Info Sheet: **N-Terminal analysis**

Determination of N-/C-Terminal Sequence by MALDI MS

General description:
Top-down-sequencing of pure intact proteins via MALDI-MS offers an emerging analytical method and is a valuable replacement of the traditional Edman sequence technology. Terminal post-translational modification, e.g., N-terminal pyroglutamyl modifications, may be detectable.

The workflow consists of:

- Mixing of 0.5-1 µl of protein solution with matrix solution on a MALDI-plate
- MALDI-MS analysis using in-source decay mode (ISD)
- Data analysis: software Biotools or Mascot protein identification.
- Expected turnaround time (after physical arrival of samples):
    - 3-5 days

Requirements and considerations:

- Volume: >5 µl;
- Minimal concentration: 5-10 pmol/µl; optimal concentration: 10-500 pmol/µl
    - rule of thumb concentrations of (mass in kDa) x 1 pmol/µl are optimal)
- Dissolve sample in volatile solvents / free of non-volatile buffers, salts, detergents, glycerol, etc.
- salt and buffer containing samples may be submitted (we will desalt them on ZipTips prior to the analysis)

Terminal Amine Isotopic Labeling of Substrates (TAILS)

General description:
TAILS is a novel multiplex quantitative proteomics platform for the determination of N-terminomes and the system-wide identification of protease substrates and cleavage sites in complex biological samples.

The workflow consists of:

- Blocking of all natural and cleaved N-termini and lysines in a proteome (exposed or not exposed to a protease) by differential isotopic labelling
- Pooling of samples and trypsin digestion
- Complexity reduction with an aldehyde-derivatized amine-reactive polymer that removes all unlabeled peptides.
- LC-MS/MS analysis
- Bioinformatics
    - Identification and quantification of proteins and N-Termini
    - Statistical analysis and report (with plots)
- Expected turnaround time (after physical arrival of samples):
    - Pilot (QC): 2 weeks
    - Main experiment: 4-6 weeks

Requirements and considerations:

- Samples should not contain free amines, that could react with the isotope labels
- A typical setup compares 5 vs. 5 replicates (e.g. WT vs. KO; treated vs. mock)