of the over 300 currently known PTMs are extensively investigated in proteomics studies. Among them are phosphorylation, glycosylation, ubiquitination, methylation, acetylation and disulphide bridge formation.

Requirements and considerations:

- The overall workflow for PTM analysis is the same as for protein identification, with additional enrichment steps. Please contact us at proteomics@fgcz.ethz.ch for further information.
- Given the low stoichiometry of the PTM, higher starting protein amounts are required.