Information about TMT experiments (Isobaric Mass Tagging)

This labeling technique is performed on the peptide level, we can use almost any starting material provided that material in the microgram range can be prepared.
We can analyse a maximum of 18 samples/repeats in one experiment. For solid statistical analysis, each sample should be submitted as 3 biological replicates.
20 to 50 ug of sample per condition/repeat are optimal for a standard relative quantification of the proteome, for relative quantification of PTMs 10 times more material is required.

Cost estimation:

Our facility buys the TMT reagents (mainly as 18 plex reagents) in a larger batch size and aliquots them for single experiments. We charge the user the reagent costs per experiment, one aliquot including 18 channels costs approx. 600 Euro. For one proteome analysis we need 0.5 to 1 aliquot per channel, for a phosphoproteome analysis 1-2 aliquots are required, which is due to the higher amounts of total protein needed. One experiment will need several days of measurement time on an MS instrument.

Duration of the experiment:

Depending on the input material, which can be very diverse, being cell culture cells, different tissues, organoids or plant tissue, different sample preparation methods have to be adapted and applied. After the cell extract preparation and determination of the protein concentration, the samples are reduced, alkylated and digested in parallel. Thereafter, the samples are desalted and labelled with one of the TMT reagents. The labelling efficiency is checked by MS and the different samples are unified to one sample. In case a global phosphoproteome analysis is performed, the modified peptides are enriched at this point. Subsequently, to reduce the sample complexity, it is fractionated by either strong cation exchange chromatography or by high pH reversed phase chromatography. Fractions are collected and about 20-30 fractions (each taking about 3h measurement time by MS) are analyzed by LC-MS/MS. The recorded MS files are analyzed using the Proteome Discoverer software package applying different home-made software tools and including a bioinformatic analysis, which has to be adapted for each project. A proteome project will take 6 weeks, a phosphoproteome project 8-10 weeks in total including sample preparation and bioinformatic analysis (if everything runs perfect). Sample preparation is a critical issue and must be adapted for each project individually.

Relative Quantification by TMT *:
To understand the functions of individual proteins and their place in complex biological systems, it is often necessary to measure changes in protein abundance relative to changes in the state of the system. These measurements have traditionally been performed using Western blot analyses. More recently, modern proteomics has evolved to include a variety of technologies for the routine quantitative analyses of both known and unknown targets.

Discovery-based relative quantification is an analytical approach that allows the scientist to determine relative protein abundance changes across a set of samples simultaneously and without the requirement for prior knowledge of the proteins involved.

Thermo Scientific Tandem Mass Tag (TMT) reagents are isobaric chemical tags consisting of an MS/MS reporter group, a spacer arm and a reactive group. Amine-reactive NHS-ester groups covalently bind to peptide N-termini or to lysine residues. Each tag fragments during MS/MS, producing unique reporter ions. Protein quantitation is accomplished by comparing the intensities of the reporter ions.

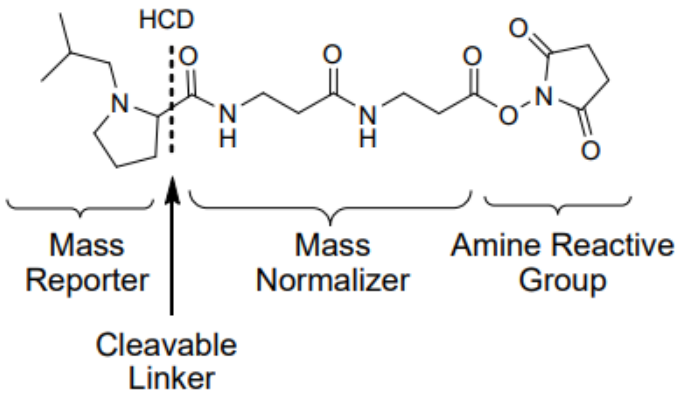
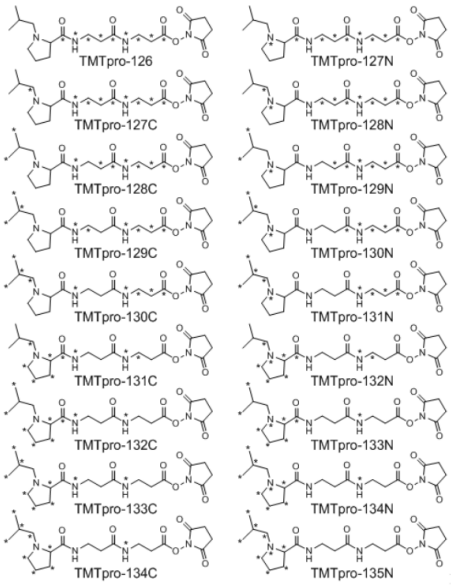


Figure 1. Chemical structures of the Thermo Scientific TMTpro Label Reagents. Each reagent within a set has the same nominal mass (i.e., isobaric). The chemical structure is composed of an amine-reactive NHS-ester group, a spacer arm and a mass reporter. A dashed line represents the MS/MS fragmentation site by higher-energy collision dissociation (HCD).

Table 2 Mass information and chemical structure for TMTpro Label Reagents

Label reagent	HCD Monoisotopic Reporter Mass[1]	Chemical structures and ^{13}C and ^{15}N stable isotope positions (*)
TMTpro-zero[2]	126.127726	—
TMTpro-126[3]	126.127726	
TMTpro-127N[3]	127.124761	
TMTpro-127C[3]	127.131081	
TMTpro-128N[3]	128.128116	
TMTpro-128C[3]	128.134436	
TMTpro-129N[3]	129.131471	
TMTpro-129C[3]	129.137790	
TMTpro-130N[3]	130.134825	
TMTpro-130C[3]	130.141145	
TMTpro-131N[3]	131.138180	
TMTpro-131C[3]	131.144500	
TMTpro-132N[3]	132.141535	
TMTpro-132C[3]	132.147855	
TMTpro-133N[3]	133.144890	
TMTpro-133C[3]	133.151210	
TMTpro-134N[3]	134.148245	
TMTpro-134C[4]	134.154565	
TMTpro-135N[4]	135.151600	

[1] HCD is a collisional fragmentation method that generates eighteen unique reporter ions from 126 to 135 Da
[2] Molecular formula = C19H30N4O6, molecular weight = 410.46 Da, modification formula = C15H25N3O3, modification mass (monoisotopic) = 295.1896.
[3] Molecular formula = C12[13C]7H30N2[15N]2O6, molecular weight = 419.4 Da, modification formula = C8[13C]7H25N[15N]2O3, modification mass (monoisotopic) = 304.2071.
[4] Molecular formula = C12[13C]8H30N3[15N]O6, molecular weight = 419.4 Da, modification formula = C7[13C]8H25N2[15N]O3, modification mass (monoisotopic) = 304.2135.

Figure2. TMTpro18plex reagent structures and isotope positions (*= C13 or N15). Sum of mass reporter and mass normalizer added to each peptide is 304 Dalton for each reagent. Upon MS/MS fragmentation by HCD distinct reporter masses from 126 to 134 can be detected.

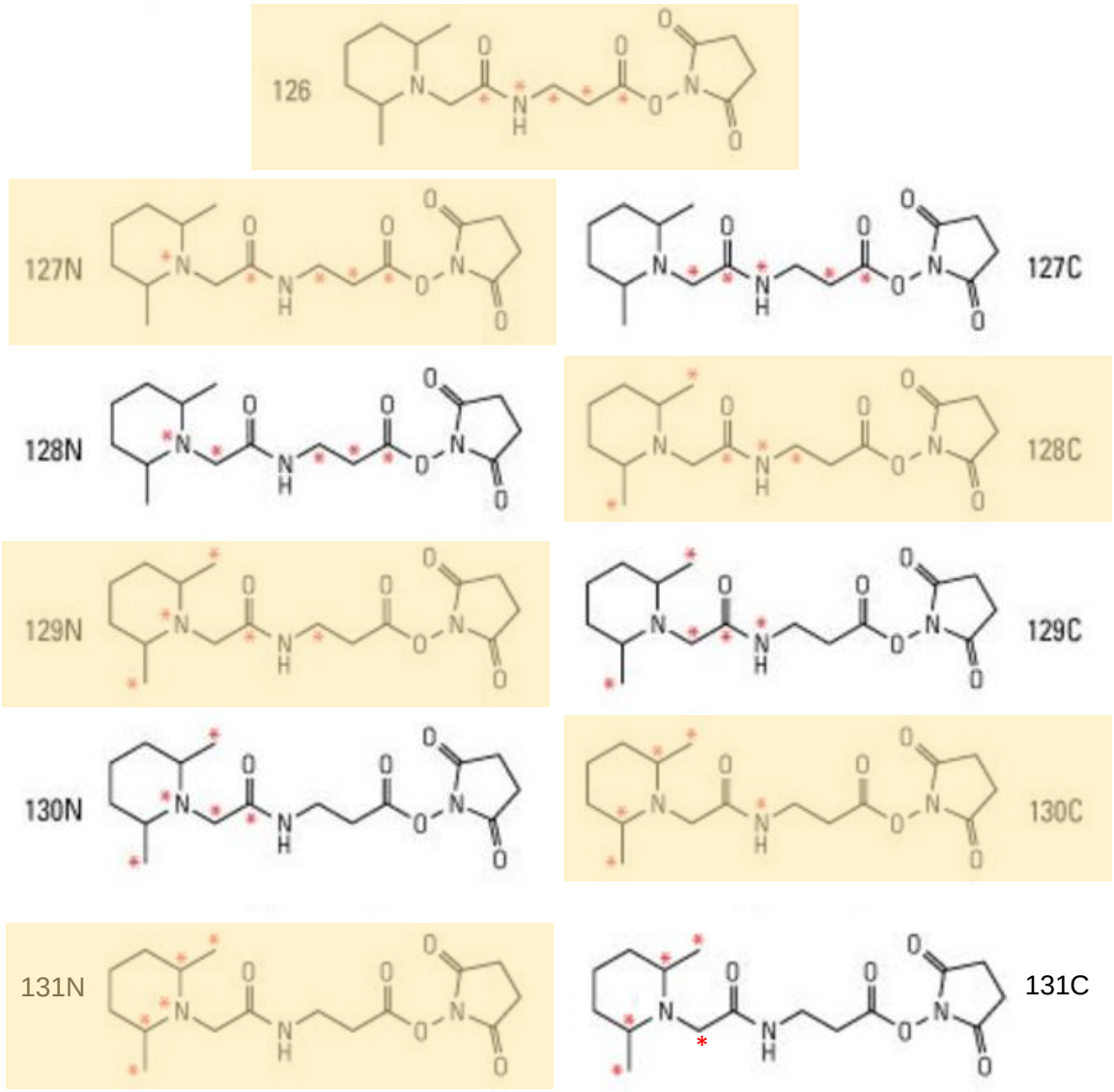


Figure3. TMT11plex reagent structures and isotope positions (*= C13 or N15). Sum of mass reporter and mass normalizer added to each peptide is 229 Dalton for each reagent. Upon MS/MS fragmentation by HCD distinct reporter masses from 126 to 131 can be detected. The TMT6plex reagent consists of the 6 yellow shaded structures.

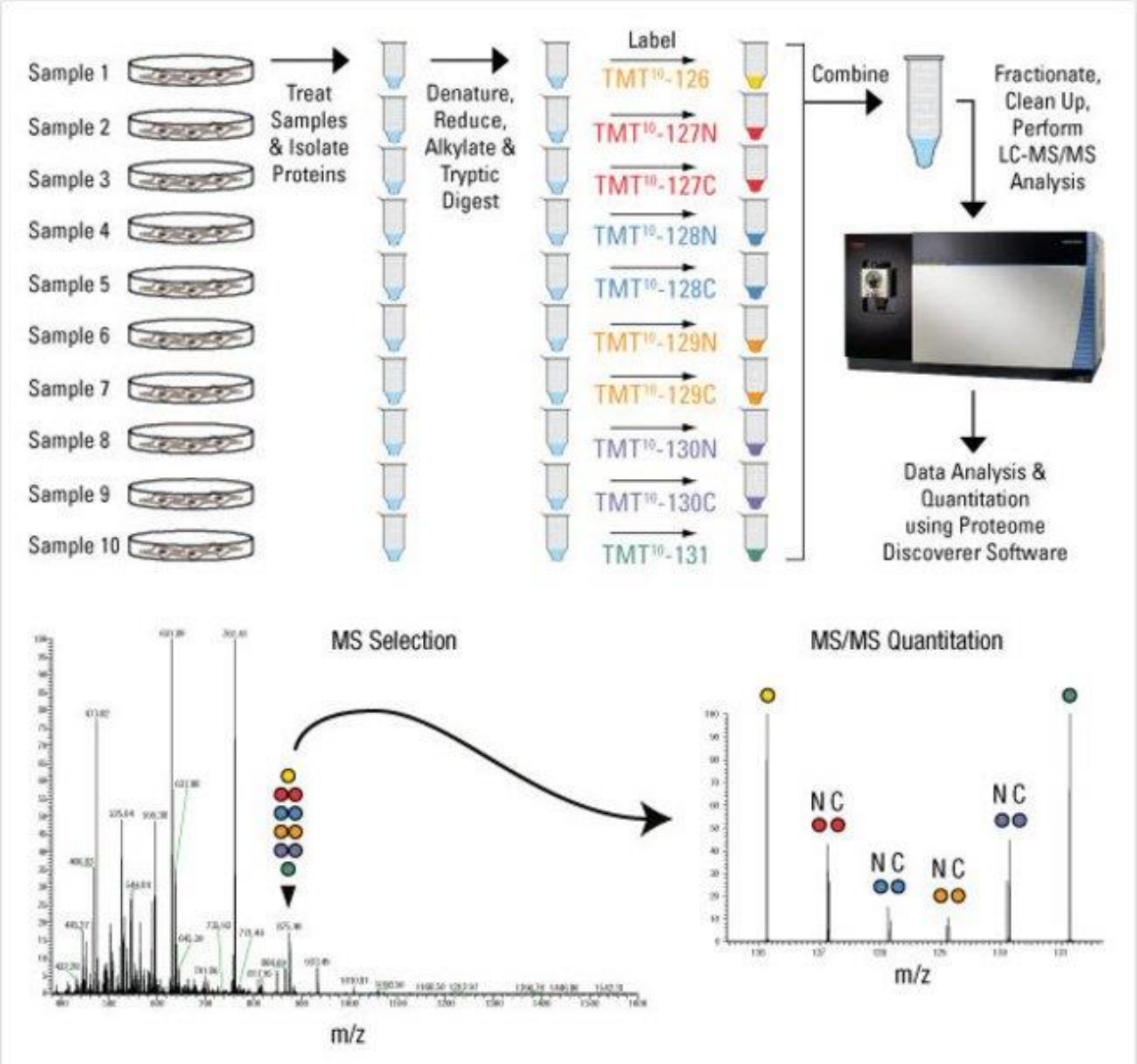


Figure 4. Procedure summary for MS experiments with TMT Isobaric Mass Tagging Reagents
Protein extracts isolated from cells or tissues are reduced, alkylated and digested overnight. Samples are labeled with the TMT Reagents and then mixed before sample fractionation and clean up. Labeled samples are analyzed by high resolution Orbitrap LC-MS/MS before data analysis to identify peptides and quantify reporter ion relative abundance. In the first MS analysis the corresponding peptides from each sample labelled with one of the 10 reagents are indistinguishable from each other, they are isobaric and their intensities sum up to one signal for each peptide. After the MS1 scan peptides are selected for fragmentation by MS/MS, peptide fragmentation results in sequence-specific ions used for identification and the fragmentation of the tag generates reporter ions unique for each sample. The relative abundance of the peptide in ten different samples is easily measured by comparing the respective reporter ion intensities.

*Text and pictures extracted and adapted from
https://www.thermofisher.com/at/en/home/life-science/protein-biology/protein-mass-spectrometry-analysis/protein-quantitation-mass-spectrometry/tandem-mass-tag-systems.html?cid=fl-tmtpro