Proteomics Course

LECTURE-25
Quantitative Proteomics: iTRAQ and TMT

Dr. Sanjeeva Srivastava
IIT Bombay

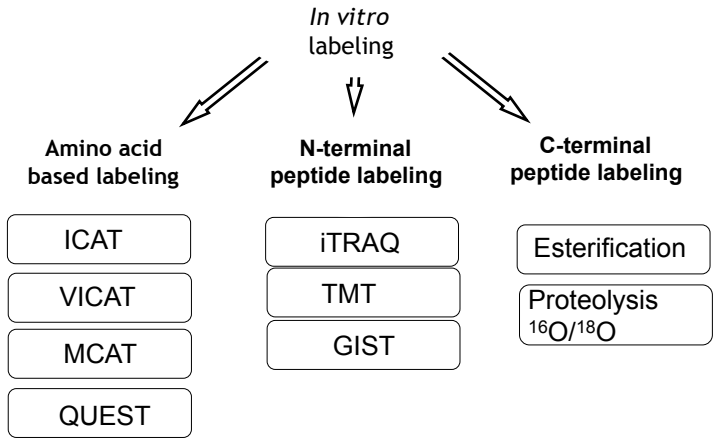
Today's lecture

- Quantitative proteomics: *in vitro* labeling
- iTRAQ method
- iTRAQ reagent
- iTRAQ experimental procedure
- Comparison of iTRAQ and ICAT
- Tandem Mass Tag (TMT)
- Comparison of iTRAQ and TMT

Quantitative Proteomics: *In vitro* labeling methods

Dass 2006; Robert et al. 2008

In vitro labeling



Isobaric Tagging for Relative and Absolute Quantification (iTRAQ)

Ross et al. 2004

iTRAQ method

- iTRAQ reagents are set of multiplexed, amine-specific, stable isotope reagents
- It enables simultaneous identification and quantitation, both relative and absolute
- There are two types of iTRAQ Reagents
  - 4-plex - for processing up to 4 samples
  - 8-plex - for processing up to 8 samples

iTRAQ method (2)

- In iTRAQ all derivatized peptides of *a given sequence* are isobaric and co-elute
  - *derived from control and treatment biological samples*
- Upon collision induced dissociation (CID) in MS/MS experiments, it provides reporter ions (signature) that differ in m/z value
  - Reporter ions can be used to monitor the relative quantitation for proteins

iTRAQ Reagent

iTRAQ reagent

- Components of iTRAQ multiplexed isobaric tagging chemistry

(1) Reporter group based on N,N-dimethylpiperazine

(2) Mass balance carbonyl group

(3) A peptide-reactive group (ester of N-hydroxysuccinimide, NHS)

iTRAQ reagent

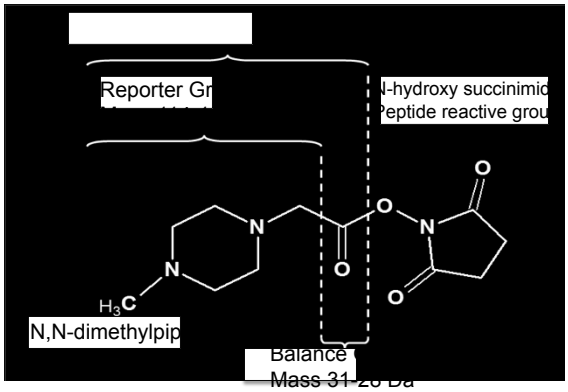
- The m/z value of the reporter group ranges from 114.1 - 117.1
- The balance group mass is 28 - 31 Da
- The overall mass of reporter plus balance components remains constant
  - 145.1 Da for all four reagents

iTRAQ reagent

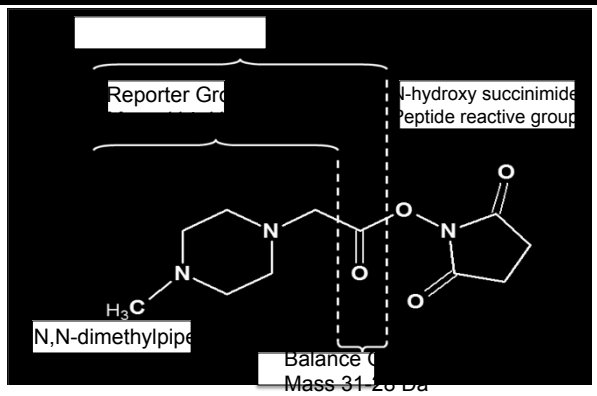
- When reacted with a peptide iTRAQ tag forms an amide linkage to any peptide amine
  - N-terminal or lysine amino group

iTRAQ reagent

- Isobaric tag consist of a reporter group, a neutral balance portion, and a peptide reactive group to give an overall mass of 145



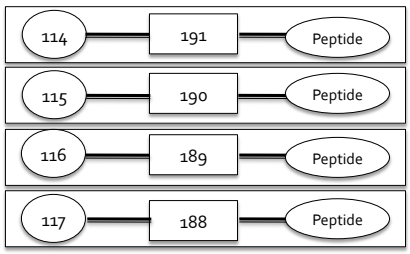
iTRAQ reagent



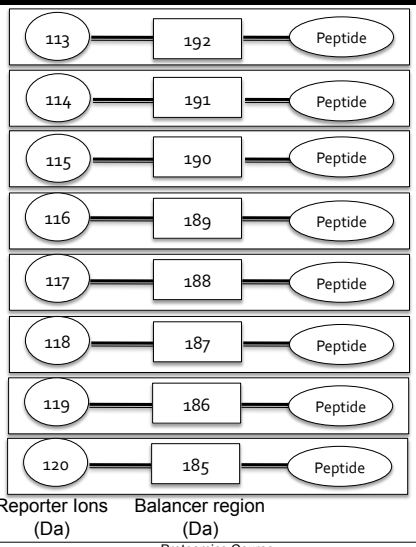
Reporter
- Provides signature ion in MS/MS
- Provides good b and y - ion series
- Maintains the charge state and ionization efficiency of peptide

Balancer
- Balances mass change of reporter to provide total mass of 145
- Neutral loss in MS/MS

iTRAQ reagent: 4-plex



iTRAQ reagent: 8-plex



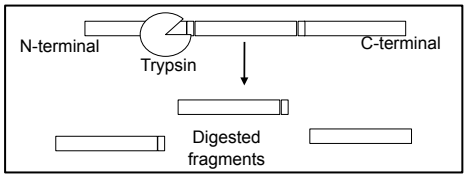
iTRAQ Experiment

Sample Preparation:
Protein reduction and cysteine blocking

- Dissolve protein sample in 0.5 M triethyl ammonium bicarbonate, pH 8.5
- Reduction step by adding a reducing agent
- Incubate samples at 60°C for 1H
- Block cysteine by adding a Cysteine Blocking Reagent

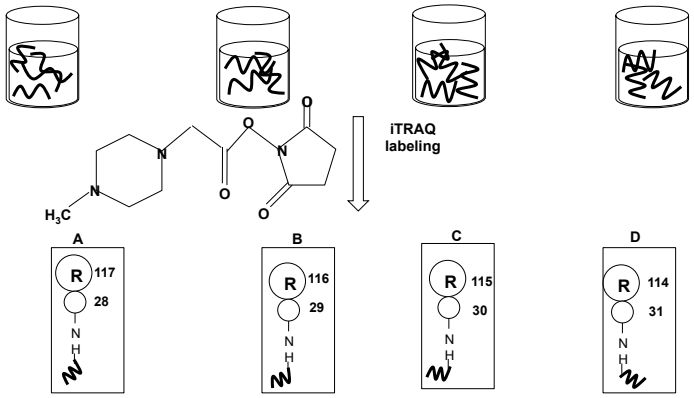
Sample Preparation:
Protein digestion

- Add trypsin solution
- Incubate overnight at 37°C
- Clean-up samples using ZipTip



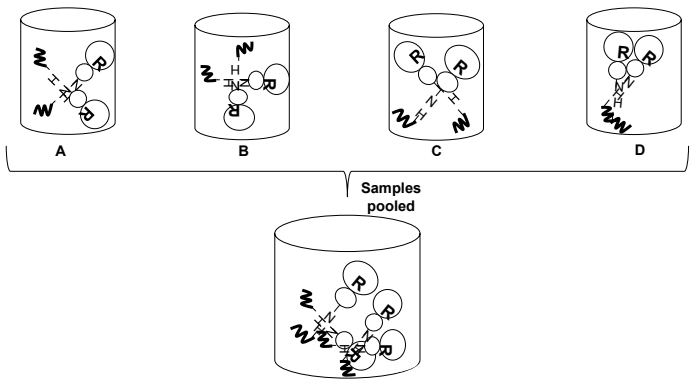
Sample Preparation:
Labeling

- Reconstitute the iTRAQ reagent in isopropanol
- Add iTRAQ reagent to digested protein sample



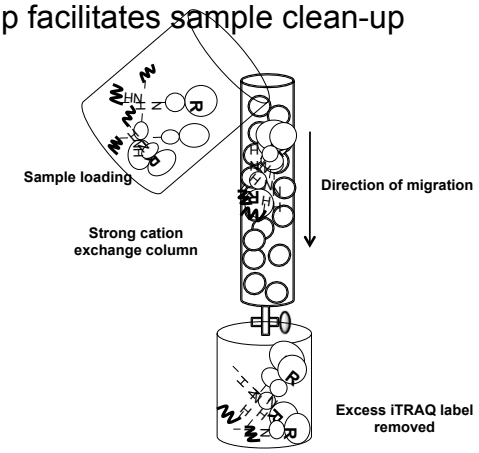
Sample Preparation:
Pooling labeled samples

- Combine labeled samples in one tube

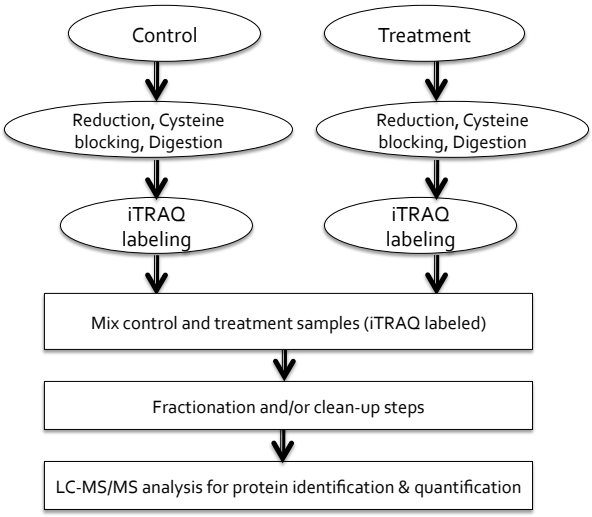


Sample Preparation: Purification

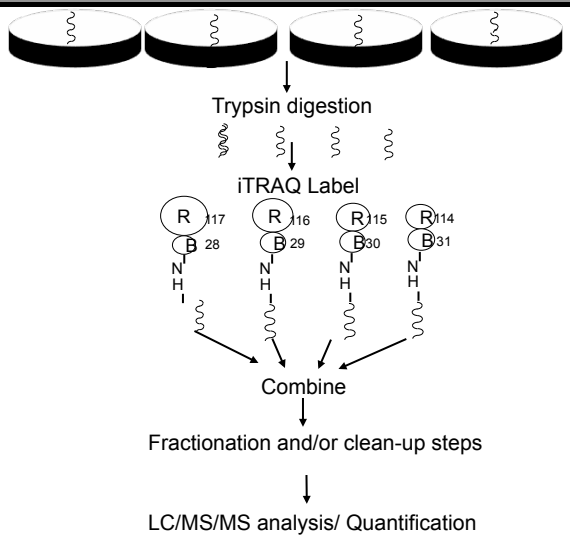
- Pooled samples are purified on a strong cation exchange column to remove excess unbound reagent
- This step facilitates sample clean-up



Overview of protocol



Overview of protocol

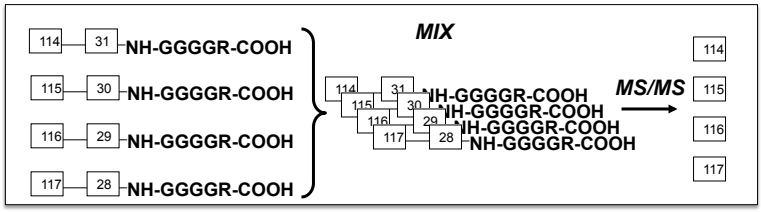


iTRAQ: MS analysis

- Peptides differentially labeled, mixed together, measured by MS
- Enables simultaneous identification and quantitation
- Labels react with N-terminus
- Reporter group is lost during fragmentation
- Used to determine relative abundance of selected peptide of interest from 4 or 8 samples

iTRAQ: MS analysis

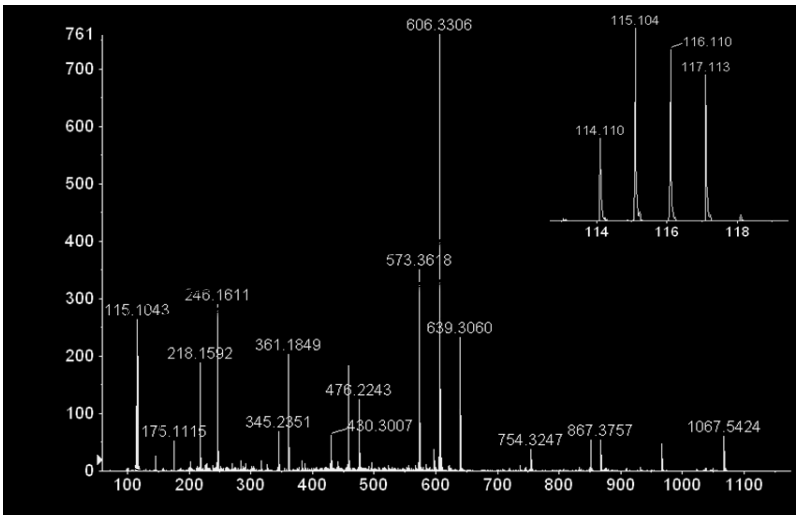
- Samples with 4 independent reagents of same mass (145) give rise to four unique reporter ions (m/z = 114–117) in MS/MS, and subsequently used to quantify different samples



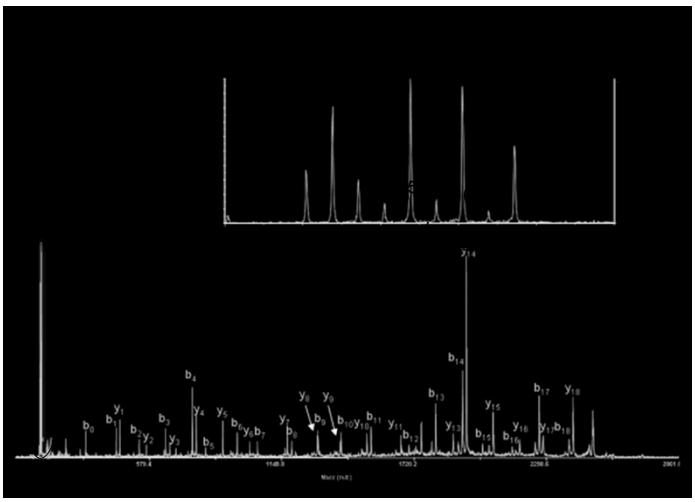
iTRAQ: MS analysis

- Quantification occurs at the level of fragment ion spectrum (MS/MS)
- Identification and quantification of peptide are achieved at MS/MS level

iTRAQ: 4-plex MS data



iTRAQ: 8-plex MS data



iTRAQ Technique
Animations

iTRAQ advantages

- Performs relative (or absolute) quantification in up to 4 or 8 samples
- Multiplexing
- Increased analytical precision and accuracy
- Expanded coverage of proteome by tagging tryptic peptides
- Eliminates limitation of ICAT for dependence on cysteine

iTRAQ disadvantages

- Possible errors in quantitation due to
  - differences in efficiency of enzymatic digestion
  - peptide pre-fractionation step
- Variability in initial protein digestion
  - tagging is performed only after individual sample processing is done, which leads to some variations

iTRAQ disadvantages (2)

- Reagents are very costly
- New search algorithms and databases required

iTRAQ vs. ICAT

- Both ICAT and iTRAQ permits identification and quantifications of proteins
- ICAT amino acid based labeling; iTRAQ on primary amine groups
- ICAT labeling has advantage to reduce sample complexity by eliminating nonlabeled/ noncysteine-containing peptides
- iTRAQ – 4 or 8-plexing; ICAT – only 2 samples

iTRAQ vs. ICAT

- iTRAQ method provides more complete coverage of original protein sequence than ICAT
- iTRAQ - increased confidence in identification
- iTRAQ - saves MS run time

iTRAQ applications
Animations

Tandem Mass Tag (TMT)

Ref: Tandem mass tags: a novel quantification strategy for comparative analysis of complex protein mixtures by MS/MS. Anal Chem. 2003 Apr 15;75(8):1895-904.

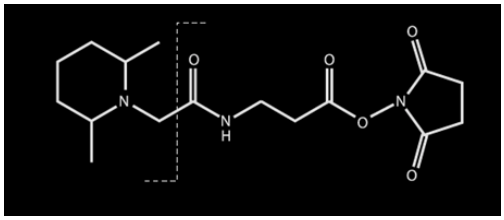
TMT

- TMTs are based on similar principle, with up to 6 possible labels
- TMT isobaric tagging technique can be used to perform absolute quantitation by adding stable isotope labeled internal standard peptides

TMT mass tags

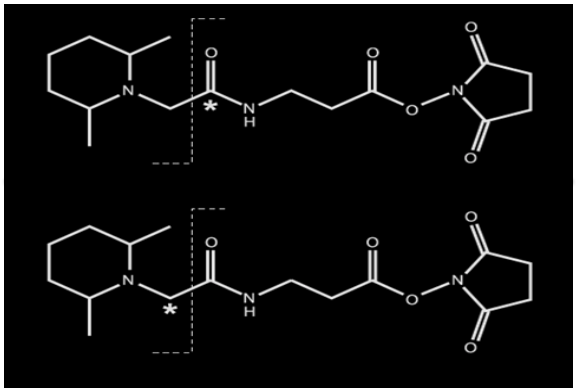
- N-terminal amine and lysine residues labeled through NHS group
- Family of chemical tags based on common structure
  - TMT^0: method development
  - TMT^2: 2-plex profiling and quantitation
  - TMT^6: 6-plex profiling and quantitation

TMT^0



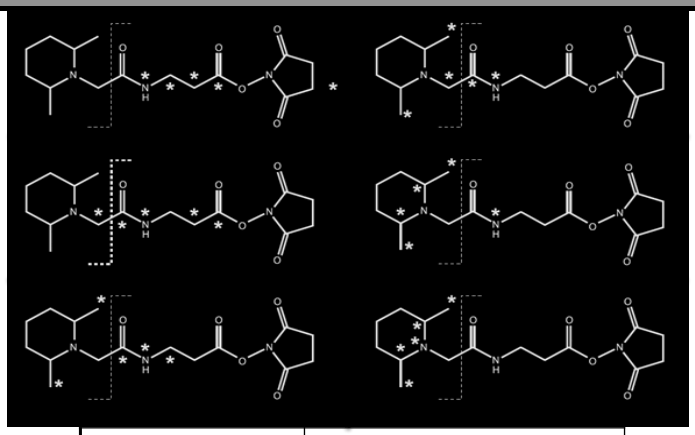
Modification	224 Da
MS/MS Reporter	126 Da

TMT^2



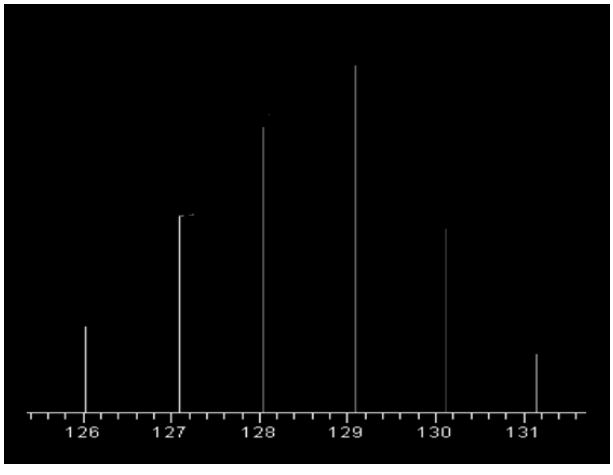
Modification	225 Da
MS/MS Reporter	126, 127 Da

TMT^6



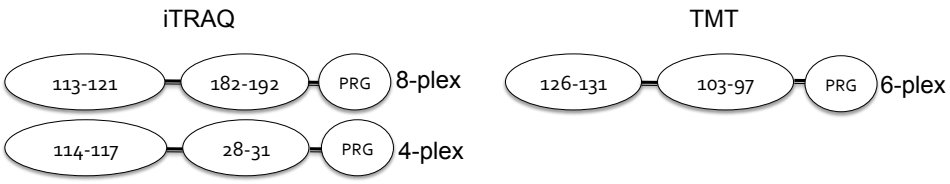
Modification	229 Da
MS/MS Reporter	126, 127, 128, 129, 130, 131 Da

TMT: MS data



MS/MS spectrum of TMT-labeled peptide, showing reporter region

iTRAQ vs. TMT



iTRAQ software
Protein Pilot
Mascot

TMT software
Proteome Discoverer
Mascot

Summary

- iTRAQ technique
- Experimental procedure
- Comparison of iTRAQ with ICAT and TMT

REFERENCES

- Robert K. Boyd, Cecilia Basic, Robert A. Bethem. 2008. Trace Quantitative Analysis by Mass Spectrometry. Chapter 11. Examples from the Literature DOI: 10.1002/9780470727140.ch11. John Wiley & Sons, Ltd
- Ross PL et al. Multiplexed protein quantitation in Saccharomyces cerevisiae using amine-reactive isobaric tagging reagents. Mol Cell Proteomics. 2004 Dec;3 (12):1154-69.
- Chhabil Dass. 2006. Fundamentals of Contemporary Mass Spectrometry. DOI: 10.1002/9780470118498.ch8. John Wiley & Sons, Inc
- Monica H. Elliott, Derek S. Smith, Carol E. Parker, 2009. Christoph Borchers. Current trends in quantitative proteomics. DOI: 10.1002/jms.1692. Journal of Mass Spectrometry. Volume 44, Issue 12, pages 1637–1660, December 2009.
- Wei Yan and Sharon S. Chen. Mass spectrometry-based quantitative proteomic profiling. Briefings in Functional Genomics and Proteomics (2005) 4 (1): 27-38.

REFERENCES

- Ross PL *et al.* Multiplexed protein quantitation in Saccharomyces cerevisiae using amine-reactive isobaric tagging reagents. Mol Cell Proteomics. 2004, 3, 1154-69.
- Thompson A *et al*. Tandem mass tags: a novel quantification strategy for comparative analysis of complex protein mixtures by MS/MS. Anal Chem. 2003, 75, 1895-904.
- Wiese S, Reidegeld KA, Meyer HE and Warscheid B. Protein labeling by iTRAQ: a new tool for quantitative mass spectrometry in proteome research. Proteomics. 2007, 7, 340-50.
- Beck F *et al*. Robust workflow for iTRAQ-based peptide and protein quantification. Methods Mol Biol. 2012, 893, 101-13. Methods Mol Biol, 2012, 893, 101-13.
- Liang S *et al*. Quantitative proteomics for cancer biomarker discovery. Comb Chem High Throughput Screen. 2012, 15, 221-31..

REFERENCES

- Kristin LM Boylan, John D Andersen, Lorraine B Anderson, LeeAnn Higgins and Amy PN Skubitz*Quantitative proteomic analysis by iTRAQ® for the identification of candidate biomarkers in ovarian cancer serum. Proteome Science 2010, 8:31
- Nobuhiro Takahashi, Toshiaki Isobe 2007. Proteomic Biology Using LC-MS: Large Scale Analysis of Cellular Dynamics and Function. Chapter 2. Proteomic Tools for Analysis of Cellular Dynamics DOI: 10.1002/9780470149652.ch2
-

ACKNOWLEDGMENT

- Mr. Sangram Patnaik, ThermoFisher for information related to TMT tags