

## 1. Sample preparation

### Qualitative

Protein identification  
Protein modifications  
Kinase Assays  
Immunoprecipitation  
Crosslinking/  
Interaction studies

### Relative Quantification

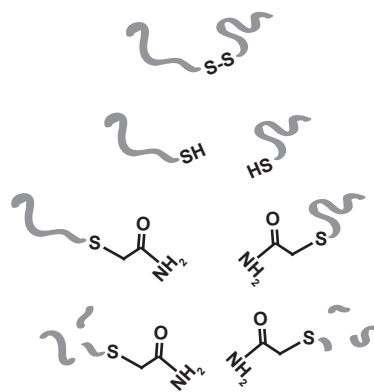
Metabolic labelling (SILAC)  
Chemical labelling (TMT)  
Unlabelled

### Absolute Quantification

comparison to a labelled peptide standard during mass spec (step 3)

## 2. Sample purification

### Paramagnetic beads (e.g. SP3)



### In-solution

↓  
**Reduction** (Dithiothreitol=DTT)  
↓  
**Alkylation** (Iodocetamide=IAA)  
↓  
**Digest** (e.g. Trypsin)  
↓  
**Cleanup** (C18, SP3, etc.)

### In-gel

↓  
Destaining

↙ ↘  
Extraction

## 3. Data analysis

### Variables

Mass spectrometer (LTQ Orbitrap, Q-Exactive, LTQ Velos, etc.)  
Chromatography, column length, ion source, length of run, mass analyser

### Software

(MASCOT, MaxQuant, Proteome Discoverer, etc.)

### Parameters (to set):

Correct Database  
Known modifications (reduction, alkylation)  
Sequence modifications (tags, mutations)  
Crosslinking parameters

### Parameters (data analysis):

Intensity, Peptide count, Coverage  
Statistical significance  
Normal distribution (proteomic data)  
Correlation (Biological replicates)