Drug Discovery Today: Technologies
Vol. 31, 2019

Protein degradation for drug discovery

Advanced proteomics approaches to unravel protein homeostasis

Paola Grandi*, Marcus Bantscheff*

Cellzome GmbH, GlaxoSmithKline, Meyerhofstrasse 1, 69117 Heidelberg, Germany

Quantitative proteomics methods are instrumental in measuring the interplay between protein synthesis and protein degradation in cells and tissues in different conditions and substantially contribute to the understanding of control mechanisms for protein homeostasis. Proteomics and chemoproteomics approaches enable the characterization of small molecule modifiers of protein degradation for therapeutic applications. Here, we review recent developments and applications of mass spectrometry-based (chemo-)proteomics methods for the study of cellular homeostasis.

Introduction

Cellular protein homeostasis or proteostasis is the dynamic equilibrium of concentration, localization and conformation of all proteins in any given cell. It enables basic cellular functions such as cell division and growth as well as cellular response to external stimuli and perturbations and assists cell differentiation and metabolic changes [5,46]. The interplay between protein synthesis and degradation governs the concentration of each protein in a cell (Fig. 1). Proteostasis is also a measure of cell health: alteration of this equilibrium is deleterious for the cell and, in vivo, has pathological consequences for tissues and organs called proteinopathies [5,48].

Cellular surveillance mechanisms ensure tight control of proteostasis and are switched on mainly by signals originated by issues with protein folding. In case of excess of misfolded proteins the heat shock response (HSR) [2] or the unfolded protein response (UPR) [78] pathways become activated and not functional proteins are degraded via the Ubiquitin Proteasome System (UPS) or by autophagy [24] (Fig. 1). In the UPS, proteins destined to degradation are labelled on lysine residues by multiple units of the 76 amino acid ubiquitin tag. This process involves multiple steps including activation of ubiquitin by conjugation to an E1 enzyme (2 enzymes known in humans), ubiquitin transfer to the E2 enzyme (ca. 35 putative enzymes in humans) and very often the transfer to an E3 ubiquitin ligase (>500–600 enzymes in humans) is necessary for attaching ubiquitin to the target protein [29]. Formation of ubiquitin chains by linking ubiquitin monomers through Lys48, signals proteins for destruction by the proteasome, a multisubunit complex rich in proteases [19,80]. Ubiquitin modifications of proteins can be removed by deubiquitinases (DUBs) a family of 80–90 peptidases which can cleave ubiquitin and process ubiquitin chains. Ubiquitination and deubiquitination, i.e. the interplay of

*Corresponding author: P. Grandi (paola.x.grandi@gsk.com), M. Bantscheff (marcus.x.bantscheff@gsk.com)

 https://doi.org/10.1016/j.ddtec.2019.02.001

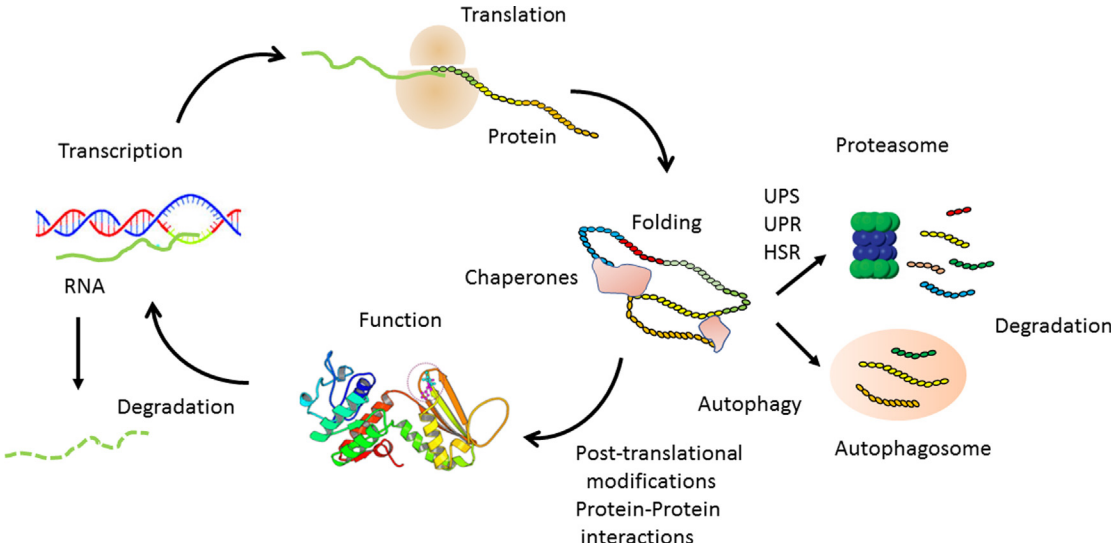

Fig. 1. Main processes which influence cellular proteostasis.
Production of mRNA (Transcription) and of proteins from mRNA (Translation) as well as removal of RNA and proteins through activation of various pathways (UPS, UPR and HSR) and degradation machineries (Proteasome and Autophagosome) ensure that proper amount of folded and functioning proteins are present in the cell. Post-translational modification of proteins as well as protein-protein interactions often contribute to the production of functional proteins.

ubiquitin ligases and DUBs, regulates most protein half-life and thus is of utmost importance for the maintenance of cellular proteostasis [36].

Similarly to the UPS regulation of autophagy, a very conserved cellular mechanism for degradation of cytoplasmic organelles, is used as quality control mechanism for not functional or aged cellular components [25,59] (Fig. 1).

The fidelity of the proteome is challenged during development and aging, and by exposure to pathogens that demand high protein folding and trafficking capacity, and hence cells use stress sensors and inducible pathways to respond to a loss of proteostatic control. These pathways make use of the transcription, translation and the protein degradation machineries available in the cell to react to stimuli and to re-establish proteostasis. Together, these systems form the so called Proteostasis Network (PN) [46]. Loss of control or of capacity of those pathways e.g. by defects in ubiquitin ligases or DUBs lead to de-regulation of protein stability, increase of misfolded or misfunctional proteins or protein aggregates or too rapid disappearance of important cellular factors i.e. tumor suppressors, and thus cause pathologies [35]. Consequently, modulation of protein degradation has become an attractive strategy in drug discovery and inhibitors of molecular chaperones [71], inhibitors of E3 ligases [45], of the proteasome and of deubiquitinases [44], PROteolysis TArgetted Chimeras [58,64,79] and molecular switches represent a rich panel of pharmacological approaches to target or use the UPS to degrade or stabilize proteins.

To understand proteome homeostasis it is important to comprehensively characterize protein synthesis and degradation rates in steady state and upon perturbations and to identify mechanisms of regulation. Protein abundances tend to be poorly correlated to mRNA expression levels [77] since post transcriptional processing as well as the expression of non-coding RNAs such as miRNAs and lincRNAs affect the stability of specific transcripts [67,72] and thus protein synthesis. In addition, protein localization, proteinprotein interactions and post translational modifications influence the rates of protein degradation [11,12,70].

Quantitative and unbiased measurements of proteome variation by mass spectrometry (MS) enable direct monitoring of cellular proteostasis following physiological stimuli, during disease development and after drug treatment [53,66]. Recent advances in mass spectrometry instrumentation, methods and reagents enabled the quantitative assessment of proteomes at unprecedented depth, sensitivity and throughput [1,57]. Dynamic and pulsed stable isotope labeling techniques can be employed either in isolation or in combination with isobaric mass tag reagents for the multiplexed proteome-wide analysis of protein turnover and degradation rates and to elucidate the effects of stimuli or compounds on protein synthesis and degradation [20,66,70,81].

In addition, a plethora of proteomics and chemoproteomics techniques to study ubiquitination has emerged and has been extensively reviewed elsewhere [10,37,38].

Proteomics approaches to measure protein homeostasis based on metabolic labelling

To measure protein homeostasis and understand dynamic adaptation to external factors it is fundamental to distinguish degradation-based processes from synthesis-based

alterations of protein levels. Historically, this has been achieved through pulse-chase experiments measuring the incorporation of a tracer into proteins in the pulse and the concomitant loss of this tracer in the chase. Initial tracers contained radioactive isotopes of carbon, hydrogen or sulfur incorporated into essential amino acids such as methionine which were added to the cell cultivation medium (Fig. 2). The time-dependent increase in incorporated radioactivity in the protein pool during the pulse and the disappearance of radioactivity of the protein pool during the chase were monitored via differential 1D or 2D gel electrophoresis followed by scintillation spectroscopy for radioactivity measurement and mass spectrometry for protein identification [15,33] and enabled determination of synthesis or degradation rates of individual proteins.

Measuring radioactively labelled proteins is a highly sensitive method, but, due to sub-stoichiometric enrichment of unstable isotopes, the application of the approach is limited to situations where individual proteins can be monitored with high confidence. In complex samples, multiple proteins can be identified even in a single 2D gel spot and determination of protein synthesis and degradation rates for individual proteins is highly challenging [26,32]. In addition, the risk associated with the use of radioactivity limited the application of these methods. The use of protein synthesis or protein degradation inhibitors (i.e. cycloheximide and/or MG132) followed by MS or by immunodetection, in principle, allows monitoring of the synthesis and degradation of proteins without the need of a tracer [39]. This latter could be applied almost in proteomic scale in yeast by using genomic tagging of proteins [11] but also in human cells by Global Protein Stability profiling [84]. However, the fact that the use of translation or protein degradation inhibitors induces cellular stress [55,87] and the awareness that protein tags as well as ectopic expression of proteins might influence protein turnover, called out for

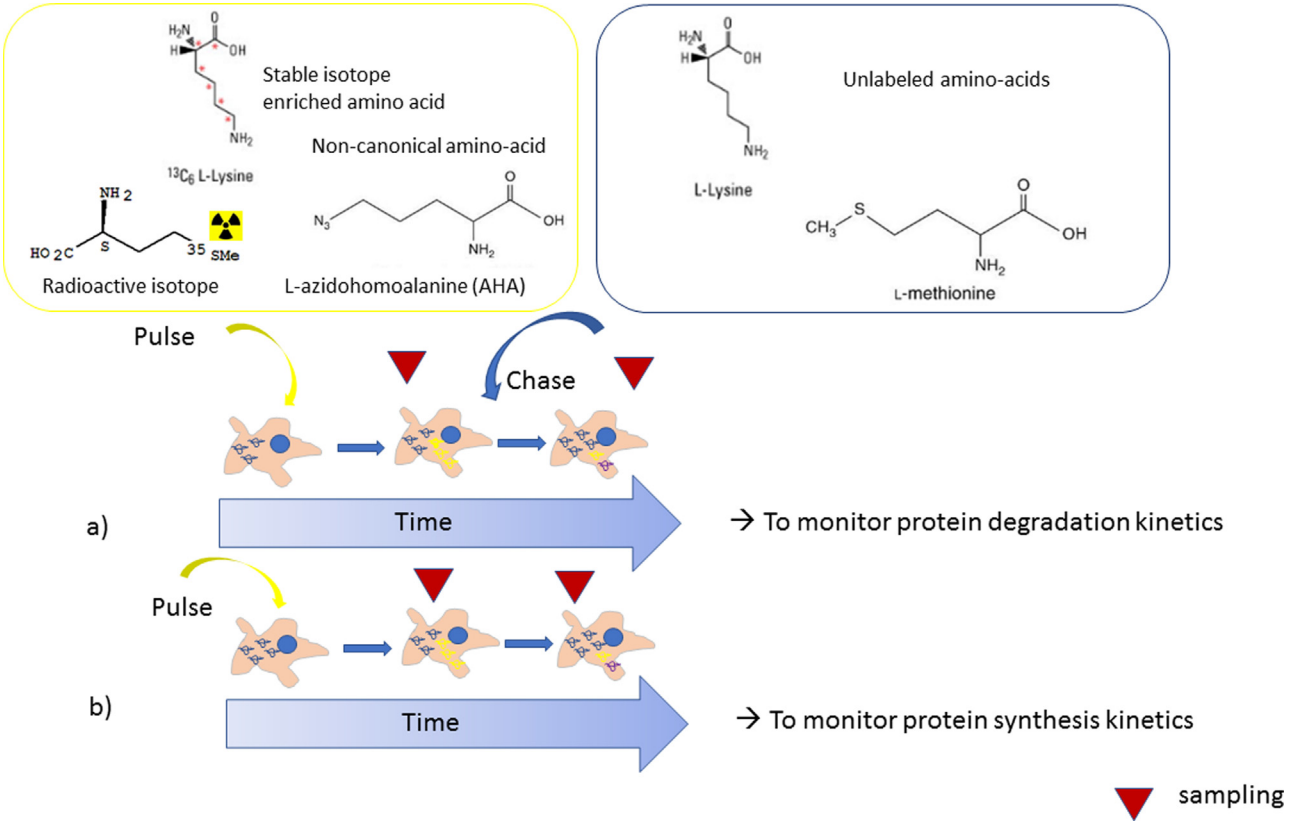

Fig. 2. sotope labelled and chemically modified tracers in kinetic experiments.Schematic representation of how labelled amino acids (radioactive, non-canonic (AHA), stable isotope) can be used to monitor protein degradation (a) or protein synthesis dynamics (b). The labelled amino acids are added to the cell culture in the absence of the unlabeled counterparts (pulse) and will be incorporated in the nascent proteins. Sampling at predetermined time intervals followed by identification of the labelled proteins allows to monitor protein synthesis kinetics (b). Substitution of the labelled amino acid with the unlabeled one (chase) and sampling after that, enables to follow the degradation of the labelled proteins with time (a).IFig. 2gr2sotope labelled and chemically modified tracers in kinetic experiments.

Schematic representation of how labelled amino acids (radioactive, non-canonic (AHA), stable isotope) can be used to monitor protein degradation (a) or protein synthesis dynamics (b). The labelled amino acids are added to the cell culture in the absence of the unlabeled counterparts (pulse) and will be incorporated in the nascent proteins. Sampling at predetermined time intervals followed by identification of the labelled proteins allows to monitor protein synthesis kinetics (b). Substitution of the labelled amino acid with the unlabeled one (chase) and sampling after that, enables to follow the degradation of the labelled proteins with time (a).

new, less invasive, unbiased methods with high sensitivity and larger applicability.

The use of stable isotope tracers for metabolic processes was pioneered by Schoenheimer as early as in 1935 and established the concept of continual breakdown and re-synthesis of proteins as an ongoing metabolic process that truly reflects ''The Dynamic State of Body Constituents'' [82]. More recently the combination of stable isotope labeling of proteins with mass spectrometry has enabled quantitative proteomics methodologies [7] such as Stable-Isotope Labelling by Amino Acids in cell culture, SILAC [61]. By substituting the (essential) amino acids in the growth medium such as Arginine, Lysine and Leucine with identical molecules enriched in stable isotopes of ^{2}H, ^{13}C, and ^{15}N the entire proteome over time becomes ''heavy'' labelled. Differential analysis of 'heavy' vs 'light' labelled cell systems has enabled a wide range of proteomics applications as high resolution mass spectrometers resolve differentially labelled isotope clusters of proteolytic peptides and relative signal abundances represent well the ratios of the corresponding peptides in heavy and light conditions [60].

SILAC approaches monitoring the rate of incorporation of isotope labelled amino acids into proteins are more versatile than radioactive pulse-chase experiments as they can be performed as pulse- or chase-alone and in combination and uniquely allow for the sensitive and multiplexed analysis of all quantifiable proteins in the investigated cell system. To minimize metabolic disturbance induced by cultivating cells in dialyzed serum with the addition of the labelled amino acids, purified growth factors or low amounts of normal serum might be added [31]. The dynamic SILAC labeling approach introduced by Pratt et al. [63] allows for the comprehensive analysis of protein turnover in many cell systems growing in culture [27]. In this approach ''heavy'' isotope labelled cellular proteins are typically transferred to normal medium for specific time intervals and, thus, the degradation rates for proteins can be determined by the rate of decay of heavy labelled peptide signals [27]. This study also analysed the influence that physical and functional parameters of proteins have on their turnover rate debunking previously reported links with molecular weight, isoelectric point and stabilizing or destabilizing N-terminal residues but showing slower degradation of proteins lacking a PEST sequence motif or disordered regions. The determination of translation rates on a proteome-wide scale can be achieved by a complementary pulsed SILAC approach using two heavy isotope labels [69]. The accurate determination of turnover and translation rates in cell culture is facilitated by the fact that the majority of newly synthesized proteins use the extracellular, non-limiting, source of amino acid rather than recycling amino acids from degraded proteins or using internal stores [17]. It should be noted though that up to 10% of the proteome do not strictly follow the experimental decay model that is generally assumed. In a recent study many non-exponentially degraded proteins were identified as subunits of complexes that are produced in super-stoichiometric amounts relative to their exponentially degraded interaction partners [56].

In order to determine turnover in primary cell systems that are short lived in culture, accurate determination of low levels of amino acid incorporation is key to extrapolate accurate protein half-lives. Recent algorithmic improvements enabled the accurate determination of global protein turnover in five mammalian primary cells from different tissue origin [53]. The half-lives of more than 9.600 proteins ranging from a few hours to above 120 days could be accurately determined. This work represents the most extensive catalogue of protein turnover in non-dividing cells and further corroborates the previous observation [17] that subunits of protein complexes tend to have coherent turnover. Analysis of proteasome and nuclear pore complexes suggests that the turnover rates of core components are lower than those of regulatory or cargo-interfacing subunits [53]. Pulsed SILAC experiments followed by biochemical separation of organelles revealed differential turnover of proteins dependent on protein function and localization [12]. As turnover rates dictate responsiveness to metabolic shifts, the proteins with the highest rates of turnover are expected to be regulatory. Conversely, the high abundance proteins often have the lowest rates of turnover and carry out more ''housekeeping'' roles in the cell. It should be noted though, that these proteins nonetheless consume a large proportion of the energy budget of protein turnover because of the scale of the flux through these protein pools [70].

Proteomics-based turnover measurements are not necessarily limited to isolated cell systems. Proteome dynamics can also be assessed by whole animal metabolic labelling with stable isotope labelled amino acids [21] or deuterated water [40].

A number of strategies have been reported combining dynamic or pulsed SILAC with chemical labeling with isobaric mass tags to multiplex turnover experiments (Fig. 3). Jayapal et al. combined iTRAQ labeling and SILAC with arginine to measure protein turnover without the assumptions of steady-state intracellular protein concentrations by monitoring both rates of incorporation and loss of the 'heavy' SILAC label over time in a pulse-chase experiment while measuring changes in overall protein levels using non-arginine-containing peptides [43]. Multiplexing dynamic or pulsed SILAC experiments with isobaric mass tags typically follows one out of two fundamental schemes: multiplexing across time points or multiplexing across different treatment conditions (Fig. 3a and b). Multiplexing along the time dimension affords more time efficient experiments and avoids missing values (Fig. 3a). In a recent report tandem

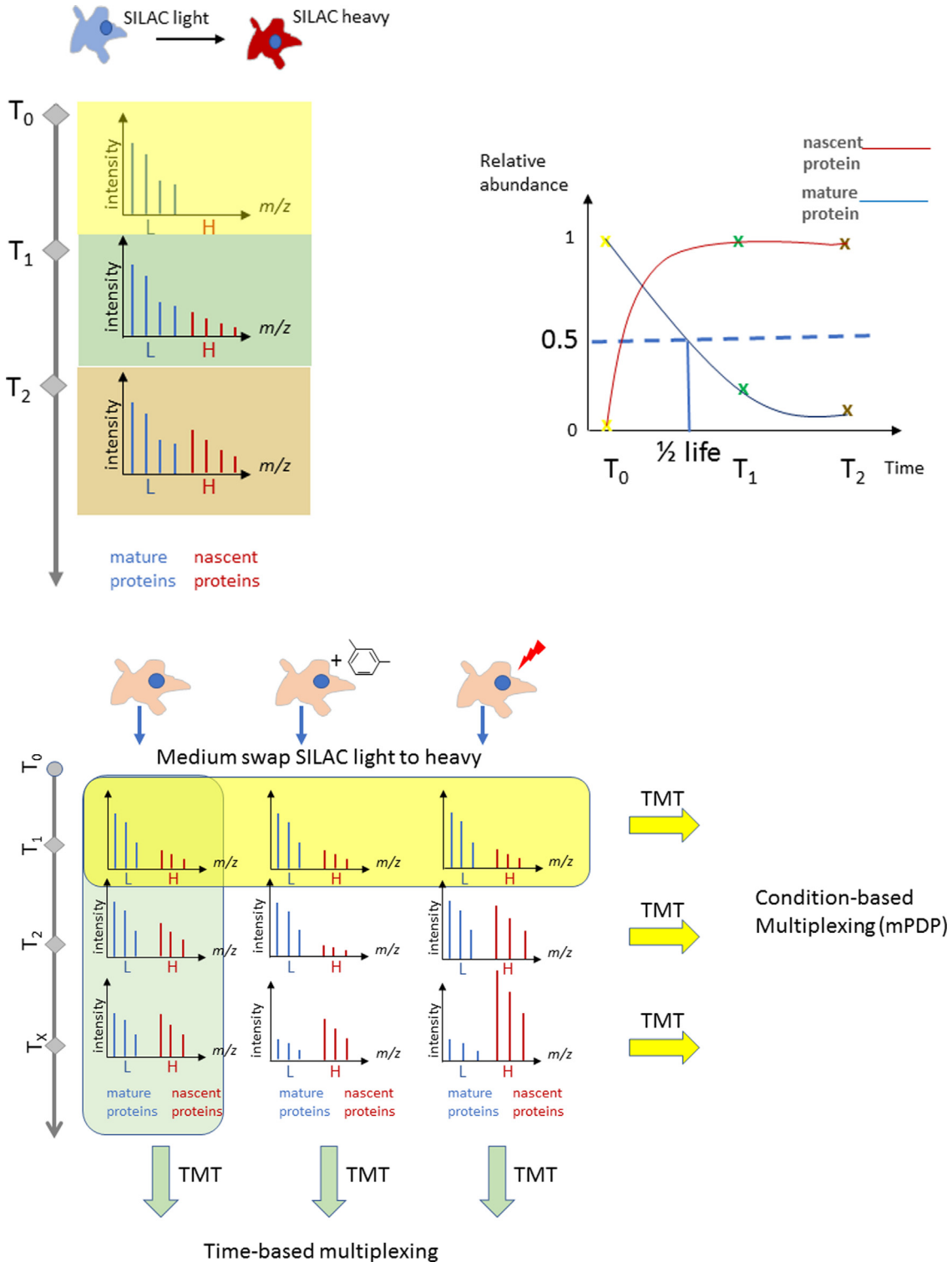

Fig. 3. Dynamic SILAC and chemical labelling.
(a) Time dependence and determination of protein half-life: schematic representation of dynamic SILAC labelling. Cells are grown in SILAC light medium and switched to SILAC heavy medium (or vice versa) to label newly synthesized proteins (nascent proteins). Samples are taken at time intervals (T0, T1, T2) and analyzed by high resolution mass spectrometry. The plot on the right illustrates how in the same experiment nascent and mature proteins can be quantified (SILAC heavy and light) at each time point and thus enabling calculation of protein half-lives.
(b) In a multiplexed SILAC/TMT proteome dynamic experiment, TMT labels can be used to label proteins at different time points from the switch of SILAC medium (Time-based multiplexing) or different treatment conditions (Condition-based multiplexing). The effect of time or of treatment on the production of newly synthesized proteins and on the stability of mature proteins can then be monitored by MS based detection of labelled peptides.

mass tag (TMT)-labeling of ten pulse time-points in a single experiment allowed recording turnover data in a single mass spectrometry experiment [85]. Application of the SPS (Synchronous Precursor Selection) fragmentation technique yielded accurate quantification and consequently the data showed a high concordance to the standard dynamic SILAC method while yielding more comprehensive data (6000 proteins on average). The accurate peptide level quantification achieved with this approach further revealed isoform and post-translational modification dependent effects on protein turnover [85]. In a comparison of protein turnover in resting versus dividing human fibroblast, time-based multiplexing of turnover measurements revealed that, for long-lived proteins, the increase in degradation is the proteostatic measure that quiescent cells adopt to avoid massive concentration [81].

In contrast to the above techniques ''multiplexed proteome dynamics profiling'' (mPDP) is designed to enable the discovery of dynamically synthesis and degradation mechanisms in cellular systems [66]. This is achieved by TMT-based multiplexing of different treatment conditions in biological replicates (Fig. 3b). Multiplexing of dynamic light-to-heavy and heavy-to-light SILAC labelled samples allows for two biological replicates for each treatment condition to be analyzed in parallel. In one replicate, cells are first grown in light SILAC medium and switched to the heavy SILAC medium prior to addition of a compound, whereas in the other replicate heavy SILAC converted cells are switched to the light SILAC medium prior to compound addition. Cell samples are extracted, followed by an optional enrichment step, e.g. for protein kinases [8]. Protein samples are digested with trypsin and peptides are labelled with TMT reagents, mixed, and subjected to multiplexed analysis by mass spectrometry. Because of the two-way dynamic SILAC labelling at common time points, this strategy enables the quantification of multiplexed isobaric signals from mature proteins, that already existed when the SILAC labels were swapped, as well as nascent proteins that were synthesized after the label swap. As light and heavy labelled peptide ion signals will have identical intensities, mature and nascent protein components from both biological replicates will be identified and quantified to the same extent. When analyzing small molecule compounds affecting cellular proteostasis by different mechanisms, it was found that this approach allows detecting more subtle effects on protein degradation than conventional dynamic SILAC studies. The approach revealed an unanticipated mechanism of a targeted degrader of the BET bromodomain family, elucidated distinct modes of action of estrogen receptor modulators, and provided the first comprehensive classification HSP90 clients based on their requirement for HSP90 constitutively or during synthesis.

By combining dynamic SILAC labeling with dimethyl labeling of Lysine residues and N-termini, the study further provided a comprehensive analysis of HSP90 clients in primary T-cells and classified clients in the presence or absence of activated TCR pathway [66].

Proteomics approaches to measure protein homeostasis in combination with chemical enrichment techniques

A technical difficulty associated with the pulsed SILAC approach is the fact that the newly synthesized proteins are clearly under-represented compared to existing proteins and, thus, the detection of the former pool by MS is challenging, especially for low abundant proteins. The use of non-canonical amino acids containing small reactive groups amenable to click-chemistry enables affinity-enrichment of newly synthesized proteins circumventing this sensitivity issue and allows the detection of nascent proteins after very short pulses (Fig. 2).

By using the methionine surrogate azidohomoalanine (AHA) in the cell culture medium followed by biotin attachment with a click reaction and affinity purification on streptavidin beads, Dieterich et al. identified newly synthesized proteins after pulsed labeling with the modified amino acid for as short as 2 h [23]. The ease of the recovery with no apparent bias for methionine content, modification, localization or expression level of the modified protein makes this method, called BONCAT for BioOrtogonal NonCanonical Amino acid Tagging, an attractive alternative to stable isotope labelling [23]. Homopropargylglycine (HPG) is an alternative to AHA as it contains an alkyne instead of an azido group on the methionine-like structure to allow the biorthogonal labelling reactions and has been used successfully for the time-resolved analysis of protein synthesis and degradation in mammalian and bacterial cells [9,22,28]. The possibility of attaching a fluorophore to such modified amino acids enabled the localization of the newly synthesized proteins in cells [74,86] including primary cells [23] and has been used in vivo to study protein turnover in murine tissues after injection of the clickable amino acid directly in the animal [16].

Besides the fact that this method is blind for the 6% of the expressed proteome which either does not carry a single methionine residue or where the only, N-terminal methionine is removed posttranslationally, the biggest concern in this approach is that the folding of proteins and thus their stability might be affected as shown by the upregulation of HSPs and chaperones in samples labelled with tagged non-canonical amino acids, [4]. The different labelling methodologies have specific advantages but also suffer from specific limitations: the possibility of using two labelling methods at the same time would gain the collective method advantages and compensate each method limitations.

Combination of BONCAT with pSILAC methods enabled monitoring protein turnover with highly accurate MS quantification for a much larger number of proteins compared to the use of the single approaches [4] and is particularly suitable for accurate determination of short lived proteins. The combination with SILAC labelling allowed to control for the effects of the azide modified amino acid in protein abundancies and in stimulation of heat shock response and showed that the use of a 30:1 mix of AHA and Met reduced substantially both biases. McShane et al. used such a combined SILAC and pulsed AHA labeling approach to discover that up to 10% of the proteome is degraded following a non-exponential decay model suggesting that for these proteins degradation is faster when they are freshly synthesized and decay is slower once these proteins are fully matured and part of protein complexes [56]. Pulsed labeling of cells with a chemically modified lipid precursor and stable isotope enriched amino acids was recently applied to study the dynamics of post-translational modification and protein turnover [86] demonstrating the versatility of approaches combining pulsed or dynamic amino acid labeling with protein or post translational modification-specific reporters.

Proteomics approaches to understand mechanism of action of drugs interfering with protein homeostasis

The elucidation of the biological processes and the identification of the molecular players in ubiquitin-mediated protein degradation, has offered opportunities for pharmacological intervention to treat several diseases characterized by altered proteolysis. These endeavors though, have not been straightforward because of the lack of classical catalytic pockets in the various ubiquitin ligases and the fact that most of the activity is mediated by protein-protein interactions [41]. Most efforts have been directed against the proteasome, E3 ligases in virtue of their higher specificity and the DUBs because of their direct link to disease [58]. Thus far, most success has come from targeting the proteasome with e.g. Bortezomib being approved for the treatment of multiple myeloma in 2003 [75] and thalidomide and its second-generation derivatives lenalidomide and pomalidomide, collectively known as immune-modulatory drugs (IMiDs), used in the treatment of hematological malignancies [30]. Originally introduced as a sedative used to prevent nausea during pregnancy in the late 1950s Thalidomide was withdrawn in 1961 due to teratogenicity and neuropathy [73]. In 2010 Ito et al. applied a chemoproteomics approach performing affinity enrichment experiments from human HeLa cell extracts with the carboxylic thalidomide derivative FR259625 covalently conjugated to ferriteglycidyl methacrylate beads and identified CRBN, the substrate receptor of a Cullin-RING ubiquitin ligase ($CRL4^{CRBN}$) complex [42] as the efficacy target. Thalidomide and its derivatives prevent the CRBN receptor from engaging an endogenous substrate [18]. IMiD binding to CRBN further induces recruitment and degradation of the Ikaros/Aiolos transcription factors and Casein Kinase 1 alpha (Ck1α) [47,52]. These factors probably represent neo-substrates, which are exclusively degraded by $CRL4^{CRBN}$ in the presence of the drug and molecules with different substituents to the phthalimide ring have been shown to degrade other proteins with similar structure motifs [54]. Such molecular glues directed to degrade proteins are of great interest for developing selective degraders and expression proteomics and proteome dynamics profiling techniques are invaluable to understand their specificity.

A related approach that has recently gained traction is based on bifunctional degraders commonly known as PROteolysis-TArgeting Chimeras (PROTACs) [3,64]. These molecules are comprised of two ligands, one recruiting an E3 ligase and the other for engaging the target, that are connected by a flexible linker. By exchanging either of the ligands different targets can be degraded and different E3 ligases can be engaged [14,49,62]. Assessing the selectivity of such molecules is not trivial as both ligands may bind to more than one protein at relevant doses and whilst some off-targets may only be bound, others might be functionally affected and another subset may be degraded [13,14,66]. Apart from standard biochemical assay panels, chemoproteomics approaches based on either affinity enrichment methods [6] or based on structural features such as Thermal Proteome Profiling [65] or limited proteolysis (Lip-MS) [68] enable the proteome-wide assessment of ligand selectivity (Fig. 4). Expression proteomics or proteome dynamics profiling enlights on the degraded target proteins and downstream regulation of protein levels, thus informing on the mechanism of action of the PROTACs [13,66,83]. Proteome dynamics profiling methods such as mPDP are of particular value as they enable distinguishing degradation targets from proteins with reduced synthesis due to downstream effects (Fig. 4). For example, the TREX complex adapter protein FYTTD1 regulating mRNA export from the nucleus was identified by thermal proteome profiling as an off-target of the BET bromodomain inhibitor JQ1. However, binding of the compound did not lead to functional consequences. A JQ1-PROTAC, on the other hand, was found to degrade FYTTD1, thus causing a strong molecular phenotype due to mRNA accumulation in the nucleus leading to an almost complete arrest of protein synthesis [66]. Understanding the total selectivity of the compounds enables a more comprehensive understanding of PROTAC-MoA and the amplified contribution of polypharmacology to phenotypic effects due to off-target degradation. The proteomic profiling techniques summarized in this chapter offer exquisite tools to investigate such effects and to guide compound optimization for enhanced selectivity.

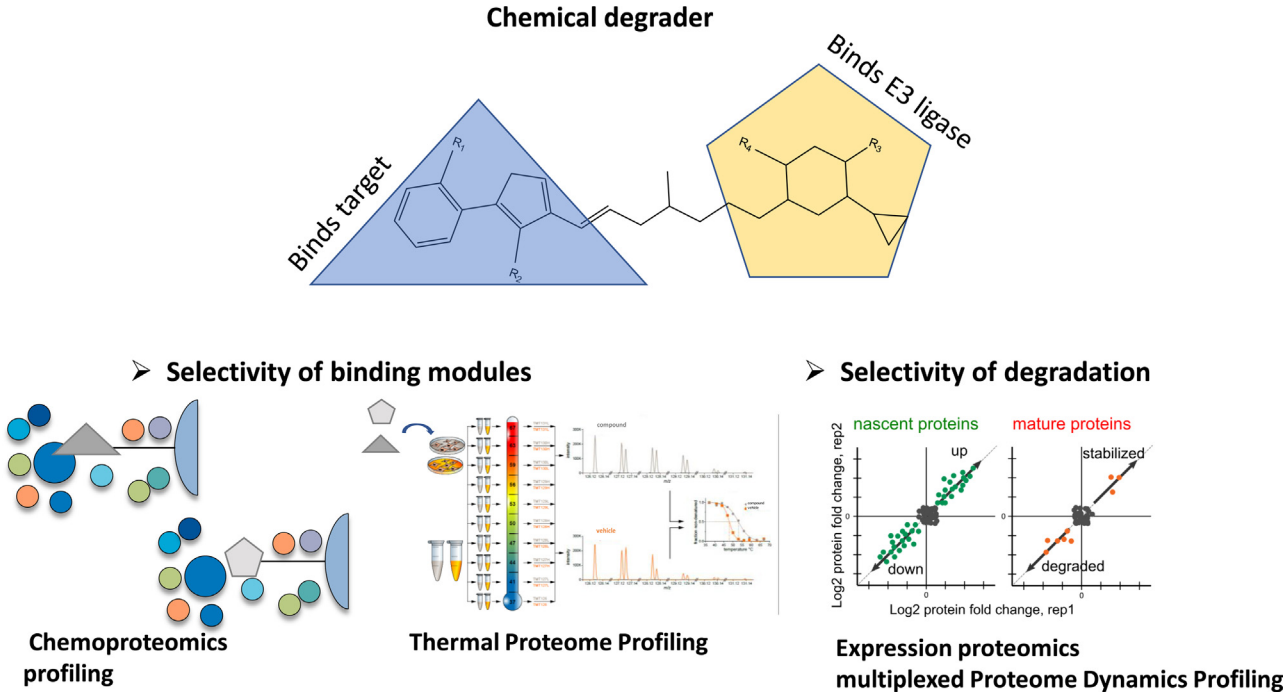

Fig. 4. Proteomics and chemoproteomics approaches to characterize chemical degraders.
The selectivity of the target- and of the E3 ligase- ligand of bifunctional chemical degraders (as PROTACs, molecular glues, IMiDs) can be assessed by a chemoproteomics approach: the ligand is immobilized on a solid phase used to capture proteins from cell lysate which are subsequently identified by mass spectrometry. Alternatively, Thermal Proteome Profiling can be used, which measures the change in thermal stability of proteins after treatment of the cells with the ligand.
The selectivity of the degradation effect induced by the bifunctional molecule is measured by mass spectrometry-based quantification of the changes in expression levels of all proteins in the cells: in a multiplexed protein dynamic experiment the effect of the chemical degrader on nascent and mature proteins can be distinguished.

Conclusions

Accurate determination of protein synthesis and degradation rates at a global scale is absolutely required to understand regulation of proteostasis under physiological and pathological conditions as well as in response to drug treatment. The combination of different labelling methods with quantitative mass spectrometry has enabled unbiased studies of protein homeostasis at the proteome level. Integration of other unbiased and large scale approaches such as transcriptomics, ribosome profiling, or metabolomics is necessary to understand mechanism and consequences of changes in protein homeostasis [50,51].

Monitoring and quantifying changes at the level of the proteome further reveals selectivity and downstream consequences of drugs which cause protein degradation. In particular, target and off-target proteolysis induced by chemical degraders might affect cell homeostasis in a different way than just inhibiting those proteins. In addition, affinity ligands present in PROTACs, molecular glues and hydrophobic patches might perturb the activity of endogenous E3 ligases or HSPs inducing additional changes in the proteome then those dependent on the targeted proteins.

Single cell gene expression studies revealed that eukaryotic cells modify transcriptional burst in relation to cell size to keep a constant concentration of mRNA species [76]. The mechanisms by which this control is achieved are not known nor it is clear whether a cell size dependent control is exerted at the level of global protein homeostasis via i.e. dynamic translation rates as demonstrated by an imaging approach [34].

Development and application of proteomic methods at single cell resolution will enable understanding cell to cell heterogeneity in controlling protein homeostasis and provide the ultimate guide for developing safe and efficacious chemical knockdown-based targeted therapies.

References

[1] Aebersold R, Mann M. Mass-spectrometric exploration of proteome structure and function. Nature 2016;537:347–55.
[2] Åkerfelt M, Morimoto RI, Sistonen L. Heat shock factors: integrators of cell stress, development and lifespan. Nat Rev Mol Cell Biol 2010;11:545–55.
[3] An S, Fu L. Small-molecule PROTACs: an emerging and promising approach for the development of targeted therapy drugs. EBioMedicine 2018;36:553–62.
[4] Bagert JD, Xie YJ, Sweredoski MJ, Qi Y, Hess S, Schuman EM, Tirrell DA. Quantitative, time-resolved proteomic analysis by combining bioorthogonal noncanonical amino acid tagging and pulsed stable isotope labeling by amino acids in cell culture. Mol Cell Proteomics 2014;13:1352–8.
[5] Balch WE, Morimoto RI, Dillin A, Kelly JW. Adapting proteostasis for disease intervention. Science 2008;319:916–9.

[6] Bantscheff M, Drewes G. Chemoproteomic approaches to drug target identification and drug profiling. Bioorg Med Chem 2012;20:1973–8.
[7] Bantscheff M, Schirle M, Sweetman G, Rick J, Kuster B. Quantitative mass spectrometry in proteomics: a critical review. Anal Bioanal Chem 2007;389:1017–31.
[8] Bantscheff M, Eberhard D, Abraham Y, Bastuck S, Boesche M, Hobson S, et al. Quantitative chemical proteomics reveals mechanisms of action of clinical ABL kinase inhibitors. Nat Biotechnol 2007;25:1035–44.
[9] Beatty KE, Xie F, Wang Q, Tirrell DA. Selective dye-labeling of newly synthesized proteins in bacterial cells. J Am Chem Soc 2005;127:14150–51.
[10] Beaudette P, Popp O, Dittmar G. Proteomic techniques to probe the ubiquitin landscape. Proteomics 2016;16:273–87.
[11] Belle A, Tanay A, Bitincka L, Shamir R, O'Shea EK. Quantification of protein half-lives in the budding yeast proteome. Proc Natl Acad Sci USA 2006;103:13004–09.
[12] Boisvert F-M, Ahmad Y, Gierliński M, Charrière F, Lamont D, Scott M, et al. A quantitative spatial proteomics analysis of proteome turnover in human cells. Mol Cell Proteomics 2012;11. M111.011429.
[13] Bondeson DP, Mares A, Smith IED, Ko E, Campos S, Miah AH, et al. Catalytic in vivo protein knockdown by small-molecule PROTACs. Nat Chem Biol 2015;11:611–7.
[14] Bondeson DP, Smith BE, Burslem GM, Buhimschi AD, Hines J, Jaime-Figueroa S, et al. Lessons in PROTAC design from selective degradation with a promiscuous warhead. Cell Chem Biol 2018;25. 78–87. e5.
[15] Bonifacino JS. Biosynthetic labeling of proteins. Curr Protoc Immunol 2001;41(1). 8.12.1-8.12.9.
[16] Calve S, Witten AJ, Ocken AR, Kinzer-Ursem TL. Incorporation of non-canonical amino acids into the developing murine proteome. Sci Rep 2016;6.
[17] Cambridge SB, Gnad F, Nguyen C, Bermejo JL, Krüger M, Mann M. Systems-wide proteomic analysis in mammalian cells reveals conserved, functional protein turnover. J Proteome Res 2011;10:5275–84.
[18] Chamberlain PP, Lopez-Girona A, Miller K, Carmel G, Pagarigan B, Chie-Leon B, et al. Structure of the human Cereblon-DDB1-lenalidomide complex reveals basis for responsiveness to thalidomide analogs. Nat Struct Mol Biol 2014;21:803–9.
[19] Ciechanover A. The ubiquitin-proteasome pathway: on protein death and cell life. EMBO J 1998;17:7151–60.
[20] Claydon AJ, Beynon R. Proteome dynamics: revisiting turnover with a global perspective. Mol Cell Proteomics 2012;11:1551–65.
[21] Claydon AJ, Thom MD, Hurst JL, Beynon RJ. Protein turnover: measurement of proteome dynamics by whole animal metabolic labelling with stable isotope labelled amino acids. Proteomics 2012;12:1194–206.
[22] Deal RB, Henikoff JG, Henikoff S. Genome-wide kinetics of nucleosome turnover determined by metabolic labeling of histones. Science 2010;328:1161–4.
[23] 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;103:9482–7.
[24] Dikic I. Proteasomal and autophagic degradation systems. Annu Rev Biochem 2017;86:193–224.
[25] Dikic I, Elazar Z. Mechanism and medical implications of mammalian autophagy. Nat Rev Mol Cell Biol 2018;19:349–64.
[26] Doherty MK, Beynon RJ. Protein turnover on the scale of the proteome. Expert Rev Proteomics 2006;3:97–110.
[27] Doherty MK, Hammond DE, Clague MJ, Gaskell SJ, Beynon RJ. Turnover of the human proteome: determination of protein intracellular stability by dynamic SILAC. J Proteome Res 2009;8:104–12.
[28] Eichelbaum K, Winter M, Diaz MB, Herzig S, Krijgsveld J. Selective enrichment of newly synthesized proteins for quantitative secretome analysis. Nat Biotechnol 2012;30:984–90.
[29] Eletr ZM, Wilkinson KD. Regulation of proteolysis by human deubiquitinating enzymes. Biochim Biophys Acta BBA - Mol Cell Res 2014;1843:114–28.
[30] Elkinson S, McCormack PL. Pomalidomide: first global approval. Drugs 2013;73:595–604.
[31] Gehrmann ML, Hathout Y, Fenselau C. Evaluation of metabolic labeling for comparative proteomics in breast cancer cells. J Proteome Res 2004;3:1063–8.
[32] Görg A, Weiss W, Dunn MJ. Current two-dimensional electrophoresis technology for proteomics. Proteomics 2004;4:3665–85.
[33] Greenbaum D, Colangelo C, Williams K, Gerstein M. Comparing protein abundance and mRNA expression levels on a genomic scale. Genome Biol 2003;8.
[34] Han K, Jaimovich A, Dey G, Ruggero D, Meyuhas O, Sonenberg N, et al. Parallel measurement of dynamic changes in translation rates in single cells. Nat Methods 2014;11:86–93.
[35] Hanna J, Guerra-Moreno A, Ang J, Micoogullari Y. Protein degradation and the pathologic basis of disease. Am J Pathol 2018;189(1):94–103.
[36] Harrigan JA, Jacq X, Martin NM, Jackson SP. Deubiquitylating enzymes and drug discovery: emerging opportunities. Nat Rev Drug Discov 2017;17:57–78.
[37] Heap RE, Gant MS, Lamoliatte F, Peltier J, Trost M. Mass spectrometry techniques for studying the ubiquitin system. Biochem Soc Trans 2017;45:1137–48.
[38] Hewings DS, Flygare JA, Bogyo M, Wertz IE. Activity-based probes for the ubiquitin conjugation–deconjugation machinery: new chemistries, new tools, and new insights. FEBS J 2017;284:1555–76.
[39] Hinkson IV, Elias JE. The dynamic state of protein turnover: it's about time. Trends Cell Biol 2011;21:293–303.
[40] Holmes WE, Angel TE, Li KW, Hellerstein MK. Chapter seven – dynamic proteomics: in vivo proteome-wide measurement of protein kinetics using metabolic labeling. In: Metallo CM, editor. Methods in enzymology. Academic Press; 2015. p. 219–76.
[41] Huang X, Dixit VM. Drugging the undruggables: exploring the ubiquitin system for drug development. Cell Res 2016;26:484–98.
[42] Ito T, Ando H, Suzuki T, Ogura T, Hotta K, Imamura Y, et al. Identification of a primary target of thalidomide teratogenicity. Science 2010;327:1345–50.
[43] Jayapal KP, Sui S, Philp RJ, Kok Y-J, Yap MGS, Griffin TJ, et al. Multitagging proteomic strategy to estimate protein turnover rates in dynamic systems. J Proteome Res 2010;9:2087–97.
[44] Kaushal K, Antao AM, Kim K-S, Ramakrishna S. Deubiquitinating enzymes in cancer stem cells: functions and targeted inhibition for cancer therapy. Drug Discov Today 2018;23(12):1974–82.
[45] Kitagawa K, Kitagawa M. The SCF-type E3 ubiquitin ligases as cancer targets. Curr Cancer Drug Targets 2016;16(2):119–29.
[46] Klaips CL, Jayaraj GG, Hartl FU. Pathways of cellular proteostasis in aging and disease. J Cell Biol 2018;217:51–63.
[47] Krönke J, Fink EC, Hollenbach PW, MacBeth KJ, Hurst SN, Udeshi ND, et al. Lenalidomide induces ubiquitination and degradation of CK1α in del(5q) MDS. Nature 2015;523:183–8.
[48] Labbadia J, Morimoto RI. The biology of proteostasis in aging and disease. Annu Rev Biochem 2015;84:435–64.
[49] Lai AC, Toure M, Hellerschmied D, Salami J, Jaime-Figueroa S, Ko E, et al. Modular PROTAC design for the degradation of oncogenic BCR-ABL. Angew Chem Int Ed Engl 2016;55:807–10.
[50] Latonen L, Afyounian E, Jylhä A, Nättinen J, Aapola U, Annala M, et al. Integrative proteomics in prostate cancer uncovers robustness against genomic and transcriptomic aberrations during disease progression. Nat Commun 2018;9(1):1176–89.
[51] Liu Y, Borel C, Li L, Müller T, Williams EG, Germain P-L, et al. Systematic proteome and proteostasis profiling in human Trisomy 21 fibroblast cells. Nat Commun 2017;8(1):1212–27.
[52] Lu G, Middleton RE, Sun H, Naniong M, Ott CJ, Mitsiades CS, et al. The myeloma drug lenalidomide promotes the cereblon-dependent destruction of Ikaros proteins. Science 2014;343:305–9.
[53] Mathieson T, Franken H, Kosinski J, Kurzawa N, Zinn N, Sweetman G, et al. Systematic analysis of protein turnover in primary cells. Nat Commun 2018;9.
[54] Matyskiela ME, Lu G, Ito T, Pagarigan B, Lu C-C, Miller K, et al. A novel cereblon modulator recruits GSPT1 to the $CRL4^{CRBN}$ ubiquitin ligase. Nature 2016;535:252–7.

[55] Mazroui R, Di Marco S, Kaufman RJ, Gallouzi I-E. Inhibition of the ubiquitin-proteasome system induces stress granule formation. Mol Biol Cell 2007;18:2603–18.
[56] McShane E, Sin C, Zauber H, Wells JN, Donnelly N, Wang X, et al. Kinetic analysis of protein stability reveals age-dependent degradation. Cell 2016;167. 803–815. e21.
[57] Meier F, Geyer PE, Winter SV, Cox J, Mann M. BoxCar acquisition method enables single-shot proteomics at a depth of 10,000 proteins in 100 minutes. Nat Methods 2018;15:440.
[58] Morrow JK, Lin H-K, Sun S-C, Zhang S. Targeting ubiquitination for cancer therapies. Future Med Chem 2015;7:2333–50.
[59] Nakatogawa H, Suzuki K, Kamada Y, Ohsumi Y. Dynamics and diversity in autophagy mechanisms: lessons from yeast. Nat Rev Mol Cell Biol 2009;10:458–67.
[60] Ong S-E. The expanding field of SILAC. Anal Bioanal Chem 2012;404:967–76.
[61] Ong S-E, 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;1:376–86.
[62] Ottis P, Toure M, Cromm PM, Ko E, Gustafson JL, Crews CM. Assessing different E3 ligases for small molecule induced protein ubiquitination and degradation. ACS Chem Biol 2017;12:2570–8.
[63] Pratt JM, Petty J, Riba-Garcia I, Robertson DHL, Gaskell SJ, Oliver SG, et al. Dynamics of protein turnover, a missing dimension in proteomics. Mol Cell Proteomics 2002;1:579–91.
[64] Sakamoto KM, Kim KB, Kumagai A, Mercurio F, Crews CM, Deshaies RJ. Protacs: chimeric molecules that target proteins to the skp1-cullin-F box complex for ubiquitination and degradation. Proc Natl Acad Sci U S A 2001;98:8554–9.
[65] Savitski MM, Reinhard FBM, Franken H, Werner T, Savitski MF, Eberhard D, et al. Tracking cancer drugs in living cells by thermal profiling of the proteome. Science 2014;346.
[66] Savitski MM, Zinn N, Faelth-Savitski M, Poeckel D, Gade S, Becher I, et al. Multiplexed proteome dynamics profiling reveals mechanisms controlling protein homeostasis. Cell 2018;173. 260–274. e25.
[67] Schmiedel JM, Klemm SL, Zheng Y, Sahay A, Blüthgen N, Marks DS, et al. Gene expression. MicroRNA control of protein expression noise. Science 2015;348:128–32.
[68] Schopper S, Kahraman A, Leuenberger P, Feng Y, Piazza I, Müller O, et al. Measuring protein structural changes on a proteome-wide scale using limited proteolysis-coupled mass spectrometry. Nat Protoc 2017;12:2391–410.
[69] Schwanhäusser B, Gossen M, Dittmar G, Selbach M. Global analysis of cellular protein translation by pulsed SILAC. Proteomics 2009;9:205–9.
[70] Schwanhäusser B, Busse D, Li N, Dittmar G, Schuchhardt J, Wolf J, et al. Global quantification of mammalian gene expression control. Nature 2011;473:337–42.
[71] Shrestha L, Bolaender A, Patel H. Heat shock protein (HSP) drug discovery and development: targeting heat shock proteins in disease. Curr Top Med Chem 2016;16:2753–64.
[72] Sin O, Nollen EAA. Regulation of protein homeostasis in neurodegenerative diseases: the role of coding and non-coding genes. Cell Mol Life Sci CMLS 2015;72:4027–47.
[73] Speirs AL. Thalidomide and congenital abnormalities. Lancet 1962;279:303–5.
[74] Tcherkezian J, Brittis PA, Thomas F, Roux PP, Flanagan JG. Transmembrane receptor DCC associates with protein synthesis machinery and regulates translation. Cell 2010;141:632–44.
[75] Twombly R. First proteasome inhibitor approved for multiple myeloma. J Natl Cancer Inst 2003;95:845.
[76] Vargas-Garcia CA, Ghusinga KR, Singh A. Cell size control and gene expression homeostasis in single-cells. Curr Opin Syst Biol 2018;8:109–16.
[77] Vogel C, Marcotte EM. Insights into the regulation of protein abundance from proteomic and transcriptomic analyses. Nat Rev Genet 2012;13:227–32.
[78] Walter P, Ron D. The unfolded protein response: from stress pathway to homeostatic regulation. Science 2011;334:1081–6.
[79] Wang P, Zhou J. Proteolysis targeting chimera (PROTAC): a paradigm-shifting approach in small molecule drug discovery. Curr Top Med Chem 2018;18:1354–6.
[80] Weissman AM. Themes and variations on ubiquitylation. Nat Rev Mol Cell Biol 2001;2:169–78.
[81] Welle KA, Zhang T, Hryhorenko JR, Shen S, Qu J, Ghaemmaghami S. Time-resolved analysis of proteome dynamics by tandem mass tags and stable isotope labeling in cell culture (TMT-SILAC) hyperplexing. Mol Cell Proteomics 2016;15:3551–63.
[82] White A. The dynamic state of body constituents. Yale J Biol Med 1942;14:677.
[83] Winter GE, Buckley DL, Paulk J, Roberts JM, Souza A, Dhe-Paganon S, et al. Phthalimide conjugation as a strategy for in vivo target protein degradation. Science 2015;348:1376–81.
[84] Yen H-CS, Xu Q, Chou DM, Zhao Z, Elledge SJ. Global protein stability profiling in mammalian cells. Science 2008;322. 7.
[85] Zecha J, Meng C, Zolg DP, Samaras P, Wilhelm M, Kuster B. Peptide level turnover measurements enable the study of proteoform dynamics. Mol Cell Proteomics 2018;17:974–92.
[86] Zhang MM, Tsou LK, Charron G, Raghavan AS, Hang HC. Tandem fluorescence imaging of dynamic S-acylation and protein turnover. Proc Natl Acad Sci USA 2010;107:8627–32.
[87] Zinck R, Cahill MA, Kracht M, Sachsenmaier C, Hipskind RA, Nordheim A. Protein synthesis inhibitors reveal differential regulation of mitogen-activated protein kinase and stress-activated protein kinase pathways that converge on Elk-1. Mol Cell Biol 1995;15:4930–8.