



Recent advancements in mass spectrometry–based tools to investigate newly synthesized proteins

Wouter van Bergen^{1,2}, Albert J. R. Heck^{1,2} and Marc P. Baggelaar^{1,2}

Abstract

Tight regulation of protein translation drives the proteome to undergo changes under influence of extracellular or intracellular signals. Despite mass spectrometry–based proteomics being an excellent method to study differences in protein abundance in complex proteomes, analyzing minute or rapid changes in protein synthesis and abundance remains challenging. Therefore, several dedicated techniques to directly detect and quantify newly synthesized proteins have been developed, notably puromycin-based, bio-orthogonal non-canonical amino acid tagging–based, and stable isotope labeling by amino acids in cell culture–based methods, combined with mass spectrometry. These techniques have enabled the investigation of perturbations, stress, or stimuli on protein synthesis. Improvements of these methods are still necessary to overcome various remaining limitations. Recent improvements include enhanced enrichment approaches and combinations with various stable isotope labeling techniques, which allow for more accurate analysis and comparison between conditions on shorter timeframes and in more challenging systems. Here, we aim to review the current state in this field.

Addresses

¹Biomolecular Mass Spectrometry and Proteomics, Bijvoet Center for Biomolecular Research and Utrecht Institute for Pharmaceutical Sciences, University of Utrecht, Padualaan 8, Utrecht, 3584 CH, the Netherlands

²Netherlands Proteomics Center, Padualaan 8, Utrecht, 3584 CH, the Netherlands

Corresponding author: Baggelaar, Marc P. (m.p.baggelaar@uu.nl)

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Keywords

Newly synthesized proteins, Protein synthesis, Protein dynamics, Mass spectrometry, Proteomics, Bio-orthogonal noncanonical amino acid

tagging (BONCAT), Puromycin, Stable isotope labeling by amino acids in cell culture (SILAC).

Abbreviations

AHA, *L*-azidohomoalanine; ANL, *L*-azidonorleucine; BONCAT, Bio-orthogonal non-canonical amino acid tagging; HPG, *L*-homo-propargylglycine; iTRAQ, Isobaric tag for relative and absolute quantitation; LC, Liquid chromatography; LPS, Lipopolysaccharide; mePROD, Multiplexed enhanced protein dynamics; MITNCAT, Multiplex isobaric tagging/non-canonical amino acid tagging multiplex; mPDP, Multiplexed proteome dynamics profiling; mRNA, Messenger ribonucleic acid; MS, Mass spectrometry; NCAA, Non-canonical amino acid; NSP, Newly synthesized protein; OPP, O-propargyl-puromycin; pSILAC, pulsed SILAC; PUNCH–P, Puromycin-associated nascent chain proteomics; QuaNCAT, Quantitative non-canonical amino acid tagging; RNA, Ribonucleic acid; SILAC, Stable isotope labeling by amino acids in cell culture; TMT, Tandem mass tag; TNF- α , Tumor necrosis factor alpha; tRNA, Transfer-ribonucleic acid.

Introduction

Protein translation is the gatekeeper between the genome and the proteome by serving as the final regulatory layer before gene expression. This multistep process comprises initiation, elongation, termination, and ribosome recycling. Protein synthesis is a fundamental process that is strongly connected to cell growth (Figure 1). Protein synthesis is generally upregulated in tumor cells, and consequently, inhibition of protein synthesis is an attractive strategy for cancer treatment [1,2].

In addition to proteome expansion to facilitate cell growth, spatiotemporal control of the translational machinery on protein synthesis by translation of newly transcribed mRNA or pre-existing mRNA pools orchestrates rapid adaptations to environmental cues and internal signals. Thereby, protein synthesis underlies many crucial physiological processes such as learning and memory formation, T cell activation, and inflammation [3].

Although the importance of protein synthesis is well recognized, quantitative analysis of protein synthesis has proven to be challenging. The rate of protein synthesis can be indirectly inferred from mRNA levels measured by RNA-sequencing or ribosome profiling. However, mRNA levels show limited correlation to protein synthesis because translation is, as the final regulatory layer of the proteome, a highly and tightly regulated process. This allows the cell to rapidly synthesize specific proteins

under stress or other extracellular or intracellular influences [4,5]. Direct analysis of synthesis at the protein level is therefore indispensable to investigate alterations in the proteome over time or in response to stimuli. Here, we review recent progress in the development of mass spectrometry (MS)-based tools to investigate, at a proteome-wide level, protein synthesis.

Mass spectrometry-based proteomics

Continuous advances in the capabilities and performance of high-resolution mass spectrometers allow the study of the proteome with increasing depth and detail. Currently, thousands of proteins and their post-translational modifications in complex biological samples can be analyzed in parallel. Quantitative techniques, such as stable isotope labeling by amino acids in cell culture (SILAC) and tandem mass tags (TMTs), allow to accurately determine changes in protein abundance over time and between different conditions, even within a single liquid chromatography (LC)-MS run [6,7]. Nevertheless, minor changes in protein abundance are challenging to detect and are often overlooked in bottom-up analyses. Therefore, targeted detection of newly synthesized proteins (NSPs) is a requisite to elucidate minute differences in protein synthesis. Consequently, dedicated MS-based methods have been developed to study protein synthesis (Figure 2).

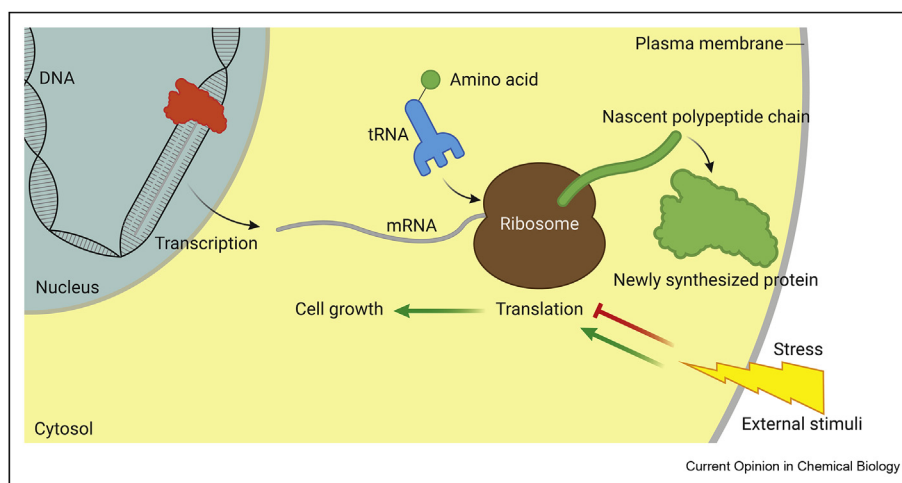
Puromycin-based approaches

Puromycin is an aminonucleoside antibiotic, produced by *Streptomyces alboniger*, and has served as inspiration for the development of powerful molecular tools to study NSPs [8]. Puromycin inhibits protein synthesis by ribosome-catalyzed incorporation into the C-terminus of nascent polypeptide chains, preventing further

polypeptide chain extension and causing premature termination of protein translation. The development of various puromycin-based reagents with additional features, such as fluorophores, photocaging groups, and radiolabels, has allowed spatiotemporal visualization of protein synthesis with applications both *in vitro* and *in vivo* [8]. In contrast to radiolabeling and fluorescence microscopy, the use of puromycin-based derivatives in combination with MS-based proteomics allows proteome-wide analysis of protein synthesis at an individual protein level resolution.

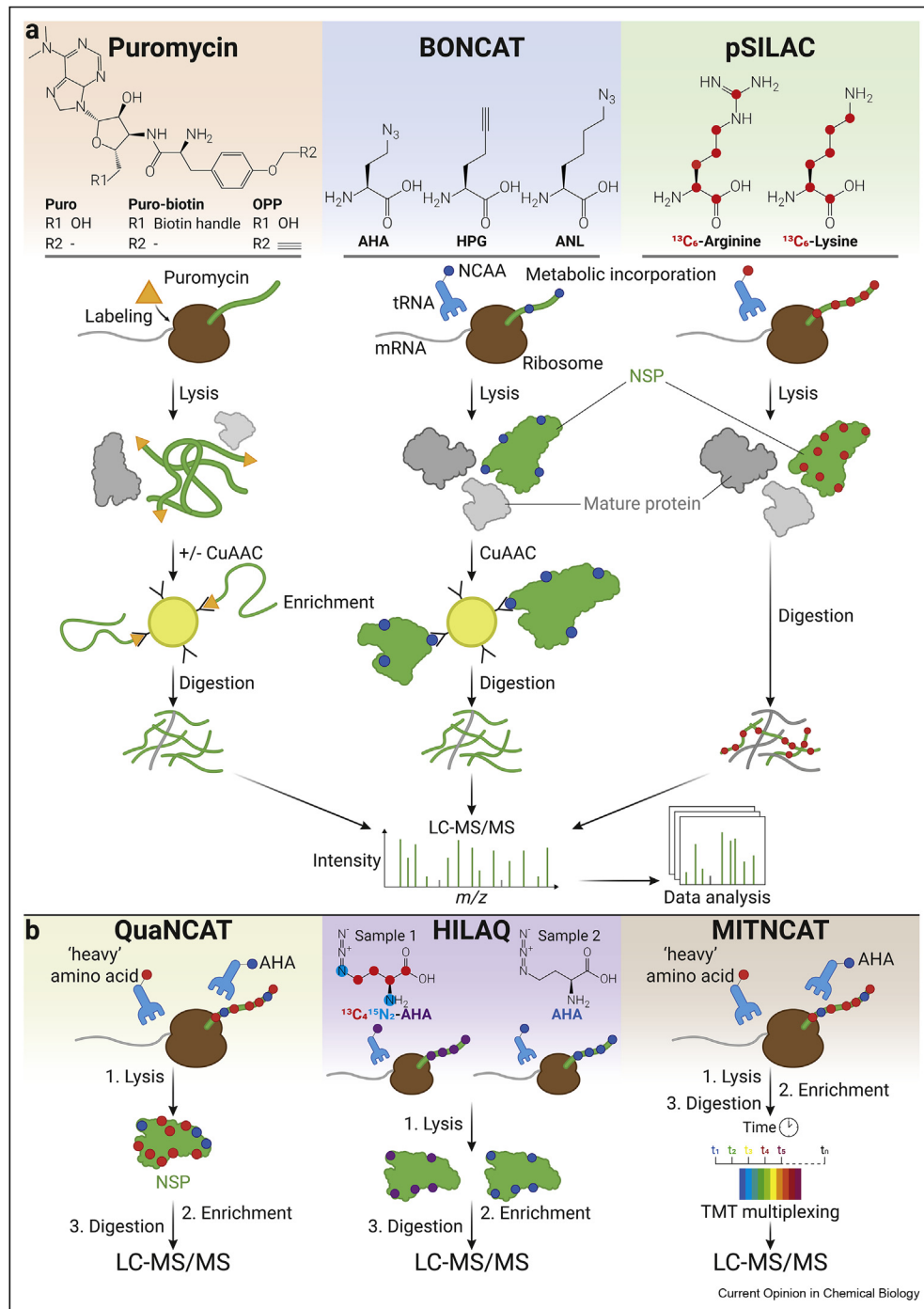
A biotin conjugate of puromycin has recently been introduced to facilitate puromycin-associated nascent chain proteomics (PUNCH-P) [9,10]. PUNCH-P allowed the detection of thousands of NSPs derived from cells or tissue. Cell cycle-specific fluctuations in protein synthesis in cell lines were monitored, and the so-called ‘translatome’ of a whole mouse brain could be charted [10]. Because puromycin-biotin presents poor cell permeability, ribosome isolation is required before puromycin-biotin labeling. In contrast, the puromycin analog O-propargyl-puromycin (OPP), that is puromycin conjugated with an alkyne, is cell-permeable and thus compatible with live-cell labeling and has been used to analyze NSPs in living early erythroid progenitor cells [11]. OPP was subsequently used in combination with pulsed SILAC (pSILAC), which is the pulsed metabolic incorporation of stable isotope-labeled amino acids in NSPs. The addition of pSILAC allows controlling for the background caused by nonspecific binders during enrichment, providing a more accurate quantification of protein synthesis [12]. The cell-permeable feature of OPP is a significant advantage over PUNCH-P, where subtleties of the cellular context may become lost on

Figure 1



Protein translation overview. mRNA is transcribed from DNA in the nucleus before it is transported to the cytosol where it is translated into protein by ribosomes. tRNA molecules serve as the link between mRNA and the ribosome by presentation of mRNA-encoded amino acids to the ribosome for elongation of the nascent polypeptide chain, generating a newly synthesized protein.

Figure 2



Mass spectrometry-based methods for analysis of newly synthesized proteins. (a) Currently used MS-based methods to analyze NSPs can be categorized into three main strategies. 1. The puromycin-based strategy relies on the aminonucleoside antibiotic, puromycin (Puro), which inhibits protein synthesis and couples to the C-terminus of nascent polypeptide chains (NPCs). Biotinylated and alkynylated variants of puromycin enable targeted enrichment and measurement of NPCs by LC-MS; 2. BONCAT-based methods rely on the methionine surrogates AHA, HPG, or ANL to enrich for NSPs. After their metabolic incorporation in NSPs, a copper (I)-catalyzed alkyne-azide cycloaddition (CuAAC) can be used to functionalize labeled NSPs with affinity handles, such as biotin, to enable enrichment; 3. SILAC relies on metabolic labeling of NSPs with isotopically labeled amino acids. The strategy does not contain an enrichment step, but NSPs can be identified by LC-MS/MS detection of the isotopically labeled amino acids. (b) Combinations of BONCAT with other quantitative techniques to enhance the accuracy and temporal resolution of NSP analysis. QuaNCAT combines BONCAT with pSILAC, to discriminate between *bona fide* NSPs and false positives. Heavy isotope-labeled AHA quantification (HILAQ) uses stable isotope-labeled AHA and nonlabeled AHA for relative quantification of two different conditions. MITNCAT (multiplex isobaric tagging/noncanonical amino acid tagging) combines QuaNCAT and TMT multiplexing to reduce labeling time and allows detection of small changes in protein synthesis in short timeframes.

ribosome isolation. However, extended *in situ* labeling times with OPP results in the accumulation of truncated puromycin-bound peptides, and inhibition of protein translation can affect the cellular processes under investigation. To alleviate these issues, Tong et al. [13] developed quantitative OPP tagging, a combination of OPP with TMT labeling which enabled a substantial reduction in labeling time, to 15 min, and the quantitation of over 3000 NSPs. Quantitative OPP tagging could track dynamic changes in protein synthesis in THP-1 macrophages after lipopolysaccharide treatment. Photocaged puromycin analogs, which do exist but have not been implemented for MS applications yet, have the potential to provide an additional level of spatial resolution in studying protein synthesis [8].

Puromycin-based approaches *in vivo*

Although incorporation of puromycin analogs in nascent polypeptide chains is a powerful approach, it can be challenging to apply *in vivo* because prolonged puromycin incubation time causes toxicity in animals because of inhibited translation and truncated puromycinylated peptides. Despite previous success in studying protein synthesis in tissue and hematopoietic stem cells by fluorescence microscopy and western blot, coupling *in vivo* puromycin labeling to MS-based proteomics to study protein synthesis at a proteome-wide level at the resolution of single proteins remains a significant challenge [14–16].

BONCAT

Bio-orthogonal noncanonical amino acid tagging (BONCAT) relies on pulsed metabolic incorporation of noncanonical amino acids (NCAAs) in NSPs. Alkyne or azide ligation handles in NCAAs enable bio-orthogonal ligation to reporter groups and subsequent enrichment of NSPs. Multiple *L*-methionine analogs have been used for BONCAT, such as *L*-azidohomoalanine (AHA), *L*-homopropargylglycine (HPG), and *L*-azidonorleucine (ANL) (Figure 2) [17–20]. From these reagents, AHA is the most used and translationally active with incorporation rates of 400 times lower than methionine, whereas HPG has an incorporation rate that is 500 times lower than methionine, and ANL is not incorporated in wild-type cells and requires expression of a mutant methionine-tRNA synthetase for incorporation [17,19,21].

Since its introduction in 2006 as an MS-based approach to measure protein synthesis, BONCAT has been widely used to investigate protein synthesis in physiological and disease processes. BONCAT has been deployed to analyze protein synthesis in tumor necrosis factor alpha- and interleukin 1 beta-dependent inflammatory response, T cell activation, oxytosis, and in neurons

[22–27]. BONCAT was also used successfully *in vivo* in *Caenorhabditis elegans*, zebrafish, and *Xenopus* [28–30]. Despite the wide recognition and success of BONCAT-based methods, some challenges remain. About 6% of the proteome is undetectable for BONCAT, as these proteins do not contain any methionine residues or solely a methionine which is directly cleaved after release from the ribosome [20,31]. In addition, enrichment with streptavidin for biotinylated proteins is prone to undesired binders, such as endogenously biotinylated proteins, hampering unambiguous identification of *bona fide* NSPs [32]. Finally, measurement of protein synthesis with high temporal resolution or in challenging systems with low metabolic rates is challenging when using BONCAT because of the often low levels of tagged proteins.

Direct detection of labeled peptides

To alleviate the problem of falsely identified NSPs due to enrichment of endogenously biotinylated proteins and nonspecific binding to immobilized streptavidin, direct detection of NCAA-containing peptides should be used. Detection of NCAA-labeled peptides can be achieved using desthiobiotin, biotinylation site identification technology, or direct detection of biotin-containing tags, which allow the elution and analysis of (desthio)biotinylated peptides by LC-MS [32–34]. In addition, various cleavable biotin linkers have been introduced which could directly detect BONCAT-labeled peptides derived from NSPs [20,35]. Alkynylated resin presents a viable option as well [36]. A novel enrichment approach, PhosID, for the enrichment of AHA-labeled peptides was recently reported [37]. The phosphonic acid handle, inspired on an enrichable crosslinker for MS termed PhoX, enables automated Fe³⁺-immobilized metal affinity chromatography enrichment and direct analysis of labeled peptides by LC-MS/MS [38]. A total of 176 NSPs were found to be significantly regulated by treatment of interferon- γ in HeLa cells, of which many had been previously reported to be interferon responsive genes [37].

Combining BONCAT with stable isotope labeling techniques

Another strategy to differentiate between *bona fide* NSPs and nonspecific binders is the combination of BONCAT and pSILAC, analogous to the combination of OPP and pSILAC [12]. In this strategy, both NCAAs and stable isotope-labeled amino acids are metabolically incorporated in the cell in a pulsed fashion. BONCAT combined with pSILAC labeling, termed quantitative NCAA tagging (QuaNCAT), has been used to reveal alterations in protein synthesis upon T cell activation [23]. In addition, using a combination of pSILAC and BONCAT allowed the study of brain-derived neurotrophic factor–

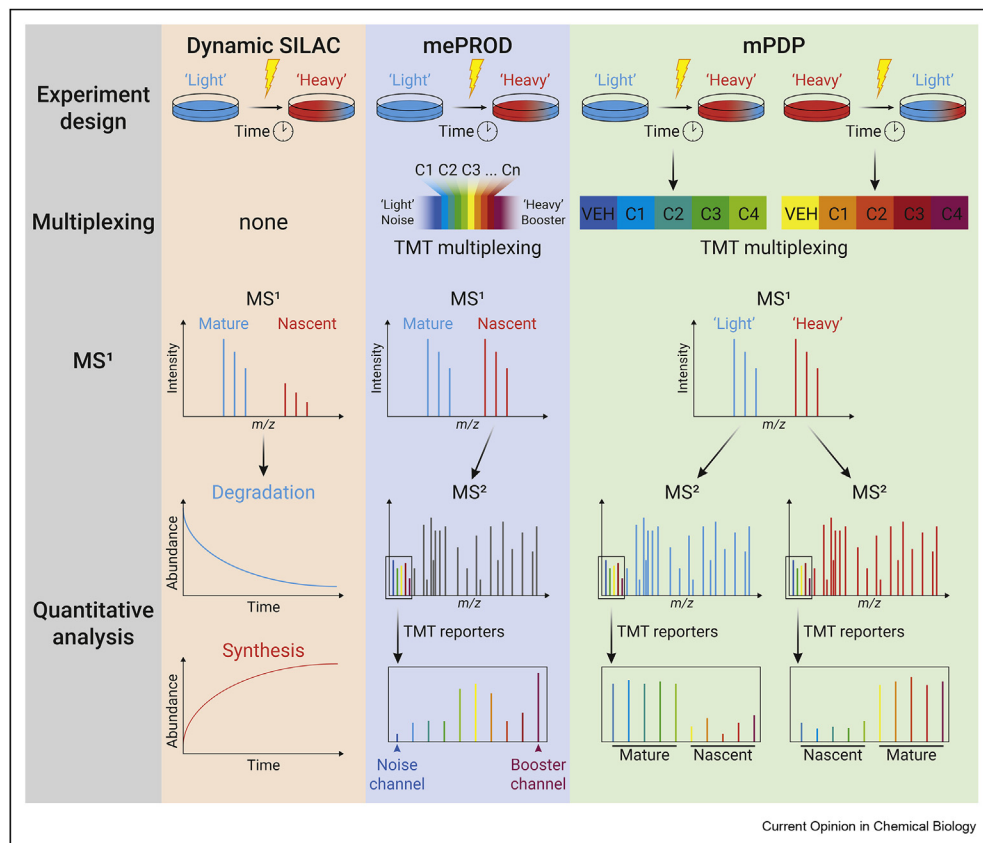
induced protein synthesis in hippocampal mouse brain slices [39]. As an alternative, heavy isotope-labeled AHA quantification was found to be more sensitive than QuaNCAT, as the biotinylated peptides were directly detected by means of the direct detection of biotin-containing tags protocol [24]. The heavy labeled AHA allowed for relative quantification of NSPs expressed during oxytosis in HT22 and HEK293T cells. Of note, combining QuaNCAT with TMT labeling enables highly multiplexed quantitative measurements of protein synthesis across multiple time points (multiplex isobaric tagging/noncanonical amino acid tagging). Using this additional quantitative technique allowed to study the rate of protein synthesis over time upon epidermal growth factor stimulation with a 15 min resolution [40]. Another study combined pSILAC with BONCAT and TMT labeling to investigate the half-lives of NSPs in MCF-7 cells after bortezomib (proteasome inhibitor) or 3-methyladenine (lysosome inhibitor) treatment [41]. These innovations demonstrate that stable isotope

labeling techniques combined with BONCAT enable higher sensitivity, temporal resolution, and produce fewer false positives.

BONCAT in illustrative challenging systems

The high sensitivity of the current mass spectrometers now enables the analysis of NSPs in systems with low metabolic rates and consequently low protein synthesis. A combination of BONCAT with isobaric tags for relative and absolute quantitation was used to monitor protein synthesis in the parasite *Leishmania mexicana* during starvation to uncover the underlying molecular mechanisms facilitating adaptation to stressful conditions [42]. The starvation time-dependent increase of expression of several proteins that potentially play crucial roles in the endoplasmic reticulum stress response pathways in the parasite was identified. These data show that BONCAT is suited to probe the response of parasites to external stimuli and can aid the discovery of new drug targets in parasites. Van Gelder *et al.* [26] studied

Figure 3



Dynamic SILAC-based methods. Dynamic SILAC uses pulsed SILAC to identify isotopically labeled proteins as NSPs, and the decrease of nonlabeled is proportional to protein degradation. Multiplexed enhanced protein dynamics (mePROD) and multiplexed proteome dynamics profiling (mPDP) are both methods that combine TMT multiplexing with dynamic SILAC. mePROD also includes a 'heavy' booster and 'light' 'noise' channel for MS¹ triggering and to improve the accuracy of quantification. mPDP creates signal amplification in the MS¹ channel by using both a 'light' to 'heavy' and a 'heavy' to 'light' switch for each condition, resulting in robust detection and quantification of synthesis and degradation by means of the individual TMT channels.

protein synthesis dependent on metabotropic glutamate receptor activation in primary hippocampal neurons. It is anticipated that the study of protein synthesis in even more challenging systems is within reach using BONCAT-based approaches, combined with stable isotope labeling, especially as mass spectrometers become even more sensitive.

Cell type-specific protein synthesis

In contrast to AHA or HPG, labeling with ANL provides the opportunity to retain some spatial information in MS-based NSP analysis, as a prerequisite for the metabolic incorporation of ANL is the (cell-specific) expression of a mutant methionine-tRNA ligase that recognizes ANL. Several studies have used this technique to monitor cell types of interest *in vivo* [43,44]. A tumor-specific proteome was consequently labeled and identified by using ANL *in vivo* [44]. Importantly, with this method, synthesis events in cell types of interest could be studied as well. This has been demonstrated by Alvarez-Castelao et al. [45] and Evans et al. [46] who performed *in vivo* cell type-specific labeling of NSPs in hippocampal neurons in mice. Cell type-specific NSP analysis is the first step toward obtaining spatial resolution in BONCAT experiments, and subcellular expression of tRNA-synthetase mutants might provide further spatial information of synthesis events.

Dynamic SILAC-based approaches

Protein levels are regulated by the interplay of protein synthesis and degradation. Therefore, to obtain a comprehensive view of protein dynamics, it is also important to monitor protein degradation and measure the half-life of individual proteins. Compared with the aforementioned techniques, labeling of cells or

organisms using exclusively pSILAC is less invasive to the biological system under investigation. After pulsed labeling of nonlabeled proteomes with isotopically labeled amino acids, labeled proteins can be identified as NSPs, and the concurrent decrease of nonlabeled proteins is proportional to protein degradation. Thereby, pSILAC allows *in vitro* and *in vivo* monitoring of both protein synthesis and degradation simultaneously, termed 'dynamic SILAC' (Figure 3). A limitation of this method is the inability to enrich for these isotope-labeled proteins or peptides. Therefore, substantially longer labeling time are required compared to BONCAT- or puromycin-based approaches, and consequently, rapid synthesis and degradation events are challenging to monitor with this method.

Dörrbaum et al. [47] used dynamic SILAC to investigate protein synthesis and degradation in drug-induced homeostatic upscaling or downscaling of primary cultured neurons. The study design included a stable isotope-labeled internal standard, allowing high precision quantification of small changes in protein synthesis and degradation. Consequently, a comprehensive profile of protein dynamics in neurons during upscaling or downscaling was obtained [47]. Furthermore, pSILAC was used to investigate translation of proteins in neuronal injury and consequent regenerative axon regrowth [48]. Of note, pSILAC is challenging in these nondividing cell lines as the labeling was barely sufficient to distinguish between injured cells and the control groups.

Combining dynamic SILAC with isobaric labeling

Multiplexed enhanced protein dynamics adds multiplexing by TMT labeling to allow the analysis of acute

Table 1

Discussed approaches and their characteristics.

Strategy	Method	Invasiveness	Labeling time	Enrichment	Multiplexing	Degradation analysis	Spatial resolution	Reference
Puromycin-based	PUNCH-P	–	15 min	+	No	–	--	[10]
	OPP	–	2 h	+	No	–	--	[11]
	OPP-pSILAC	–	2 h	+	No	–	--	[12]
	QOT	+	15 min	+	Yes	–	--	[13]
BONCAT-based	AHA/HPG	++	Hrs/days	+	No	+	--	[17]
	ANL	–	Days (<i>in vivo</i>)	+	No	+	+	[43–46]
	QuaNCAT	++	2–4 h	+	No	+	--	[23]
	MITNCAT	++	15 min	+	Yes	+	--	[40]
	HILAQ	++	1 h	++	No	+	--	[24]
	PhosID	++	4–24 h	++	No	+	--	[37]
SILAC-based	Dynamic SILAC	+++	6 h/days	N/A	No	++	--	[47,48]
	mPDP	+++	3–48 h	N/A	Yes	++	--	[50]
	MePROD	+++	2 h	N/A	Yes	++	--	[49]

QOT, quantitative OPP tagging; MITNCAT, multiplex isobaric tagging/noncanonical amino acid tagging; HILAQ, heavy isotope-labeled AHA quantification; mPDP, multiplexed proteome dynamics profiling; MePROD, multiplexed enhanced protein dynamics.

Beneficial attributes are represented with '+', whereas limitations are indicated as '–'. 'Invasiveness' describes the adverse effect of the technique on the system under investigation.

changes in protein synthesis and enables detection of minor differences in protein synthesis after only 2 h in fewer than 100,000 cells. Signal amplification was achieved by the inclusion of an isotopically labeled booster channel for MS1 triggering (Figure 3). Although booster channels enable the identification of more peptides, high levels of booster proteome may adversely affect quantitative accuracy [49]. Multiplexed enhanced protein dynamics was used to study the eIF2 α - and mTOR-dependent pathways in the integrated stress response. Crosstalk between these two pathways was observed [50]. Savitski *et al.* used dynamic SILAC combined with TMT labeling, called ‘multiplexed proteome dynamics profiling’, to investigate the effects of estrogen receptor modulators on protein homeostasis in MCF-7 cells, and differential effects on protein synthesis and degradation between various modulators were observed [51,52]. These studies demonstrate the power and potential of combining dynamic SILAC with isobaric labeling for concomitant analysis of protein synthesis and degradation.

Conclusions

MS-based proteomics in various combinations with stable isotope labeling and affinity enrichment has led to a powerful toolbox to study NSPs. The recent advances in these methodologies, including completely novel enrichment strategies, and the application and combination of multiple (stable isotope-based) quantitative techniques allow the investigation of NSPs with increasing accuracy, temporal, and spatial resolution in ever more challenging systems. Puromycin-based approaches, BONCAT, and pulsed/dynamic SILAC are currently still the core techniques used to study NSPs. These orthogonal techniques all have their own unique strengths and weaknesses, as we aimed to summarize in Table 1. The extensive toolbox available to study NSPs allows researchers the selection of suitable methods for specific research questions. We anticipate that the field will develop further toward methods with an even higher sensitivity, temporal resolution, and spatial resolution and to the level of detail whereby newly synthesized proteoforms can also be analyzed. As studying NSPs is key to understanding changes in the proteome in health and disease and enables the discovery of new therapeutic targets, further development of such tools remains essential.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Papers of particular interest, published within the period of review, have been highlighted as:

- * of special interest
- ** of outstanding interest

1. Bhat M, Robichaud N, Hulea L, Sonenberg N, Pelletier J, Topisirovic I: **Targeting the translation machinery in cancer.** *Nat Rev Drug Discov* 2015, **14**:261–278.
 2. Ruggero D: **Translational control in cancer etiology.** *Cold Spring Harb Perspect Biol* 2013, **5**, a012336.
 3. Buxbaum AR, Haimovich G, Singer RH: **In the right place at the right time: visualizing and understanding mRNA localization.** *Nat Rev Mol Cell Biol* 2015, **16**:95–109.
 4. Schwanhäusser B, Busse D, Li N, Dittmar G, Schuchhardt J, Wolf J, Chen W, Selbach M: **Global quantification of mammalian gene expression control.** *Nature* 2011, **473**:337–342.
 5. Buccitelli C, Selbach M: **mRNAs, proteins and the emerging principles of gene expression control.** *Nat Rev Genet* 2020, **21**:630–644.
 6. Ong SE, Blagoev B, Kratchmarova I, Kristensen DB, Steen H, Pandey A, Mann M: **Stable isotope labeling by amino acids in cell culture, SILAC, as a simple and accurate approach to expression proteomics.** *Mol Cell Proteomics* 2002, **1**:376–386.
 7. Thompson A, Schäfer J, Kuhn K, Kienle S, Schwarz J, Schmidt G, Neumann T, Hamon C: **Tandem mass tags: a novel quantification strategy for comparative analysis of complex protein mixtures by MS/MS.** *Anal Chem* 2003, **75**:1895–1904.
 8. Aviner R: **The science of puromycin: from studies of ribosome function to applications in biotechnology.** *Comput Struct Biotechnol J* 2020, **18**:1074–1083.
 9. Aviner R, Geiger T, Elroy-Stein O: **Genome-wide identification and quantification of protein synthesis in cultured cells and whole tissues by puromycin-associated nascent chain proteomics (PUNCH-P).** *Nat Protoc* 2014, **9**:751–760.
 10. Aviner R, Geiger T, Elroy-Stein O: **Novel proteomic approach (PUNCH-P) reveals cell cycle-specific fluctuations in mRNA translation.** *Genes Dev* 2013, **27**:1834–1844.
 11. Forester CM, Zhao Q, Phillips NJ, Urisman A, Chalkley RJ, Oses-Prieto JA, Zhang L, Ruggero D, Burlingame AL: **Revealing nascent proteomics in signaling pathways and cell differentiation.** *Proc Natl Acad Sci U S A* 2018, **115**:2353–2358.
 12. Uchiyama J, Ishihama Y, Imami K: **Quantitative nascent proteome profiling by dual pulse labeling with O-propargyl-puromycin and stable isotope labeled amino acids.** *J Biochem* 2020, <https://doi.org/10.1093/jb/mvaa104>.
 13. Tong M, Suttapitugsakul S, Wu R: **An effective method for accurate and sensitive quantitation of rapid changes of newly synthesized proteins.** *Anal Chem* 2020, **92**:10048–10057.
- Describes quantitative O-propargyl-puromycin (OPP) tagging (QOT), *i.e.* OPP labeling combined with tandem mass tag (TMT) multiplexing with labeling times as low as 15 min and the quantitation of over 3000 newly synthesized proteins. QOT could track dynamic changes in protein synthesis in THP-1 macrophages after lipopolysaccharide (LPS) treatment.
14. Hidalgo San Jose L, Signer RAJ: **Cell-type-specific quantification of protein synthesis in vivo.** *Nat Protoc* 2019, **14**:441–460.
 15. Liu J, Xu Y, Stoleru D, Salic A: **Imaging protein synthesis in cells and tissues with an alkyne analog of puromycin.** *Proc Natl Acad Sci U S A* 2012, **109**:413–418.
 16. Signer RAJ, Magee JA, Salic A, Morrison SJ: **Haematopoietic stem cells require a highly regulated protein synthesis rate.** *Nature* 2014, **508**:49–54.

17. Kiick KL, Saxon E, Tirrell DA, Bertozzi CR: **Incorporation of azides into recombinant proteins for chemoselective modification by the Staudinger ligation.** *Proc Natl Acad Sci U S A* 2002, **99**:19–24.
18. Van Hest JCM, Kiick KL, Tirrell DA: **Efficient incorporation of unsaturated methionine analogues into proteins in vivo.** *J Am Chem Soc* 2000, **122**:1282–1288.
19. Ngo JT, Champion JA, Mahdavi A, Tanrikulu IC, Beatty KE, Connor RE, Yoo TH, Dieterich DC, Schuman EM, Tirrell DA: **Cell-selective metabolic labeling of proteins.** *Nat Chem Biol* 2009, **5**:715–717.
20. Dieterich DC, Link AJ, Graumann J, Tirrell DA, Schuman EM: **Selective identification of newly synthesized proteins in mammalian cells using bioorthogonal noncanonical amino acid tagging (BONCAT).** *Proc Natl Acad Sci U S A* 2006, **103**:9482–9487.
21. Calve S, Witten AJ, Ocken AR, Kinzer-Ursem TL: **Incorporation of non-canonical amino acids into the developing murine proteome.** *Sci Rep* 2016, **6**.
22. Choi KYG, Lippert DND, Ezzatti P, Mookherjee N: **Defining TNF- α and IL-1 β induced nascent proteins: combining bio-orthogonal non-canonical amino acid tagging and proteomics.** *J Immunol Methods* 2012, **382**:189–195.
23. Howden AJM, Geoghegan V, Katsch K, Efstathiou G, Bhushan B, Boutourea O, Thomas B, Trudgian DC, Kessler BM, Dieterich DC, et al.: **QuaNCAT: quantitating proteome dynamics in primary cells.** *Nat Methods* 2013, **10**:343–346.
24. Ma Y, McClatchy DB, Barkallah S, Wood WW, Yates JR: **HILAQ: a novel strategy for newly synthesized protein quantification.** *J Proteome Res* 2017, **16**:2213–2220.
25. Schanzenbächer CT, Sambandan S, Langer JD, Schuman EM: **Nascent proteome remodeling following homeostatic scaling at hippocampal synapses.** *Neuron* 2016, **92**:358–371.
26. van Gelder CA, Penning R, Veth T, Catsburg LA, Hoogenraad CC, MacGillavry HD, Altelaar M: **Temporal quantitative proteomics of mGluR-induced protein translation and phosphorylation in neurons.** *Mol Cell Proteomics* 2020, **17**. mcp.RA120.002199.
27. Koren SA, Gillett DA, D'alton SV, Hamm MJ, Abisambra JF: **Proteomic techniques to examine neuronal translational dynamics.** *Int J Mol Sci* 2019, **20**.
28. Ullrich M, Liang V, Chew YL, Banister S, Song X, Zaw T, Lam H, Berber S, Kassiou M, Nicholas HR, et al.: **Bio-orthogonal labeling as a tool to visualize and identify newly synthesized proteins in *Caenorhabditis elegans*.** *Nat Protoc* 2014, **9**:2237–2255.
29. Hinz FI, Dieterich DC, Tirrell DA, Schuman EM: **Noncanonical amino acid labeling in vivo to visualize and affinity purify newly synthesized proteins in larval zebrafish.** *ACS Chem Neurosci* 2012, **3**:40–49.
30. Shen W, Liu HH, Schiapparelli L, McClatchy D, He HY, Yates JR, Cline HT: **Acute synthesis of CPEB is required for plasticity of visual avoidance behavior in *Xenopus*.** *Cell Rep* 2014, **6**:737–747.
31. Ma Y, Yates JR: **Proteomics and pulse azidohomoalanine labeling of newly synthesized proteins: what are the potential applications?** *Expert Rev Proteomics* 2018, **15**:545–554.
32. Schiapparelli LM, McClatchy DB, Liu H-H, Sharma P, Yates JR, Cline HT: **Direct detection of biotinylated proteins by mass spectrometry.** *J Proteome Res* 2014, **13**:3966–3978.
33. Kim DI, Cutler JA, Na CH, Reckel S, Renuse S, Madugundu AK, Tahir R, Goldschmidt HL, Reddy KL, Haganir RL, et al.: **BioSITE: a method for direct detection and quantification of site-specific biotinylation.** *J Proteome Res* 2018, **17**:759–769.
34. Hirsch JD, Eslamizar L, Filanoski BJ, Malekzadeh N, Haugland RP, Beechem JM, Haugland RP: **Easily reversible desthiobiotin binding to streptavidin, avidin, and other biotin-binding proteins: uses for protein labeling, detection, and isolation.** *Anal Biochem* 2002, **308**:343–357.
35. Szychowski J, Mahdavi A, Hodas JLL, Bagert JD, Ngo JT, Landgraf P, Dieterich DC, Schuman EM, Tirrell DA: **Cleavable biotin probes for labeling of biomolecules via azide-alkyne cycloaddition.** *J Am Chem Soc* 2010, **132**:18351–18360.
36. Zhang G, Bowling H, Hom N, Kirshenbaum K, Klann E, Chao MV, Neubert TA: **In-depth quantitative proteomic analysis of de novo protein synthesis induced by brain-derived neurotrophic factor.** *J Proteome Res* 2014, **13**:5707–5714.
37. Kleinpenning F, Steigenberger B, Wu W, Heck AJR: **Fishing for newly synthesized proteins with phosphonate-handles.** *Nat Commun* 2020, **11**:1–10.
- Introduces PhosID, a novel strategy for analysis of newly synthesized proteins (NSPs) by bio-orthogonal non-canonical amino acid tagging (BONCAT). The phosphonic acid affinity tag enabled automated Fe³⁺-IMAC enrichment and direct analysis of labeled peptides by mass spectrometry. 176 NSPs were found to be significantly regulated by treatment of interferon- γ in HeLa cells.
38. Steigenberger B, Pieters RJ, Heck AJR, Scheltema RA: **PhoX: an IMAC-enrichable cross-linking reagent.** *ACS Cent Sci* 2019, **5**:1514–1522.
39. Bowling H, Bhattacharya A, Zhang G, Lebowitz JZ, Alam D, Smith PT, Kirshenbaum K, Neubert TA, Vogel C, Chao MV, et al.: **BONLAC: a combinatorial proteomic technique to measure stimulus-induced translational profiles in brain slices.** *Neuropharmacology* 2016, **100**:76–89.
40. Rothenberg DA, Taliaferro JM, Huber SM, Begley TJ, Dedon PC, White FM: **A proteomics approach to profiling the temporal translational response to stress and growth.** *iScience* 2018, **9**:367–381.
- Describes multiplex isobaric tagging/non-canonical amino acid tagging (MITNCAT) for analysis of newly synthesized proteins with a temporal resolution of 15 min. Translation dynamics after epidermal growth factor stimulation could be tracked with this method.
41. Tong M, Smeekens JM, Xiao H, Wu R: **Systematic quantification of the dynamics of newly synthesized proteins unveiling their degradation pathways in human cells.** *Chem Sci* 2020, **11**:3557–3568.
42. Kalesh K, Denny PW: **A BONCAT-iTRAQ method enables temporally resolved quantitative profiling of newly synthesized proteins in *Leishmania mexicana* parasites during starvation.** *PLoS Neglected Trop Dis* 2019, **13**, e0007651.
43. Alvarez-Castelao B, Schanzenbächer CT, Hanus C, Glock C, Dieck ST, Dörrbaum AR, Bartnik I, Nassim-Assir B, Ciirdaeva E, Mueller A, et al.: **Cell-type-specific metabolic labeling of nascent proteomes in vivo.** *Nat Biotechnol* 2017, **35**:1196–1201.
44. Azizian NG, Sullivan DK, Nie L, Pardo S, Molleur D, Chen J, Weintraub ST, Li Y: **Selective labeling and identification of the tumor cell proteome of pancreatic cancer in vivo.** *J Proteome Res* 2020, <https://doi.org/10.1021/acs.jproteome.0c00666>.
45. Alvarez-Castelao B, Schanzenbächer CT, Langer JD, Schuman EM: **Cell-type-specific metabolic labeling, detection and identification of nascent proteomes in vivo.** *Nat Protoc* 2019, **14**:556–575.
46. Evans HT, Bodea L-GG, Götz J, Rgen Gö Tz J: **Cell-specific non-canonical amino acid labelling identifies changes in the de novo proteome during memory formation.** *Elife* 2020, **9**.
- Demonstrates cell-specific labeling with azidonorleucine (ANL) of newly synthesized proteins (NSPs) during spatial long-term memory formation in hippocampal neurons in vivo. After 16 h of labeling, 156 NSPs were found to be significantly altered and highly interconnected with each other. Proteins associated with glutamate receptor recycling were increased and altered synthesis of mRNA splicing proteins was observed.
47. Dörrbaum AR, Alvarez-Castelao B, Nassim-Assir B, Langer JD, Schuman EM: **Proteome dynamics during homeostatic scaling in cultured neurons.** *Elife* 2020, **9**.
48. Rozenbaum M, Rajman M, Rishal I, Koppel I, Koley S, Medzihradsky KF, Oses-Prieto JA, Kawaguchi R, Amieux PS, Burlingame AL, et al.: **Translatome regulation in neuronal injury and axon regrowth.** *eNeuro* 2018, **5**.

49. Cheung TK, Lee CY, Bayer FP, McCoy A, Kuster B, Rose CM: **Defining the carrier proteome limit for single-cell proteomics.** *Nat Methods* 2021, **18**:76–83.
50. Klann K, Tascher G, Münch C: **Functional translome proteomics reveal converging and dose-dependent regulation by mTORC1 and eIF2 α .** *Mol Cell* 2020, **77**:913–925. e4.
- Introduces multiplexed enhanced protein dynamics (mePROD), a combination of dynamic SILAC and TMT multiplexing. mePROD includes an isotopically labeled booster channel for MS1 triggering as signal amplification to measure acute changes in protein synthesis in less than 100,000 cells. Crosstalk between eIF2 α - and mTOR-pathways was observed during integrated stress response.
51. Savitski MM, Zinn N, Faelth-Savitski M, Poeckel D, Gade S, Becher I, Muelbaier M, Wagner AJ, Stroemer K, Werner T, *et al.*: **Multiplexed proteome dynamics profiling reveals mechanisms controlling protein homeostasis.** *Cell* 2018, **173**: 260–274. e25.
52. Grandi P, Bantscheff M: **Advanced proteomics approaches to unravel protein homeostasis.** *Drug Discov Today Technol* 2019, **31**:99–108.