Quantitative Proteomics

EMBnet course

29 Jan, 2008

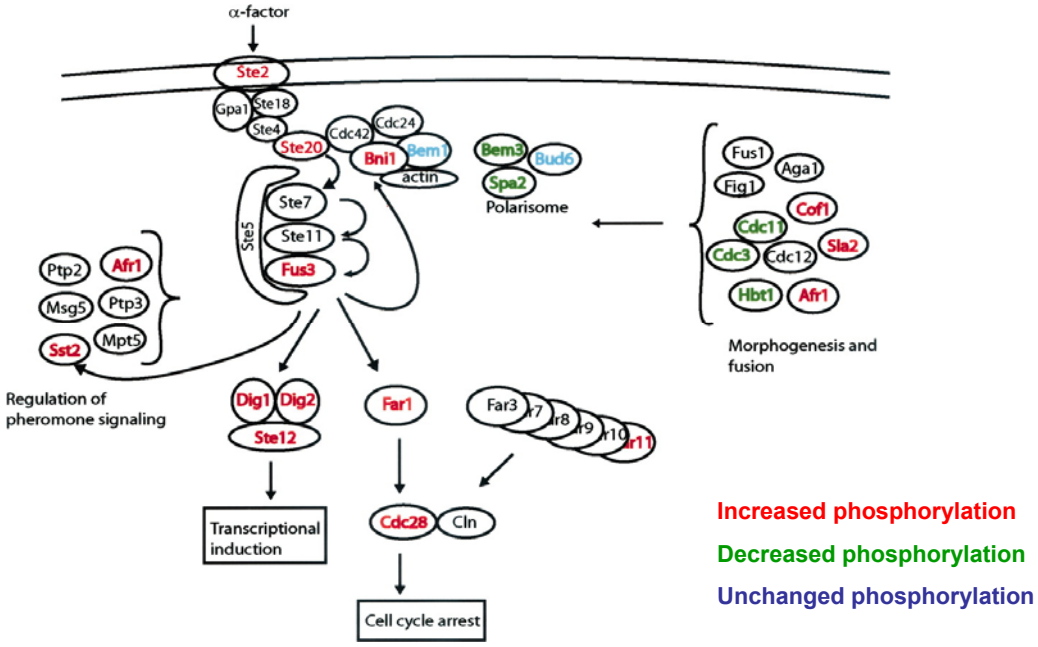
Why quantitative proteomics?

Table 1. Published studies using quantitative proteomics methods for identification of cancer biomarkers

Quantitative proteomic method	Cancer type	Features	References
SILAC-based quantitation	Prostate cancer	Differential proteomics for metastasis markers	Everley *et al.* [2]
	Breast cancer	Differential proteomics of membrane and membrane bound proteins	Liang *et al.* [4]
	Pancreatic cancer	Identification of secreted proteins as biomarkers	Gronborg *et al.* [3]
2-D PAGE	Hepatocellular carcinoma	Cleavage products of ER proteins as potential biomarker in serum	Chignard, *et al.* [55]
	Bladder cancer	Adipocyte-type fatty acid-binding protein expression associated with both tumor stage and tumor grade	Ohlsson *et al.* [14]
	Esophageal cancer	Combination of laser capture microdissection and DIGE approach to identify cancer specific proteins	Zhou *et al.* [56]
	Pancreatic cancer	Serum biomarker analysis using immunoaffinity followed by DIGE	Yu *et al.* [57]
iTRAQ labeling	Leukemia	Dysregulated proteins in bone marrow leukemic stem cells	Seshi [58]
	Lung cancer	Biomarkers for tumor metastasis	Keshamouni *et al.* [22]
cICAT labeling	Breast cancer	Differentially expressed proteins in nipple aspirate fluid	Pawlik *et al.* [59]
	Endometrial cancer	Biomarker identification from tumor tissue	DeSouza *et al.* [60]
	Liver cancer	Quantitative profiling of microdissected tissues	Li *et al.* [61]
	Pancreatic cancer	Quantitative analysis of pancreatic juice for candidate biomarkers	Chen *et al.* [17]
^{18}O labeling	Lung tumor	Low molecular weight proteins as biomarkers from serum of mice bearing cancer xenografts	Hood *et al.* [62]
	Breast cancer	Biomarkers in doxorubicin resistant and sensitive breast cancer cell lines	Brown and Fenselau [63]
	Breast cancer	Biomarker analysis of laser capture microdissected tissues from normal breast and ductal carcinoma.	Zang *et al.* [20]
Absolute quantitation	Prostate cancer	Serum analysis of PSA as biomarker	Barnidge *et al.* [28]
Label-free quantitation	Breast cancer	Semiquantitative study of patient sera	Ru *et al.* [64]
	Breast cancer	Accurate mass tag strategy	Patwardhan *et al.* [65]
Antibody arrays	Prostate cancer	Validation of thrombospondin (TSP-1) as a biomarker	Shafer *et al.* [37]
Bead-based arrays	Cervical cancer	Multiplexed analysis of serum	Waterboer *et al.* [46]
	Ovarian cancer	Quantitation of circulating IL-8 and anti-IL-8 autoantibodies	Lokshin *et al.* [66]
Aptamer arrays	Breast, lung, and colorectal cancers	RNA aptamers for estimation of VEGF as a biomarker	Li *et al.* [38]

Quantitative proteomics for identification of cancer biomarkers

Why do we need to quantify protein phosphorylation changes?



Quantitative phosphoproteomics applied to the yeast pheromone signaling pathway

How quantitative proteomics?

1. **Label-free**

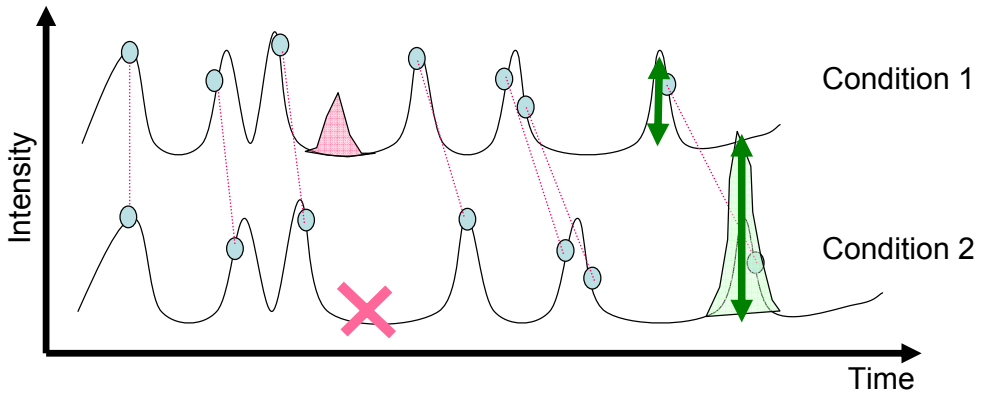
2. **SILAC (Stable Isotope Labelling with Amino acids in Cell culture)**

3. **iTRAQ (isobaric Tags for Relative and Absolute Quantitation)**

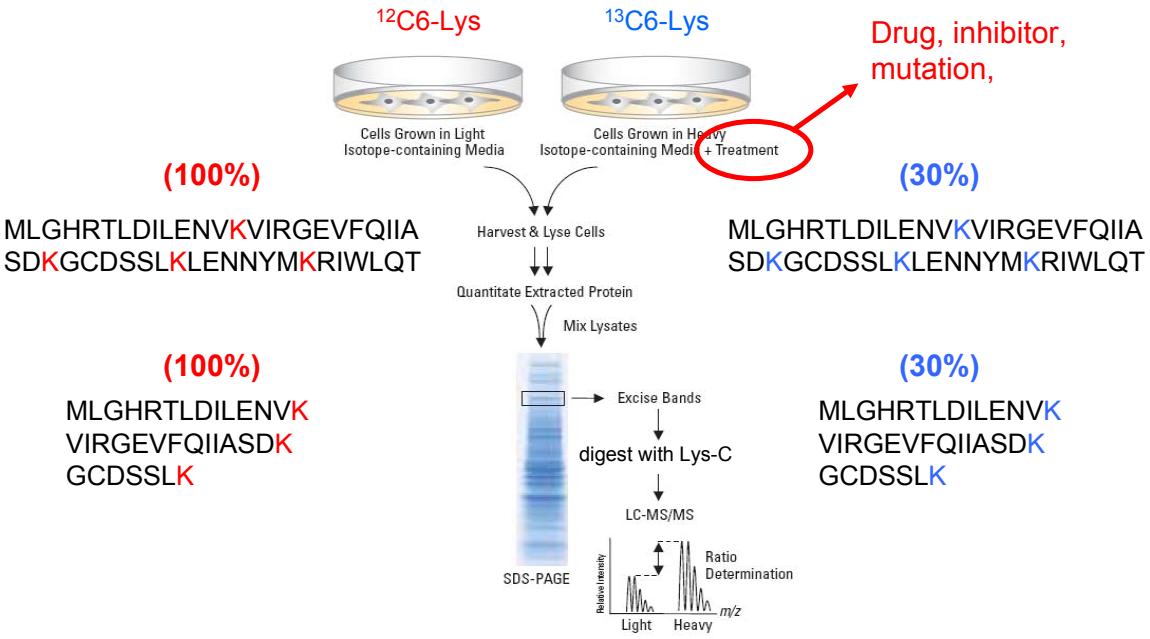
4.

Label-free approach

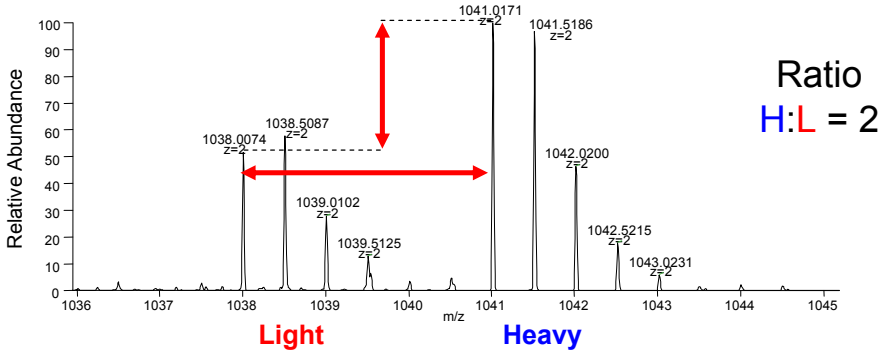
- Integration of extracted ion chromatogram peaks (Higgs et al., 2005)
- Spectral counting (Pang et al., Gao et al.)



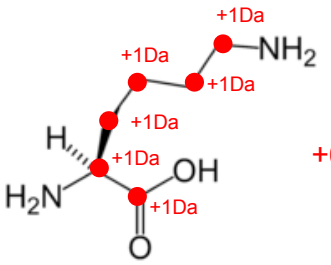
SILAC approach



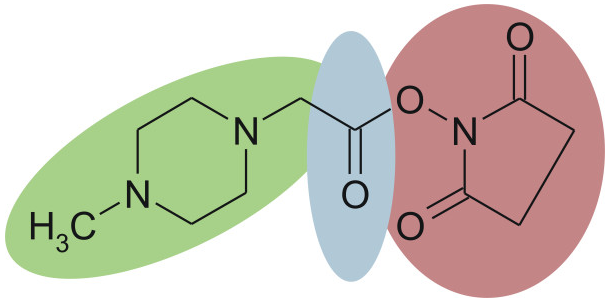
Representative MS spectrum generated using SILAC



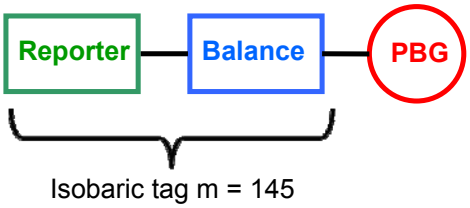
Ratio
H:L = 2



iTRAQ reagent structure



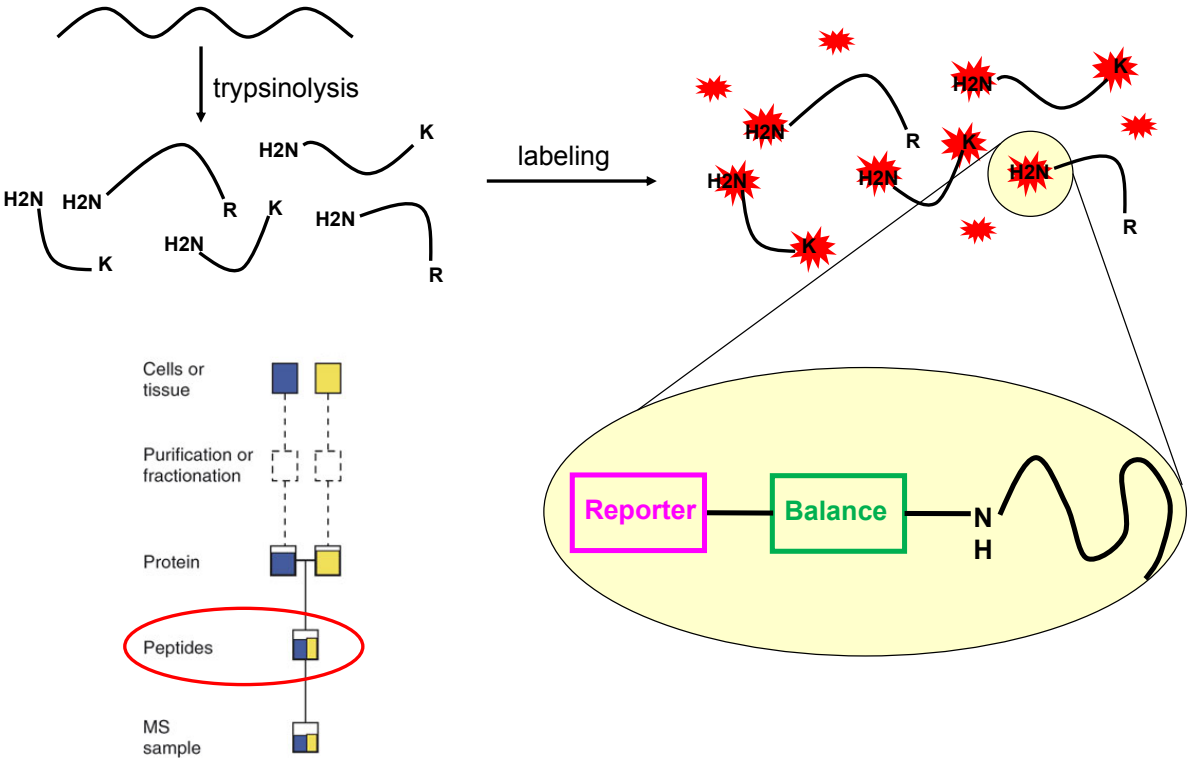
m = 114, 117 m = 31, 28



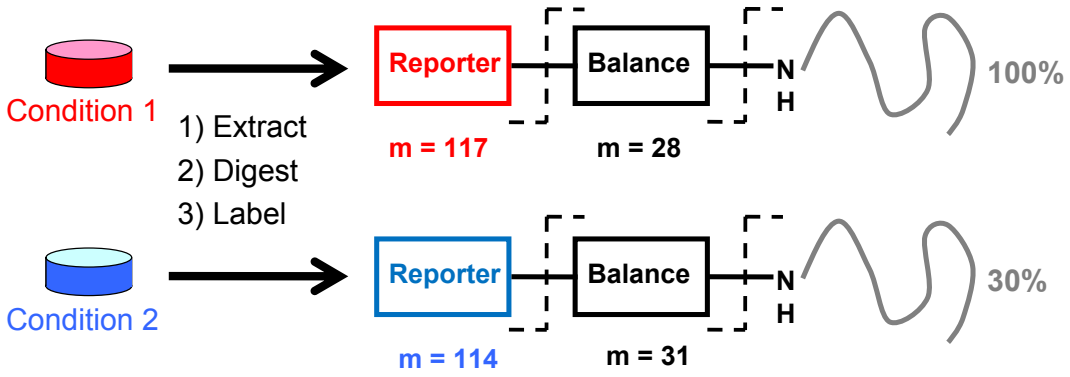
Isobaric tag m = 145

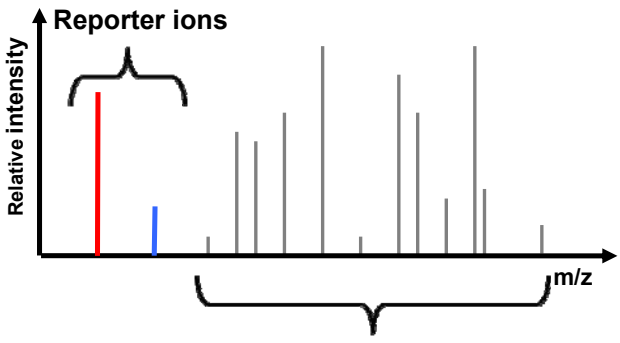
4 different iTRAQ reagents, 4 different reporters, same total mass, same behavior in MS

iTRAQ principle



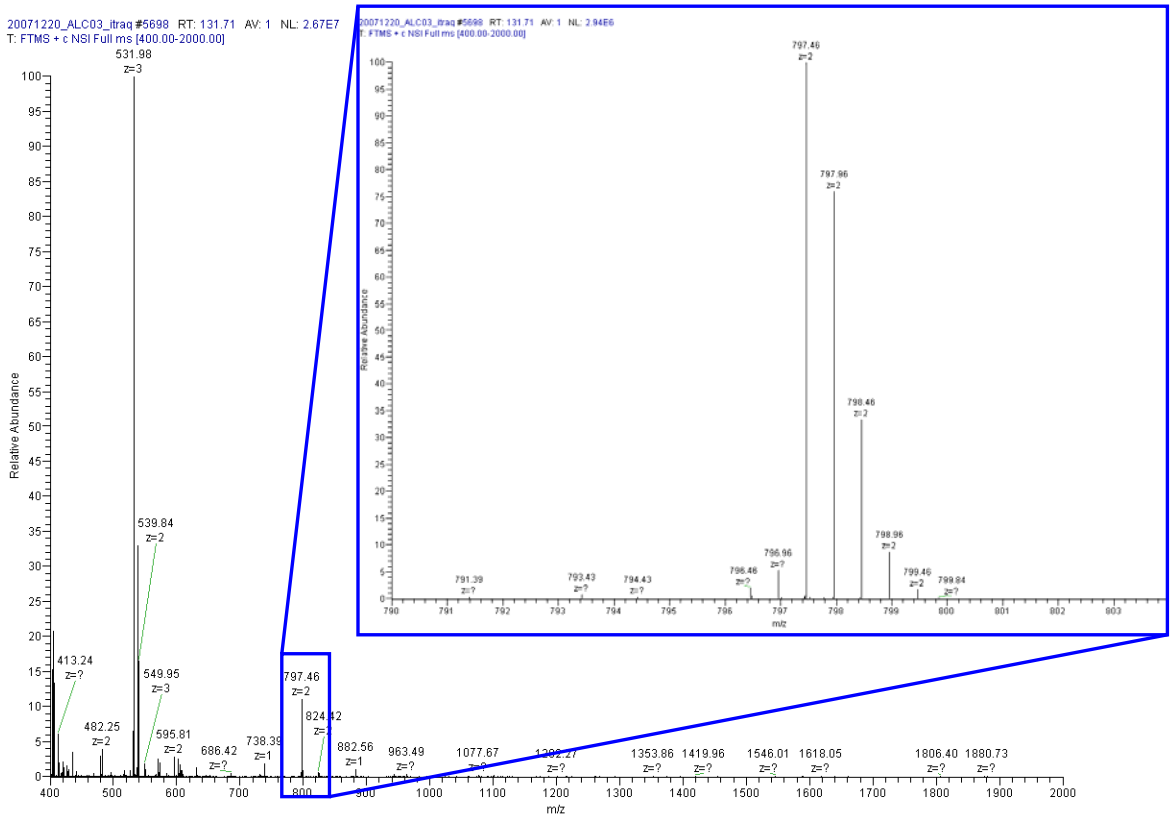
iTRAQ principle



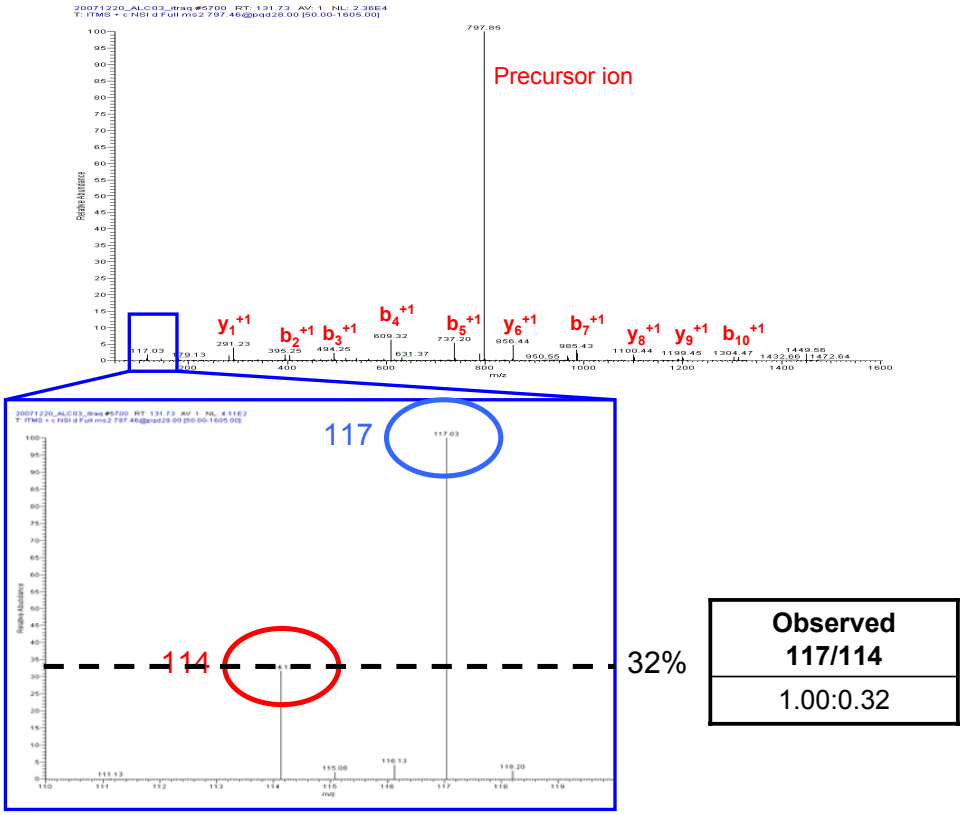


b- and y-ions with additive intensities

Representative MS spectrum generated using iTRAQ



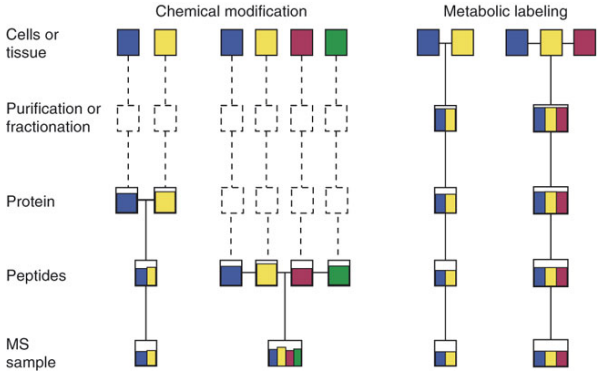
Representative MS² spectrum generated using iTRAQ



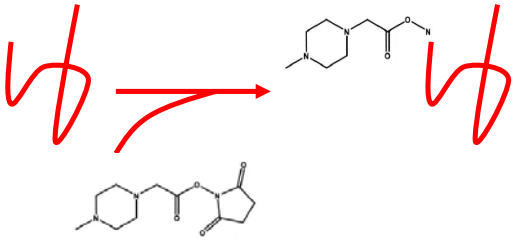
Observed 117/114
1.00:0.32

Which is the best approach?

SILAC requires living (and auxotrophic) organisms

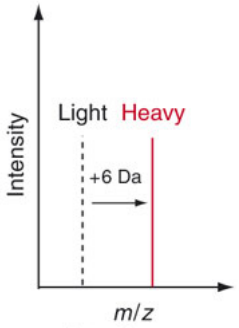


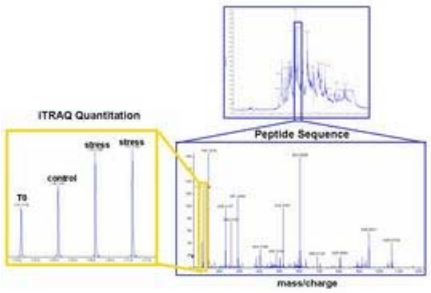
Fractionation and purification steps before iTRAQ labeling might introduce errors in quantitation



iTRAQ involves a derivatization step that might not be complete or introduce side products

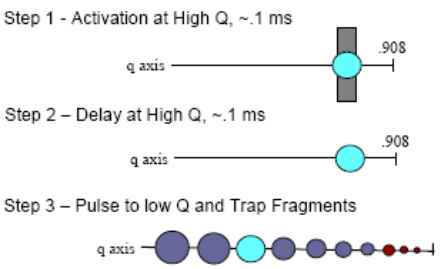
Which is the best approach?



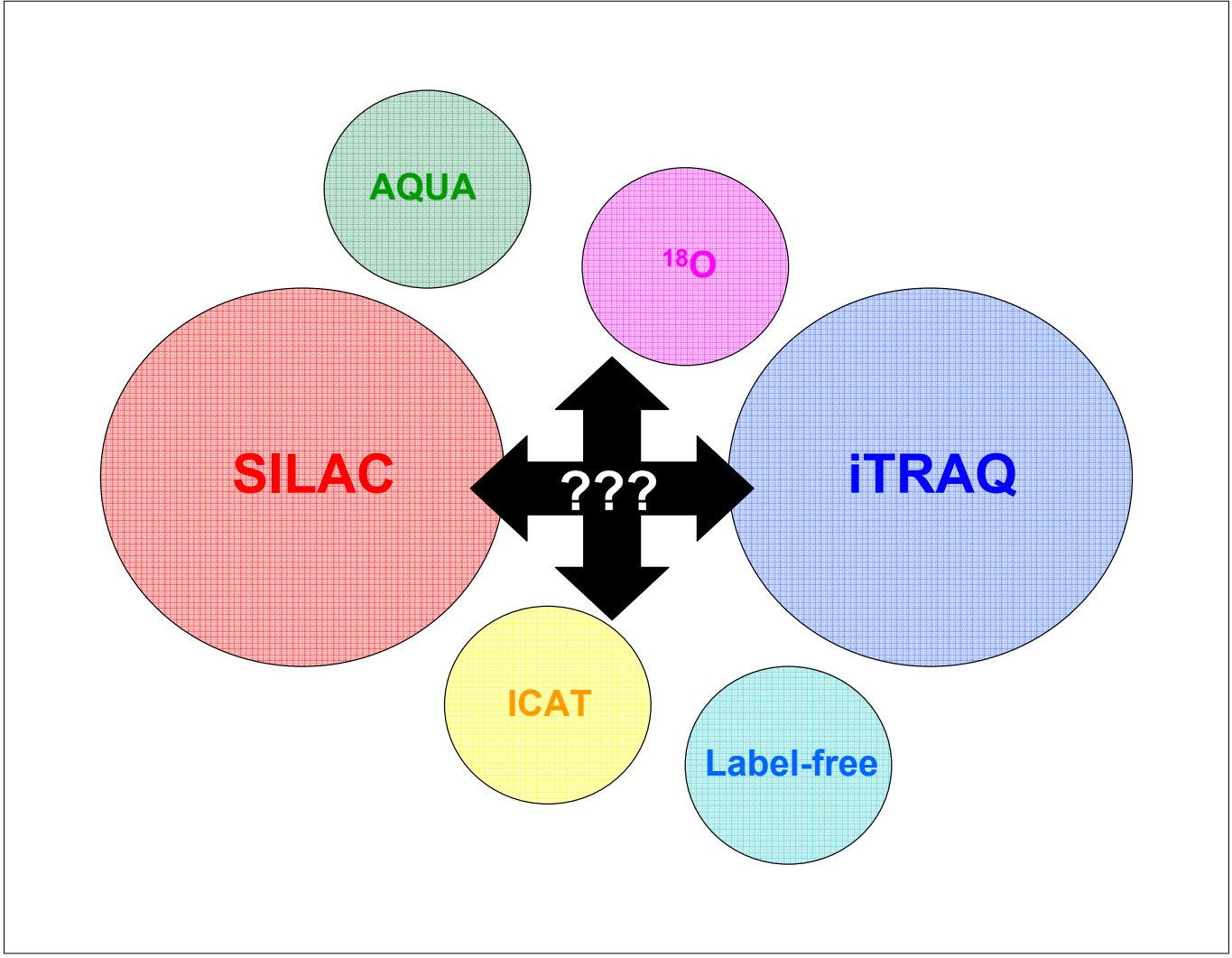


SILAC: quantitation in full MS, identification in MS/MS

iTRAQ: identification and quantitation in MS/MS



iTRAQ requires a new fragmentation mechanism (PQD)


AQUA
^{18}O
SILAC
???
iTRAQ
ICAT
Label-free