Info Sheet: **Targeted protein quantification**

We distinguish three tiers of targeted proteomics analysis:

- Tier 1 measurements satisfy the highest analytical standards and generally correspond to diagnostic laboratory tests that **quantify** a single or a small number of peptides/proteins across large sample cohorts.
- Tier 2 measurements deliver research quality data and typically **quantify** peptides/proteins in a highly multiplexed fashion.
- Tier 3 measurements are typically used for early-stage biological studies; enable **repeatable measurement** of the same sets of peptides/proteins across samples.

Tier 1 and 2 measurement **always** include internal standard peptides for each targeted analyte and are executed using *Selected* or *Parallel Reaction Monitoring* (*SRM*/*PRM*). The usage of internal standards ensures confident and precise quantification. Both data acquisition schemes require assay development prior to data recording and their data output is strictly limited to the targeted molecules! Both Tiers differ in their requirements for assay characterization (see next section).

Tier 3 measurement employ *Data Independent Acquisition* (*DIA*) strategies that do not require assay development prior to data recording. Instead, they aim to record maps comprising all analyte species contained in the sample. These maps are then queried post data recording (*in silico*) using targeted data extraction. *DIA* measurements typically lack internal standard peptides, leading to less confident and uncalibrated data. Done with care, *DIA* can identify differentially abundant peptides and proteins.

Assay development

The following steps need to be completed:

- Selection of proteotypic peptides for the proteins of interest (~3 peptides/protein)
- Purchase of synthetic stable isotope labeled peptides
- LC-MS method setup and assay characterization
  - Tier 1: Response Curve (LOD, LOQ), Mini-Validation of Repeatability, Selectivity, Stability and Reproduceable Detection of endogenous Analyte
  - Tier 2: Response curve (LOD, LOQ) and Mini-validation of Repeatability.

Tier 3 experiments do **not** require assay development in a classical sense. Extraction is instead guided by spectral or chromatogram libraries that can be generated from the same samples, downloaded from public repositories or predicted based on peptide sequences.

Requirements and considerations:

All Tiers require a reference proteome, preferable from Uniprot or a similar source.

LC-MS Quantitation of target proteins

This experiment consists in the application of the developed assay to a (large) set of samples. Every change in targets or type of samples requires a new optimization.