TECHNOLOGIES AND TECHNIQUES

The emergence of proteome-wide technologies: systematic analysis of proteins comes of age

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Abstract | During the lifetime of a cell proteins can change their localization, alter their abundance and undergo modifications, all of which cannot be assayed by tracking mRNAs alone. Methods to study proteomes directly are coming of age, thereby opening new perspectives on the role of post-translational regulation in stabilizing the cellular milieu. Proteomics has undergone a revolution, and novel technologies for the systematic analysis of proteins have emerged. These methods can expand our ability to acquire information from single proteins to proteomes, from static to dynamic measures and from the population level to the level of single cells. Such approaches promise that proteomes will soon be studied at a similar level of dynamic resolution as has been the norm for transcriptomes.

System-level analysis has revolutionized cell research by enabling a conceptual shift from the focus on a single particle, complex or pathway to a holistic view of all cellular components and their dynamic crosstalk. The era of bioinformatics and advanced technologies brought about a diversity of large-scale methodologies that enable such systematic measurements of the various cellular molecules — such as DNA (genomics)[1,2], mRNA (transcriptomics)[3,4], proteins (proteomics)[5–9], post-translational modifications (such as glycomics[10,11] and phosphoproteomics[12,13]), lipids (lipidomics)[14–16] and small molecules (metabolomics)[17–19] — as well as their functional[20,21] and physical interactions[22]. Endeavours to combine these data reveal unappreciated modularity and flexibility in biological organization[23].

Cells must constantly integrate external and internal signals to recalculate ever-changing circumstances and respond appropriately. How such adaptation occurs is not yet completely understood for any perturbation. Therefore, investigating the spatial and temporal alterations of cellular components is crucial for a mechanistic understanding of living systems. With the advent of microarrays, and later deep sequencing, such analyses have been extensively undertaken for RNA expression profiles[3,24]. These efforts resulted in a large gain of knowledge of the transcriptional regulation dynamics in many organisms. However, proteins carry out most cellular functions. Although it is often assumed that mRNA levels reflect the abundance and activity of their respective proteins, systematic quantification of proteins shows, in almost every organism that has been examined so far, that transcript abundance is not a good predictor of protein level either in steady state or in response to stress[23,25–27]. In fact, it has been shown that proteins that are crucial for the response to environmental stimuli often show no altered regulation at the mRNA level[21]; thus, they most probably undergo post-translational regulation[23,25–30]. Several evolutionary studies comparing data from humans, chimpanzees and rhesus macaques have shown that levels of protein expression seem to be much more tightly conserved across species than are levels of mRNA transcripts[31–33]. Stemming from this observation is the idea that protein levels are tightly controlled for functional consequences, whereas the regulation of mRNA levels has evolved with less constraints[34]. Indeed, the cellular protein pool is maintained by a dynamic and complex balance of interconnected post-transcriptional and post-translational processes: localization, processing and degradation of mRNAs, as well as the translation, localization, modification and degradation of the proteins themselves[23] (FIG. 1). The biggest challenge in the systematic analysis of proteins is therefore to obtain an accurate assessment of the rates and determinants of each of these regulatory steps, as well as their relative contribution to protein abundance. Hence, overcoming technical barriers to obtain accurate and dynamic measurements of proteins should give rise to an increasingly realistic image of how a biological unit functions.

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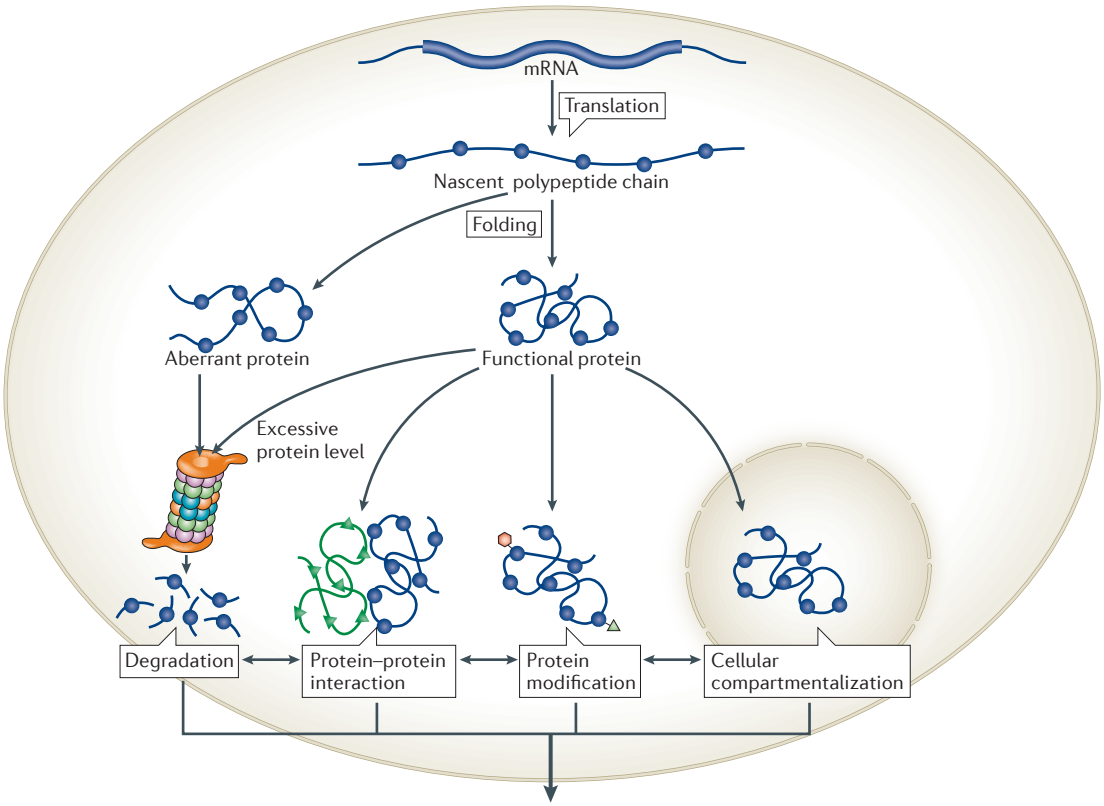


Figure 1 | **Modes of post-transcriptional regulation to control the functional protein pool of the cell.** The synthesis of a fully mature and functional protein according to its encoding mRNA transcript is a complex process of interconnected nodes of regulation. To maintain an appropriate level of functional proteins in the cell, several major tiers of regulation exist — the translation of the nascent peptide, its correct folding and maturation. Maturation includes the creation of physical interactions with additional protein partners, proper compartmentalization within the cell and the acquisition of molecular modifications such as phosphorylation and ubiquitylation. To maintain the protein pool at the appropriate level according to the inputs received by the cell, a tightly regulated crosstalk between all of these processes must occur. Excessive amounts of protein or aberrant proteins can be degraded.

With the emergence of enabling technologies (TABLE 1), the past few years brought about the first systematic measurements of proteins under basal growth conditions[5,7,9,35,36] (described below). However, it is obvious that these studies are only the 'tip of the iceberg', and that important information will be obtained from studying protein dynamics in response to fluctuating cues, namely growth conditions, external factors or genetic mutations. Our Review provides an overview of the technological advances in different strategies to measure various aspects of protein regulation in a systematic manner. We discuss three major developments that such new approaches facilitate: the move from single-protein, low-throughput studies to whole-proteome, high-throughput analyses; the shift from population-level proteomic analyses to studies at single-cell resolution; and the expansion of the proteomics scope from a single-time point, static view of the cell into a complex and dynamic vision.

Measuring protein abundance

Measuring the abundance of all cellular proteins is important for a true representation of the cellular milieu. Moreover, by comparing protein to mRNA levels under various conditions it is possible to detect both post-transcriptional and post-translational regulation. Several approaches enable the acquisition of such information.

Population-level approaches. Immunodetection by western blot analysis (TABLE 1) has been used to measure the abundance of the yeast proteome. For this purpose, a specialized collection of yeast strains (library) was constructed, in which each open reading frame was fused to a tandem affinity purification epitope[36] (TAP epitope) in its native chromosomal context and under the control of its endogenous promoter. Through immunodetection of this common tag, a census of the repertoire and absolute levels of all proteins expressed during logarithmic growth in a standard laboratory medium was obtained (covering 80% of the proteome). Levels of proteins, many of which were detected for the first time, ranged from fewer than 50 copies to more than 10^6 molecules per cell[36]. These results imply that the remaining 20% of the proteome is only expressed under specific conditions (although the lack of detection might also have been due to the detection threshold or problems with the tagging procedure). In humans, in whom tagging of all genes

Tandem affinity purification epitope
(TAP epitope). An epitope, against which specific and high-affinity antibodies exist, that can be used for purification and western blot analysis.

Table 1 | **Common assays used in proteomic studies**

Assay	Application	Advantages	Disadvantages
Antibody labelling by western blot analysis	• Abundance • Degradation rate (by cycloheximide chase)	Does not require a special infrastructure	• Laborious • Depends on genetic tagging • Low sensitivity to small changes • Population-level resolution • Single time point measurement
Antibody labelling by immunohistochemistry	Abundance	Does not require a special infrastructure	• Laborious in terms of genetic tagging or production of an antibody specific for the native protein • Fixation can distort readout • Single time point measurement • Cross-reactivity between different proteins and isoforms
Mass spectrometry	• Abundance • Post-translational modification (by purification or enrichment of the modification of interest) • Localization (by subcellular fractionation) • Protein–protein interaction (by affinity purification of complexes)	• Can be applied to any organism • Sensitive • Quantitative	• Requires a special infrastructure • Sensitive to sample preparation artefacts • Currently only functions at population-level resolution
Flow cytometry	• Abundance • Half-life (by cycloheximide chase or tFT)	• Quantitative • Single-cell resolution	• Requires a special infrastructure • Depends on genetic tagging for proteome-wide measurements • Low sensitivity • Single time point measurement
Microscopy	• Localization • Abundance • Half-life (by tFT or bleach–chase) • Protein–protein interaction (by split-YFP assay)	• Quantitative • Single-cell resolution	• Requires a special infrastructure • Depends on genetic tagging for proteome-wide measurements • Single time point measurement for proteome-wide measurements
Microfluidics	• Localization • Abundance	• Quantitative • Single-cell resolution • Enables dynamic measurements in a controllable medium	• Requires a special infrastructure • Depends on genetic tagging for proteome-wide measurements
Deep sequencing	Translation rate (by ribosome profiling)	• Readily applied to any organism • Parallel measurements in single experiment	• Requires a special infrastructure • Population-level resolution • Single time point measurement
Protein microarray	• Protein–protein interaction • Post-translational modification	Readily applied to any organism	• Requires a special infrastructure • Population-level resolution • Proteins lose subcellular compartmentalization
Automated growth rate analysis	• Genetic interaction • Protein–protein interaction (by yeast two-hybrid screen, split-DHFR assay or split-ubiquitin assay)	Does not require a special infrastructure	• Depends on genetic tagging • Population-level resolution

DHFR, dihydrofolate reductase; tFT, tandem fluorescent protein timer.

is difficult, a heroic venture is being undertaken by the Human Protein Atlas project[37]. This project has been set up to create affinity-purified antibodies specific for every protein in the human genome and currently covers more than 80% of all human protein-coding genes. These antibodies are then used to systematically obtain information on changes in the level of protein expression in various tissues and cancer cell types, as well as information on the subcellular localization of proteins[37,38]. An important use of this database is its integration with additional data sets, including transcriptomic data from of 27 tissues and 44 cell lines[39]. In addition, these antibodies can be used as capturing agents that, in combination with mass spectrometry detection (immuno-SILAC (stable isotope labelling with amino acids in cell culture)), enrich target peptides and substantially reduce sample complexity[40].

However, immunodetection has many disadvantages for use in systematic analyses. First, it requires either the generation of a genetically modified collection of proteins or the production of antibodies specific for each protein; both approaches are expensive and time consuming. Therefore, realistically, this method is probably not applicable to many model organisms. Second, the measurement and quantification of proteins are laborious, which makes it difficult, even for studies in yeast, to expand the immunodetection methodology to measure protein abundance at multiple time points or under many different conditions. Third, genetic modification may interfere with protein function, localization or stability, and a non-generic antibody can differ in affinity and specificity, leading to the lack of homogenous data. Fourth, detection of proteins in a whole-cell lysate means

that no live measurement can be carried out. Fifth, antibody measurements are not reliably reproducible and are not sensitive enough to detect minor differences in protein concentration or low-abundance proteins. Sixth, antibodies can cross-react not only with different proteins but most importantly with different isoforms of the same protein. Finally, whereas immunochemistry enables studies at single-cell resolution in principle, western blot analysis is, by definition, only suitable for population-level studies.

An alternative and powerful approach, which overcomes many of these disadvantages of immunodetection, is mass spectrometry, as it does not involve the creation of genetically tagged proteins or specific antibodies (TABLE 1) (reviewed in REFS 41–44). Improved methodologies using advances in computational proteomics, instrument performance and sample preparation enable the robust recognition and quantification of nearly all endogenous proteins with a single experiment. These approaches have already been demonstrated for several types of tissues, organisms and organelles following purification[44–53]. For example, the levels of all expressed proteins in haploid yeast cells were measured relative to their diploid counterparts by mass spectrometry[6]. In these extensive measurements, 4,399 proteins were identified and quantified. The measurements showed high levels of agreement with other methods (such as western blot analysis of the TAP epitope library[36] mentioned above and flow cytometry of the Yeast GFP Fusion Localization Database[9] mentioned below). The advancements in this field and the current simplicity of the entire workflow make mass spectrometry-based shotgun proteomics also applicable for studying proteome dynamics in response to stress, such as heat shock[54], in a time-dependent manner. This method can also be used to study evolutionary questions. For example, quantitative trait locus (QTL) analysis for protein measurements of more than 78 yeast strains revealed complex interactions between independent genetic loci, which suggests tight maintenance of stoichiometry for functionally related members of a pathway[47].

Due to the complexity of the human proteome, mass spectrometry approaches are still struggling to be as comprehensive as in more simple organisms. Near-complete coverage of the proteome can be obtained, as shown by recent studies in which common human cancer cell lines were used to identify more than 10,000 different proteins in a single experiment[49,52]. According to comparisons with transcriptome data, these measurements are close to being a complete count of the human proteome. However, the shift from cell lines to tissue samples is not trivial; so far, there are only a few in-depth proteomic studies with high coverage in tissue samples. Examples include the investigation of tissue-specific phosphorylation in mouse tissues[55], the characterization of the secretome of activated immune cells[45] and the identification of the proteins expressed in an entire human colon tumour[56]. Although they are very robust, mass spectrometry approaches have limitations in terms of both sample preparation requirements and the need for large amounts of clean starting material. Additional difficulties are in the detection of low-abundance, very short, very hydrophobic or very hydrophilic peptides. Current efforts are directed at overcoming technical barriers and reaching higher sensitivity and coverage, as well as at gaining meaningful functional interpretation (reviewed in REF. 41). These technological advances are now rapidly being developed and suggest that sensitive, accurate and complete proteome measurements by mass spectrometry will be possible for any biological tissue in the near future[41,57]. For example, mass spectrometry-based high-resolution isoelectric focusing (HiRIEF) enables whole-proteome coverage in plants[58], mice and humans[59]. Integration of these data with six-reading-frame translation (6FT) of the genome facilitated the definition of novel coding loci[59]. Drawbacks of HiRIEF are the necessity for costly instrumentation and expertise. Although the first attempts to achieve single-cell resolution are underway[60,61], it will probably take some time to attain a robust methodology.

Single-cell methodologies. In the past decade, it has become evident that populations of cells, even genetically identical ones, show high variability. This is exemplified by the resistance of bacteria to antibiotics[62], the response of unicellular organisms to fluctuating environments[26,63], tissue differentiation and the response of cancer cells to chemotherapy[63,64]. To understand how such variability occurs, novel approaches to track DNA, RNA and proteins at single-cell resolution[64] are essential.

One of the pioneering techniques to systematically measure protein abundance at a single-cell level was tracking fluorescence by flow cytometry (TABLE 1). A seminal study carried out in yeast obtained systematic measurements of protein abundance in single cells by investigating 2,000 yeast strains from the Yeast GFP collection[9] by flow cytometry. This collection encompasses more than 5,000 yeast strains, in each of which one yeast gene has been fused to a GFP epitope at its carboxyl terminus, thus retaining its natural chromosomal context and promoter. By measuring protein abundance under two growth conditions it was shown that yeast cells markedly modulate their proteome between growth in nutrient-rich medium and minimal medium. Importantly, many of these proteome changes could not be predicted by the changes in mRNA levels under these conditions[7]. One of the important aspects of such single-cell measurements was the possibility to extract information regarding the distribution of protein expression within the population — also known as 'noise'. Noise analyses from this study defined highly regulated 'quiet' proteins (which have low variability within the population), such as those involved in the translational machinery, versus noisy proteins (which have high variability within the population), such as stress response proteins[7] (BOX 1).

Two of the shortcomings of flow cytometry are the dependency on genetic tagging for proteome-wide measurements and the limited availability of distinguishable fluorophores, which enables the measurement of only 10–15 surface parameters in parallel. Mass cytometry overcomes the polychromatic constraints of

Shotgun proteomics
Techniques for the parallel identification of all proteins in a sample using high-performance liquid chromatography followed by mass spectrometry.

Box 1 | **Using single-cell proteomics to gain insight into cellular decision making**

New possibilities to study protein dynamics at single-cell resolution enable the acquisition of information on molecular diversity. Heterogeneity between cells can be due to the randomness of molecular processes (such as signalling, transcription and translation), but can also result from active 'bet-hedging' strategies whereby cells with identical genomes drive different proteomic landscapes to induce variable qualities.

Cellular diversity in decision making is prevalent throughout all life forms, including viruses, bacteria, yeast and lower metazoans to mammals[63,147,148]. In the budding yeast *Saccharomyces cerevisiae*, hundreds of proteins expressed from their native promoters have highly 'noisy' (REF. 7) or even bimodal[26] expression patterns. Interestingly, genes with increased noise levels were associated with stress responses, which indicates that variability in protein expression gives an advantage that is selected for under such conditions. The ability to distribute risks (both fitness for the long term and survival in the short term) in combination with cell-to-cell communication and environmental sensing are the underlying rules that govern pattern formation and development from microorganisms to mammals. Therefore, the current efforts in developing single-cell resolution methodologies to measure the various components of the cell will, in the future, help to answer a range of cell biological questions.

flow cytometry by tracking metal atoms; the detection is carried out by atomic mass spectrometry rather than by photon optics as used for flourophores[65]. There are up to 100 enriched stable isotopes of transition elements available that can be used as reporting tags and that can be measured simultaneously by the mass spectrometer at single-cell resolution. For example, 31 markers of the entire human haematopoietic system were measured to characterize the functional response to immune modulators and small-molecule drug inhibitors[66]. Hence, mass cytometry data are compatible with conventional flow cytometry analyses; however, the dimensionality is markedly increased and enables a multiparameter cellular assessment[66,67].

Due to lack of sensitivity, the least abundant two-thirds of the labelled proteins cannot be detected by existing flow cytometric approaches[7,68]. To complement these efforts, microscopic approaches (TABLE 1) were recently used to assess the abundance of a wider range of proteins in the GFP-tagged yeast library[9] under various stresses and with mutated genetic backgrounds[25–27,69]. Microscopy-based methodologies (BOX 2) can provide accurate quantification of more than 85% of the tagged yeast proteins[25,26]. For example, application of various stresses, such as DNA damage[25], reducing or oxidizing stress and starvation[26], resulted in changes to the abundance of hundreds of proteins with a poor correlation to the corresponding mRNA changes[26]. This technology is rapidly developing; however, it is still costly and laborious to implement (reviewed in REF. 70).

Systematic efforts (such as The Kahn Dynamic Proteomics Database) have also been carried out in human cell lines using a library of annotated reporter cell clones (LARC)[71]. In the LARC collection, each clone contains an endogenous protein fused to YFP, which is expressed from its natural promoter and in its normal chromosomal context. Although it is incomplete (currently 2,180 clones out of the entire human genome)[72], this library has revolutionized the scope of single-cell proteomics in humans. The main applications so far have been to study the dynamics in quantity and location of each tagged protein by means of automated time-lapse fluorescence microscopy during cell cycle stages[73] and in response to chemotherapy treatments and environmental stresses[74].

A similar YFP-tagged library of 1,018 strains has been constructed and measured in *Escherichia coli*[75,76]. The library was quantitatively imaged during growth in a microchemostat using a single-molecule fluorescence microscope[77] (BOX 2; TABLE 1). The flow chamber used was not only sensitive (enabling the detection of the low copy number of endogenous proteins)[77] but also provided rapid analysis of 16,000 cells per minute, which made it possible to quantify single-cell protein abundance. Interestingly, in this system, the mRNA of each tagged gene was detected by fluorescence *in situ* hybridization (FISH) using probes that recognized the YFP-encoding mRNA sequence, and hence parallel readouts of mRNA and protein levels were obtained. This led to the non-trivial observation that, in *E. coli*, at single-cell resolution there is no correlation between the mRNA and protein levels[77].

The recent development of high-throughput microchemostats (BOX 2) has emerged as a powerful tool for single-cell analysis. In contrast to the static measurements obtained by microscopy and flow cytometry, microchemostats enable time-lapse experiments and can assess ~1,000 strains in parallel, while maintaining a high spatiotemporal resolution[27,78]. Using this method, the abundance and localization of ~4,000 yeast proteins from the GFP library[9] were determined in response to the DNA damage inducer methyl methanesulphonate, and an additional five conditions were analysed for 576 of these proteins[27]. The superior acquisition speed of this setup relative to all other methods to date enables the measurement of stress responses at the very early stages, and in a dynamic and controllable environment.

To summarize, novel libraries of tagged molecules together with advanced acquisition tools enable protein abundances of entire proteomes to be measured in single live cells. Calculations that take into account the total number of protein copies per unit of cell volume and other known parameters, such as the protein mass relative to the composition of cells and the average protein length, estimated that there are 2–4 million proteins per cubic micrometre (that is, 10^{-15} l) in bacteria, yeast and mammalian cells[79]. However, these numbers are in apparent discrepancy to the values obtained using system-level proteomics and even more so relative to conventional biochemistry. For example, values that are

Fluorescence *in situ* hybridization
(FISH). A technique for detecting nucleic acids (DNA or mRNA) inside a single cell by use of a complementary oligonucleotide probe that is detected by fluorescence microscopy.

Box 2 | **Using microfluidics devices and microscopy platforms for proteome measurements**

Automated visualization systems are designed for the large-scale tracking of different strains in parallel. Current advances in image processing and extraction of data enable single-cell resolution measurements of a wide variety of physiological parameters in addition to fluorescence emission.

High-throughput microscopy

A large number of microscopes are now available with an automated platform to rapidly acquire images, both bright-field and fluorescent, in 96-well and 384-well formats by means of an automated stage and appropriate software. This approach can be optimized for genome-wide screens by tailoring them into an automated sample preparation platform. Using automated microscopy platforms, it is so far possible to obtain static measurements (single time point) of a large number of samples or to carry out time-lapse tracking for a smaller sample size[70].

Microchemostat

This approach enables the growth and tracking of strains in a large number of separated chambers. The major advantage of such a setup is that strains can be kept under constant conditions by generating a flux of medium. When such systems are integrated with microscopy they enable tracking of single cells in a tightly controlled environment and in a very short time frame. Another advantage over conventional microscopy setups is the ability of microchemostat-based approaches to carry out time-lapse tracking in a genome-wide manner[27].

reported for fission yeast and mammalian cells are often approximately threefold to tenfold lower[79]. These differences highlight the need to push the limits of these systems even further[79] and to carefully rescale values taking into account key physiological parameters.

As mentioned above, the measured protein level reflects the outcome of interconnected regulatory determinants, such as translation, post-translational modifications, localization, protein–protein interactions and degradation. In the following sections, we discuss systematic measurements of these processes.

Obtaining translation levels

Regulating protein translation is an important aspect of controlling protein abundance[80–82]. Ribosome profiling strategies have emerged as a powerful tool to map which mRNA transcripts are translated at any particular moment and at what efficiency, in a systematic manner[5] (TABLE 1) (reviewed in REF. 83). First demonstrated for yeast, ribosome profiling was used to infer translation levels under both nutrient-rich and starvation conditions[5], or during meiosis[84]. The great advantage of this method is that it is readily applied to any organism, including higher eukaryotes, and even to the very complex mammalian proteome[85–87]. For example, ribosome profiling of mouse embryonic stem cells[85] revealed that the translation levels of a large number of transcripts changed after differentiation. Moreover, the profiling uncovered new protein isoforms that arise from short, polycistronic ribosome-associated coding RNAs, amino-terminal extensions, truncations of known proteins and translation initiation at non-AUG codons[85]. Ribosome profiling of primary human cell lines during human cytomegalovirus infection revealed an unanticipated complexity of the coding capacity of the virus. For example, multiple distinct polypeptides can be generated from a single genomic locus by alternative start sites[86]. Recently, the widespread dynamic nature of translational regulation of hundreds of mRNAs has been discovered to underlie mammalian cell cycle progression[87], and the coordination of the translational control of mRNAs within molecular complexes dedicated to cell cycle progression, lipid metabolism, and genome integrity and organization have also been demonstrated[87]. Generally, the parameters obtained by these approaches can be used in holistic calculations aiming to understand how protein levels are regulated in the presence of a given mRNA and tRNA pool, as well as to calculate codon usage considerations[88].

Ribosome profiling
A strategy for sequencing ribosome-protected mRNA fragments to provide a genome-wide map of translated proteins.

Measuring post-translational effects

The final translated protein product is subject to additional regulatory processes, such as modifications, determination of subcellular localization, acquisition of physical interactions and degradation. Such alterations are major determinants in controlling the abundance and function of each protein.

Computing turnover rates. Protein degradation is an important determinant of absolute protein abundance (FIG. 1). The half-lives of individual proteins can range from minutes to years[89]; hence, knowing the degradation rate of a protein is very important. To systematically measure protein half-lives in yeast, the TAP epitope-tagged collection of proteins[36] was quantified by western blot as a function of time following the inhibition of protein synthesis[90]. This seminal study from 2006 is still of relevance today, despite the introduction of newer techniques such as quantitative non-canonical amino acid tagging (QuaNCAT; see below). Incorporation of half-life measurements with the assessment of mRNA abundance and translation rates showed that transcription, translation and protein degradation are tightly coordinated to achieve uniform effects on protein abundance[90]. Interestingly, clustering of 3,751 yeast proteins, according to production rates, protein abundance and degradation rates, revealed two types of protein metabolism: the first is optimized for efficient protein production, which is characterized by high production rates and abundance, and low degradation rates; the second is optimized for regulatory functions, which is characterized by low production rates and abundance, and high degradation rates[90]. Unfortunately, this approach to measure protein half-life is neither easily transferrable to other organisms nor easily applicable to study protein dynamics during stress responses for the same reasons

as mentioned above: the method is laborious, dependent on collections of tagged strains and only provides a population-level analysis.

Protein half-life measurements have also been carried out in human cell lines using bleach–chase experiments on the LARC collection[71]. Specifically, 100 clones were bleached and tracked by time-lapse fluorescence microscopy using automated image analysis at a high temporal resolution of every 20 minutes for 24 hours to obtain protein decay rates under normal conditions[91] and under stress[92].

Recently developed tandem fluorescent protein timers (tFTs) are optimal for the measurement of protein half-life. The tFTs are built from a fusion of two single-colour proteins, which have non-overlapping emission spectra, that mature with distinct kinetics[93,94]. When a tFT is fused to a native protein, the ratio of fluorescence intensities from the two fluorescent protein domains indicates the age of a protein. The fusion of tFTs to proteins of interest has enabled their longevity, segregation and inheritance to be studied, as well as their mobility between subcellular compartments over time in living yeast cells[93]. For example, these experiments revealed the stable nature and asymmetric inheritance of nuclear pore complexes[93]. Although they have the advantage of providing a high-resolution analysis, both bleach–chase experiments and tFTs have the disadvantage of requiring extensive cloning to construct artificial collections of tagged proteins and therefore cannot be easily applied to future proteome-wide studies.

An alternative strategy for measuring protein turnover rates is mass spectrometry-based detection[95–97]. Developments such as bioorthogonal non-canonical amino acid tagging (BONCAT)[98], in which metabolic labels are introduced to cells in a pulse, distinguish new proteins from old proteins. When BONCAT is coupled with pulsed SILAC (pSILAC)[99], it is known as QuaNCAT[100] and enables rapid large-scale proteomic quantification of translation and degradation by both enriching and quantifying newly synthesized proteins. Using QuaNCAT, chemoattractant-induced cytoskeleton dynamics in *Dictyostelium discoideum* cells were shown in a time resolution of seconds to minutes[101]. Application of such an approach in *Streptomyces coelicolor* cultures enabled the estimation of protein turnover rates for 115 highly abundant proteins during the transition from exponential growth to stationary phase[102]. In human cells, QuaNCAT was used to monitor the early expression changes of more than 600 proteins in primary resting T cells exposed to activation stimuli[103].

Charting post-translational modifications. Post-translational modifications of proteins, such as sumoylation, palmitoylation, ubiquitylation and phosphorylation, are highly prevalent and versatile, and can markedly affect protein stability, function, localization and physical interactions. In most cases, consensus sequences for predicting modifications are not simple to characterize, and the physiological implications of these modifications, their effect on protein fate and their contribution to cell homeostasis are yet to be understood[104]. Several strategies have been developed to systematically uncover the broad array of modifications on all proteins. The major approach is to identify peptides modified by a post-translational modification using mass spectrometry. Peptides carrying the studied modification can be enriched after labelling and purification (through a column or pull down with a specific antibody) (reviewed in REF. 11). One such example is the global analysis of protein palmitoylation (a lipid modification) in yeast[105,106]. This approach relies on an acyl–biotinyl exchange, in which biotin moieties were substituted for the acyl modification and used for subsequent purification of modified proteins and their identification by mass spectrometry[106] (TABLE 1). Using such methods enables not only the discovery of new protein substrates carrying a particular post-translational modification but also the study of the modification machinery by quantifying which substrates disappear when specific enzymes (in this case, palmitoyltransferases) are deleted[105].

Similar approaches can be used to study the phosphorylation of all proteins (phosphoproteome) in parallel in many different organisms. For example, the phosphorylation events underlying the transition to a filamentous growth form in yeast, and the kinases mediating these events, have been measured[107]. By taking the phosphorylation events and integrating them with other data, it was possible to identify and validate new proteins that affect invasive growth[107]. In another example, the phosphoproteome was characterized during yeast osmotic shock, and more than 5,000 unique phosphopeptide variants were identified, of which 15% changed more than twofold following 5 minutes of osmotic shock[8]. In *Bacillus subtilis*, phosphoproteomics uncovered Arg phosphorylation as a novel modification that is implicated in the general stress response[108]. Also, the measurement of the phosphoproteome of nine mouse tissues showed that a typical phosphoprotein is widely expressed but that it has variable, often tissue-specific, phosphorylation states that tune protein activity to the specific needs of each tissue[55]. For example, the expression of phosphorylated Tau, the hallmark of many neurodegenerative diseases, was detected in nearly all tissues, which indicates that it might have a more general role as a regulator of cytoskeletal dynamics than previously appreciated. However, the discovery of extensive brain-specific phosphorylation sites in Tau provides a potential explanation for its unique role in these cells[55]. Such approaches can also be used to identify targets of a regulatory complex directly, such as the identification of substrates of the mTOR complex in mouse embryonic fibroblasts[109]. Proteomic and phosphoproteomic profiling of human embryonic stem cells during differentiation[110] and maintenance[111], as well as their comparison to induced pluripotent stem cells, revealed differences that had a functional relevance and that highlighted signalling networks[12,112]. To enable these types of experiment to be carried out with ease, protocols for the preparation of samples are being improved, advanced acquisition by mass spectrometry is developed and comprehensive analysis programmes for the identification of protein phosphorylation sites are becoming prevalent[11].

Box 3 | **Future perspectives in proteomic studies**

Accessibility
It is essential to adapt existing proteomic technologies to be cost- and time-effective, as well as simple to use, such that every laboratory can measure proteome dynamics. This includes the creation of user-friendly and cost-effective equipment, and the convergence of high quality freeware analysis platforms. This will ensure that proteomics becomes as straightforward as microarray and deep-sequencing technologies are now.

Resolution
Studies at the population level have an averaging effect that often eliminates interesting biological information. Although some readouts can already be carried out at single-cell resolution, new technologies will have to be created to advance this ability.

Accuracy
A fundamental question concerning proteome-wide resolution is how to ensure complete coverage including protein species. Currently, mass spectrometry-based technologies are the only platform that can be considered comprehensive. However, in contrast to simple model organisms such as yeast, for which complete proteome coverage can now routinely be obtained, several developments are required to reach the same resolution for complex proteomes such as the human proteome[41]. To this end, different bottlenecks in the scheme are being addressed, such as robustness of sample preparation to cover low-abundance proteins or membrane proteins[149], creation of enriching protocols for unstudied modifications, optimization of peptide separation and analysis in the mass spectrometer, and assembly of analysis pipelines to interpret the biological relevance of the results. Broadening the detection limits and increasing the sensitivity will soon enable the streamlined, straightforward and complete analysis of mammalian proteomes.

Comparison
Although vast amounts of data have been collected for both proteomes and transcriptomes, we still lack an understanding regarding their coordination[31]. Single-cell measurements of *Escherichia coli* proteins and their corresponding transcripts demonstrate little correlation between copy numbers[77]. Hence, the next frontier is to track proteins and mRNAs in parallel in other organisms, thereby reducing the discrepancies that arise from independent measurements and enabling the discovery of the underlying principles of co-regulation.

Integration
The growing availability of systematic cellular data alongside computational work is enabling a new era of biological modelling[124,142–145,150]. Analysis pipelines will have to be developed to enable the integration of a wide variety of proteome-level data. The major challenge in this direction is the definition of protein variants for each protein-coding gene. An ultimate goal would be to incorporate additional profiling, such as glycomics, lipidomics, metabolomics and enzymology, to gain a true understanding of the crosstalk between the cellular layers of information.

An additional post-translational modification that can be analysed by mass spectrometry in a similar manner is ubiquitylation[113]. This modification is highly dynamic in response to a halt in protein translation or degradation[113]. An important drawback of this approach is that it is still challenging to distinguish ubiquitylation sites from sumoylation sites, as both leave diglycine isopeptides that can be recognized by an enriching antibody after tryptic digestion[11]. The mass spectrometry approach is applicable to any type of modification that can be enriched, such as methylation, acetylation, poly(ADP)ribosylation or glycosylation, and hence offers exciting possibilities for future discoveries. As more than 200 types of *in vivo* modifications are known, the major challenge will be to tailor the enrichment steps[11].

A novel technology that is now emerging for the analysis of protein modifications is the protein microarray. As a readout, modified products are detected by specific antibodies or other colorimetric assays[104,114] (TABLE 1). Using this methodology, mammalian cell extracts were analysed and this revealed 1,500 potential substrates of various ubiquitin-like modifications. A major disadvantage of this method is that it exposes proteins to the artificial condition of cell lysis, in which proteins and ions lose their cellular compartmentalization. However, it has the major advantage that it can be applied to additional post-translational modifications and enables analysis of the dynamics between different cellular phases or various tissues.

Protein microarray
A method to study protein modifications or physical interactions. Proteins are immobilized on glass slides and exposed to probe molecules (usually fluorescently labelled). A reaction between the probe and the arrayed protein emits a signal that is detected by a laser scanner.

Mapping changes in localization. Regulation of protein localization is often used to control processes quickly and independently of *de novo* protein synthesis[25,29,72,73,91]. For example, the 26S proteasome accumulates in proteasome storage granules upon glucose depletion[115] to tightly regulate its function and stability. However, cellular control of protein activity by modulating its compartmentalization is not easily measured on a large scale. To explore the localization dynamics of proteins on a genome-wide scale, three strategies have been used.

The first strategy involves microscopic imaging of fluorescently tagged protein collections[9,71]. For example, imaging of the GFP-tagged yeast collection enabled the systematic identification of the localization of 75% of the proteome and the classification of proteins into 22 distinct localization categories under normal growth conditions[9]. It has been shown that hundreds of changes in protein localization occur when this library is exposed to environmental stress conditions[25–27], which implies that protein relocalization under stress is a far more widely used strategy than previously appreciated. Similarly, the human LARC collection[71] was used to discover the dependence of nuclear protein localization on the cell cycle[71,73]. These methods are accurate and rapid, but they rely on the creation of fluorescently tagged protein collections.

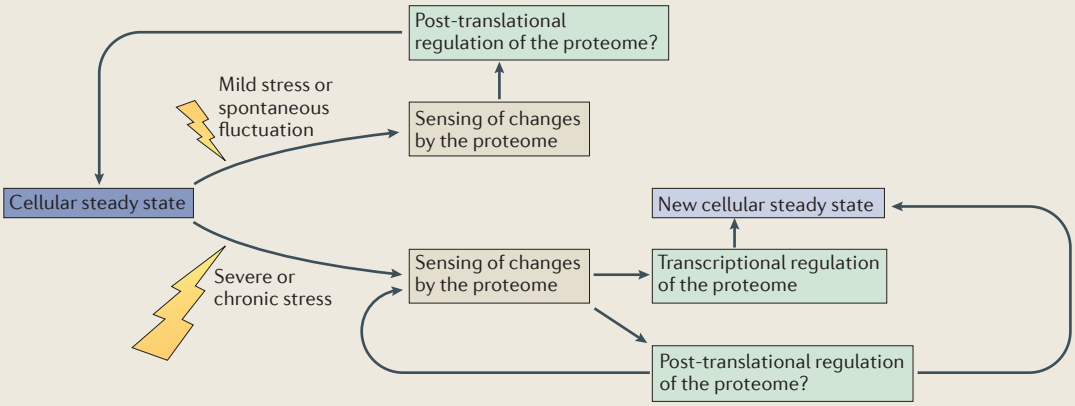
The second strategy to determine protein localization in cell culture is immunohistochemistry. The Human Protein Atlas[37] project is using this approach to identify the subcellular localization of protein-coding genes in three different cell lines in a systematic manner. The entire human proteome should be examined within the next two years. However, to analyse changes in protein localization following stress is an enormous task even for a single cell line.

The third strategy is based on the biochemical isolation of organelles followed by mass spectrometry and has been used to construct a catalogue of the protein content of subcellular structures[116,117]. Mass spectrometry-based proteomics can be used to study the dynamics of the protein content in organelles and therefore is complementary to fluorescence-based microscopy but without the need to tag genes. For example, subcellular fractionation of SILAC-labelled HeLa cells into compartments enabled an unbiased analysis of how proteins move from one cellular compartment to another in response to perturbations[116]. However, fractionation-based approaches are not yet sensitive enough to detect proteins from single cells. Moreover, as many fractionation protocols

Box 4 | **Uncovering the role of the proteome in buffering fluctuations**

Current proteomic studies, although far from having covered the entire biological landscape, already provide huge amounts of information across many organisms and conditions. An important conclusion of these data is the underestimated dynamic capacity of proteins and the role of this capacity in every aspect of cellular physiology. We now begin to uncover how cells regulate protein levels in a post-translational manner to respond robustly to ever-changing environmental conditions. Therefore, information about protein variants, modifications, levels and localization is crucial to our understanding of cellular response pathways. As proteins are the sentinels that connect a cell to the external world, sense the environment and integrate all inputs into a molecular decision, it is not surprising that the preliminary response to a change in inputs involves regulation at the protein level. Moreover, it might be the case that correct regulation of proteins may be sufficient, under some circumstances, to prevent the cell from initiating a transcriptional response, such that the post-translational response functions as a molecular buffer (see the figure).

For the proteome to function as a buffer for transcriptional activation it should have a very low threshold for detecting perturbations, while keeping a very high threshold for initiating a transcriptional response. Proteomic-level regulation must therefore be rapid and finely tuned. Hence, uncovering the scope of post-translational regulation necessitates studying conditions of mild perturbations that do not induce a transcriptional response. Biological experiments often use radical stress stimuli to enable a robust transcriptional response as the readout. Therefore, to uncover the elegance of cellular behaviour and the extent of protein buffering, small perturbations should be carried out and highly accurate methods of proteomic measurement should be used to collect information on the changes to the proteome that occur early after stimulation. This distinction between changes in the transcriptome and proteome in response to stimuli should be taken into account when modelling cellular responses.



Protein-fragment complementation assays
Assays for measuring protein–protein interactions based on the premise that when two proteins interact they also bring into proximity any protein fragments attached to them, thus enabling the fragments to complement each other and to fold into an active reporter protein.

Two-hybrid screens
Types of protein-fragment complementation assay in which each half of a split Gal4 transcription factor (the DNA-binding domain or the activation domain) is fused to one of two proteins of interest (bait and prey). Physical proximity of the two proteins enables the reconstitution of the Gal4 transcription factor, thus leading to transcriptional activation.

Split-ubiquitin assays
Types of protein-fragment complementation assay in which each half of a ubiquitin enzyme is fused to one of two proteins of interest (bait and prey). One half of the ubiquitin is also fused to a transcription factor. Physical proximity enables the reconstitution of the split protein into a ubiquitin moiety that is recognized by endogenous ubiquitin-directed proteases, which cleave between the ubiquitin and the transcription factor. The cleaved transcription factor can relocate to the nucleus and activate a reporter gene.

Split-DHFR assays
Types of protein-fragment complementation assay in which each half of a split dihydrofolate reductase (DHFR) enzyme is fused to one of two proteins of interest (bait and prey). Physical proximity enables the reconstitution of the split protein into a functional DHFR enzyme, which makes it possible for cells to grow in the presence of an inhibitor of the endogenous and essential DHFR enzyme (such as methotrexate).

only enrich for certain organelles rather than fully purifying them, care should be taken in the interpretation of such data.

Detecting physical interactions. Proteins function as a dynamic network, therefore the elucidation of protein–protein interactions provides insight into fundamental aspects of function, organization and signalling in cells. System-level interactome analysis has been extensively carried out using various protein-fragment complementation assays, such as two-hybrid screens[118], split-ubiquitin assays[119] or split-DHFR assays[22], as well as TAP followed by mass spectrometry analyses[120] and protein microarrays[121] (TABLE 1), combined with bioinformatics-based approaches[122,123]. These strategies have been applied to a wide variety of organisms, such as *E. coli*[124], the yeast *Saccharomyces cerevisiae*[22], plants (*Arabidopsis thaliana*, *Oryza sativa* and *Brachypodium distachyon*)[125], *Caenorhabditis elegans*[126], *Drosophila melanogaster*[127] and humans[128–130]. However, most approaches for mapping protein–protein interactions require cloned protein-coding sequences, thus making it laborious to create complete collections in additional organisms. Importantly, current methodologies for the investigation of protein–protein interactions all require averaging of entire populations. However, some approaches are now being developed, such as split-YFP approaches[131,132], that should enable the analysis at single-cell resolution (reviewed in REFS 133,134).

The identification of protein–protein interactions has been used for various analyses. First, it enables a protein with a completely unknown function to be placed into a functional context that is given by its binding partners with a known function[135]. In addition, by mapping the changing interactions that occur in different cellular environments, insights into functional complexity can be obtained. Understanding protein–protein interactions can help to elucidate the pathophysiology and development of many diseases[136–139], to identify novel drug targets and to understand the mechanisms of action of new therapeutic compounds[135]. For example, building the most complete interactome of proteins associated with Alzheimer's disease has revealed 200 high-confidence protein–protein interactions between eight confirmed Alzheimer's disease-related genes and 66 candidate disease-related genes. Of these candidate disease-related genes, 31 are located in chromosomal regions containing susceptibility loci related to the aetiology of late-onset Alzheimer's disease, and 17 of them have dysregulated expression patterns in patients with Alzheimer's disease.

Split-YFP approaches
Types of protein-fragment complementation assay in which each half of a split fluorescent protein (YFP, GFP or cyan fluorescent protein) is fused to one of two proteins of interest (bait and prey). Physical proximity enables the reconstitution of the split protein into a functionally fluorescent product.

Moreover, the detection of four novel direct interactions between well-characterized Alzheimer's disease-related genes (*APP* (amyloid-β (A4) precursor protein), *A2M* (α2 macroglobulin), *APOE* (apolipoprotein E), *PSEN1* (presenilin 1) and *PSEN2*) strongly validates the capacity of protein–protein interaction studies to support the formulation of molecular mechanism hypotheses and to elucidate their malfunction in disease progression[140].

Conclusions

In this Review, we have provided a summary of current technologies and their capacity to answer various biological questions. In the past, measurements of mRNA abundance were assumed to follow the basic dogma of one gene leading to one protein. However, proteomic studies showing post-translational regulation of proteins, protein modifications, protein isoforms (alternative splicing) and variability within populations indicate that this paradigm is far from accurate[141]. Thus, the next big frontier is describing this diversity by integrating data for the various parameters that can be measured, although this is computationally challenging (BOX 3). Recent studies use proteomic profiling to improve genomic annotation based on the incorporation of RNA sequencing data sets (proteogenomics)[142], to integrate multidimensional 'omics' data to characterize biological networks[143,144] and to develop personalized medicine approaches[145,146]. The determination of all expressed proteins and their changes in expression during a process of interest could revolutionize classic medical research in the entire cellular system. For example, integrative personal omics profile (iPOP), an analysis that combines genomic, transcriptomic, proteomic, metabolomic and autoantibody profiles from a single individual over a 14-month period, revealed various medical risks and uncovered extensive molecular changes across healthy and diseased conditions[146]. Therefore, this huge leap in system-level profiles and the wealth of data stemming from it can now potentially function as a valuable resource to develop personalized medical approaches. Obtaining complete dynamic profiles for diseased and healthy individuals over time will be extremely valuable in the early diagnostics, monitoring and treatment of disease states[146].

Current achievements in studying system-level proteomics provide a wide view of protein characteristics under changing conditions. The variability in protein function mediated by the post-translational events described here requires that we now shift the focus of studies in cellular responses from simple measurements and comparisons, to dissecting combinatorial and modulatory effects that fine-tune the cellular response (BOX 1). This has been extensively done in some fields in which cellular decisions are known to be modulated by post-translational effects, such as apoptosis[147], and should now be expanded to other fields. Directly studying cellular dynamics at the protein level opens up a new perspective of cell biology that could not be systematically studied before, but there is still much to be achieved (BOX 3). Recent years have brought prosperity in our proteomic scope and in our understanding of the role of proteins in maintaining homeostasis (BOX 4). Delving deeper into the behaviour of this first line of defence, mediated by the 'workers' of the cell, will paint an increasingly vivid and interesting picture of cell biology in the coming years.

1. The ENCODE Project Consortium. Identification and analysis of functional elements in 1% of the human genome by the ENCODE pilot project. *Nature* **447**, 799–816 (2007).
2. Valouev, A. *et al.* Determinants of nucleosome organization in primary human cells. *Nature* **474**, 516–520 (2011).
3. Gasch, A. P. *et al.* Genomic expression programs in the response of yeast cells to environmental changes. *Mol. Biol. Cell* **11**, 4241–4257 (2000).
4. Hughes, T. R. *et al.* Functional discovery via a compendium of expression profiles. *Cell* **102**, 109–126 (2000).
5. Ingolia, N. T., Ghaemmaghami, S., Newman, J. R. & Weissman, J. S. Genome-wide analysis *in vivo* of translation with nucleotide resolution using ribosome profiling. *Science* **324**, 218–223 (2009).
Provides the first example of ribosome profiling as a valid tool for mapping translation in yeast and leads the way for assaying additional organisms.
6. De Godoy, L. M. *et al.* Comprehensive mass-spectrometry-based proteome quantification of haploid versus diploid yeast. *Nature* **455**, 1251–1254 (2008).
7. Newman, J. R. *et al.* Single-cell proteomic analysis of *S. cerevisiae* reveals the architecture of biological noise. *Nature* **441**, 840–846 (2006).
8. Soufi, B. *et al.* Global analysis of the yeast osmotic stress response by quantitative proteomics. *Mol. Biosyst.* **5**, 1337–1346 (2009).
9. Huh, W. K. *et al.* Global analysis of protein localization in budding yeast. *Nature* **425**, 686–691 (2003).
10. Zielinska, D. F., Gnad, F., Wisniewski, J. R. & Mann, M. Precision mapping of an *in vivo* N-glycoproteome reveals rigid topological and sequence constraints. *Cell* **141**, 897–907 (2010).
11. Olsen, J. V. & Mann, M. Status of large-scale analysis of post-translational modifications by mass spectrometry. *Mol. Cell. Proteom.* **12**, 3444–3452 (2013).
12. Phanstiel, D. H. *et al.* Proteomic and phosphoproteomic comparison of human ES and iPS cells. *Nature Methods* **8**, 821–827 (2011).
13. Newman, R. H. *et al.* Construction of human activity-based phosphorylation networks. *Mol. Syst. Biol.* **9**, 655 (2013).
14. Wang, C., Wang, M., Zhou, Y., Dupree, J. L. & Han, X. Alterations in mouse brain lipidome after disruption of CST gene: a lipidomics study. *Mol. Neurobiol.* http://dx.doi.org/10.1007/s12035-013-8626-0 (2014).
15. Cimino, J. *et al.* Towards lipidomics of low-abundant species for exploring tumor heterogeneity guided by high-resolution mass spectrometry imaging. *Int. J. Mol. Sci.* **14**, 24560–24580 (2013).
16. Zhang, Q. & Wakelam, M. J. O. Lipidomics in the analysis of malignancy. *Adv. Biol. Regul.* **54**, 93–98 (2013).
17. Cooper, S. J. *et al.* High-throughput profiling of amino acids in strains of the *Saccharomyces cerevisiae* deletion collection. *Genome Res.* **20**, 1288–1296 (2010).
18. Zenobi, R. Single-cell metabolomics: analytical and biological perspectives. *Science* **342**, 1243259 (2013).
19. Rubakhin, S. S., Romanova, E. V., Nemes, P. & Sweedler, J. V. Profiling metabolites and peptides in single cells. *Nature Methods* **8**, S20–29 (2011).
20. Schuldiner, M. *et al.* Exploration of the function and organization of the yeast early secretory pathway through an epistatic miniarray profile. *Cell* **123**, 507–519 (2005).
21. Hillenmeyer, M. E. *et al.* The chemical genomic portrait of yeast: uncovering a phenotype for all genes. *Science* **320**, 362–365 (2008).
22. Tarassov, K. *et al.* An *in vivo* map of the yeast protein interactome. *Science* **320**, 1465–1470 (2008).
23. Vogel, C. & Marcotte, E. M. Insights into the regulation of protein abundance from proteomic and transcriptomic analyses. *Nature Rev. Genet.* **13**, 227–232 (2012).
24. Causton, H. C. *et al.* Remodeling of yeast genome expression in response to environmental changes. *Mol. Biol. Cell.* **12**, 323–337 (2001).
25. Tkach, J. M. *et al.* Dissecting DNA damage response pathways by analysing protein localization and abundance changes during DNA replication stress. *Nature Cell Biol.* **14**, 966–976 (2012).
26. Breker, M., Gymrek, M. & Schuldiner, M. A novel single-cell screening platform reveals proteome plasticity during yeast stress responses. *J. Cell Biol.* **200**, 839–850 (2013).
27. Dénervaud, N. *et al.* A chemostat array enables the spatio-temporal analysis of the yeast proteome. *Proc. Natl Acad. Sci. USA* **110**, 15842–15847 (2013).
Uses, together with references 25 and 26, high-throughput microscopy and microchemostats for proteome-wide quantitative measurements at single-cell resolution for both protein abundance and subcellular localization. Provide the first system-level evidence for localization changes under stress.
28. Jung, S. *et al.* Global analysis of condition-specific subcellular protein distribution and abundance. *Mol. Cell. Proteom.* **12**, 1421–1435 (2013).
29. Lee, M. V. *et al.* A dynamic model of proteome changes reveals new roles for transcript alteration in yeast. *Mol. Syst. Biol.* **7**, 514 (2011).
30. Washburn, M. P. *et al.* Protein pathway and complex clustering of correlated mRNA and protein expression analyses in *Saccharomyces cerevisiae. Proc. Natl Acad. Sci. USA* **100**, 3107–3112 (2003).

31. Khan, Z. *et al.* Primate transcript and protein expression levels evolve under compensatory selection pressures. *Science* **342**, 1100–1104 (2013).
32. Schrimpf, S. P. *et al.* Comparative functional analysis of the *Caenorhabditis elegans* and *Drosophila melanogaster* proteomes. *PLoS Biol.* **7**, e48 (2009).
33. Laurent, J. M. *et al.* Protein abundances are more conserved than mRNA abundances across diverse taxa. *Proteomics* **10**, 4209–4212 (2010).
34. Vogel, C. Evolution. Protein expression under pressure. *Science* **342**, 1052–1053 (2013).
35. Walther, T. C., Olsen, J. V. & Mann, M. Yeast expression proteomics by high-resolution mass spectrometry. *Methods Enzym.* **470**, 259–280 (2010).
36. Ghaemmaghami, S. *et al.* Global analysis of protein expression in yeast. *Nature* **425**, 737–741 (2003).
37. Uhlen, M. *et al.* Towards a knowledge-based Human Protein Atlas. *Nature Biotech.* **28**, 1248–1250 (2010).
Enables a systematic exploration of the human proteome using antibody-based proteomics, by combining high-throughput generation of affinity-purified antibodies with protein profiling in a multitude of tissues and cells assembled in tissue microarrays.
38. Navani, S. The human protein atlas. *J. Obstet. Gynecol. India.* **61**, 27–31 (2011).
39. Stadler, C. *et al.* RNA- and antibody-based profiling of the human proteome with focus on chromosome 19. *J. Proteome Res.* **13**, 2019–2027 (2014).
40. Edfors, F. *et al.* Immuno-proteomics using polyclonal antibodies and stable isotope labeled affinity-purified recombinant proteins. *Mol. Cell. Proteom.* http://dx.doi.org/10.1074/mcp. M113.034140 (2014).
41. Mann, M., Kulak, N. a, Nagaraj, N. & Cox, J. The coming age of complete, accurate, and ubiquitous proteomes. *Mol. Cell.* **49**, 583–590 (2013).
42. Altelaar, A. F. M., Munoz, J. & Heck, A. J. R. Next-generation proteomics: towards an integrative view of proteome dynamics. *Nature Rev. Genet.* **14**, 35–48 (2013).
43. Bensimon, A., Heck, A. J. R. & Aebersold, R. Mass spectrometry-based proteomics and network biology. *Annu. Rev. Biochem.* **81**, 379–405 (2012).
44. Walzthoeni, T., Leitner, A., Stengel, F. & Aebersold, R. Mass spectrometry supported determination of protein complex structure. *Curr. Opin. Struct. Biol.* **23**, 252–260 (2013).
45. Meissner, F., Scheltema, R. a, Mollenkopf, H.-J. & Mann, M. Direct proteomic quantification of the secretome of activated immune cells. *Science* **340**, 475–478 (2013).
46. Sabidó, E., Selevsek, N. & Aebersold, R. Mass spectrometry-based proteomics for systems biology. *Curr. Opin. Biotechnol.* **23**, 591–597 (2012).
47. Picotti, P. *et al.* A complete mass-spectrometric map of the yeast proteome applied to quantitative trait analysis. *Nature* **494**, 266–270 (2013).
48. Maier, T. *et al.* Quantification of mRNA and protein and integration with protein turnover in a bacterium. *Mol. Syst. Biol.* **7**, 511 (2011).
49. Beck, M. *et al.* The quantitative proteome of a human cell line. *Mol. Syst. Biol.* **7**, 549 (2011).
50. Geiger, T. *et al.* Initial quantitative proteomic map of 28 mouse tissues using the SILAC mouse. *Mol. Cell. Proteom.* **12**, 1709–1722 (2013).
51. Low, T. Y. *et al.* Quantitative and qualitative proteome characteristics extracted from in-depth integrated genomics and proteomics analysis. *Cell Rep.* **5**, 1469–1478 (2013).
52. Nagaraj, N. *et al.* Deep proteome and transcriptome mapping of a human cancer cell line. *Mol. Syst. Biol.* **7**, 548 (2011).
53. Schmidt, A. *et al.* Absolute quantification of microbial proteomes at different states by directed mass spectrometry. *Mol. Syst. Biol.* **7**, 510 (2011).
54. Nagaraj, N. *et al.* System-wide perturbation analysis with nearly complete coverage of the yeast proteome by single-shot ultra HPLC runs on a bench top orbitrap. *Mol. Cell. Proteomics* **11**, M111.013722 (2012).
55. Huttlin, E. L. *et al.* A tissue-specific atlas of mouse protein phosphorylation and expression. *Cell* **143**, 1174–1189 (2010).
56. Wiśniewski, J. R. *et al.* Extensive quantitative remodeling of the proteome between normal colon tissue and adenocarcinoma. *Mol. Syst. Biol.* **8**, 611 (2012).
57. Hebert, A. S. *et al.* The one hour yeast proteome. *Mol. Cell. Proteom.* **13**, 339–347 (2013).
58. Baerenfaller, K. *et al.* Genome-scale proteomics reveals *Arabidopsis thaliana* gene models and proteome dynamics. *Science* **320**, 938–941 (2008).
59. Branca, R. M. M. *et al.* HiRIEF LC-MS enables deep proteome coverage and unbiased proteogenomics. *Nature Methods* **11**, 59–62 (2014).
60. Passarelli, M. K. & Ewing, A. G. Single-cell imaging mass spectrometry. *Curr. Opin. Chem. Biol.* **17**, 854–859 (2013).
61. Ibáñez, A. J. *et al.* Mass spectrometry-based metabolomics of single yeast cells. *Proc. Natl Acad. Sci. USA* **110**, 8790–8794 (2013).
62. Gefen, O. & Balaban, N. Q. The importance of being persistent: heterogeneity of bacterial populations under antibiotic stress. *FEMS Microbiol. Rev.* **33**, 704–717 (2009).
63. Balázsi, G., van Oudenaarden, A. & Collins, J. J. Cellular decision making and biological noise: from microbes to mammals. *Cell* **144**, 910–925 (2011).
64. Rubakhin, S. S., Lanni, E. J. & Sweedler, J. V. Progress toward single cell metabolomics. *Curr. Opin. Biotechnol.* **24**, 95–104 (2013).
65. Tanner, S. D., Baranov, V. I., Ornatsky, O. I., Bandura, D. R. & George, T. C. An introduction to mass cytometry: fundamentals and applications. *Cancer Immunol. Immunother.* **62**, 955–965 (2013).
66. Bendall, S. C. *et al.* Single-cell mass cytometry of differential immune and drug responses across a human hematopoietic continuum. *Science* **332**, 687–696 (2011).
67. Bodenmiller, B. *et al.* Multiplexed mass cytometry profiling of cellular states perturbed by small-molecule regulators. *Nature Biotech.* **30**, 858–867 (2012).
68. Bar-Even, A. *et al.* Noise in protein expression scales with natural protein abundance. *Nature Genet.* **38**, 636–643 (2006).
Characterizes, together with reference 7, the noise profile of 43 yeast proteins during 11 environmental conditions and shows the first evidence for stress-related noisy proteins versus house-keeping proteins with low noise.
69. Handfield, L.-F., Chong, Y. T., Simmons, J., Andrews, B. J. & Moses, A. M. Unsupervised clustering of subcellular protein expression patterns in high-throughput microscopy images reveals protein complexes and functional relationships between proteins. *PLoS Comput. Biol.* **9**, e1003085 (2013).
70. Rimon, N. & Schuldiner, M. Getting the whole picture: combining throughput with content in microscopy. *J. Cell Sci.* **124**, 3743–3751 (2011).
71. Sigal, A. *et al.* Generation of a fluorescently labeled endogenous protein library in living human cells. *Nature Protoc.* **2**, 1515–1527 (2007).
72. Frenkel-Morgenstern, M. *et al.* Dynamic Proteomics: a database for dynamics and localizations of endogenous fluorescently-tagged proteins in living human cells. *Nucleic Acids Res.* **38**, D508–D512 (2010).
73. Sigal, A. *et al.* Dynamic proteomics in individual human cells uncovers widespread cell-cycle dependence of nuclear proteins. *Nature Methods* **3**, 525–531 (2006).
74. Geva-Zatorsky, N. *et al.* Protein dynamics in drug combinations: a linear superposition of individual-drug responses. *Cell* **140**, 643–651 (2010).
75. Hu, P. *et al.* Global functional atlas of *Escherichia coli* encompassing previously uncharacterized proteins. *PLoS Biol.* **7**, e96 (2009).
76. Butland, G. *et al.* Interaction network containing conserved and essential protein complexes in *Escherichia coli*. *Nature* **433**, 531–537 (2005).
77. Taniguchi, Y. *et al.* Quantifying *E. coli* proteome and transcriptome with single-molecule sensitivity in single cells. *Science* **329**, 533–538 (2010).
Profiles protein and mRNA expression levels at single-molecule sensitivity and at single-cell resolution. Also demonstrates the surprising lack of correlation between protein and mRNA levels in the same cell.
78. Maerkl, S. J. Next generation microfluidic platforms for high-throughput protein biochemistry. *Curr. Opin. Biotechnol.* **22**, 59–65 (2011).
79. Milo, R. What is the total number of protein molecules per cell volume? A call to rethink some published values. *BioEssays* **35**, 1050–1055 (2013).
80. Simpson, C. E. & Ashe, M. P. Adaptation to stress in yeast: to translate or not? *Biochem. Soc. Trans.* **40**, 794–799 (2012).
81. Besse, F. & Ephrussi, A. Translational control of localized mRNAs: restricting protein synthesis in space and time. *Nature Rev. Mol. Cell Biol.* **9**, 971–980 (2008).
82. Sonenberg, N. & Hinnebusch, A. G. Regulation of translation initiation in eukaryotes: mechanisms and biological targets. *Cell* **136**, 731–745 (2009).
83. Kuersten, S., Radek, A., Vogel, C. & Penalva, L. O. F. Translation regulation gets its "omics" moment. *Wiley Interdiscip. Rev. RNA* **4**, 617–630 (2013).
84. Brar, G. A. *et al.* High-resolution view of the yeast meiotic program revealed by ribosome profiling. *Science* **335**, 552–557 (2012).
85. Ingolia, N. T., Lareau, L. F. & Weissman, J. S. Ribosome profiling of mouse embryonic stem cells reveals the complexity and dynamics of mammalian proteomes. *Cell* **147**, 789–802 (2011).
86. Stern-Ginossar, N. *et al.* Decoding human cytomegalovirus. *Science* **338**, 1088–1093 (2012).
87. Stumpf, C. R., Moreno, M. V., Olshen, A. B., Taylor, B. S. & Ruggero, D. The translational landscape of the mammalian cell cycle. *Mol. Cell.* **52**, 574–582 (2013).
88. Gingold, H. & Pilpel, Y. Determinants of translation efficiency and accuracy. *Mol. Syst. Biol.* **7**, 481 (2011).
89. Ravid, T. & Hochstrasser, M. Diversity of degradation signals in the ubiquitin–proteasome system. *Nature Rev. Mol. Cell. Biol.* **9**, 679–690 (2008).
90. Belle, A., Tanay, A., Bitincka, L., Shamir, R. & O'Shea, E. K. Quantification of protein half-lives in the budding yeast proteome. *Proc. Natl Acad. Sci. USA* **103**, 13004–13009 (2006).
91. Eden, E. *et al.* Proteome half-life dynamics in living human cells. *Science.* **331**, 764–768 (2011).
92. Geva-Zatorsky, N. *et al.* Using bleach-chase to measure protein half-lives in living cells. *Nature Protoc.* **7**, 801–811 (2012).
93. Khmelinskii, A. *et al.* Tandem fluorescent protein timers for *in vivo* analysis of protein dynamics. *Nature Biotech.* **30**, 708–714 (2012).
Develops advanced tFTs, which elegantly overcome the methodological problems of measuring protein dynamics *in vivo*.
94. Lippincott-Schwartz, J. & Patterson, G. H. Photoactivatable fluorescent proteins for diffraction-limited and super-resolution imaging. *Trends Cell Biol.* **19**, 555–565 (2009).
95. Claydon, A. J. & Beynon, R. Proteome dynamics: revisiting turnover with a global perspective. *Mol. Cell. Proteom.* **11**, 1551–1565 (2012).
96. Claydon, A. J., Thom, M. D., Hurst, J. L. & Beynon, R. J. Protein turnover: measurement of proteome dynamics by whole animal metabolic labelling with stable isotope labelled amino acids. *Proteomics.* **12**, 1194–1206 (2012).
97. Doherty, M. K., Hammond, D. E., Clague, M. J., Gaskell, S. J. & Beynon, R. J. Turnover of the human proteome: determination of protein intracellular stability by dynamic SILAC. *J. Proteome Res.* **8**, 104–112 (2009).
98. Hodas, J. J. L. *et al.* Dopaminergic modulation of the hippocampal neuropil proteome identified by bioorthogonal noncanonical amino acid tagging (BONCAT). *Proteomics* **12**, 2464–2476 (2012).
99. Schwanhäusser, B., Gossen, M., Dittmar, G. & Selbach, M. Global analysis of cellular protein translation by pulsed SILAC. *Proteomics* **9**, 205–209 (2009).
100. Bagert, J. D. *et al.* 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. Proteom.* **13**, 1352–1358 (2014).
101. Sobczyk, G. J., Wang, J. & Weijer, C. J. SILAC-based proteomic quantification of chemoattractant-induced cytoskeleton dynamics on a second to minute timescale. *Nature Commun.* **5**, 3319 (2014).
102. Jayapal, K. P. *et al.* Multitagging proteomic strategy to estimate protein turnover rates in dynamic systems. *J. Proteome Res.* **9**, 2087–2097 (2010).
103. Howden, A. J. M. *et al.* QuaNCAT: quantitating proteome dynamics in primary cells. *Nature Methods* **10**, 343–346 (2013).
104. Merbl, Y., Refour, P., Patel, H., Springer, M. & Kirschner, M. W. Profiling of ubiquitin-like modifications reveals features of mitotic control. *Cell* **152**, 1160–1172 (2013).
Provides a new methodology for profiling post-translational modifications that is applicable to many cellular and environmental conditions.
105. Roth, A. F. *et al.* Global analysis of protein palmitoylation in yeast. *Cell* **125**, 1003–1013 (2006).
106. Wan, J., Roth, A. F., Bailey, A. O. & Davis, N. G. Palmitoylated proteins: purification and identification. *Nature Protoc.* **2**, 1573–1584 (2007).

107. Zhang, Y., Kweon, H. K., Shively, C., Kumar, A. & Andrews, P. C. Towards systematic discovery of signaling networks in budding yeast filamentous growth stress response using interventional phosphorylation data. *PLoS Comput. Biol.* **9**, e1003077 (2013).
108. Schmidt, A. *et al.* Quantitative phosphoproteomics reveals the role of protein arginine phosphorylation in the bacterial stress response. *Mol. Cell. Proteomics* **13**, 537–550 (2013).
109. Robitaille, A. M. *et al.* Quantitative phosphoproteomics reveal mTORC1 activates *de novo* pyrimidine synthesis. *Science* **339**, 1320–1323 (2013).
110. Rigbolt, K. T. G. *et al.* System-wide temporal characterization of the proteome and phosphoproteome of human embryonic stem cell differentiation. *Sci. Signal.* **4**, rs3 (2011).
111. Zoumaro-Djayoon, A. D. *et al.* Investigating the role of FGF-2 in stem cell maintenance by global phosphoproteomics profiling. *Proteomics.* **11**, 3962–3971 (2011).
112. Olsen, J. V. *et al.* Global, *in vivo*, and site-specific phosphorylation dynamics in signaling networks. *Cell.* **127**, 635–648 (2006).
113. Kim, W. *et al.* Systematic and quantitative assessment of the ubiquitin-modified proteome. *Mol. Cell.* **44**, 325–340 (2011).
114. Merbl, Y. & Kirschner, M. W. Large-scale detection of ubiquitination substrates using cell extracts and protein microarrays. *Proc. Natl Acad. Sci. USA* **106**, 2543–2548 (2009).
115. Peters, L. Z., Hazan, R., Breker, M., Schuldiner, M. & Ben-Aroya, S. Formation and dissociation of proteasome storage granules are regulated by cytosolic pH. *J. Cell Biol.* **201**, 663–671 (2013).
116. Ahmad, Y., Boisvert, F.-M., Lundberg, E., Uhlen, M. & Lamond, A. I. Systematic analysis of protein pools, isoforms, and modifications affecting turnover and subcellular localization. *Mol. Cell. Proteomics* **11**, M111.013680 (2012).
117. Boisvert, F.-M. *et al.* A quantitative spatial proteomics analysis of proteome turnover in human cells. *Mol. Cell. Proteomics* **11**, M111.011429 (2012).
118. Uetz, P. *et al.* A comprehensive analysis of protein-protein interactions in *Saccharomyces cerevisiae*. *Nature* **403**, 623–627 (2000).
119. Gisler, S. M. *et al.* Monitoring protein-protein interactions between the mammalian integral membrane transporters and PDZ-interacting partners using a modified split-ubiquitin membrane yeast two-hybrid system. *Mol. Cell. Proteom.* **7**, 1362–1377 (2008).
120. Miernyk, J. A. & Thelen, J. J. Biochemical approaches for discovering protein–protein interactions. *Plant J.* **53**, 597–609 (2008).
121. Popescu, S. C. *et al.* MAPK target networks in *Arabidopsis thaliana* revealed using functional protein microarrays. *Genes Dev.* **23**, 80–92 (2009).
122. Von Mering, C. *et al.* Comparative assessment of large-scale data sets of protein–protein interactions. *Nature* **417**, 399–403 (2002).
123. Mika, S. & Rost, B. Protein–protein interactions more conserved within species than across species. *PLoS Comput. Biol.* **2**, e79 (2006).
124. Rajagopala, S. V. *et al.* The binary protein–protein interaction landscape of *Escherichia coli*. *Nature Biotech.* **32**, 285–290 (2014).
125. Braun, P., Aubourg, S., Van Leene, J., De Jaeger, G. & Lurin, C. Plant protein interactomes. *Annu. Rev. Plant Biol.* **64**, 161–187 (2013).
126. Walhout, A. J. *et al.* Protein interaction mapping in *C. elegans* using proteins involved in vulval development. *Science* **287**, 116–122 (2000).
127. Giot, L. *et al.* A protein interaction map of *Drosophila melanogaster*. *Science* **302**, 1727–1736 (2003).
128. Goodman, S. R., Daescu, O., Kakhniashvili, D. G. & Zivanic, M. The proteomics and interactomics of human erythrocytes. *Exp. Biol. Med.* **238**, 509–518 (2013).
129. Rual, J.-F. *et al.* Towards a proteome-scale map of the human protein-protein interaction network. *Nature* **437**, 1173–1178 (2005).
130. Havugimana, P. C. *et al.* A census of human soluble protein complexes. *Cell* **150**, 1068–1081 (2012).
131. Zhang, S., Ma, C. & Chalfie, M. Combinatorial marking of cells and organelles with reconstituted fluorescent proteins. *Cell.* **119**, 137–144 (2004).
132. Sung, M.-K. & Huh, W.-K. *In vivo* quantification of protein-protein interactions in *Saccharomyces cerevisiae* using bimolecular fluorescence complementation assay. *J. Microbiol. Methods.* **83**, 194–201 (2010).
133. Johnsson, N. Analyzing protein–protein interactions in the post-interactomic era. Are we ready for the endgame? *Biochem. Biophys. Res. Commun.* **445**, 739–745 (2014).
134. Rao, V. S., Srinivas, K., Sujini, G. N. & Kumar, G. N. S. Protein–protein interaction detection: methods and analysis. *Int. J. Proteom.* **2014**, 147648 (2014).
135. Ngounou Wetie, A. G. *et al.* Protein-protein interactions: switch from classical methods to proteomics and bioinformatics-based approaches. *Cell. Mol. Life Sci.* **71**, 205–228 (2013).
136. Vlasblom, J., Jin, K., Kassir, S. & Babu, M. Exploring mitochondrial system properties of neurodegenerative diseases through interactome mapping. *J. Proteomics* **100**, 8–24 (2013).
137. Hagen, N., Bayer, K., Roesch, K. & Schindler, M. The intra viral protein interaction network of hepatitis C virus. *Mol. Cell. Proteom.* http://dx.doi.org/10.1074/mcp.M113.036301 (2014).
138. Moya, I. M. & Halder, G. Discovering the Hippo pathway protein-protein interactome. *Cell Res.* **24**, 137–138 (2014).
139. Guney, E. & Oliva, B. Analysis of the robustness of network-based disease-gene prioritization methods reveals redundancy in the human interactome and functional diversity of disease-genes. *PLoS ONE* **9**, e94686 (2014).
140. Soler-López, M., Zanzoni, A., Lluís, R., Stelzl, U. & Aloy, P. Interactome mapping suggests new mechanistic details underlying Alzheimer's disease. *Genome Res.* **21**, 364–376 (2011).
141. Cox, J. & Mann, M. Quantitative, high-resolution proteomics for data-driven systems biology. *Annu. Rev. Biochem.* **80**, 273–299 (2011).
142. Woo, S. *et al.* Proteogenomic database construction driven from large scale RNA–seq data. *J. Proteome Res.* **13**, 21–28 (2014).
143. Shi, Z., Wang, J. & Zhang, B. NetGestalt: integrating multidimensional omics data over biological networks. *Nature Methods* **10**, 597–598 (2013).
144. Talwar, P. *et al.* Genomic convergence and network analysis approach to identify candidate genes in Alzheimer's disease. *BMC Genomics* **15**, 199 (2014).
145. Borchers, C. H. *et al.* The Human Proteome Organization Chromosome 6 Consortium: integrating chromosome-centric and biology/disease driven strategies. *J. Proteom.* **100**, 60–67 (2014).
146. Chen, R. *et al.* Personal omics profiling reveals dynamic molecular and medical phenotypes. *Cell* **148**, 1293–1307 (2012).
147. Rubinstein, A. D. & Kimchi, A. Life in the balance — a mechanistic view of the crosstalk between autophagy and apoptosis. *J. Cell Sci.* **125**, 5259–5268 (2012).
148. Zeng, L. *et al.* Decision making at a subcellular level determines the outcome of bacteriophage infection. *Cell* **141**, 682–691 (2010).
149. Wiśniewski, J. R., Zougman, A., Nagaraj, N. & Mann, M. Universal sample preparation method for proteome analysis. *Nature Methods* **6**, 359–362 (2009).
150. Gorini, G., Nunez, Y. O. & Mayfield, R. D. Integration of miRNA and protein profiling reveals coordinated neuroadaptations in the alcohol-dependent mouse brain. *PLoS ONE* **8**, e82565 (2013).

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Competing interests statement
The authors declare no competing interests.

DATABASES
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Max-Planck Unified (MAPU) Proteome Database: http://www.mapuproteome.com
PeptideAtlas: http://www.peptideatlas.org
The Human Protein Atlas: http://www.proteinatlas.org
The Kahn Dynamic Proteomics Database: http://www.weizmann.ac.il/mcb/UriAlon/DynamProt/
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