BMG 744 Proteomics-Mass Spectrometry

Quantitative analysis of the proteome

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Proteomics Data Standards

- 2005 MCP - Paris guidelines
- 2008 HUPO – MIAPE and mzML
- 2008 NCI - Amsterdam principles (6)
- 2011 NCI – Sydney
  - For users of public data
  - Reviewers of journals
  - Multi-site projects with unpublished data

Kissinger et al MCP 10:1-9, 2011

Proteomics Data Standards

- Common descriptive terms
- Sufficient experimental description
- Data format
- Data quality
  - Mass accuracy (evidence of calibration)
  - Repeatability (technical and biological replicates)
  - False discovery rate (MRM and pseudoMRM)
  - Degeneracy of MRM
  - # of peptides to make a match
- Reference materials

Kissinger et al MCP 10:1-9, 2011

Quantitative proteomics

Use of isotopes

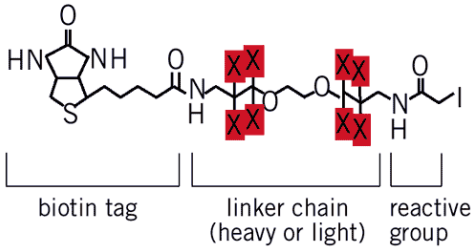
- ICAT (d_o/d_8) and ICAT $^{13}C_0/^{13}C_8$
- d_0/d_{10} propionic anhydride (N-terminal labeling)
- $^{15}N/^{14}N$ (whole cell labeling)
- $^{18}O/^{16}O$ (trypsin)
- iTRAQ labeling

- Non-isotope methods
  - Peptide coverage
  - Classical triple quadrupole methods

Isotope-coded affinity technology

Isotope-Coded Affinity Tags

heavy reagent: D8-ICAT Reagent (X=deuterium)
light reagent: D0-ICAT Reagent (X=hydrogen)



This reagent reacts with cysteine-containing proteins (80-85% of proteome)

Labeling can be replacement of hydrogens (X) with deuterium, or better to exchange ^{12}C with ^{13}C in the linker region (this avoids chromatography issues)

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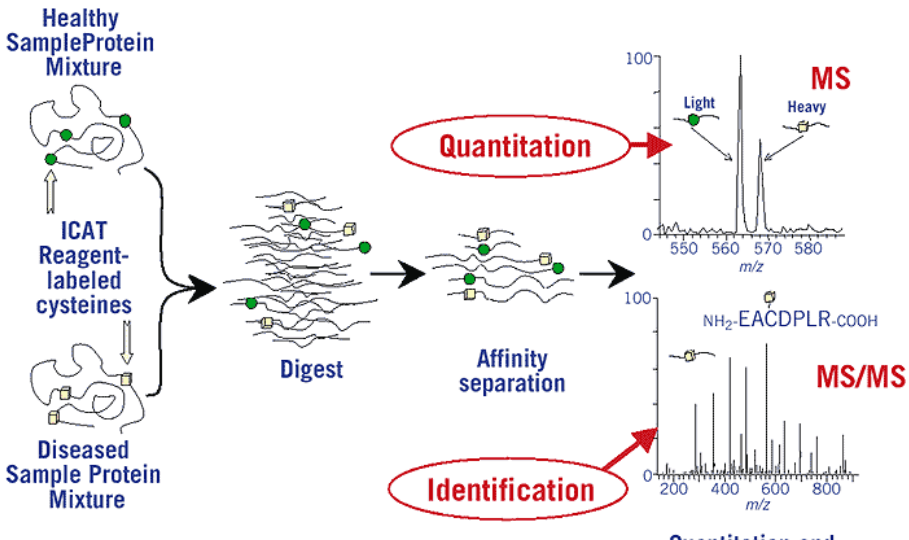

Credit: Dr. Ruedi Aebersold
Institute for Systems Biology, Seattle, WA

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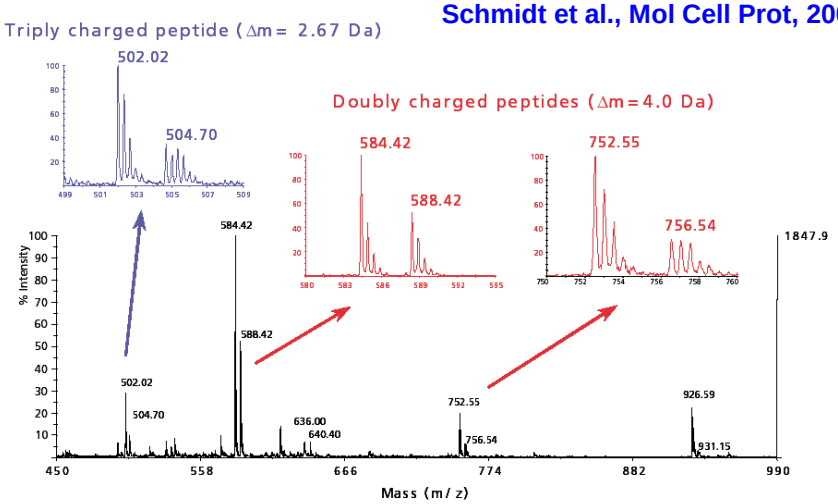
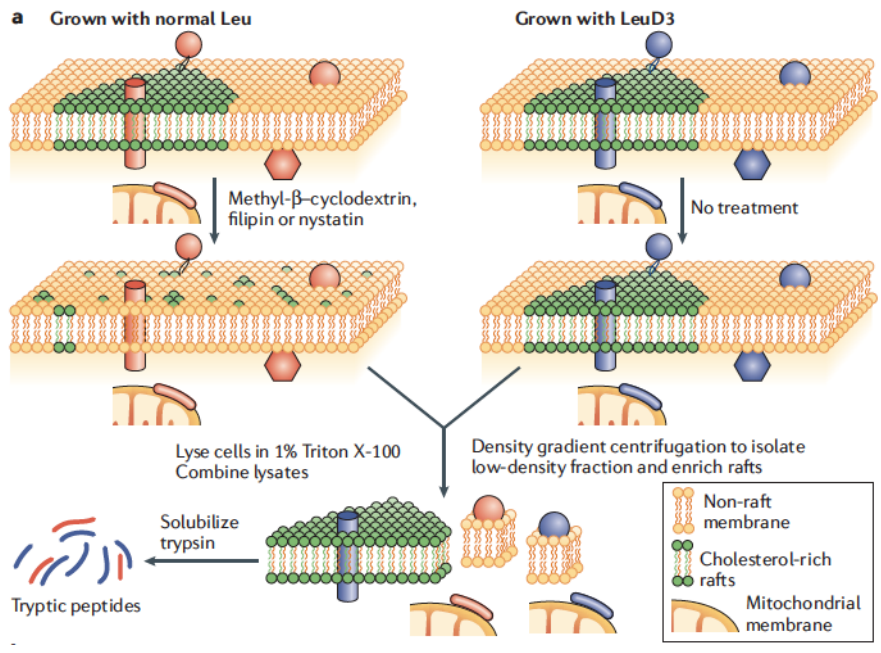
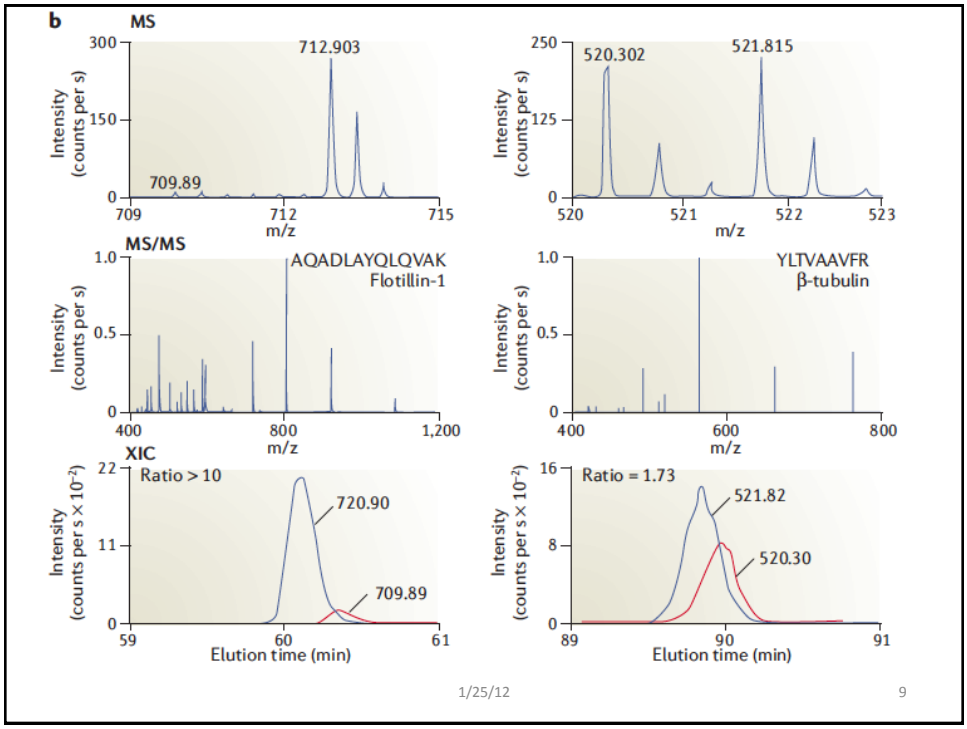
Quantification from ESI-mass spectrum

Schmidt et al., Mol Cell Prot, 2003






b
MS
712.903
709.89
Intensity (counts per s)
m/z
520.302
521.815
MS/MS
AQADLAYQLQVAK
Flotillin-1
YLTVAAVFR
β-tubulin
XIC
Ratio > 10
720.90
709.89
Ratio = 1.73
521.82
520.30
Intensity (counts per s × 10⁻²)
Elution time (min)
1/25/12
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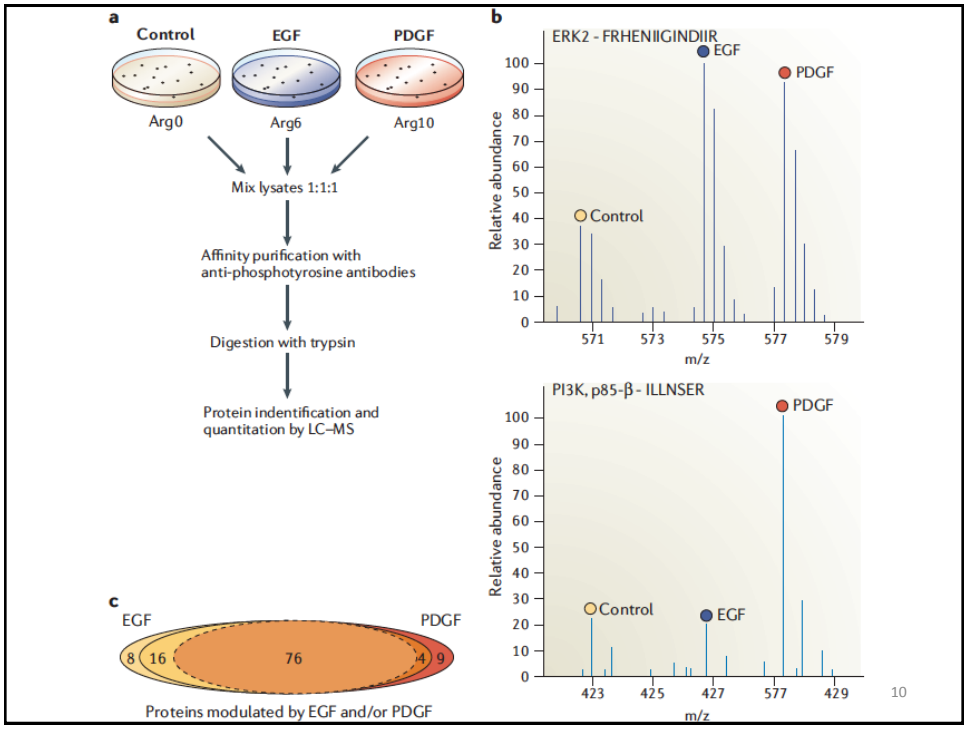
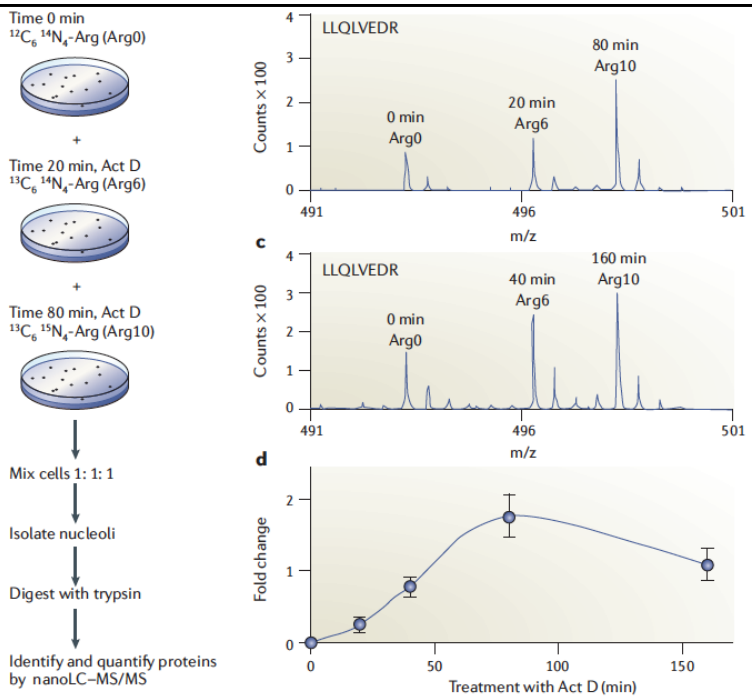

a
Control
EGF
PDGF
Arg0
Arg6
Arg10
Mix lysates 1:1:1
Affinity purification with anti-phosphotyrosine antibodies
Digestion with trypsin
Protein indentification and quantitation by LC–MS
b
ERK2 - FRHENIIGINDIIR
EGF
PDGF
Control
Relative abundance
m/z
PI3K, p85-β - ILLNSER
c
EGF
PDGF
8
16
76
4
9
Proteins modulated by EGF and/or PDGF
10



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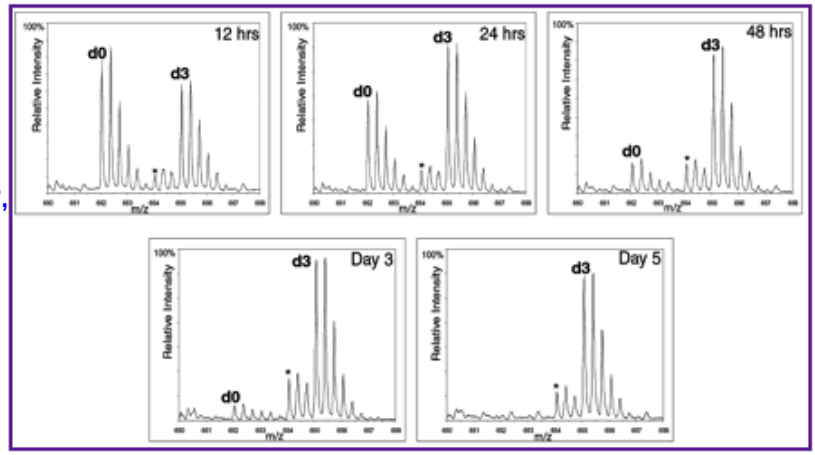
Time-dependent leucine incorporation with SILAC

Ong et al., MCP 1:367, 2002



The cells are pre-labeled with leucine-d_0. Leucine-d_3 is added to the medium and cells sampled at various times later. The peaks annotated with d_0 and d_3 are the triply charged peaks of the peptide VAPEEHPVLLTEAPLNPK, which contains three leucines.

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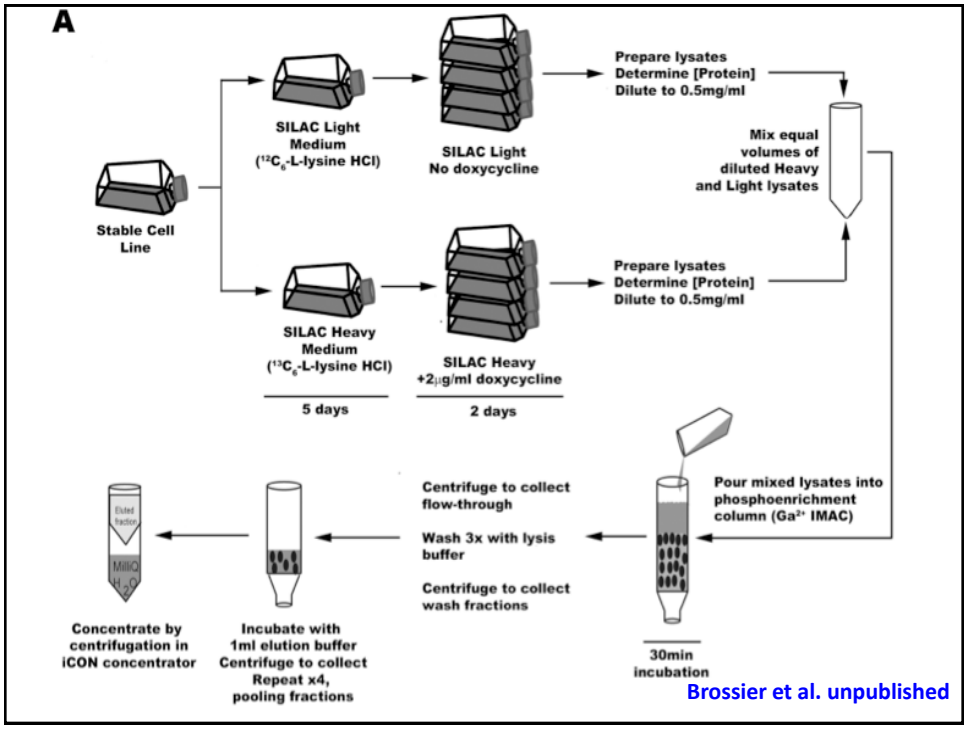
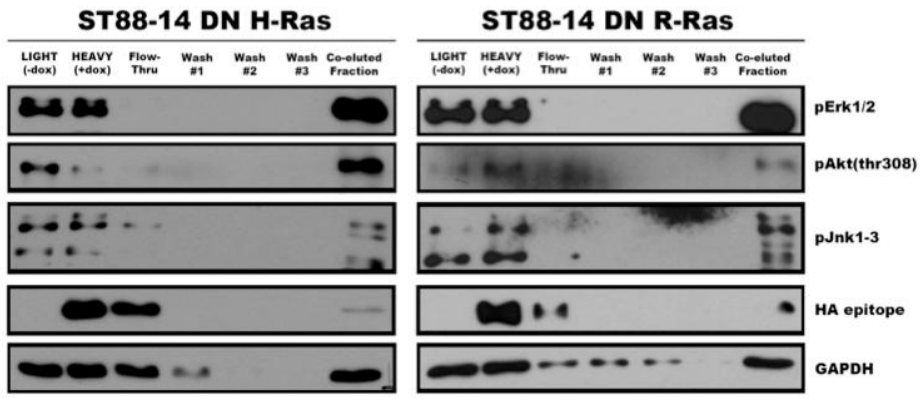
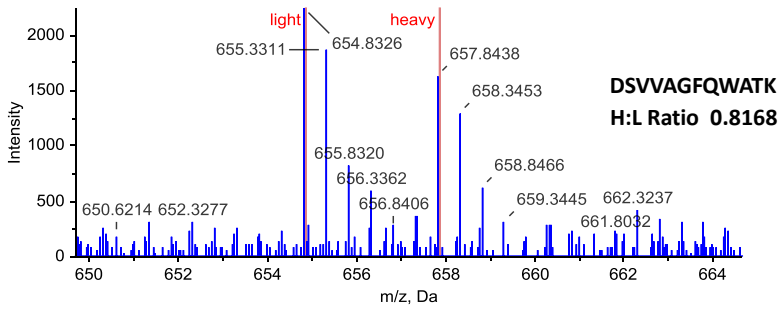


Brossier et al. unpublished

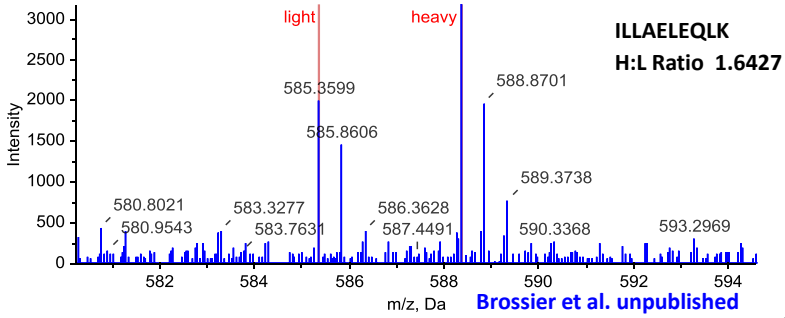
Verifying absorption of phosphoproteins onto IMAC



Brossier et al. unpublished



Elongation factor 2 OS=Homo sapiens GN=EEF2 PE=1 SV=4



Vimentin OS=Homo sapiens GN=VIM PE=1 SV=4

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^{18}O-labeling

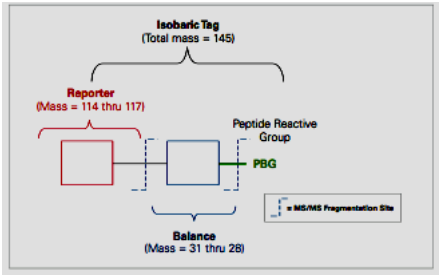
- Trypsin catalyzes the transfer of ^{18}O in ^{18}O-enriched water to both the carboxylate oxygens of the C-terminus of tryptic peptides

  $$R\text{-}COOH \longrightarrow R\text{-}C^{18}O_2H$$

- The peptides have an increase in mass of 4 Da
- Generally not considered a large enough mass difference

iTRAQ quantification

- The iTRAQ™ reagents
  - React with Lys amino groups and each one adds 145 Da to the molecular weight of the peptide
  - Fragmentation produces reporter ions from *m/z* 114, 115, 116 and 117
  - Current iTRAQ kit contains 8 forms with reporter fragment ions of *m/z* 114, 115, 116, 117, 118, 119 and 121



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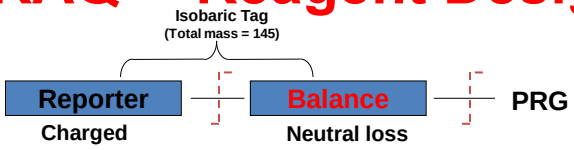
iTRAQ™ Reagent Design

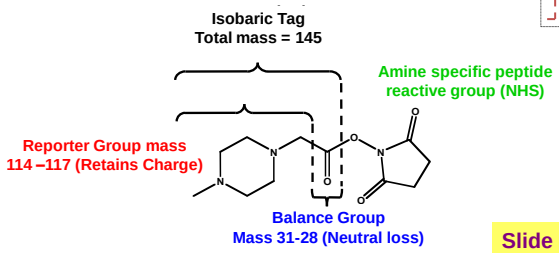


- Gives strong signature ion in MS/MS
- Gives good b- and y-ion series
- Maintains charge state
- Maintains ionization efficiency of peptide
- Signature ion masses lie in quiet region

- Balance changes in concert with reporter mass to maintain total mass of 145
- Neutral loss in MS/MS

Amine specific

= MS/MS Fragmentation Site



Slide provided by Applied Biosystems

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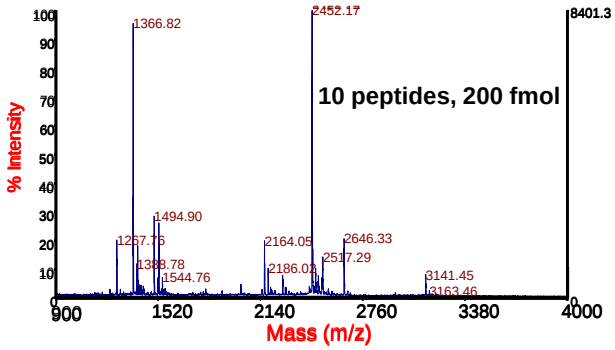
Other non-isotopic quantitative methods in proteomics

The coverage (the number of peptides observed for a protein) is sensitive to the amount of the protein

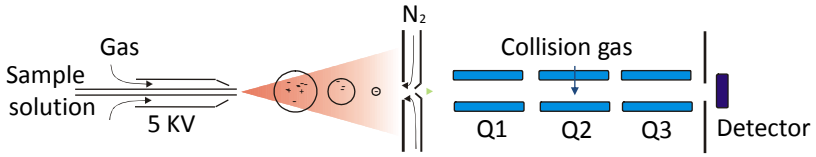
- **This can be used to calculate whether a treatment affects the abundance of a protein where fold-change > 2**
- **Applies to LC-MS (MUDPIT methods)**



Triple quad MRM analysis

Peptides of interest can be analyzed like small molecules

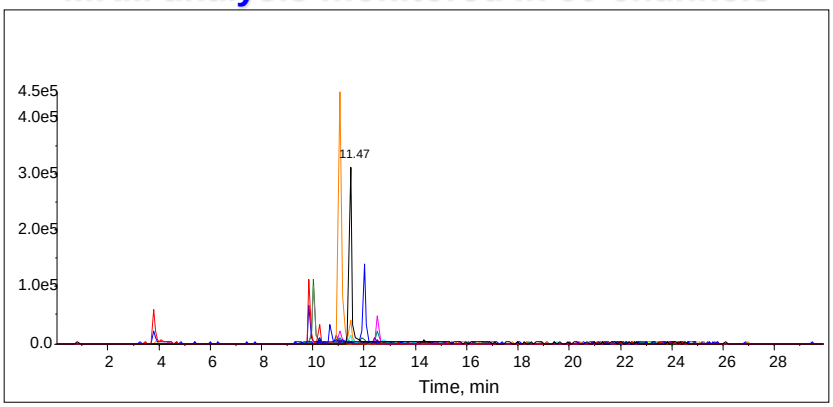
- **Choose the parent molecular ion, collide with argon gas and select a unique fragment**



- **Multiple reaction ion scanning**
  - **First filter the [M-H]- molecular ion of the analyte (Q1)**
  - **Fragment the molecular ion with N_2 gas (Q2)**
  - **Select a specific (and unique) fragment ion (Q3)**

Quantitation experiment for biotinylated cytochrome c

MRM analysis monitored in 50 channels



Each colored peak represents a different biotinylated peptide

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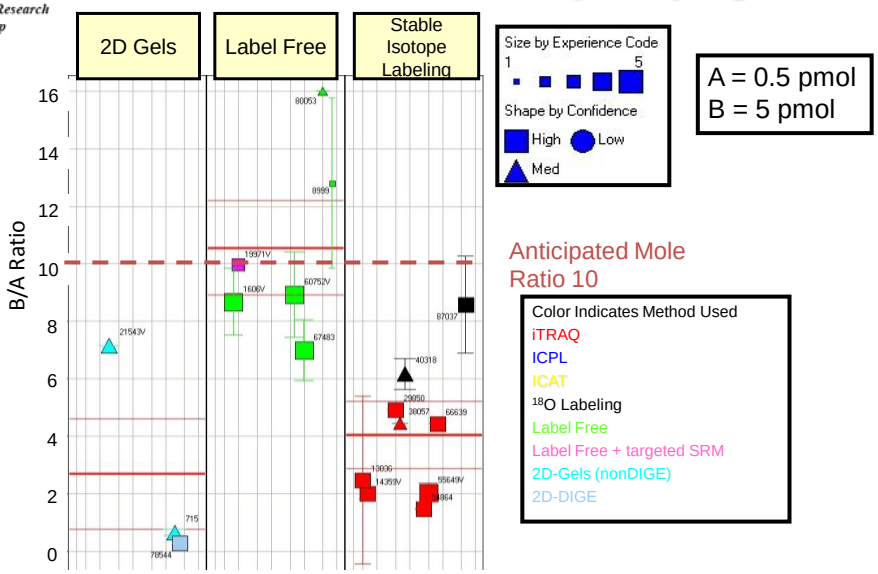
Quantitative Accuracy: Myoglobin

Proteomics Research Group



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2008 ABRF Study - identification of three truncated peptides

ABRF Proteomics Research Group

Conclusions

- Proteomics offers a wide arrange of approaches for the qualitative analysis of proteins
- Many methods and approaches were used successfully identify and sequence the truncated sites
- In many cases, the combination of two complementary approaches (e.g., 1D SDS PAGE to resolve protein components followed by LC-MS/MS for sequence information) gave a higher success rate than use of a single experimental approach.
- As expected, experience remains a key factor in this study

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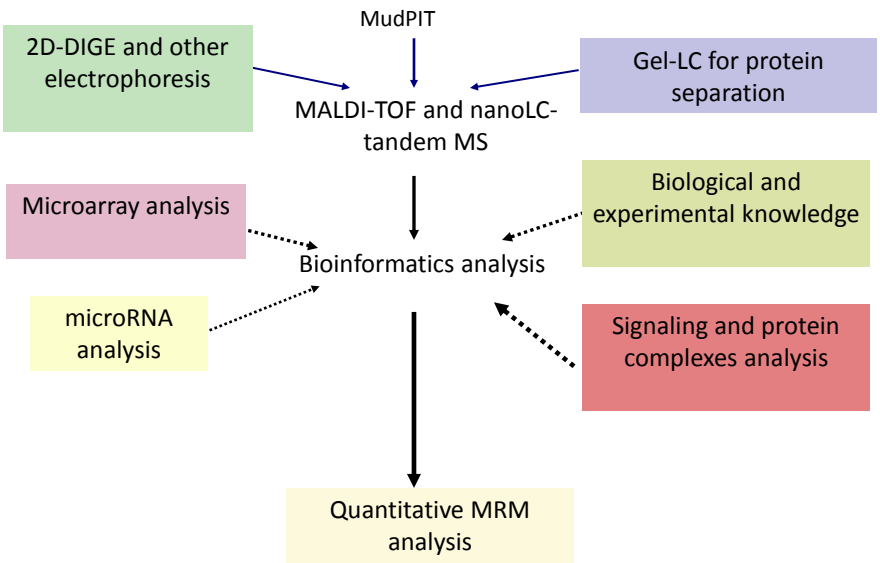
Workflow for generation of proteomics data



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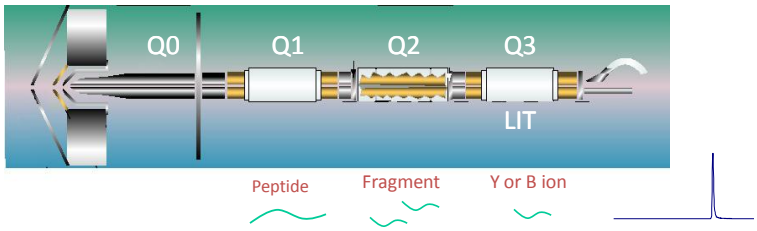
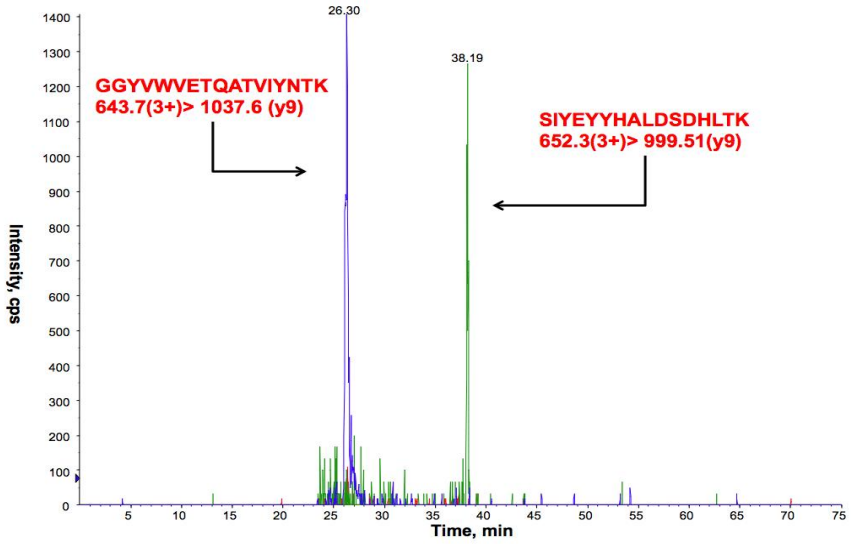
Multiple Reaction Monitoring



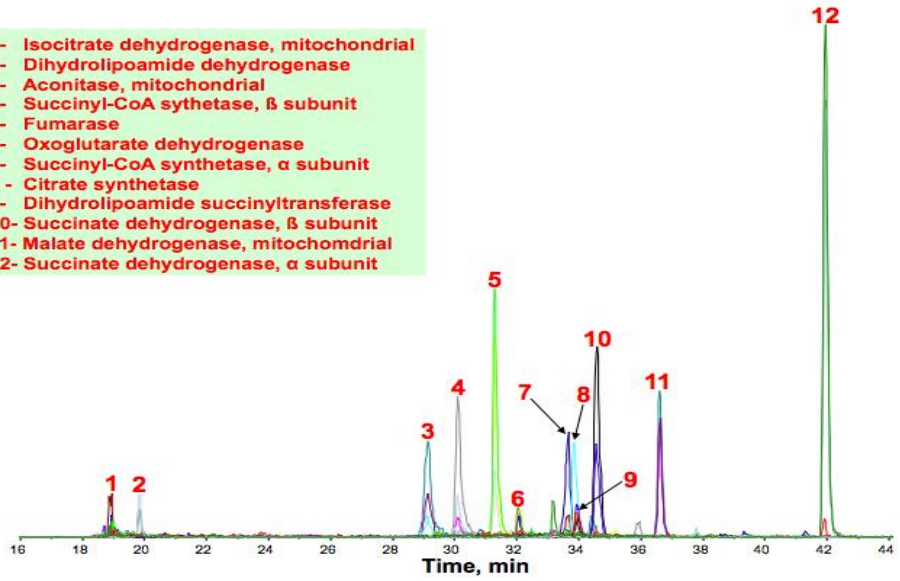
- MRM methods are the gold standard for quantitative analysis of small molecules
  - Currently performed on a triple quadrupole instrument
  - Each tryptic peptide ion is isolated in Q1, fragmented by collision in Q2 and a specific fragment measured after filtration in Q3
- Proteotypic peptides can represent proteins (like oligonucleotides for DNA)
  - Generally a 8-aa peptide is unique
  - Multiple channels - 10-20 msec per channel

HIF-1α in kidney cytosol by LC-MRM-MS



Multiple reaction ion monitoring of Krebs cycle enzymes

1- Isocitrate dehydrogenase, mitochondrial
2- Dihydrolipoamide dehydrogenase
3- Aconitase, mitochondrial
4- Succinyl-CoA sythetase, ß subunit
5- Fumarase
6- Oxoglutarate dehydrogenase
7- Succinyl-CoA synthetase, α subunit
8 - Citrate synthetase
9- Dihydrolipoamide succinyltransferase
10- Succinate dehydrogenase, ß subunit
11- Malate dehydrogenase, mitochomdrial
12- Succinate dehydrogenase, α subunit



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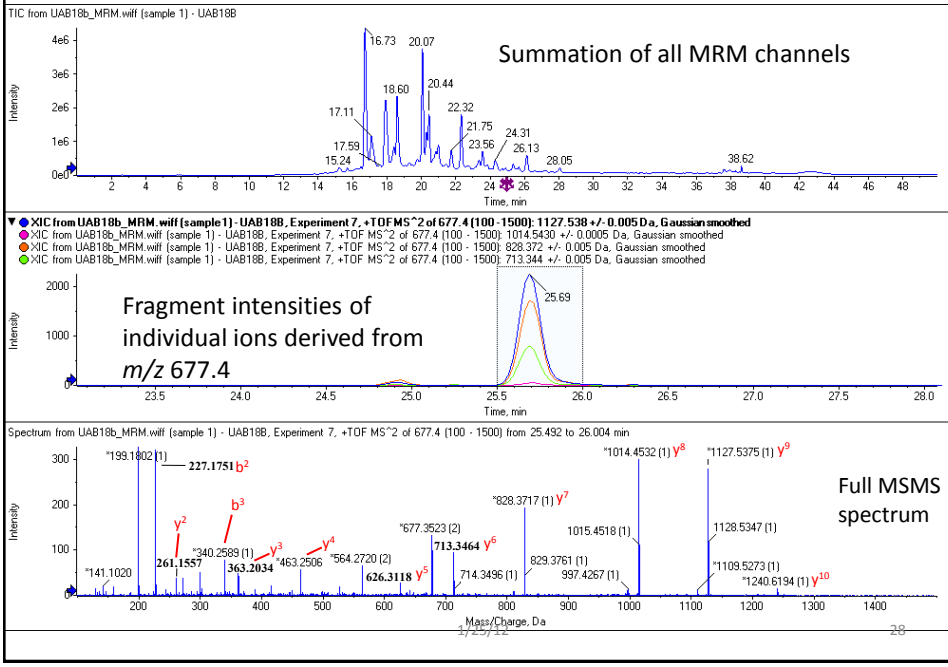
MS/MS Fragmentation of **LIIWDSYTTNK**
Found in **gi|11055998**, guanine nucleotide-binding protein, beta-4 subunit [Homo sapiens]



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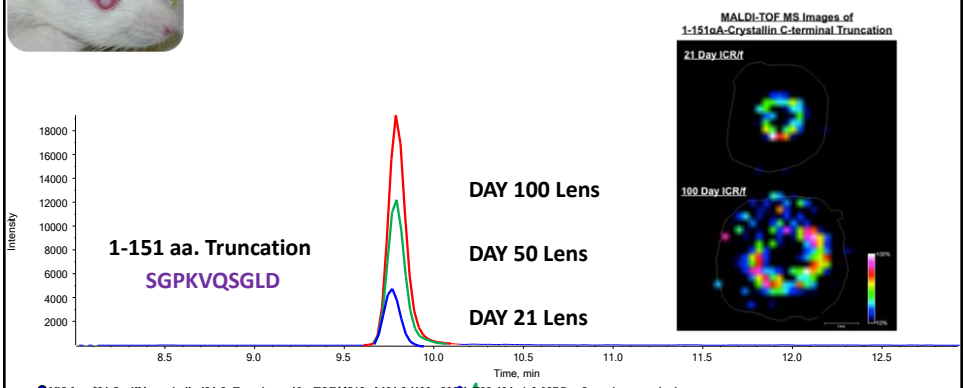
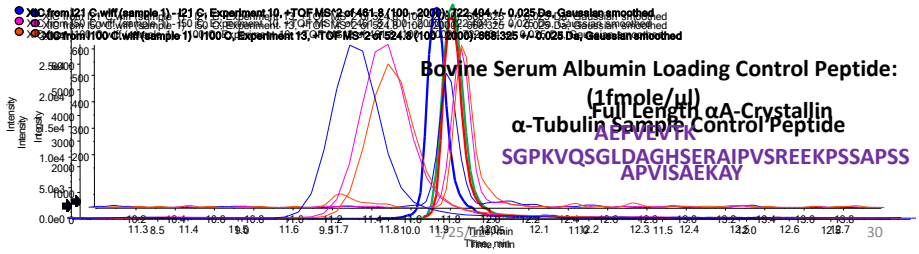
Verifying and quantifying C-truncation

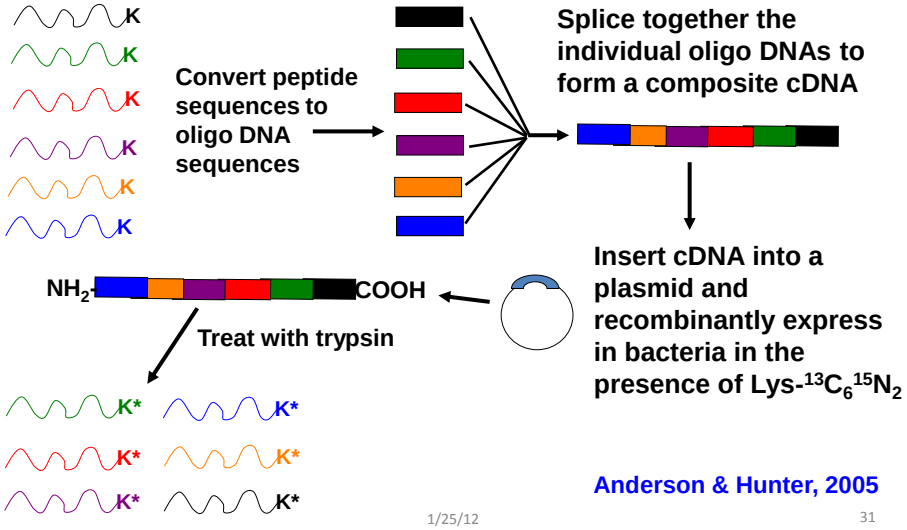
- αA crystallin is supposedly processed to a 173aa form from the 196aa translated product. Interestingly, what we see is the removal of an interior 23aa peptide, so it must be differential splicing, not posttranslational processing.
- Processed rat αA crystallin has a chymotrypsin cleavage site at ^{141}Phe
- This peptide can be observed as a triply charged peptide
  - FSGPKVQSGLDAGHSERAIPVSREEKPSSAPSS
- The C-truncations observed by mass spectrometry imaging are the following:
  - SGPKVQSGLD (truncation at 151)
  - SGPKVQSGLDAGHSE (truncation at 156)
  - SGPKVQSGLDAGHSER (truncation at 157)
  - SGPKVQSGLDAGHSERAIPVSR (truncation at 163)
  - SGPKVQSGLDAGHSERAIPVSREEKPS (truncation at 168)

Truncation of αA-crystallin in ICR/F rat





Concatenation - making ^{13}C-labeled peptide internal standards



Anderson & Hunter, 2005

Quantitative peptide MRM-MS

- The albumin-depleted plasma proteome is mixed with the composite ^{13}C,^{15}N-labeled protein internal standard and then treated with trypsin
- The molecular ions (doubly charged) and the specific y ions for each peptide and its labeled form are entered into the MRM script one channel at a time
- A single run may consist of 30 peptides in 60 channels
- Sensitivity is compromised by "sharing out" measurement time, but can be compensated for by carrying out nanoLC

Advantage of a C-terminal labeled lysine

	186	301	448	505	642	755	886	987	1115	b ions
A	D	E	F	G	H	I	M	T	K	
1133	1062	948	833	686	629	492	379	248	147	y ions

With the labeled lysine is at the C-terminus, only the b_{10} ion contains the isotope atoms

	186	301	448	505	642	755	886	987	1123	b ions
A	D	E	F	G	H	I	M	T	K*	
1141	1070	956	841	694	637	500	387	256	155	y ions

References for these talks (1)

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- Schwanhäusser B, Busse D, Li N, Dittmar G, Schuchhardt J, Wolf J, Chen W, Selbach M. Global quantification of mammalian gene expression control. *Nature* 473: 337-342 (2011).