
Preface

Since its introduction about a decade ago, SILAC—stable isotope labeling by amino acids in cell culture—combined with mass spectrometry has not merely been integrated into the spectrum of quantitative methods applied in proteomics and protein research laboratories. In fact, it has become a prime tool of functional proteomics research, which allows us to address important questions in the fields of biology, biotechnology, medicine, and beyond.

SILAC could obtain this status by virtue of its versatility. This book tries to provide a synopsis of the large array of different SILAC methods by presenting a set of protocols that have been established by renowned scientists and their working groups. These protocols describe basic applications such as the labeling of various model organisms but also highly advanced strategies relying on SILAC, e.g., for the analysis of protein interactions, the mapping of posttranslational modifications, or the characterization of subcellular proteomes.

The book aims at applicability, and so all chapters entail step-by-step instructions that are easy to follow.

Chapter 1 provides a historical overview in which Matthias Mann outlines, from a very personal perspective, important steps in the development and implementation of SILAC as well as further significant innovations in the use of this unique method over the last 12 years. The main feature of the SILAC technology is the potential to label the entire proteome with defined combinations of stable isotopes during an organism's growth. At first applied to mammalian cell lines, its applicability to various other organisms has since been demonstrated as well. Chapter 2 is about a protocol for the effective labeling of both Gram positive and negative bacteria using only lysine in its “light” and “heavy” version. Chapter 3 continues with the description of an experimental procedure for complete SILAC labeling of the yeast *Saccharomyces cerevisiae*, a widely used model system for higher eukaryotes, including details on the generation of auxotrophic strains and the SILAC-based analysis of membrane protein complexes. SILAC applied to the protozoan *Trypanosoma brucei*, a further unicellular eukaryotic organism, enables new studies on the parasite's unique biology. A straightforward protocol for the metabolic labeling of both the procyclic and the bloodstream form of *T. brucei* with SILAC amino acids in a cell culture system can be found in Chapter 4.

Since the complete incorporation of SILAC amino acids into proteomes requires approximately five cell doublings, issues of partial labeling arise when working with nondividing cells. Chapter 5 addresses this problem by introducing an elegant multiplex SILAC labeling approach. It allows for the quantification of partially labeled proteins from nondividing cell types as exemplified here by primary neurons.

The following four chapters deal with the applicability of SILAC to the metabolic labeling of multicellular organisms including the higher plant model system *Arabidopsis thaliana* as well as *Drosophila melanogaster*, *Mus musculus*, and *Caenorhabditis elegans*. In Chapter 6, limitations of the SILAC technology in plant cells are discussed and an alternative protocol for labeling whole *A. thaliana* plants using ^{15}N salts is provided. SILAC labeling of *D. melanogaster*, as described in Chapter 7, relies on feeding “heavy” lysine-labeled yeast to flies, which can easily be implemented for quantitative analyses. Chapter 8 informs about the details how to generate SILAC mice and how to utilize them as spike-in standard

for quantitative proteomics studies of organs. And Chapter 9 presents an innovative protocol for SILAC-based quantitative phosphoproteome analyses in *C. elegans* along with RNAi-mediated gene knockdown.

The high potential SILAC offers for the global study of signaling networks is highlighted in Chapter 10 providing the reader with state-of-the-art knowledge and practical information about how to conduct large-scale quantitative and time-resolved phosphoproteomics studies using SILAC. Detailed experimental procedures for the global analysis of dynamic changes in protein ubiquitination and methylation are described in Chapters 11 and 12, respectively.

A further important field of application for SILAC is the study of protein interactomes, in which SILAC-based protein quantification provides an effective measure to reliably distinguish between specific interaction partners and co-purified background binders in affinity-based protein purification or coimmunoprecipitation experiments. Complementary to Chapter 3, the potential and versatility of SILAC approaches for the study of protein–protein interactions are discussed in Chapters 13–16, i.e., the comparative analysis of human protein complexes (Chapter 13), the identification of stable and dynamic interaction partners exemplified by the human 26S proteasome complex (Chapter 14), the characterization of nuclear protein–protein interactions in mammalian cells (Chapter 15), and the delineation of dynamic processes involved in the assembly of the human spliceosome (Chapter 16).

Chapter 17 turns to a protocol for protein interaction studies in autotrophic organisms, in which $^{14}\text{N}/^{15}\text{N}$ labeling combined with coimmunoprecipitation and antigen competition is used.

At first introduced as a label-free approach, the original protein correlation profiling method has been refined using SILAC. Dynamic aspects of protein interactomes and organellar proteomes can be analyzed by such spike-in SILAC standard-enhanced variations, for which protocols are presented in Chapters 18 and 19, respectively.

An important innovation of the SILAC technology has been its implementation in the study of cancer tissues, which is based on the generation of SILAC-labeled reference proteomes used as spike-in standards for relative protein quantification. In Chapter 20, the reader finds detailed information about how to properly design and successfully conduct such super-SILAC experiments for quantitative proteome analyses of tissue samples. Chapter 21 provides an optimized protocol for SILAC labeling of *D. melanogaster*, both in cell culture and in tissues, which allows tackling various questions in genetics and developmental biology. As outlined in Chapter 22, SILAC was also applied to the global study of secreted proteins, a new promising approach to identify protein biomarker candidates for human diseases. Furthermore, employed in pulse experiments, SILAC is a powerful technology for the large-scale analysis of protein turnover rates. Chapter 23 is about a protocol for pulsed SILAC that enables the identification of microRNA-mediated changes in the synthesis rates of proteins. The protocol is complemented by the description of computational and experimental approaches for the analysis of potential microRNA targets identified in a pulsed SILAC study.

The SILAC technology is intimately linked to high-resolution mass spectrometry facilitating the generation of a wealth of data about both the identity and the abundance of proteins in biological samples. Efficient processing and computational analysis of large SILAC-encoded mass spectrometry datasets is therefore a key step in quantitative proteomics studies. Chapter 24 presents an easy-to-follow protocol for the quantitative analysis of SILAC-based proteomics data using the freely available software MaxQuant.

In sum, the methods and experimental strategies described in this volume hopefully give an impression of the amazing diversity of SILAC applications. Smartly combined with different molecular and cell biology or biochemical techniques, SILAC is and will be a most valuable and multifunctional tool for many research studies aiming at a better understanding of cellular organization, protein interaction, and signaling networks as well as protein translation, turnover, and expression changes in health and disease.

The chapters also provide important information about practical aspects of sample fractionation, enrichment, and/or further processing steps as well as—to different extent—details on how to conduct LC/MS and downstream data analyses. In doing so, the book hopefully will serve students and experienced scientists alike as a valuable reference of how to make use of the SILAC technology for their own research.

I would like to thank the editors of the book series for their initial suggestion and their ongoing support in all editorial matters and also members of my group, especially Silke Oeljeklaus and Ida Suppanz, for providing much needed and welcomed assistance.

Finally and most importantly, I would like to cordially thank all contributors for sharing their knowledge!

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Stable Isotope Labeling by Amino Acids in Cell Culture
(SILAC)

Methods and Protocols

Warscheid, B. (Ed.)

2014, XIV, 372 p. 65 illus., 27 illus. in color., Hardcover

ISBN: 978-1-4939-1141-7

A product of Humana Press