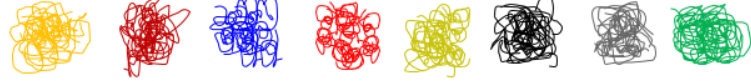


Isobaric labeling Quantification (iTRAQ/TMT/iodoTMT)

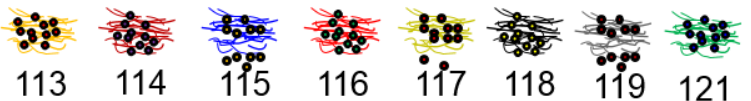
8 accurately quantified protein samples (iTRAQ 8plex)



Reduced, alkylated, and trypsin digestion



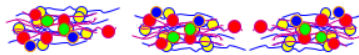
Label each peptide pool with an iTRAQ reagent



Pool peptide samples



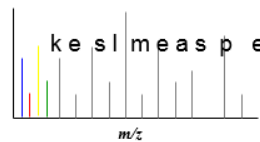
Samples are dried, desalted with C18 column, dried again and strong cation exchange fractionated usually around 12-18 fractions



nanoLC-MS-MS 2 hours for each cation fraction



Quantification and identification



Data analysis
ProteinPilot5.0/Mascot2.6/
Proteome Discoverer2.2
ProteolQ2.8/Scaffold Q+S
MaxQuant/TPP
processing software

iTRAQ technology uses different chemical tags which allows multiplexing of for up to eight samples and produces identical MS/MS sequencing ions for all four versions of the same derivatized tryptic peptide. The optimum sample amount is approximately 50-100 μ g per sample. The labeling is achieved at the peptide level. All the peptides are labeled. Quantitation is achieved by comparison of the peak areas and resultant peak ratios for the reporter ions in the MS/MS spectra, which reflect the relative abundance of the proteins.

Advantage of iTRAQ/TMT/SILAC:

- Avoid potential problems with 2D gel approach.
- Wider diversity of proteins analysed- hydrophilic, hydrophobic, acidic and basic proteins.
- Quantification at the peptide level.
- iTRAQ or TMT combined with multidimensional LC can analyze complex samples.
- Up to eight samples can be analyzed simultaneously.