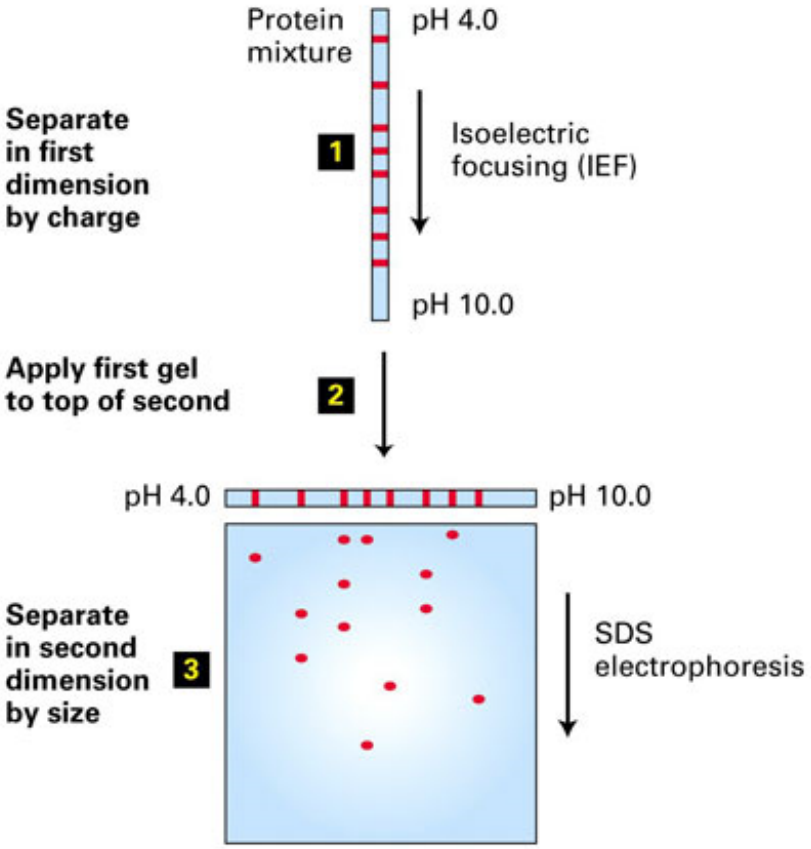
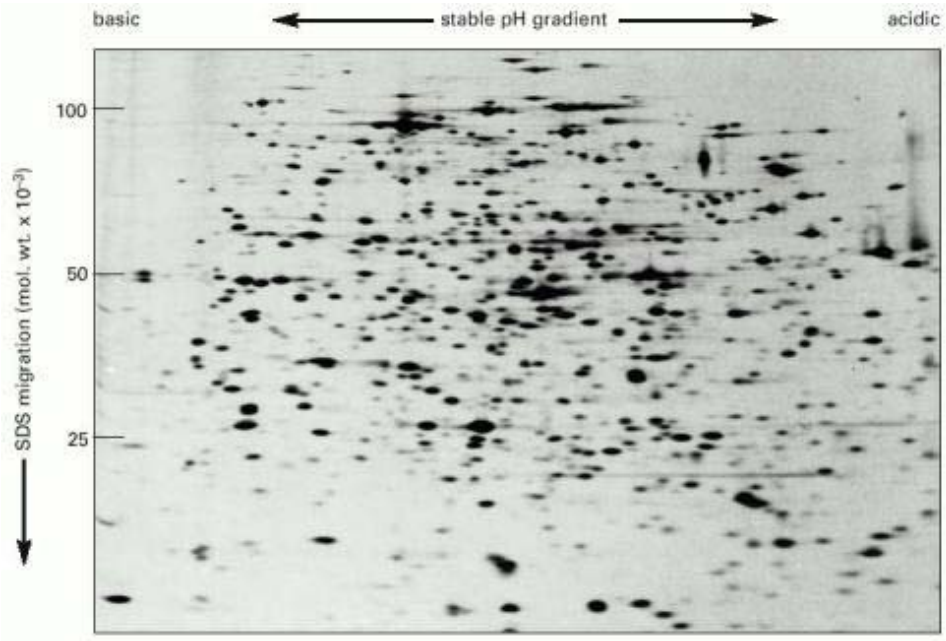
Quantitative proteomics:
Principles & Strategies

2DE-based approach





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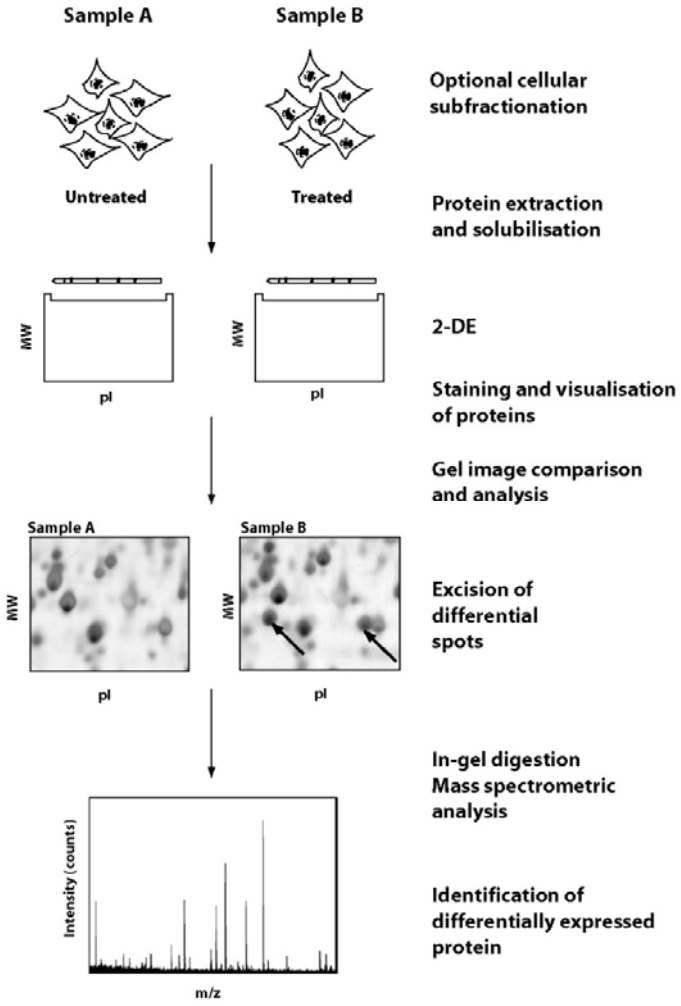
2DE-based approach



"I see 1,000 spots, but identify 50 only."

Why ??

Sensitivity of silver stained gel spot ~ **1 ng**
(low digestion efficiency of in-gel digestion, ~ < 5%)

Best sensitivity of **LC-MS** ~ 1 attomole of protein = **~ 40 fg**

Almost >10,000-fold in sensitivity

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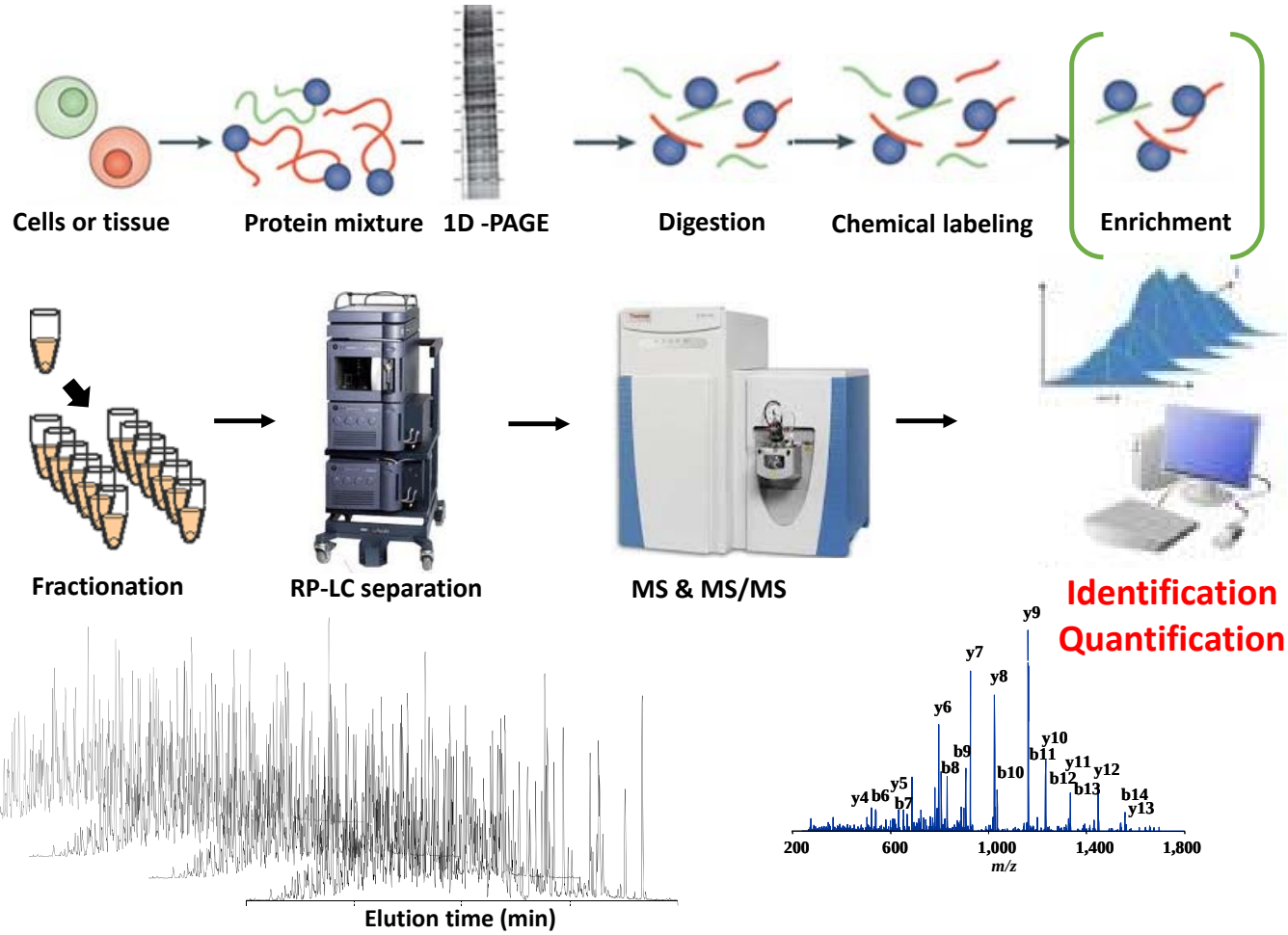
Quantitative proteomics workflow (LC-MS/MS based)

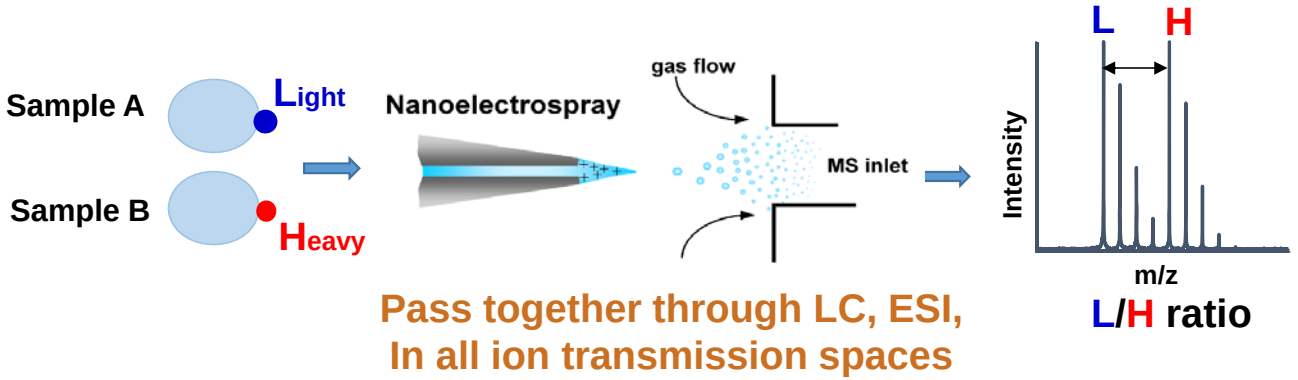


LC-MS/MS based quantification strategies

- Label-free (MS intensity)
- Spectral counting (Semi label-free)
- **Stable isotope labeling**
  - **Metabolic labeling (SILAC)**
  - Chemical labeling
  - Enzymatic labeling
- Synthetic heavy peptides (AQUA)
- Fully heavy protein (PSAQ)

Stable isotope labeling

✓ Light (^{12}C, ^{16}O, ^{14}N) and heavy isotope (^{13}C, ^{18}O, ^{15}N) labeled peptide pairs have <u>exactly identical</u> chemical properties



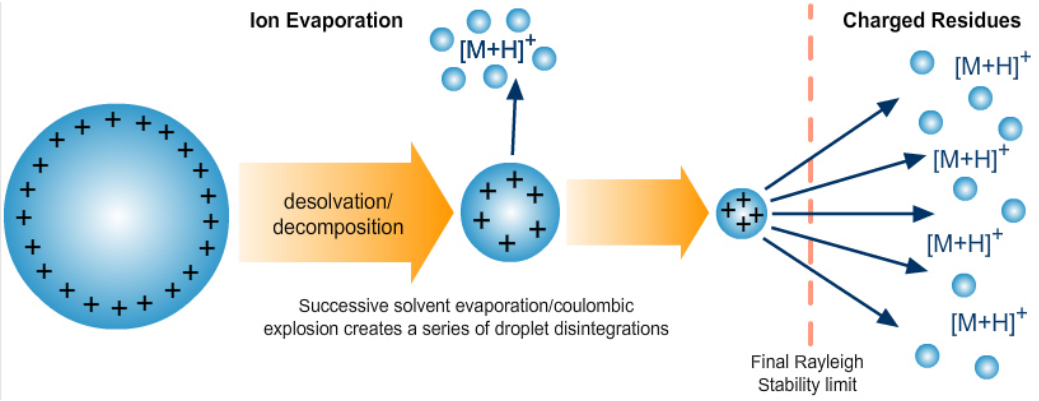
- **<u>Metabolic labeling</u> (SILAC)**
- <u>Chemical labeling</u> (**TMT/iTRAQ, Dimethylation**)
- <u>Enzymatic labeling</u> (**^{18}O-labeling**)

LC-MS/MS based quantification

- **Heavy reference** (stable isotope labeled peptide) is necessary for accurate quantification
- **Relative quantification** within a single peptide with same sequence
- Why ???

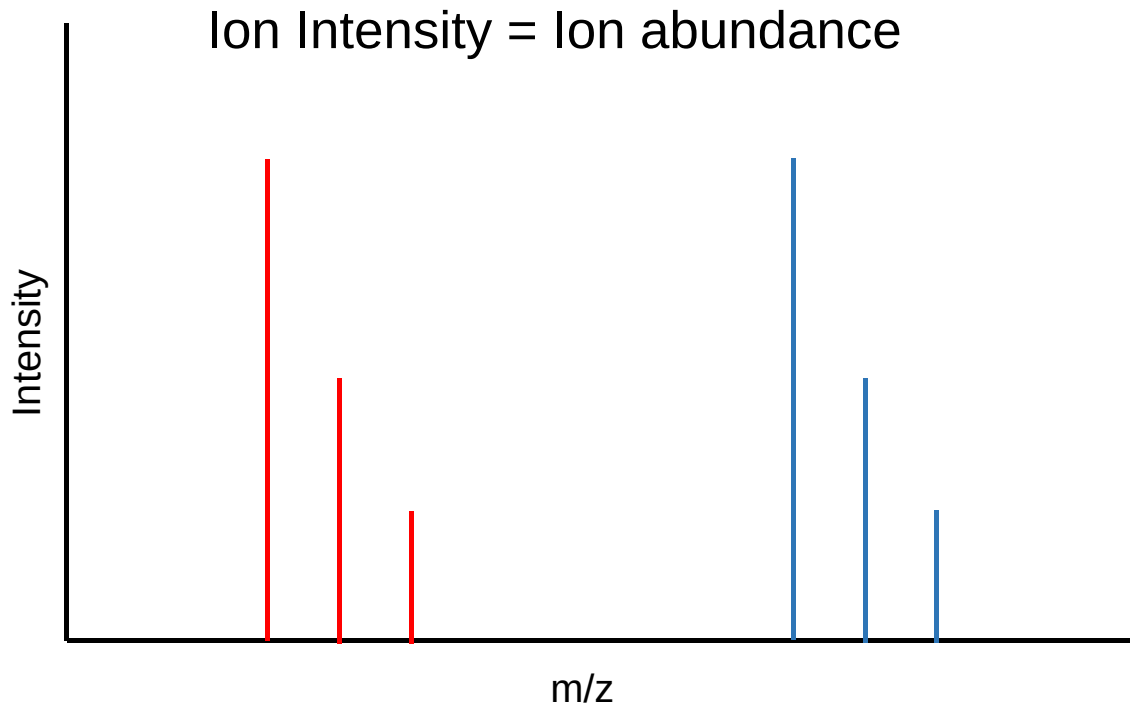
  Different ionization efficiency

  - basicity (# of charge state)
  - hydrophobicity (desolvation)
  - solvent composition in eluent



Stable Isotope Labeling by Amino acids in Cell culture, SILAC

MS-based quantitation at MS1 level



- Double or triple sample complexity, i.e. decrease in identification rate
- Better quantification dynamic range and accuracy
- Low multiplexing power

Stable Isotope Labeling by Amino acids in Cell culture, SILAC

(A sort of history of SILAC)

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Instrumental in establishing emerging MS imaging capability at EMSL. As a part of this effort, the world's first C60 SIMS FTICR mass spectrometer was built at EMSL in 2011; this unique instrument couples the high spatial resolution of SIMS with the high mass performance of an FTICR MS for more accurate molecular characterization. Key contributor in the development of PNNL's pioneering high-throughput accurate mass and time (AMT) tag strategy for ultra-sensitive proteomics that capitalized on the high resolution and accuracy afforded by the LC-FTMS platforms established in EMSL. Among the first (in 1999) to use stable isotope labeling methods, now central to quantitative proteomics, to impart a specific isotope signature into proteins, an initial top-down proteomics endeavor that led to significant advancements in the field.

J. Am. Chem. Soc. **1999,** *121,* 7949−7950

High Throughput Proteome-Wide Precision Measurements of Protein Expression Using Mass Spectrometry

Ljiljana Paša-Tolić, Pamela K. Jensen, Gordon A. Anderson, Mary S. Lipton, Kim K. Peden, Suzana Martinović, Nikola Tolić, James E. Bruce, and Richard D. Smith*

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We have developed an approach utilizing organisms cultured in stable-isotope labeled media (e.g., rare-isotope depleted and normal) to provide effective "internal calibrants" for *all* detected proteins, thus enabling precise proteome-wide measurement of changes in protein abundances resulting from cellular perturbations. The two (or more) isotopically distinctive cell populations are mixed prior to sample processing steps, eliminating all experimental variables associated with cell lysis, separation, and mass spectrometric analysis. Changes in relative protein abundances are thus precisely reflected by the ratio of two isotopically different and resolvable versions of each protein.

Isotopically depleted (~99.95% 12C, ~99.99% 14N, and >99.995% 1 H)
13C: 1.07 % → 0.05 %
15N: 0.368 % → 0.01 %
2H: 0.0115 % → 0.005 %

SHORT COMMUNICATION

J Am Soc Mass Spectrom 2000, 11, 78–82

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Accepted September 24, 1999

Proteome Analysis Using Selective Incorporation of Isotopically Labeled Amino Acids

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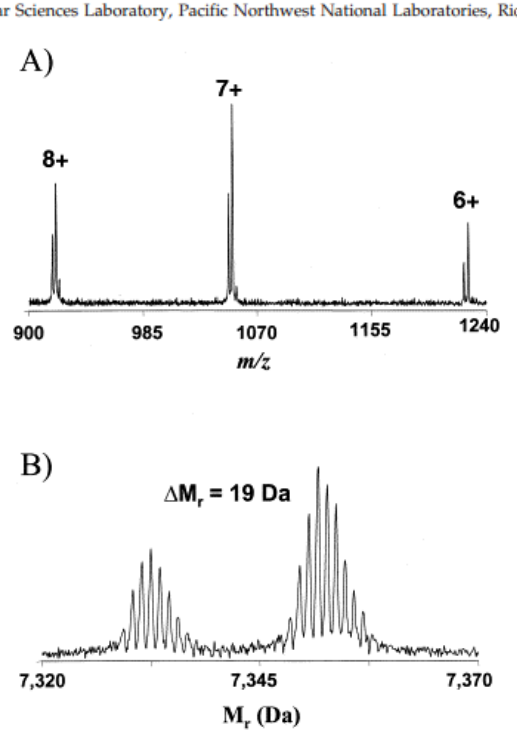
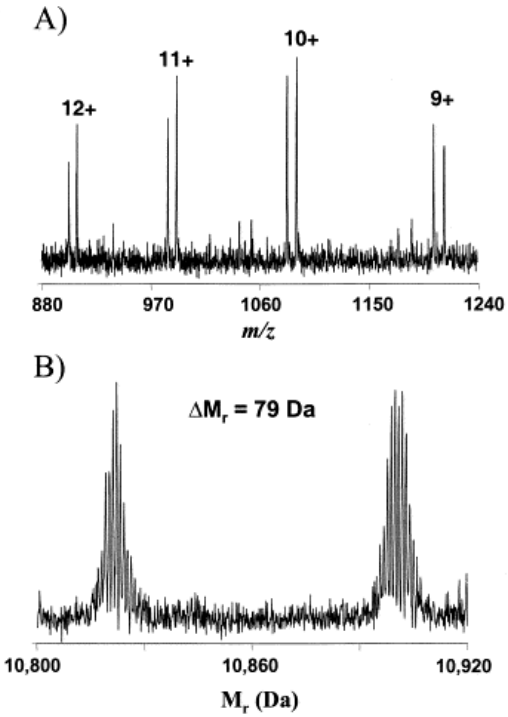
Environmental and Molecular Sciences Laboratory, Pacific Northwest National Laboratories, Richland, Washington, USA

Leu-d10





JOURNAL OF MASS SPECTROMETRY
J. Mass Spectrom. 2002; **37**: 99–107

JMS

Selective incorporation of isotopically labeled amino acids for identification of intact proteins on a proteome-wide level

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Received 17 July 2001; Accepted 18 October 2001; Published online 14 December 2001

Leucine (Leu-D_{10}), isoleucine (Ile-D_{10}), phenylalanine (Phe-D_8), arginine (Arg-$^{13}C_6$), histidine (His- $^{13}C_6$) or lysine (Lys- $^{13}C_6$)

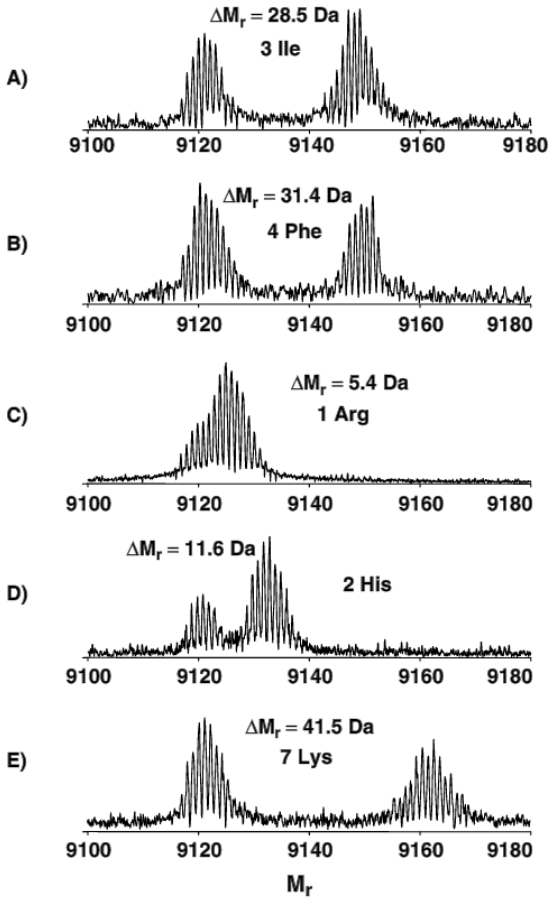

Figure 2. Zero charge state spectra of the *E. coli* PTS system protein HPr (M_r = 9119.4 Da) detected during on-line CIEF/FTICR analysis from *E. coli* grown in minimal medium combined with cells grown in minimal medium containing 0.1 mg ml^{-1} of (A) Ile-D_{10}, (B) Phe-D_8, (C) Arg-$^{13}C_6$, (D) His-$^{13}C_6$ or (E) Lys-$^{13}C_6$.

research articles Journal of proteome research

Leu-d10

Quantitative Analysis of the Yeast Proteome by Incorporation of Isotopically Labeled Leucine

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Received March 27, 2002

Quantitative comparison of protein expression levels in 2D gels is complicated by the variables associated with protein separation and mass spectrometric responses. Metabolic labeling allows cells from different experiments to be mixed prior to analysis. This approach has been reported for prokaryotic cells. Here, we demonstrate that metabolic labeling can also be successfully applied to the eukaryote *Saccharormyces cerevisiae*. Yeast leucine auxotrophs grown on synthetic complete media containing natural abundance Leu or D_{10}-Leu were mixed prior to 2D gel separation and MALDI analysis of the digested proteins. D_{10}-Leu labeling provided an effective internal calibrant for peptide MS analysis, and the number of Leu residues yielded an additional parameter for peptide identification at low mass resolution (1000). Metabolic incorporation of D_{10}-Leu into yeast proteins was found to be quantitative since the intensities of the peptide peaks corresponded to those expected on the basis of the percent label in the media. Thus, D_{10}-Leu labeling should provide reliable data for comparing proteomes both quantitatively and qualitatively from wild-type and nonessential-gene-null-mutant strains of *S. cerevisiae*. Given the central role played by yeast in our understanding of eukaryotic gene and protein expression, it is anticipated that the quantitative expressional proteomic method outlined here will have widespread applications.

Keywords: quantitative proteomics • metabolic labeling • *S. cerevisiae* • D_{10}-Leu • 2DE • MALDI-TOF-MS • relative expression levels

RAPID COMMUNICATIONS IN MASS SPECTROMETRY
Rapid Commun. Mass Spectrom. 2002; **16**: 2115–2123
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/rcm.831

RCM

Amino acid residue specific stable isotope labeling for quantitative proteomics‡

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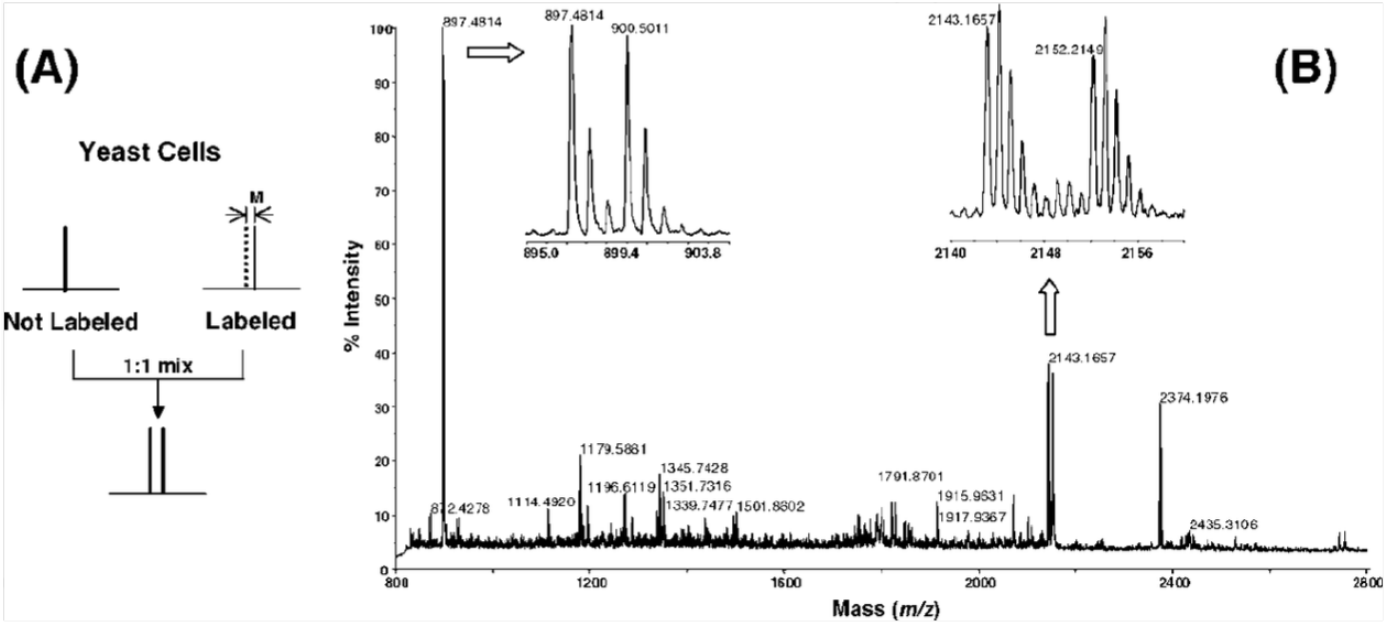


Leu-d3

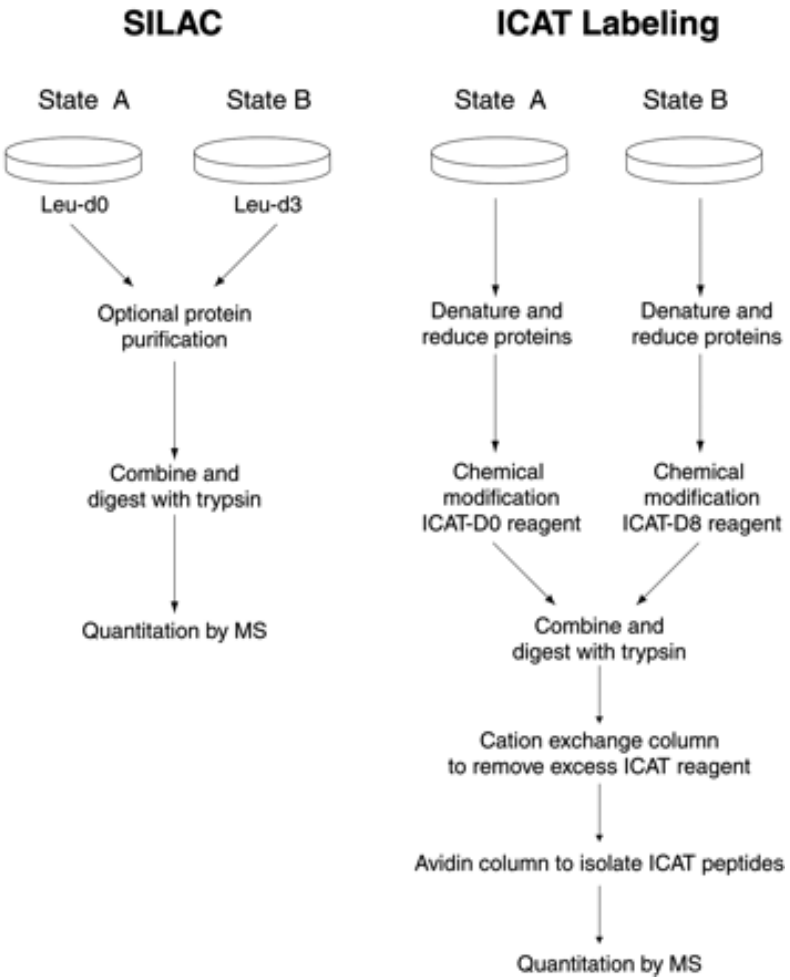
Stable Isotope Labeling by Amino Acids in Cell Culture, SILAC, as a Simple and Accurate Approach to Expression Proteomics*

Shao-En Ong‡, Blagoy Blagoev‡, Irina Kratchmarova‡, Dan Bach Kristensen§, Hanno Steen‡¶, Akhilesh Pandey‡‖, and Matthias Mann‡**

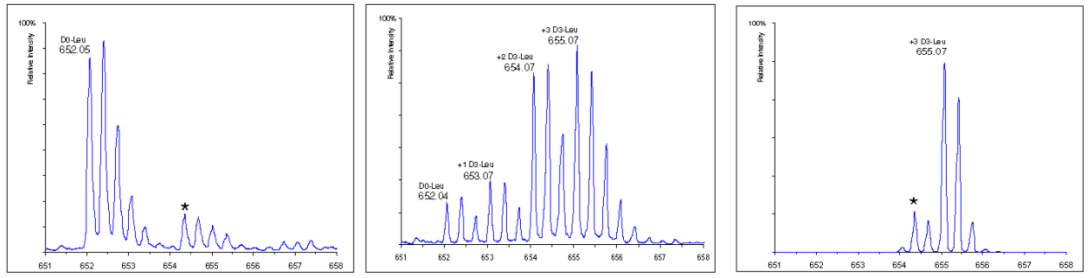
Received, April 13, 2002, and in revised form, May 17, 2002
Published, MCP Papers in Press, May 20, 2002, DOI 10.1074/mcp.M200025-MCP200

Deuterated leucine (**Leu-d3**)

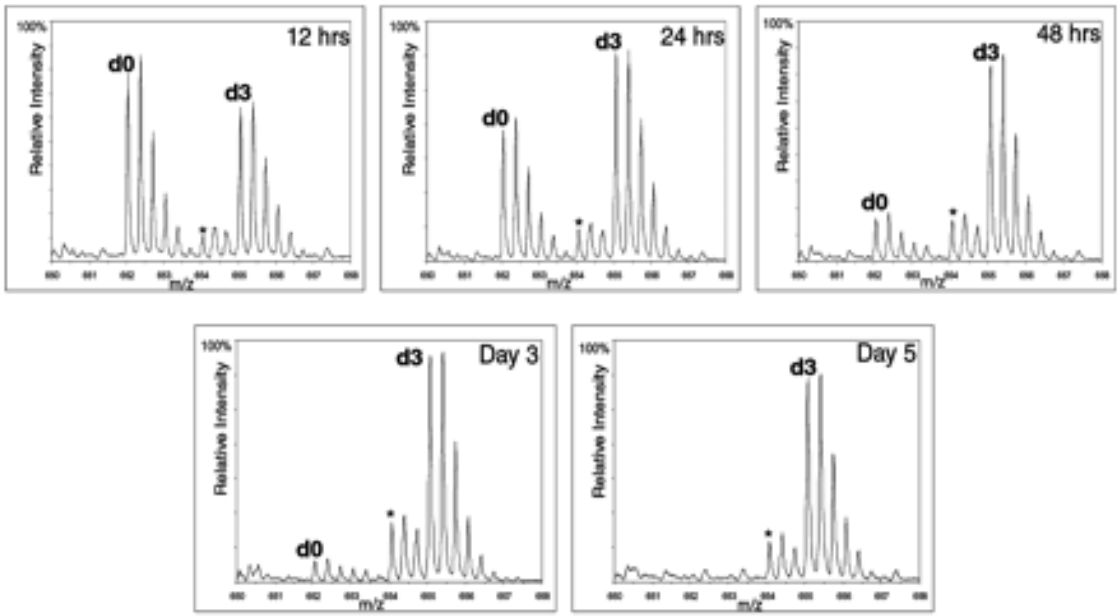
- Complete incorporation after five cell doublings
- No differences in cell morphology, doubling times, and ability of the cells to differentiate

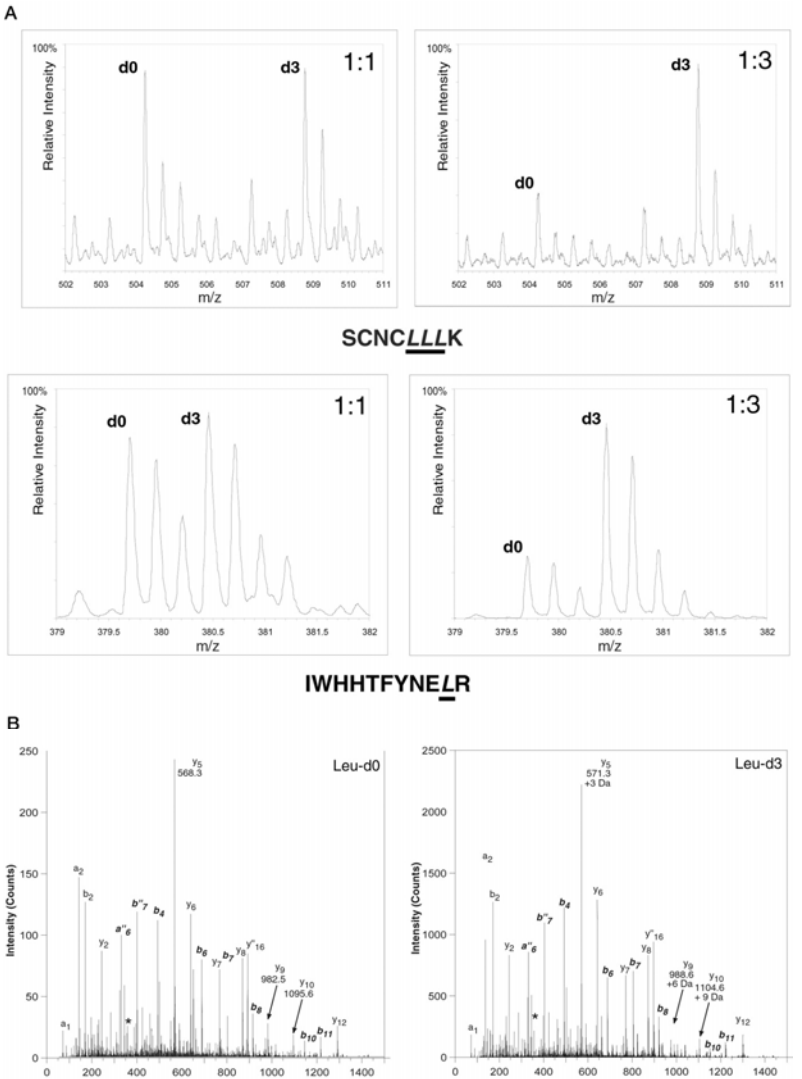


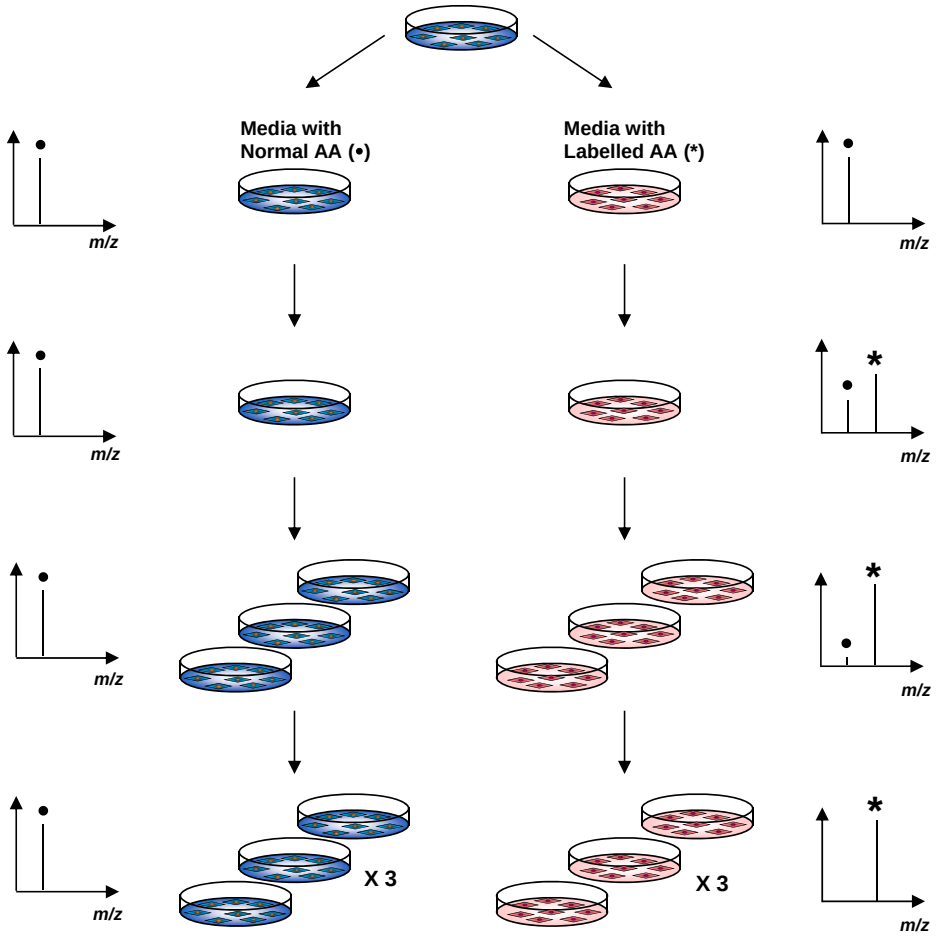
Importance of Dialyzed Serum



- non-dialzed serum contains free (unlabeled) amino acids!


12 hrs
24 hrs
48 hrs
Day 3
Day 5
d0
d3
Relative Intensity
m/z


A
1:1
1:3
d0
d3
Relative Intensity
m/z
SCNCLLLK
IWHHTFYNELR
B
Leu-d0
Leu-d3
Intensity (Counts)



Cells in normal culture media

Start SILAC labelling by growing cells in labelling media

(labelled AA / dialized serum)

Passage cells to allow incorporation of labelled AA

By 5 cell doublings cells have incorporated

Grow SILAC labelled cells to desired number of cells for experiment

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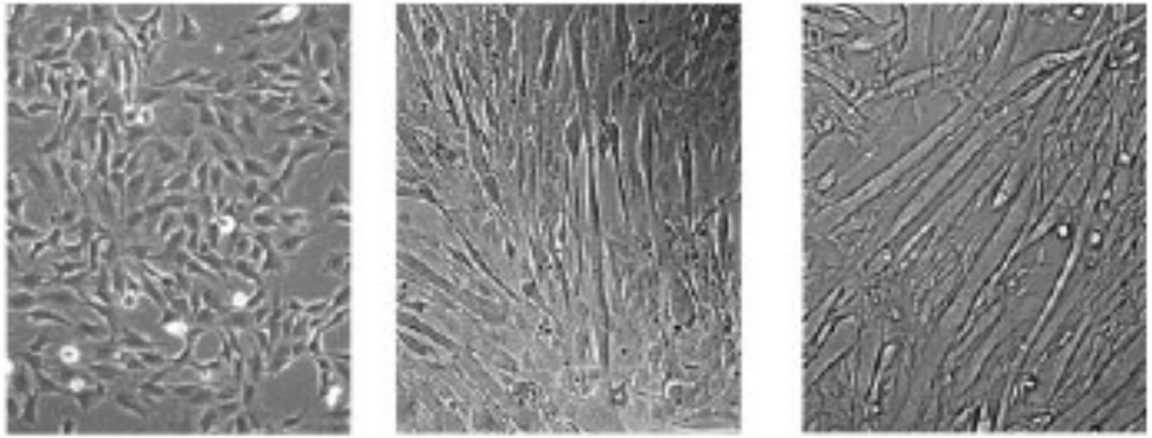
No alterations to cell phenotype

C2C12 myoblast cell line



Labeled cells behaved as expected under differentiation protocols