For absolute quantitation of target proteins from a complex sample matrix, we typically use a strategy which relies on **isotopically labeled synthetic peptides** as internal standards. The workflow consists of the following steps:

1. Selection of Target Peptides (Performed by the Customer, with Core Consultation as Needed)

- Identify unique peptides (typically **tryptic peptides**) from the target protein as surrogates for protein quantitation
- Peptide selection criteria:
    - Must be **unique** to the target protein.
    - Should **not contain known post-translational modifications (PTMs)**.
    - Should **avoid reactive amino acids** (e.g., methionine; cysteine if possible).
    - Should demonstrate **good LC-MS sensitivity** (check **PeptideAtlas** for reference. More ideally, if the protein standard is available, an LC-MS analysis is preferred to confirm detectability.).
- Synthesize stable **isotope-labeled (heavy, e.g., ^{13}C-labeled) peptide analogs** for quantitation.
    - In most case uses 13C-6 arginine or lysine as the C-term residue

2. Proteomic Analysis (Performed by the Core Facility)

- **Method Development (Targeted MS Analysis)**
    - Develop and optimize a **customized targeted MS method** to enhance sensitivity and robustness.
        - Needs the isotope-labeled peptide standards and test samples (a positive control is needed) from the customer
        - Cost depends on **personnel time** and **instrument time** and may vary depending on the target protein.
- Analysis of the real samples
    - Perform sample digestion, spike in the heavy peptide standards, run LC-MS
    - **Perform quantitative comparison** of endogenous (light) and spiked-in isotope-labeled (heavy) peptides to calculate the absolute protein concentration based on the known amount of spiked-in peptides.