
Preface

Recombinant DNA technologies have revolutionized the way biologists study and manipulate proteins. The ability to produce chimeric proteins by inserting a peptide sequence before, after, or within a protein through genetic manipulation has led to the development of a multitude of techniques that render a protein of interest unique merely by adding an encoded label. Prominent examples are the introduction of small epitopes for immunolabeling, the use of affinity tags for protein purification, and the fusion to fluorescent proteins for imaging. The power of those approaches lies in the simplicity and absolute specificity of genetic encoding. However, the genetically encodable tags are a priori limited by the 20 proteogenic amino acids, which cover a very limited part of the chemical space.

This limitation is overcome by techniques that allow the covalent functionalization of a protein of interest with a synthetic probe, which includes fluorescent dyes, radiolabels, chemical cross-linkers, photoactivatable molecules, pharmacologically active compounds, toxins, synthetic biosensors, or nanoparticles [1, 2]. The application of such artificial synthetic objects in living cells or living organisms opens new avenues for studying and manipulating protein function in living systems. The issue of labeling specificity becomes critical for labeling in situ in a physiological context or in the cases where well-defined chemically modified biomolecules are desired. Classical reactive labeling techniques, however, are usually not selective enough for this purpose. This problem has been overcome over the last 15 years based on the pioneering work of Roger Y. Tsien and his group, and today various covalent labeling techniques are available that are perfectly site-specific and can be applied in the context of cells and organisms.

Today, the field as a whole is at an exciting stage: while some site-specific labeling approaches are now fully mature and well adopted by the molecular and cell biology community, new approaches and ingenious ways of applying existing approaches continue to emerge. The creative application of site-specific protein labeling techniques in cell biology beyond simple fluorescent labeling requires both a biologist's knowledge of biological problems and an organic chemist's understanding of the opportunities and problems involved in generating a custom label for the problem in question. *Methods of Site-Specific Protein Labeling* is directed at scientists from all fields that want to get a better understanding of labeling techniques. In particular, it aims at providing researchers interested in such techniques with advice on how to choose the most appropriate labeling method for their biological question and information on general considerations and problems involved in the design, the generation, and the application of the corresponding organic molecules used for the labeling step.

The first chapters deal with the background and basic considerations of site-specific protein labeling. As often, the historical perspective is insightful: In Chapter 1, B. Albert Griffin, Stephen R. Adams, and Roger Y. Tsien provide a highly interesting recollection of why and how they came to invent the FAsH-tag. Chapter 2, written from the industrial perspective by Lukas Leder from Novartis, provides an overview of applications of labeled proteins in assays that are common in the industry, and Lukas Leder shares experiences that his laboratory made with adopting site-specific protein labeling. Chapter 3 was motivated

The chapters that follow cover the most relevant methods of site-specific protein labeling with selected applications. The techniques described include tag-based methods (which can be further subdivided), methods that rely on the incorporation of unnatural amino acids during protein translation, and methods that work specifically on native, untagged proteins.

Developed by Roger Y. Tsien and coworkers, the archetype of a self-labeling tag is the retracysteine tag which can specifically react with biarsenical compounds [3]. A recently developed self-labeling tag is described in the contribution of Lina Cui and Jianghong Rao (Chapter 5), which presents how a single terminal cysteine can be exploited for site-specific labeling with cyanobenzothiazole derivatives. The contribution of Thomas K. Berger and Ehud Y. Isacoff (Chapter 6) demonstrates additionally how well positioned cysteines within a cell-membrane receptor can be functionalized with thiol-linked environment-sensitive dyes to measure protein motion in ion channels in real time.

The size of the added tag sometimes being a concern, strategies combining the small size of a short peptide sequence with the speed and high specificity of protein-catalyzed labeling have also been designed. In these methods, the labeling reaction is trimolecular and involves a transferase enzyme, the molecule used for labeling, and the recognition (acceptor) peptide sequence. Here, the transferase enzyme can be added in medium or needs to be coexpressed if intracellular labeling is required. The enzyme-mediated labeling of tags is described for Sfp-mediated labeling—applied in phage display—by Bo Zhao et al. (Chapter 11), for BirA-mediated labeling by Michael Fairhead and Mark Howarth (Chapter 12), and for Sortase-mediated labeling by Max Popp (Chapter 13).

Fusing a peptide or protein tag to the protein of interest is not required in techniques relying on unnatural amino acid incorporation during protein synthesis. The inserted unnatural amino acid plays the role of the molecular anchor in this case. Since the size of the side chain of the unnatural amino acid can be limited by the cell's protein translation machinery, often a small chemical functionality is introduced to which a chemical probe can be tethered in a second step using various bioorthogonal chemical “click” reactions. Using this methodology, the contribution of Peter Landgraf, Elmer R. Antileo, Erin M. Schuman, and Daniela C. Dieterich (Chapter 14) illustrates how metabolic labeling can be used to mark newly synthesized proteomes. The contribution of Kathrin Lang, Lloyd Davis, and Jason W. Chin (Chapter 15) describes the recent development of methods to fully genetically encode these unnatural “anchor” amino acids in order to be able to selectively label a single protein at a specific residue in living mammalian cells.

The “Holy Grail” in protein labeling is to be able to specifically target any native, non-tagged protein with a chemical probe in a physiological context. The two final chapters are reserved for this topic and are written by Itaru Hamachi with coworkers Tomonori Tamura (Chapter 16) and Shinya Tsukiji (Chapter 17), respectively. They describe two related approaches to how native protein labeling can be achieved by relying on labeling probes made of three parts, (1) a recognition moiety, binding selectively to the native protein of interest, (2) the probe to be attached, and (3) a reactive group, which can react with nucleophilic residues on the protein surface. While this reactive group is in principle capable of labeling any protein in a mixture, selectivity is achieved due to close proximity of the reactive group to the protein of interest, enforced by the recognition moiety.

In putting together this edition, we have attempted to include what we perceive as the currently most relevant and best established labeling methods across the different general methodologies. A number of important techniques are not presented, however, because detailed reviews and protocols have been recently published elsewhere. This includes the tetracysteine tag [3], lipoic acid-mediated labeling [4], labeling based on the genetically encoded aldehyde tag [5], and transglutaminase-based labeling [6]. While we have not attempted to include examples for every possible application of site-specific protein labeling, the chapters are nonetheless designed to provide guidance on the limits and possibilities of each technique and references to applications that have been described in the literature. For more information on applications and a comparative analysis of the various techniques, as well as introductions to other labeling methods not included here, we invite the readers to consult recent reviews on site-specific labeling [1, 2].

Finally, we thank all the authors that have contributed to this edition of *Methods in Molecular Biology*. We hope that both authors and readers will find this compendium useful and that it will support the further development of creative ideas in the field and facilitate making site-specific protein labeling a standard, widely used lab technique.

Paris, France
Munich, Germany

Arnaud Gautier
Marlon J. Hinner

References

1. O'Hare H, Johnsson K, Gautier A (2007) Chemical probes shed light on protein function. *Curr Opin Struct Biol* 17:488–494. doi: [10.1016/j.Sbi.2007.07.005](https://doi.org/10.1016/j.Sbi.2007.07.005)
2. Hinner MJ, Johnsson K (2010) How to obtain labeled proteins and what to do with them. *Curr Opin Biotechnol* 21:766–776. doi: [10.1016/j.copbio.2010.09.011](https://doi.org/10.1016/j.copbio.2010.09.011)

3. Hoffmann C, Gaietta G, Zürn A, Adams SR, Terrillon S, Ellisman MH, Tsien RY, Lohse MJ (2010) Fluorescent labeling of tetracysteine-tagged proteins in intact cells. *Nat Protoc* 5:1666–1677. doi: [10.1038/nprot.2010.129](https://doi.org/10.1038/nprot.2010.129)
4. Uttamapinant C, Sanchez MI, Liu DS, Yao JZ, Ting AY (2013) Site-specific protein labeling using PRIME and chelation-assisted click chemistry. *Nat Protoc* 8:1620–1634. doi: [10.1038/nprot.2013.096](https://doi.org/10.1038/nprot.2013.096)
5. Rabuka D, Rush JS, deHart GW, Wu P, Bertozzi CR (2012) Site-specific chemical protein conjugation using genetically encoded aldehyde tags. *Nat Protoc* 7:1052–1067. doi: [10.1038/nprot.2012.045](https://doi.org/10.1038/nprot.2012.045)
6. Dennler P, Schibli R, Fischer E (2013) Enzymatic antibody modification by bacterial transglutaminase. *Methods Mol Biol* 1045:205–215. doi: [10.1007/978-1-62703-541-5_12](https://doi.org/10.1007/978-1-62703-541-5_12)

Site-Specific Protein Labeling

Methods and Protocols

Gautier, A.; Hinner, M.J. (Eds.)

2015, XII, 267 p. 72 illus., 34 illus. in color., Hardcover

ISBN: 978-1-4939-2271-0

A product of Humana Press