



Properties of ^{13}C -Substituted Arginine in Stable Isotope Labeling by Amino Acids in Cell Culture (SILAC)

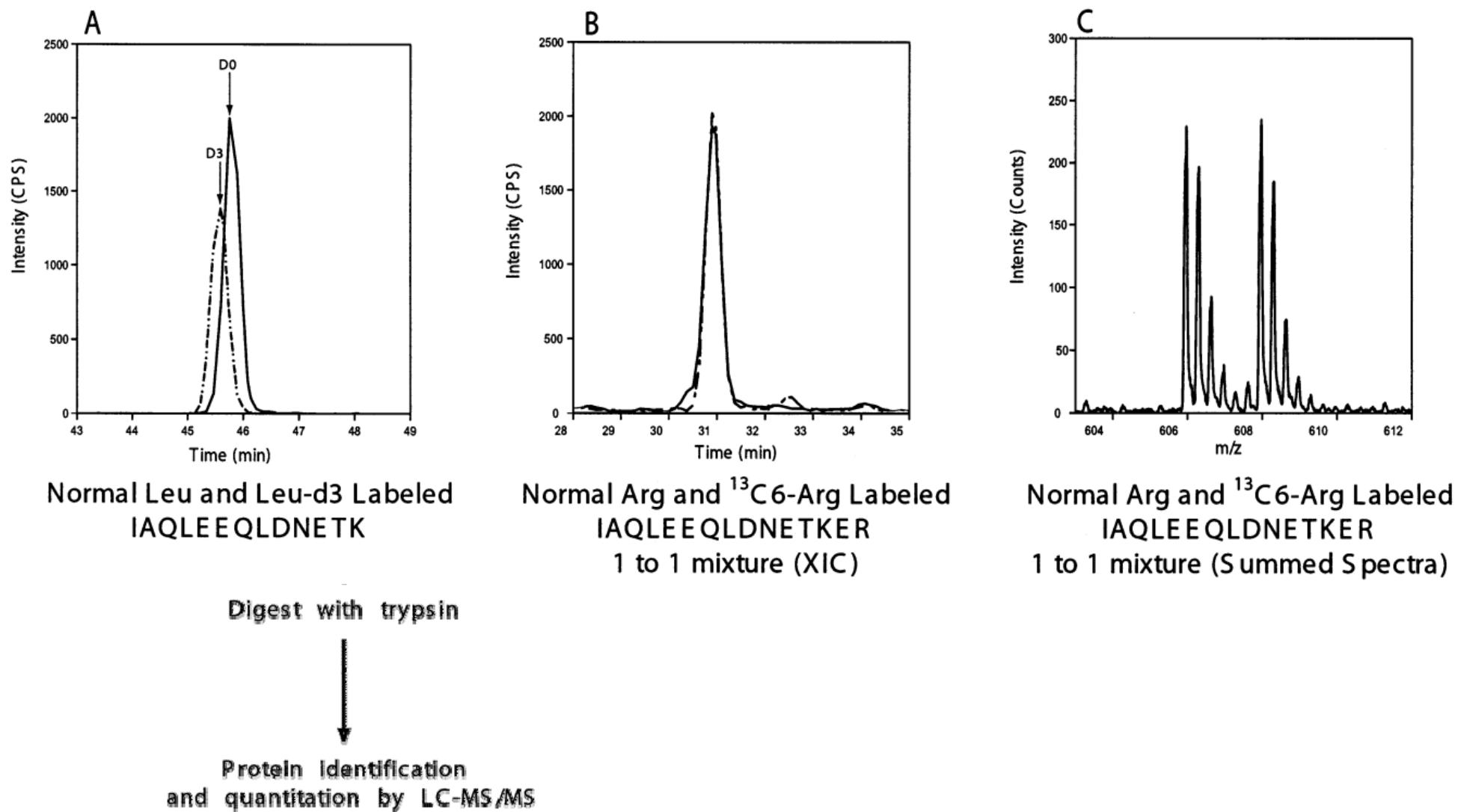
Shao-En Ong, Irina Kratchmarova, and Matthias Mann*

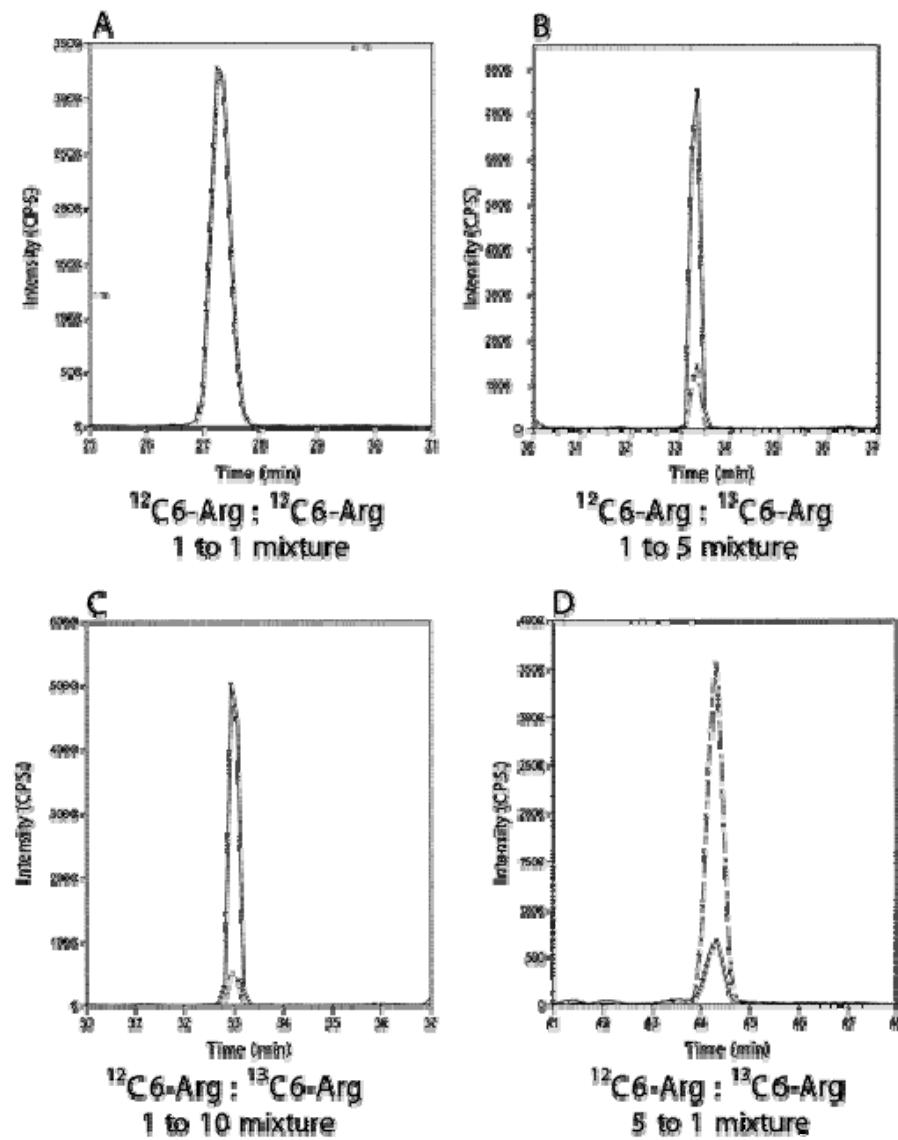
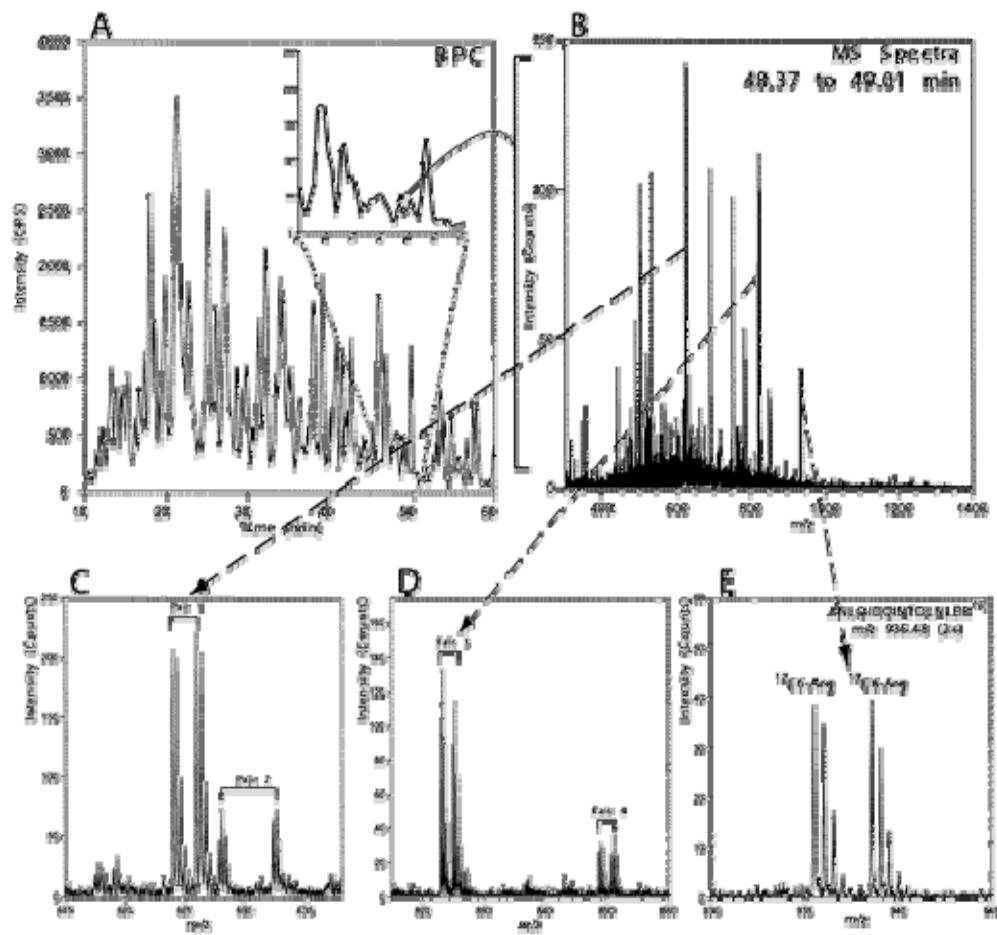
*Center for Experimental BioInformatics (CEBI), Department of Biochemistry and Molecular Biology,
University of Southern Denmark, Campusvej 55, DK-5230 Odense M, Denmark*

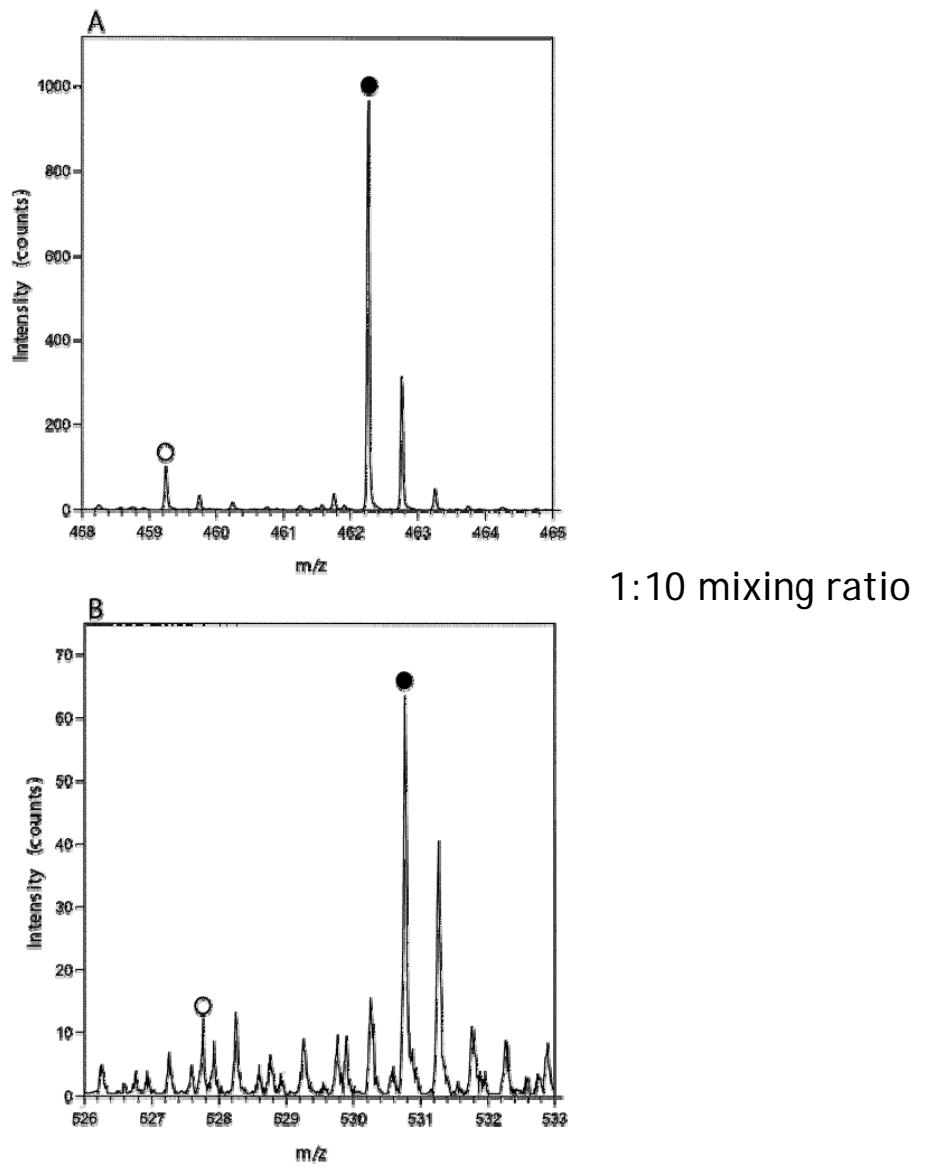
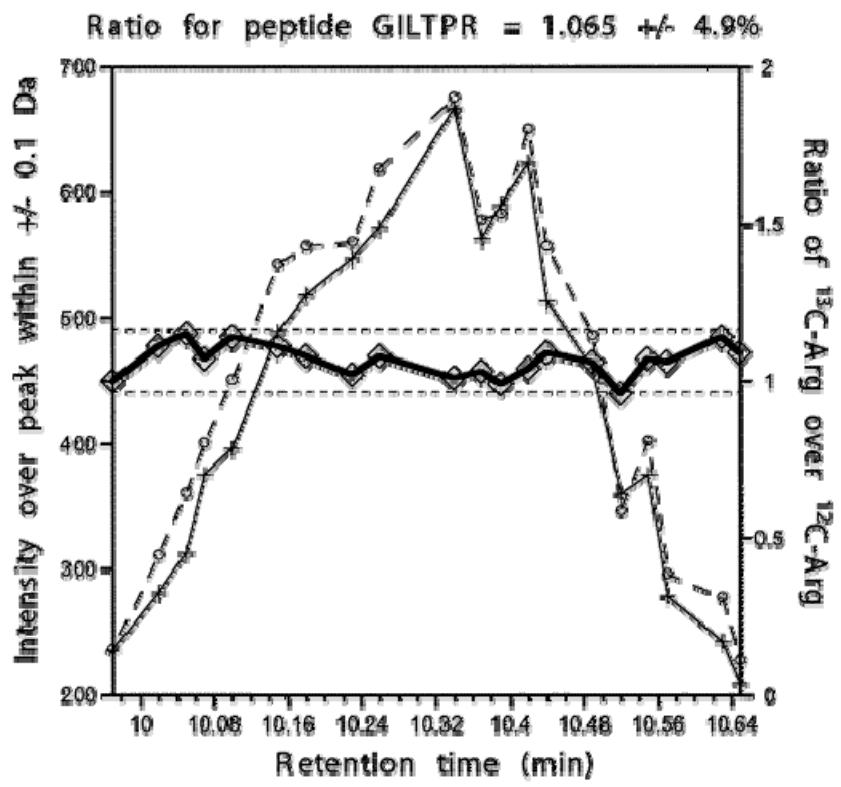
Received September 19, 2002

We have recently described a method, stable isotope labeling by amino acids in cell culture (SILAC) for the accurate quantitation of relative protein abundances. Cells were metabolically labeled with deuterated leucine, leading to complete incorporation within about five cell doublings. Here, we investigate fully substituted ^{13}C -labeled arginine in the SILAC method. After tryptic digestion, there is a single label at the C-terminal position in half of the peptides. Labeled and unlabeled peptides coelute in liquid chromatography–mass spectrometric analysis, eliminating quantitation error due to unequal sampling of ion profiles. Tandem mass spectrum interpretation and database identification are aided by the predictable shift of the y-ions in the labeled form. The quantitation of mixtures of total cell lysates in known ratios resolved on a one-dimensional SDS–PAGE gel produced consistent and reproducible results with relative standard deviations better than five percent under optimal conditions.

Keywords: quantitative proteomics • peptide sequencing • protein identification • LC–MS/MS • cell culture • SILAC



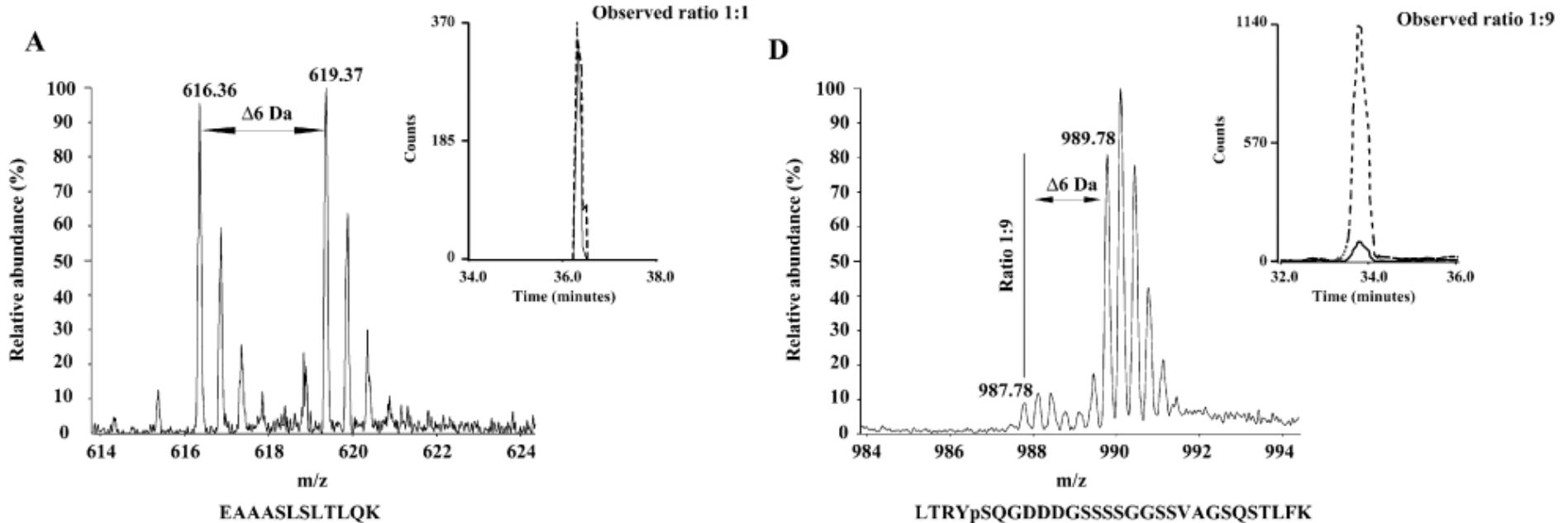




A Proteomic Approach for Quantitation of Phosphorylation Using Stable Isotope Labeling in Cell Culture

L-lysine ($^{13}\text{C}_6$)
or/and
L-arginine ($^{13}\text{C}_6$)

Nieves Ibarrola,[†] Dario E. Kalume,[†] Mads Gronborg,^{†,‡} Akiko Iwahori,[†] and Akhilesh Pandey^{*,†}



Triple SILAC

Arg0, Arg6, Arg10

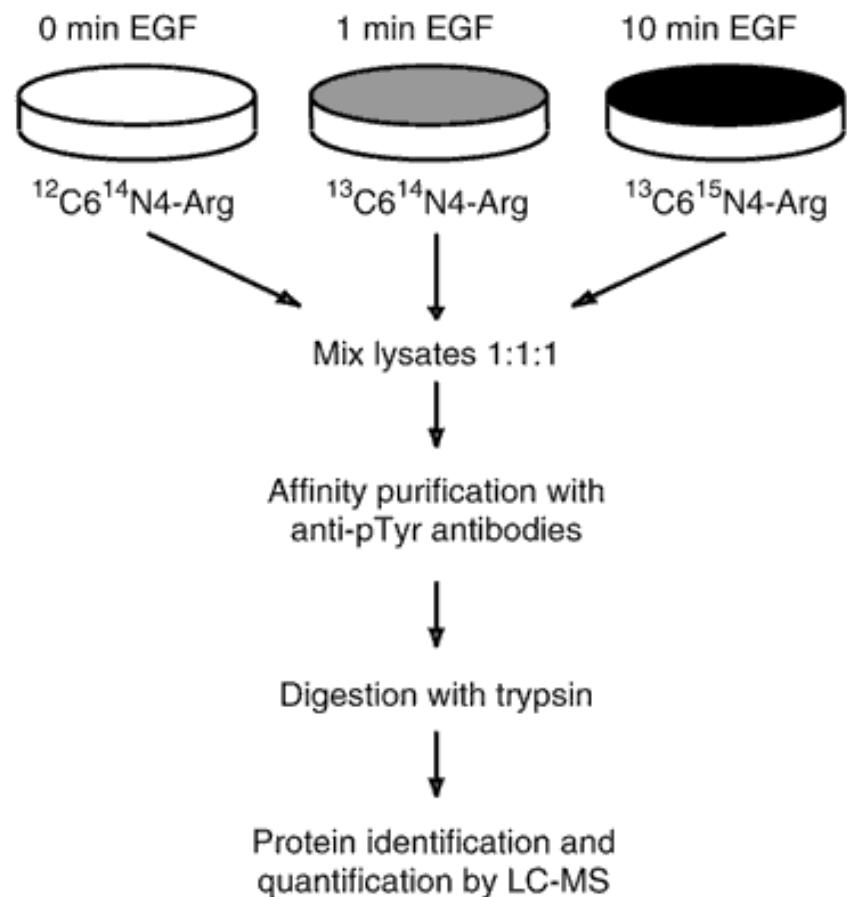
LETTERS

nature
biotechnology

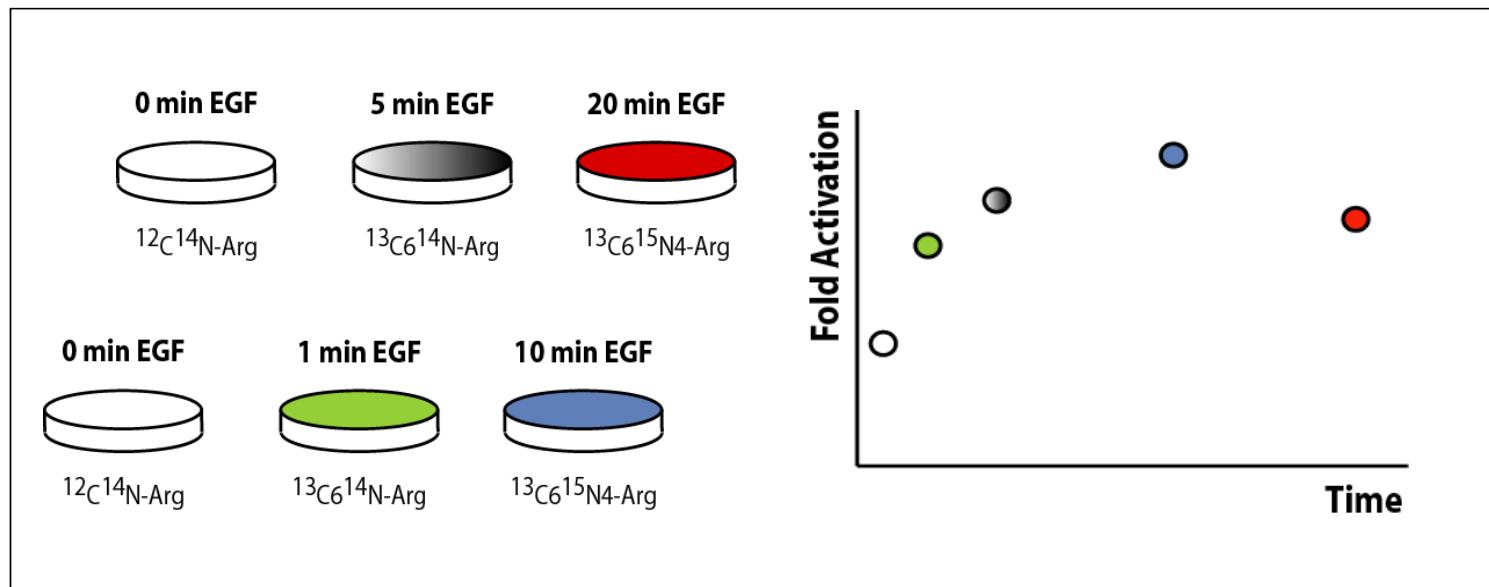
Temporal analysis of phosphotyrosine-dependent signaling networks by quantitative proteomics

Blagov Blagoev^{1,2}, Shao-En Ong^{1,2}, Irina Kratchmarova¹ & Matthias Mann¹

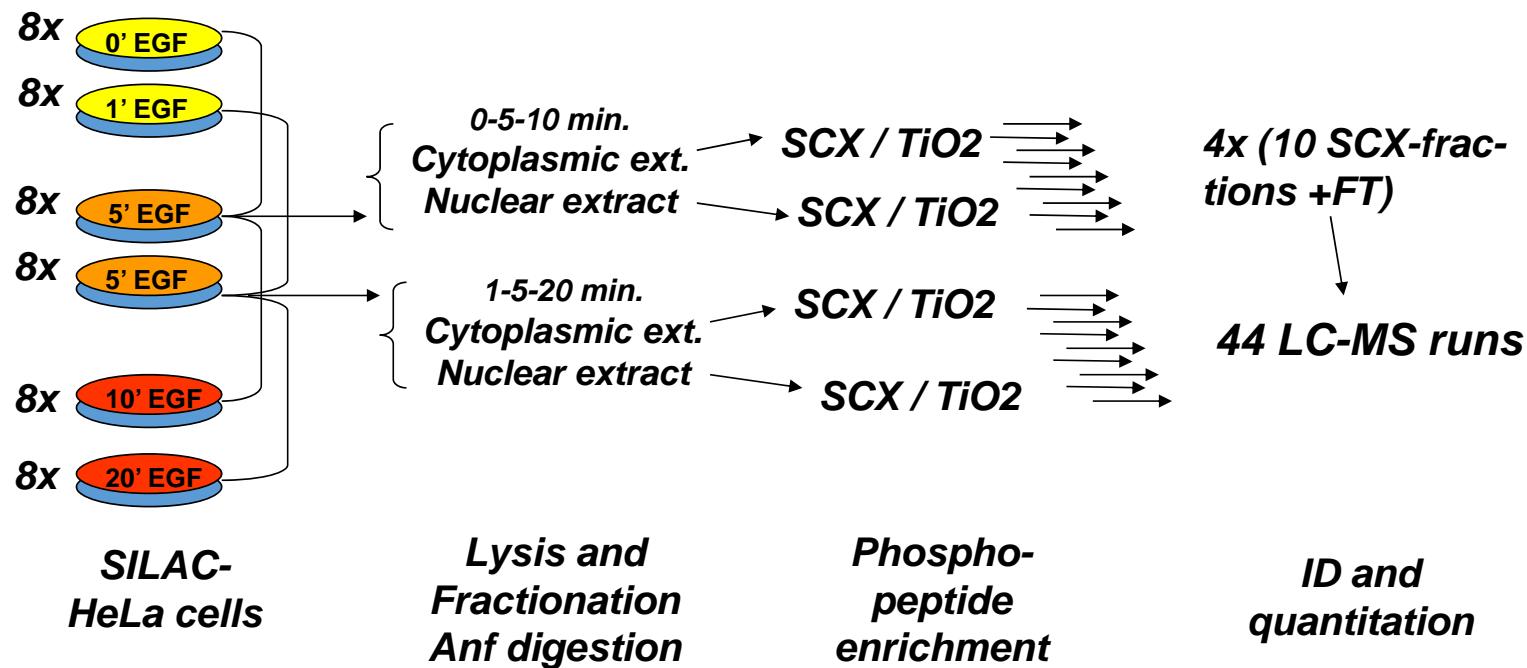
NATURE BIOTECHNOLOGY VOLUME 22 NUMBER 9 SEPTEMBER 2004



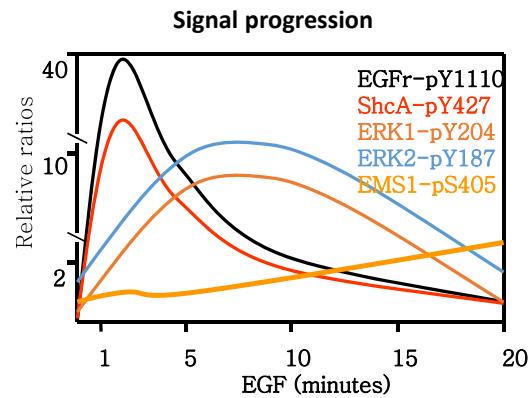
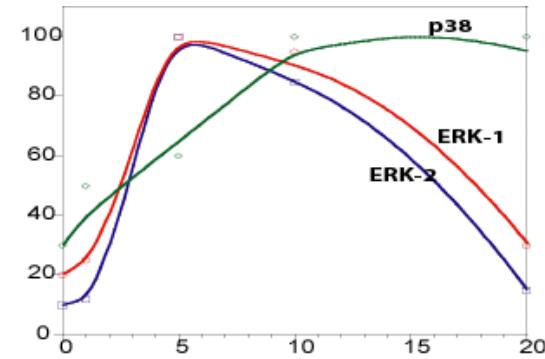
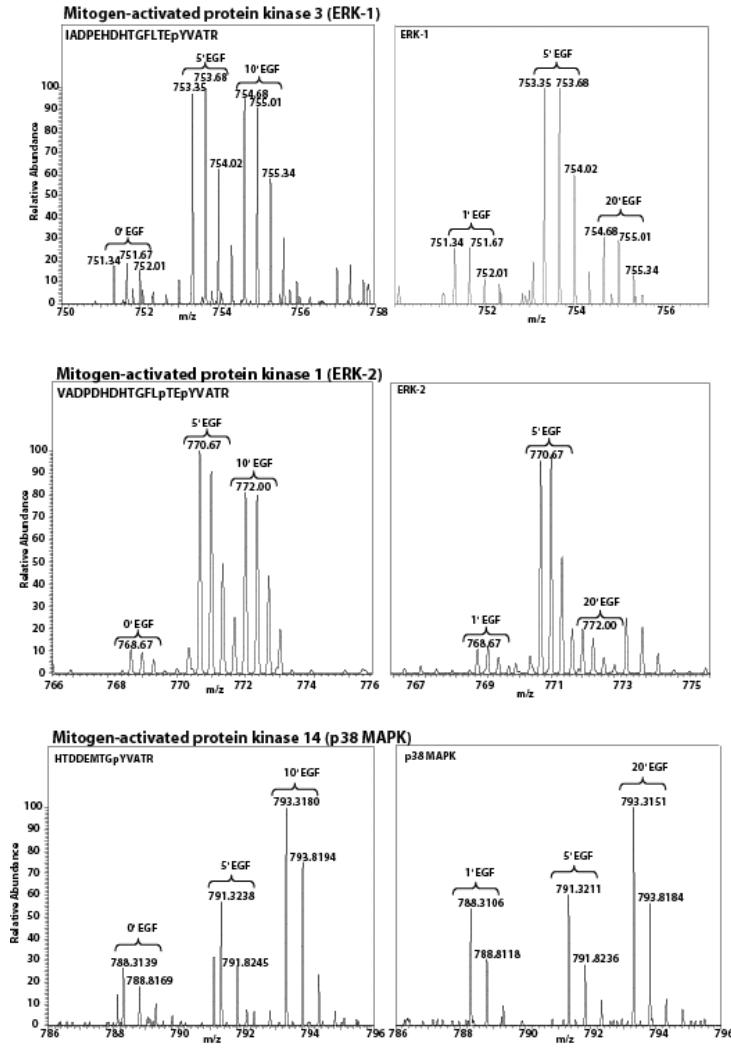
Five time-point “multiplexing” profile



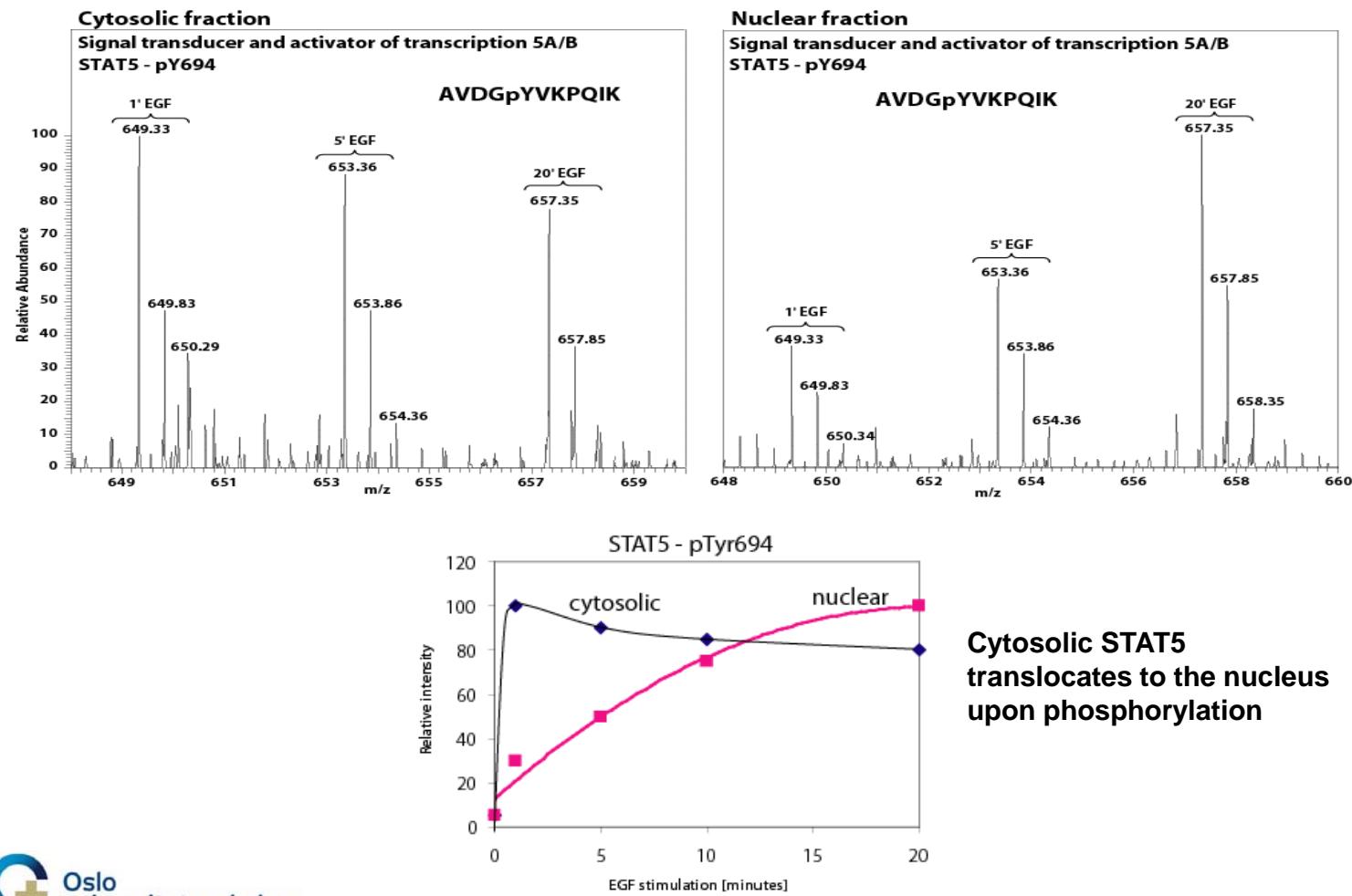
Quantitative phosphoproteomics in EGFR signaling



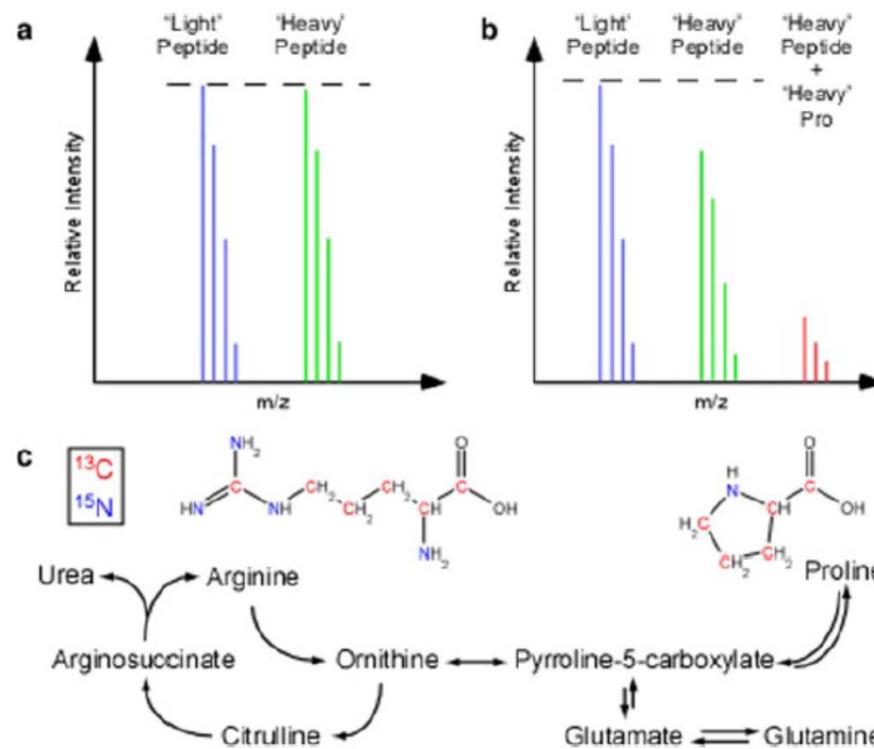
MAP kinases activation



Spatial distribution of phosphorylation dynamics



Proline Conversion!



Mol Cell Proteomics. 2008 Sep;7(9):1587-97

A simple solution for proline conversion issue

An experimental correction for arginine-to-proline conversion artifacts in SILAC-based quantitative proteomics

Research

x Author's Choice

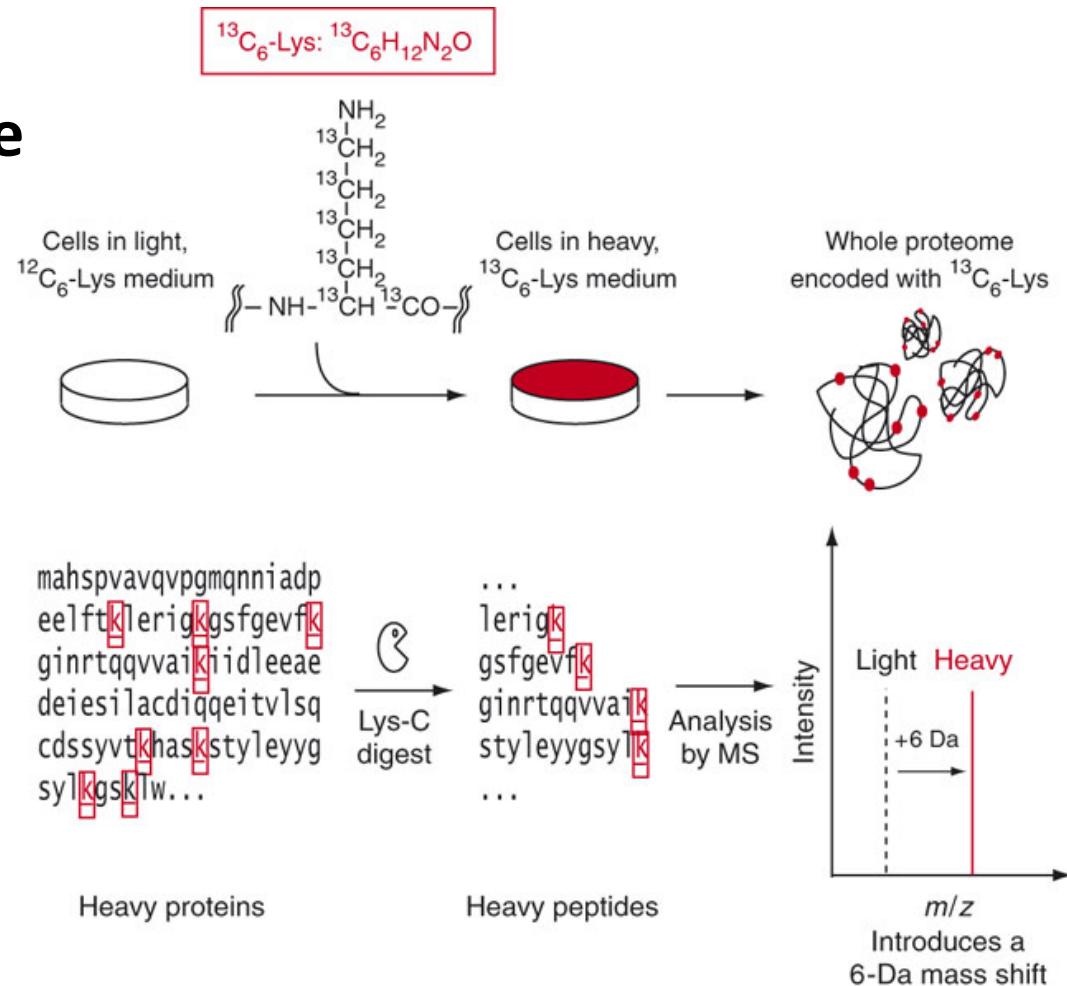
A Genetic Engineering Solution to the “Arginine Conversion Problem” in Stable Isotope Labeling by Amino Acids in Cell Culture (SILAC)*

Claudia C. Bicho‡, Flavia de Lima Alves§, Zhuo A. Chen§, Juri Rappaport¶, and Kenneth E. Sawin||

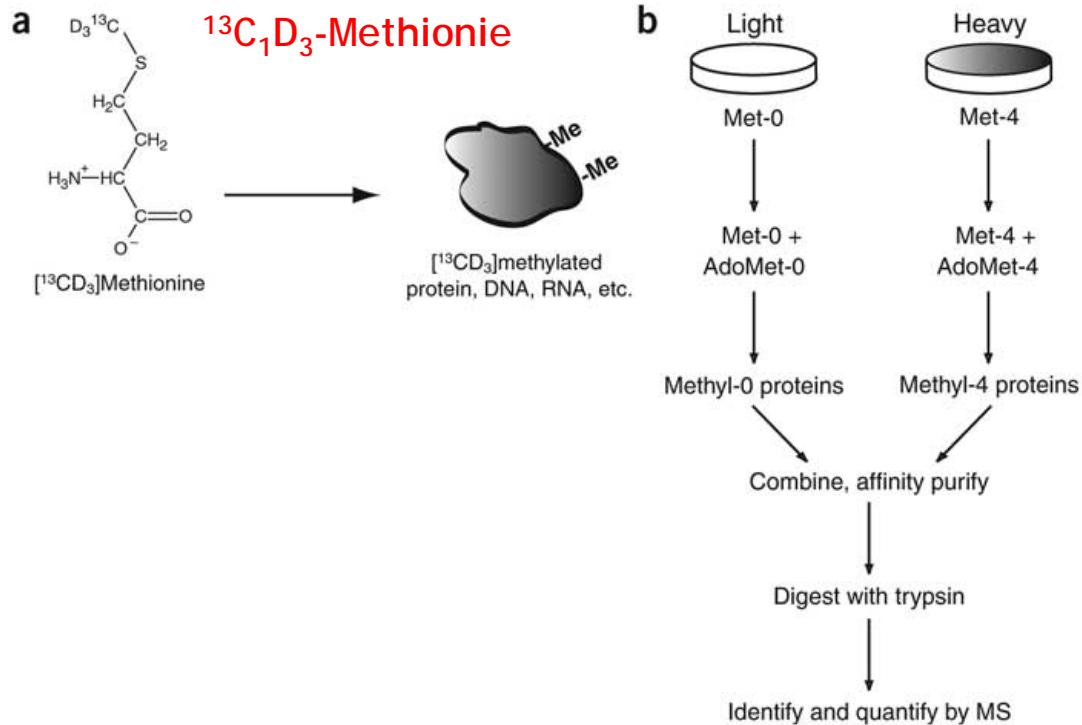
Research

Prevention of Amino Acid Conversion in SILAC Experiments with Embryonic Stem Cells*

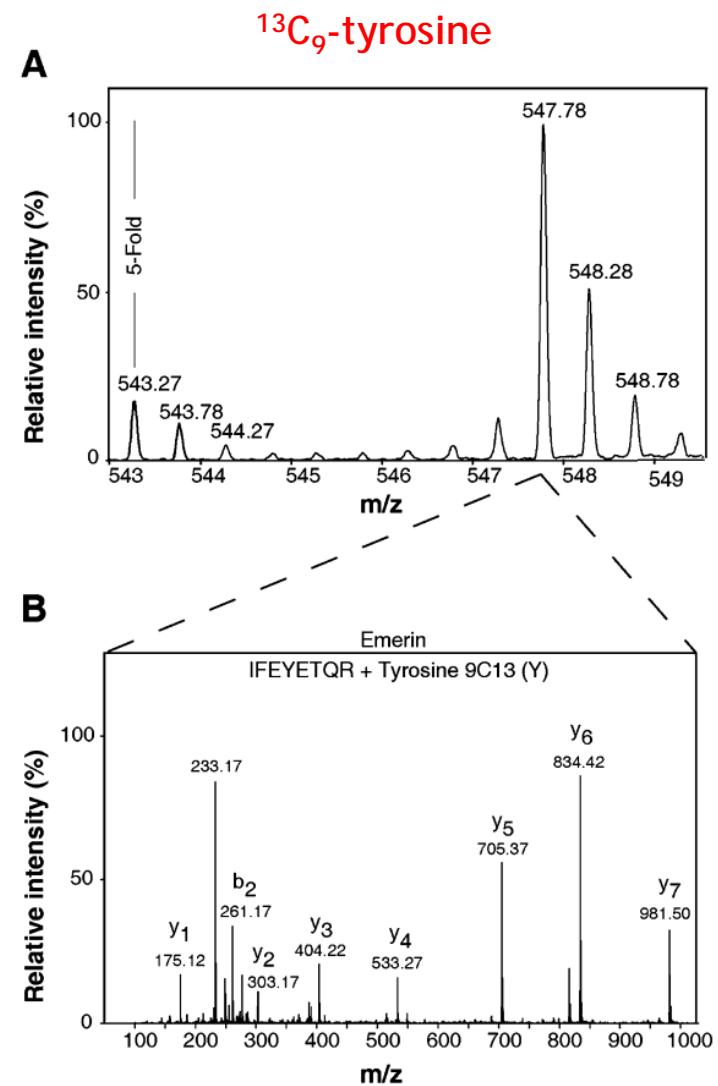
Sean C. Bendall‡§¶||, Chris Hughes‡¶||, Morag H. Stewart§||, Brad Doble§, Mickie Bhatia§, and Gilles A. Lajoie‡**



Other amino acids

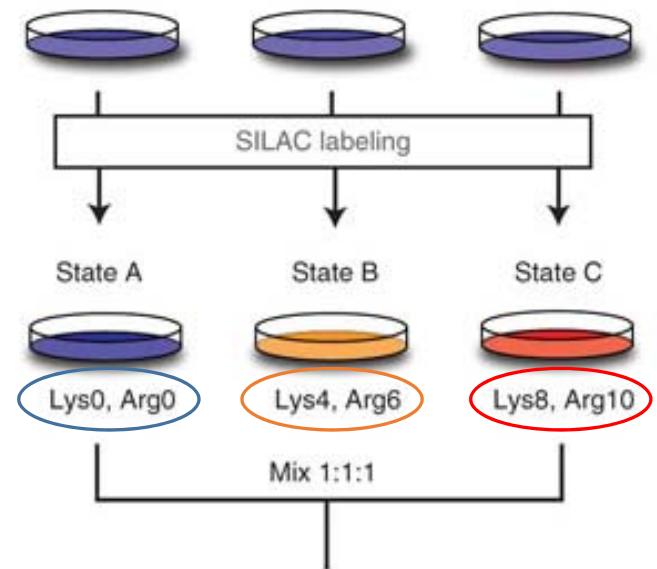


Nature Methods - 1, 119 - 126 (2004)

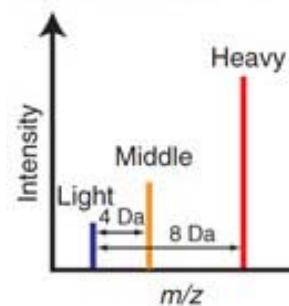


J. Proteome Res., 2005, 4 (5), pp 1661–1671

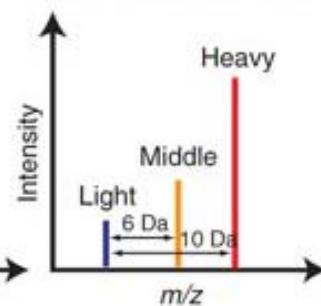
Current classical SILAC methods



Lys-containing peptide



Arg-containing peptide



SILAC ratios

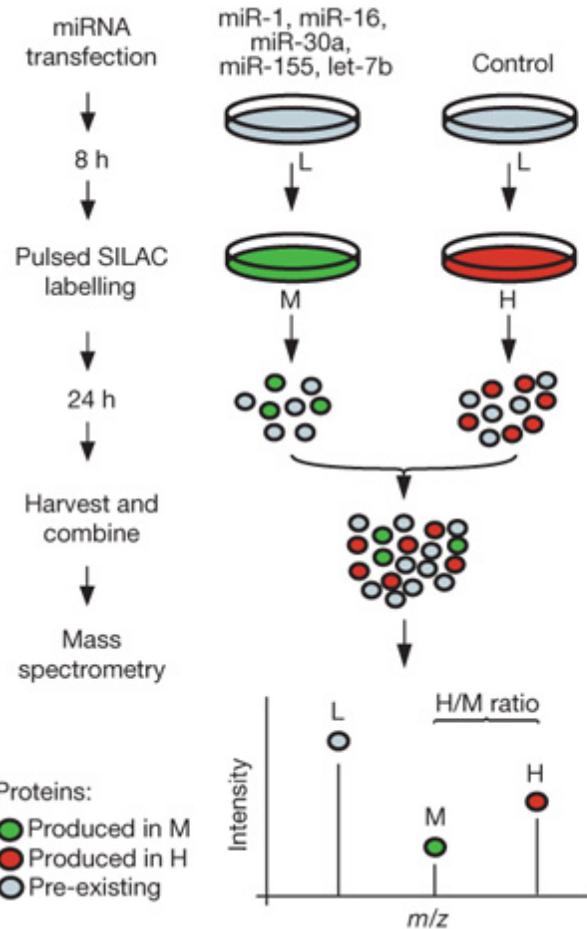
$$\text{Ratio}_1 = \frac{\text{Heavy (State C)}}{\text{Light (State A)}}$$

$$\text{Ratio}_2 = \frac{\text{Heavy (State C)}}{\text{Middle (State B)}}$$

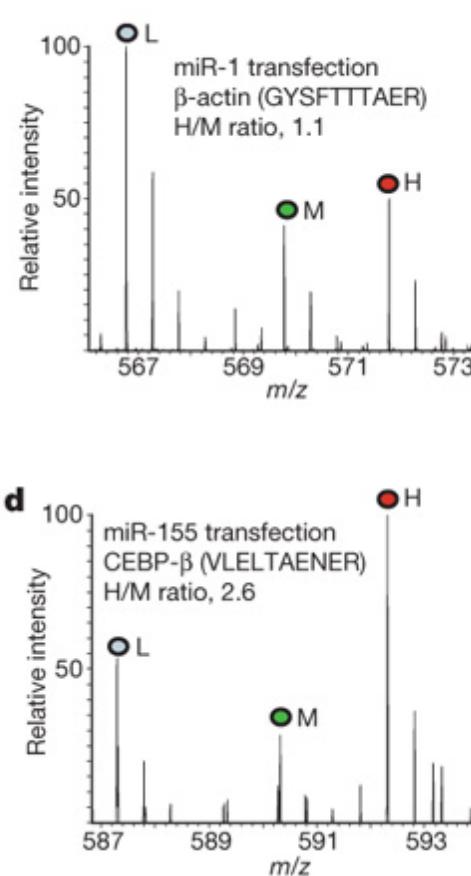
$$\text{Ratio}_3 = \frac{\text{Middle (State B)}}{\text{Light (State A)}}$$

Pulsed SILAC (pSILAC)

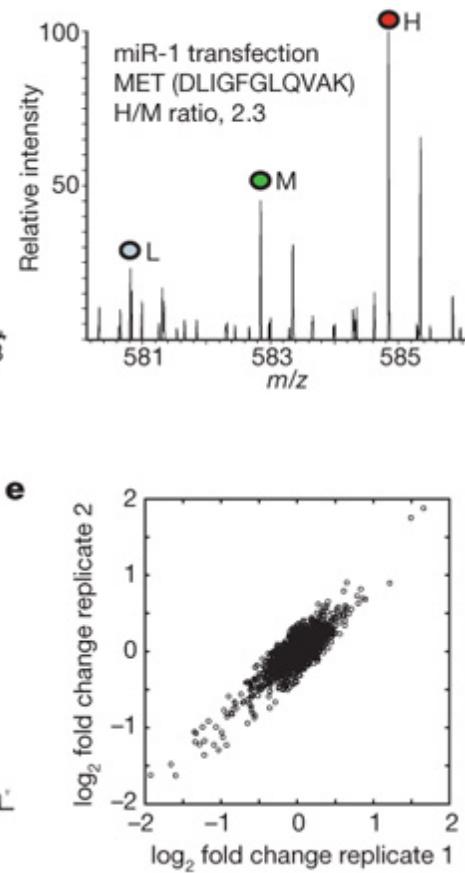
a



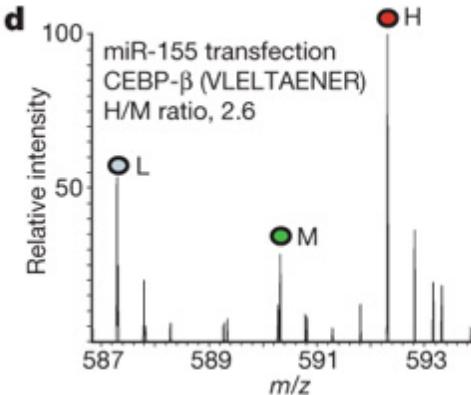
b



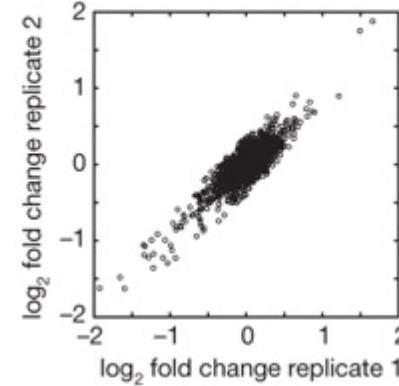
c



d



e

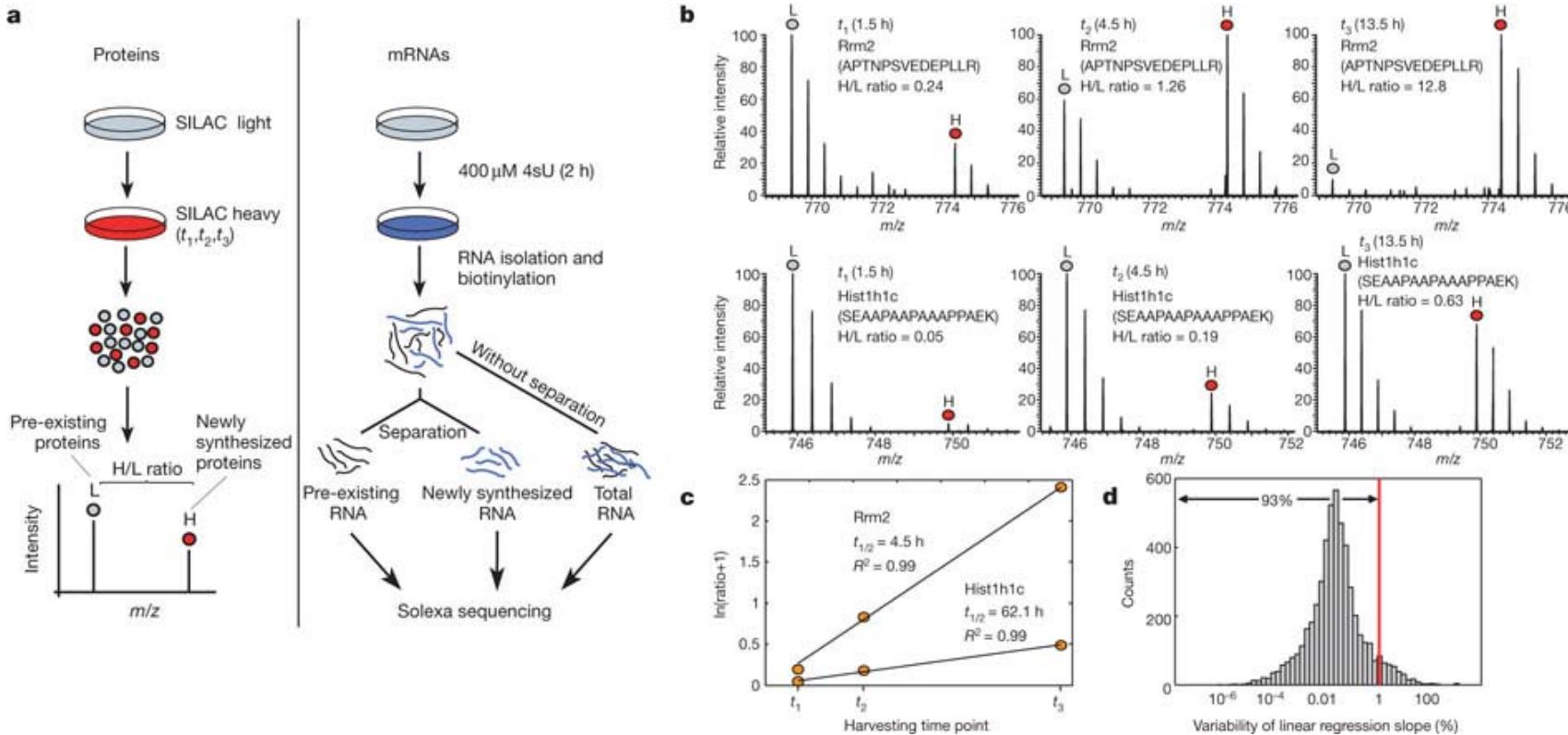


Matthias Selbach



Nikolaus Rajewsky

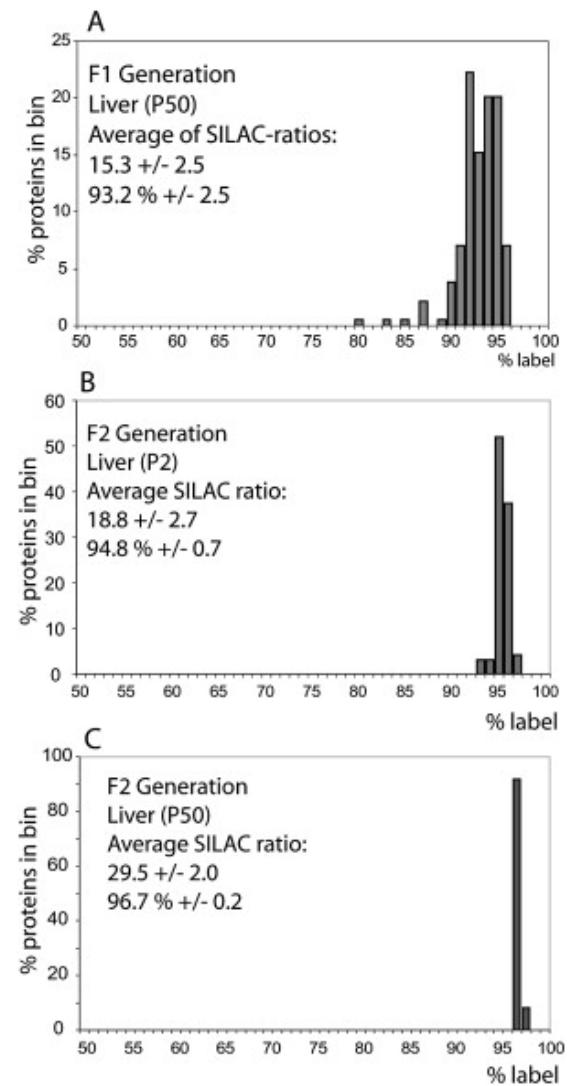
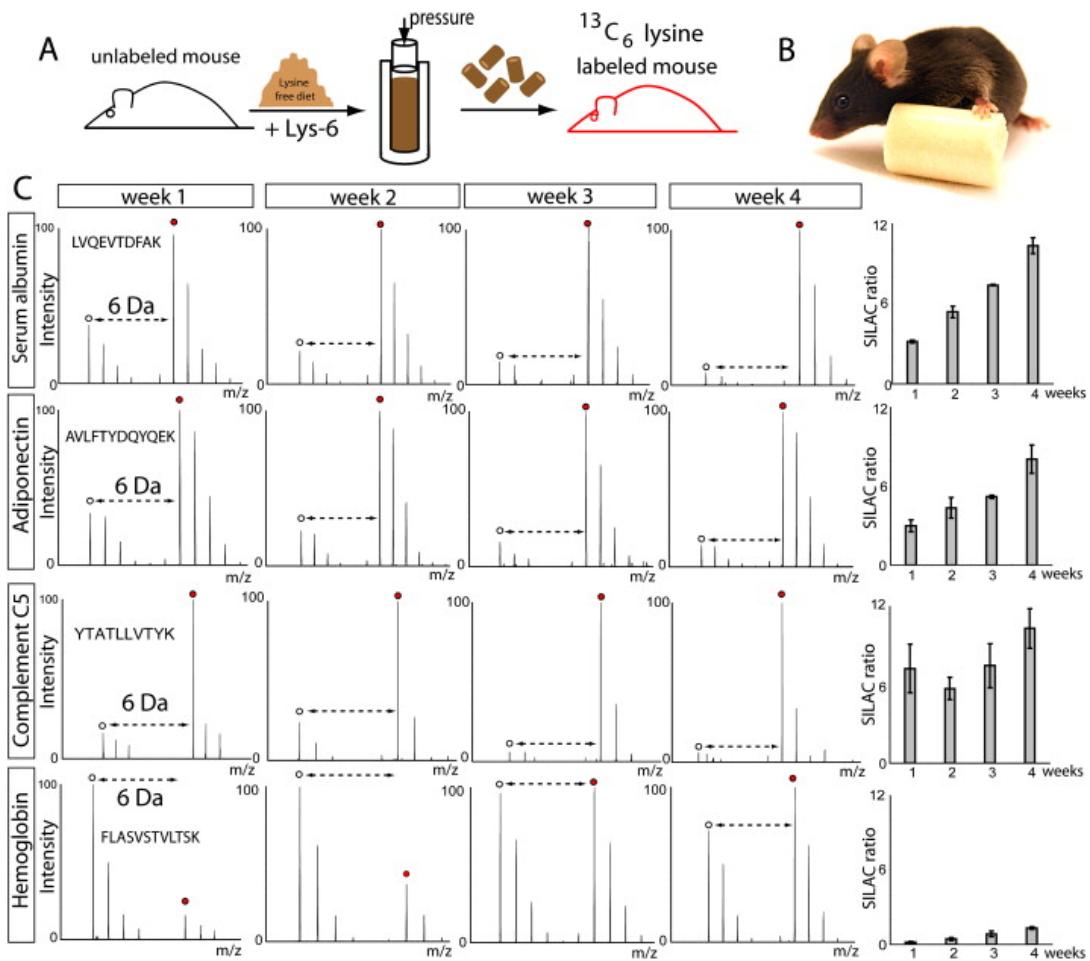
Pulsed SILAC (pSILCA)



Matthias Selbach

Parallel quantification of mRNA and protein turnover and levels

SILAC-mouse

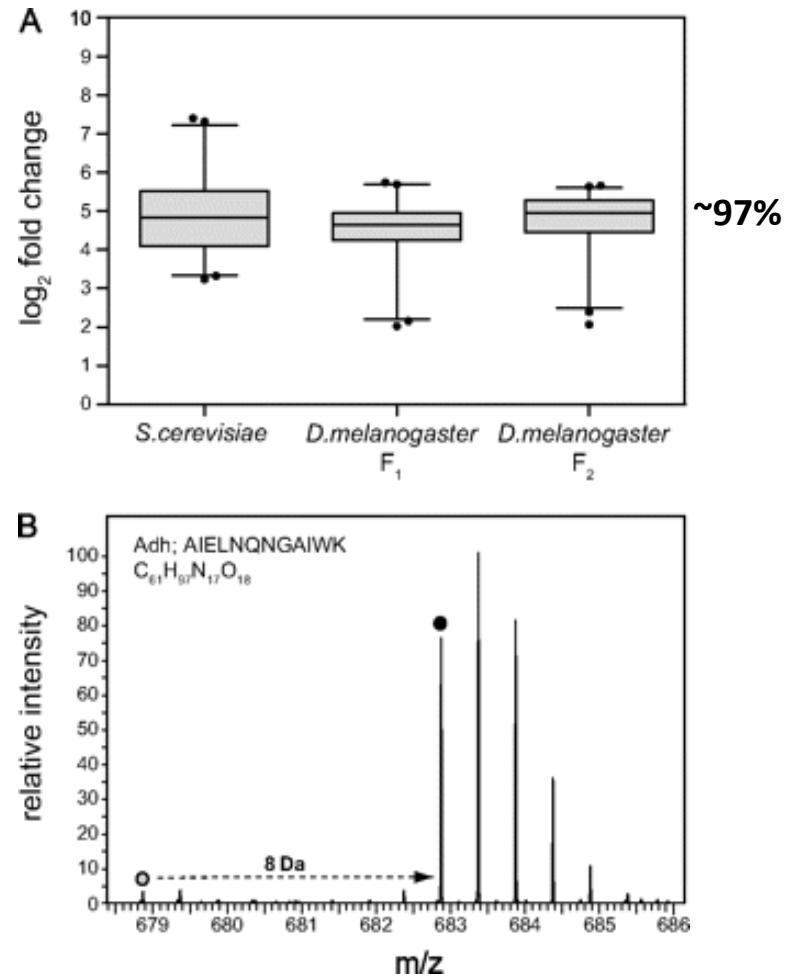
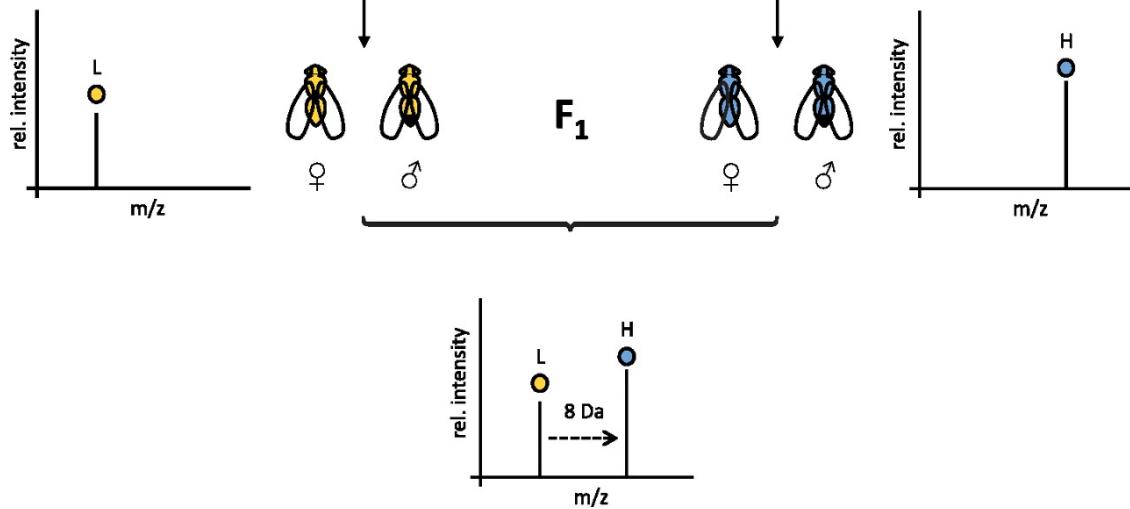


Cell. 2008 Jul 25;134(2):353-64

SILAC-fly



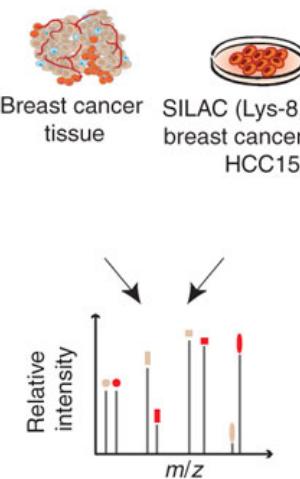
Matthias Selbach



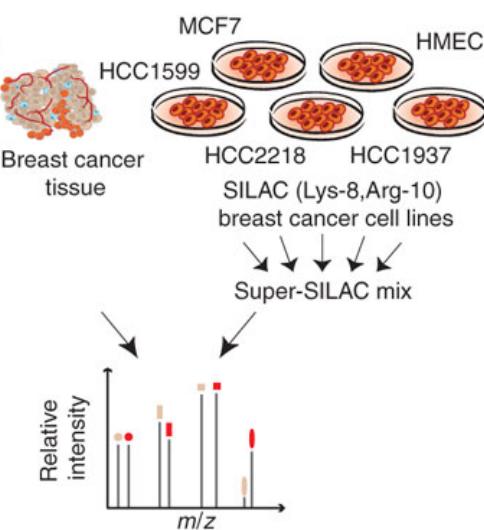
[Mol Cell Proteomics.](#) 2010 Oct;9(10):2173-83

Super-SILAC

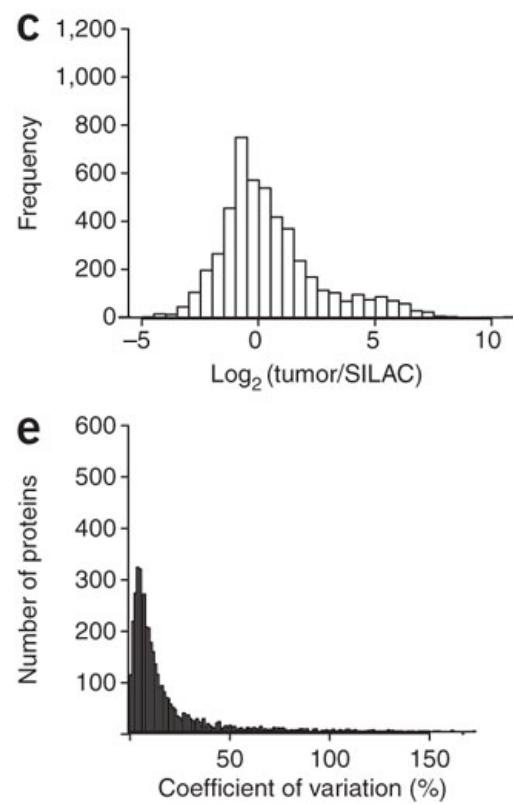
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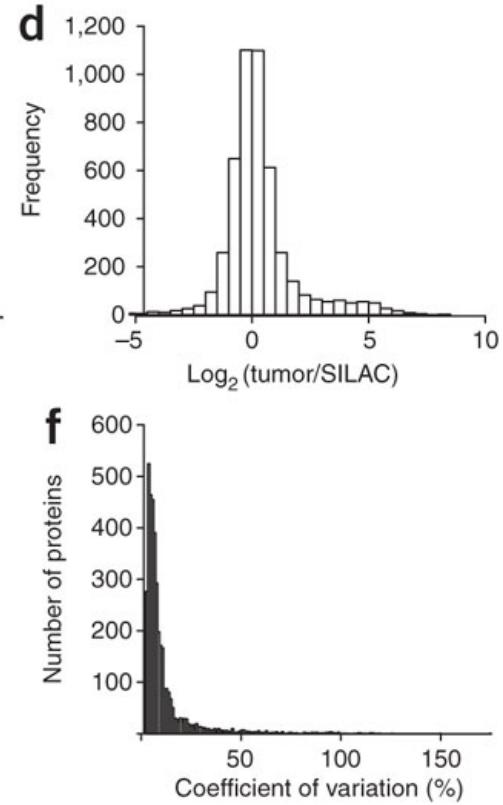
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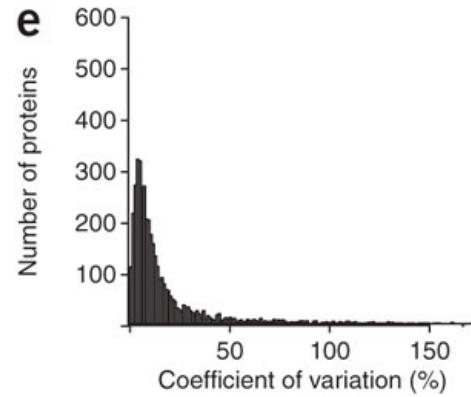
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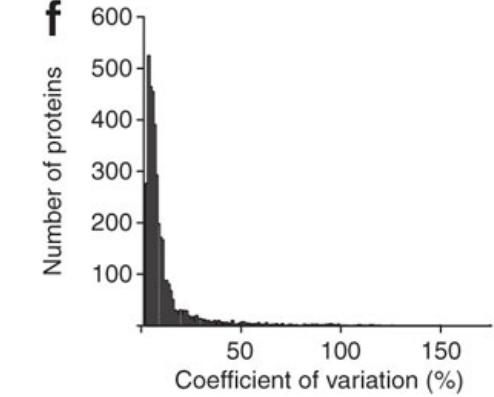
d



e



f



Spike-in SILAC

