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Analysis of Organelle Dynamics by Quantitative Mass Spectrometry Based Proteomics

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1. Introduction

A major goal of cell biology is to understand the dynamic interplay between different reactions in the cell. In eukaryotes, compartmentalization of the cytoplasm into organelles facilitates the coordinated execution of many cellular functions. To understand how this is achieved, it is important to know the protein composition of the different organelles, and to determine how it may change over time. In addition, the activity of many proteins is regulated by often reversible and dynamic **p**ost-**t**ranslational **m**odifications (PTMs). In recent years, proteomics has matured into a staple technique for cell biology. Modern approaches of proteomics rely on mass-spectrometry. Here, we build on several reviews (Aebersold and Mann, 2003; Choudhary and Mann, 2010; Walther and Mann, 2010) to summarize and highlight contemporary applications of MS-based proteomics to the analysis of organelle dynamics.

2. MS-based quantitative proteomics approaches for proteins and their post-translational modifications

Most proteomics studies aim to not just identify proteins but to quantitate their abundance in different samples. In the past few years, several quantitative proteomics approaches have been developed to accomplish this task.

2.1 MS-based quantitative proteomics approaches

2.1.1 Stable isotope labeling by amino acids in cell culture (SILAC)

The most commonly used method to quantitate proteins is **s**table **i**sotope **l**abeling by **a**mino acids in cell **c**ulture (SILAC; (Ong et al., 2002)) in combination with **l**iquid **c**hromatography (LC) and tandem high resolution mass spectrometry (LC-MS/MS). Cells are labeled with non-radioactive heavy labeled amino acids, typically arginine and /or lysine. After cell lysis, extracts from differently labeled and differently treated cells are mixed and digested with a protease that cuts after the labeled amino acids, such as trypsin for the case of arginine/lysine. The resulting peptide mixture is fractionated by LC on a C18 column and analyzed in a high resolution mass spectrometer. The mass shift between labeled and unlabeled peptides allows the quantification of intensities of peptides, and based on that of

proteins, derived from cells differentially labeled and subjected to different conditions. Since chemically identical peptides are quantitated in the same spectrum, the accuracy of this methodology is very high. In addition, mixing samples directly after lysis limits the chance for experimental errors. However, in its simplest rendition, SILAC-based proteomics is limited to samples that can be metabolic labeled, including cells and model organisms (ranging from yeast to mice) (de Godoy et al., 2008; Kruger et al., 2008). Recently developed "spike-in" approaches that use isotope labeled cell extracts as standards for analysis of samples of interests from sources that cannot be labeled, such as patient samples, are compared. In case a single cell extract does not adequately represent a particular tissue or sample, several extracts can be mixed to obtain a "super-SILAC" standard (Geiger et al., 2010). This approach of SILAC reference standards is not limited to quantitation of protein abundance but can also be applied to quantify changes in PTMs, such as phosphorylation. In an example of such an analysis, a phosphopeptide standard combining untreated or insulin treated mouse liver cell lines were spiked into samples derived from the liver of insulin treated or untreated mice. This method led to the identification of over 15,000 and quantitation of 10,000 phosphosites (Monetti et al., 2011).

2.1.2 Isobaric tags for relative and absolute quantification (iTRAQ)

Chemically labeling of proteins in different samples can also be used for their quantitation. One such technique uses **i**sobaric **t**ags for **r**elative and **a**bsolute **q**uantification (iTRAQ). In iTRAQ experiments, different chemical groups modify the primary amino group of either the N-terminus or lysine side chains of peptides in different samples (Ross et al., 2004). These differentially labeled peptides are pooled and analyzed by LC-MS/MS setup. Each of the labels has the same mass and therefore each peptide is visible in a single peak in the MS spectrum. However, fragmentation of that peak leads to formation of a low molecular mass reporter ion characteristic for each tag in the MS/MS spectrum that is used to quantify the relative amounts of the corresponding peptides and proteins. It is very important for this technique to distinguish peptides which have a similar mass and elute at the same time because this would lead to false ratios as both peptides contribute to the abundance of the same reporter ions (Ow et al., 2009; Zhang et al., 2010). In addition, it is crucial to ensure complete labeling of the sample. Moreover, side reactions of chemical labeling may be unavoidable, but can lead to false positive identifications of PTMs. For example, alkylation of a peptide mixture with iodoacetamide can produce a 2-acetamidoacetamide covalent adduct to lysine. This has the same atomic composition as a diglycine adduct of a ubiquitinated peptide after tryptic digest (Nielsen et al., 2008).

2.1.3 Label free approaches

In addition to the described labeling methods, so called "label-free" approaches that directly compare the abundance of peptides and proteins between samples are very attractive to analyze complex protein mixtures. Such approaches enable the analysis of samples which cannot be easily labeled. One type of label free quantitation approaches uses alignments of separate LC-MS/MS runs to compare peptide intensities between different samples. Recent advances in computational proteomics enable quantification of peptides from less complex samples by this approach (Mueller et al., 2008; Wong and Cagney, 2010). However, the accuracy of this approach is still somewhat lower compared to measurements from metabolic labeled samples. The analysis is particularly challenging for PTMs. Post

translational modified peptides are generally of low abundance and even small changes can have important effects on the cell.
Sometimes only a few modified peptides are of interest for the question under consideration. In these cases, proteomics can be targeted to sequencing to a subset of previously identified peptides (Schmidt et al., 2009). One method to achieve this, called **m**ultiple **r**eaction **m**onitoring (MRM), is performed on so called triple quadrupole mass analyzers. In the first quadrupole, peptides of interest are isolated by their mass. The second quadrupole is a collision cell where the peptides are fragmented. The last quadrupole is set to some specific fragments that are characteristic for the peptide. The advantage of MRMs over unbiased approaches is the high sensitivity and speed (Kitteringham et al., 2009; Malmstrom et al., 2009; Wolf-Yadlin et al., 2007). However, false positive rates in these experiments can be high due to limited resolution of the quadrupole instruments compared to orbitraps. In alternative approaches using high resolution orbitrap instruments, the specific m/z of peptides of interest are written in an "inclusion list" (Jaffe et al., 2008). Whenever a peptide of this m/z is found in the MS spectrum it is selected for fragmentation and MS/MS analysis. If higher sensitivity of the instrument is required, **s**elected **i**on **m**onitoring (SIM) scans can be used to survey one or several pre-defined ranges of the m/z spectrum rather than a full spectrum during the chromatography run (Michalski et al., 2011).

2.2 Posttranslational modifications

Quantitative proteomics can detect changes of the abundance of proteins in a whole proteome from cells in different conditions. In addition biological activity of proteins varies and is often regulated by PTMs. Therefore, it is important to measure changes of PTMs spatially and temporarily. MS is ideal to study PTMs because it can detect specific mass shifts due to the modification and assign its exact position in the amino acid sequence.

2.2.1 Phosphorylation

The most studied PTM is phosphorylation of the amino acids serine, threonine or tyrosine. Phosphorylation is important for many cellular processes. Protein phosphorylation conventionally is analyzed by ^{32}P labeling, band shifts on SDS-PAGE gels or the detection of phosphorylated residues by site-specific antibodies. While these techniques yielded great insights, they focus usually on just one or a few proteins at a time. To study the complexity of signaling networks, systematic methods to study phosphorylation of many proteins at the same time are required. MS-based methods analyzing phosphorylation of proteins by detecting the phosphorylated peptides resulting from their digestion require enrichment of phosphorylated peptides to overcome their low abundance in cells. Several different methods have been established to enrich phosphorylated peptides. Antibodies specific for a specific phosphorylated amino acid, e.g., phospho-tyrosine, are used. These enrichment methods are very useful to study a specific phosphorylation species. A different enrichment method is **i**mmobilized **m**etal (e.g., Fe^{3+}) **a**ffinity **c**hromatography (IMAC) (Corthals et al., 2005; Muszynska et al., 1992). With IMAC, all phosphopeptides are enriched due to the interaction between negatively charged phosphate groups and the immobilized positive metal ions. A similar technique uses TiO_2 to complex phosphorylated peptides on a resin by their charge (Pinkse et al.,

2004). In contrast to IMAC, phosphopeptide enrichment by TiO_2 requires a competitor for the binding sites, such as **d**i**h**ydro**b**enzoic acid (DHB) or lactic acid, to exclude unphosphorylated negatively charged peptides (Larsen et al., 2005). A drawback of these competitors is possible contamination of the MS instruments by the competitor. In organisms of relatively low proteome complexity, such as *Saccharomyces cerevisiae,* this setup was sufficient to identify 5534 phosphosites (Soufi et al., 2009). However, due to the much higher complexity of the mammalian phosphoproteome, some studies use a pre-fractionation step of peptides before phosphopeptides enrichment, e.g., by **s**trong **c**ation e**x**change chromatography (SCX) (Villen and Gygi, 2008).

After enrichment of phosphorylated peptides, samples are analyzed by LC-MS/MS. To identify phosphorylated peptides and assign the localization of the phosphorylation site with high confidence, distinct fragmentation methods have been used. **C**ollision **i**nduced **d**issociation (CID) fragmentation often results in a neutral loss because the phosphoester bond is relatively fragile. The resulting lost ions of 98 or 80 Da were used to scan specifically for phosphorylated peptides. However, this phenomenon often dominates the MS/MS scans (Tholey et al., 1999) and leads to reduced backbone fragmentation. For efficient identification of phosphorylated peptides in ion traps, the neutral loss signal can be isolated after MS/MS and subjected to additional CID to yield a MS^3 spectrum (Jin et al., 2004). Multistage activation virtually combines MS^2 and MS^3 by parallel excitation and fragmentation (Schroeder et al., 2004). Even with multistage activation, fragmentation of phosphopeptides can be insufficient to identify the peptide sequence or to assign the phosphorylation site correctly with high confidence. Recent technical developments allow for different fragmentation techniques. **E**lectron **c**apture **d**issociation (ECD) or **e**lectron **t**ransfer **d**issociation (ETD) lead to sole backbone fragmentation between N and C bonds thereby generating c and z ions (Syka et al., 2004; Zubarev et al., 2000). PTMs unstable during CID fragmentation therefore stay intact with ETD fragmentation and make site specific assignment easier. **H**igher **c**ollision **e**nergy dissociation (HCD) uses the same principle of CID but higher collision energies, thus efficiently fragmenting the peptide backbone even in the presence of a low energy bond to a PTM. A recent study used a LTQ OrbitrapVelos to analyze both the precursor ion and its peptide fragments after HCD with high resolution in an orbitrap. This so called "high-high" technique yielded up to 16,000 identified phosphorylation sites with high confident assignments (Nagaraj et al., 2010).

Data quality is a particularly important issue for large scale PTM studies. For example, it is possible to identify a phosphorylated peptide with high confidence (>99%), but it is sometimes impossible to assign its site with high confidence between two adjacent serines. Therefore large scale datasets should always contain a peptide identification score and a PTM localization score (Beausoleil et al., 2006; Gnad et al., 2011; Olsen et al., 2006).

2.2.2 Glycosylation

Although phosphorylation is by far the most studied PTM, MS can also be used to study other PTMs. Another example for a prominent PTM is N-glycosylation of asparagine residues occurring in the endoplasmic reticulum. N-glycosylation plays an important role in the assembly of complex organelles and is involved in many cellular processes, such as apoptosis and the immune response (Varki, 2009). Due to the complexity of sugar moieties it is very challenging to analyze N-glycosylated proteins or peptides by MS approaches. Additionally the abundance of N-glycosylated proteins is usually very low in comparison to

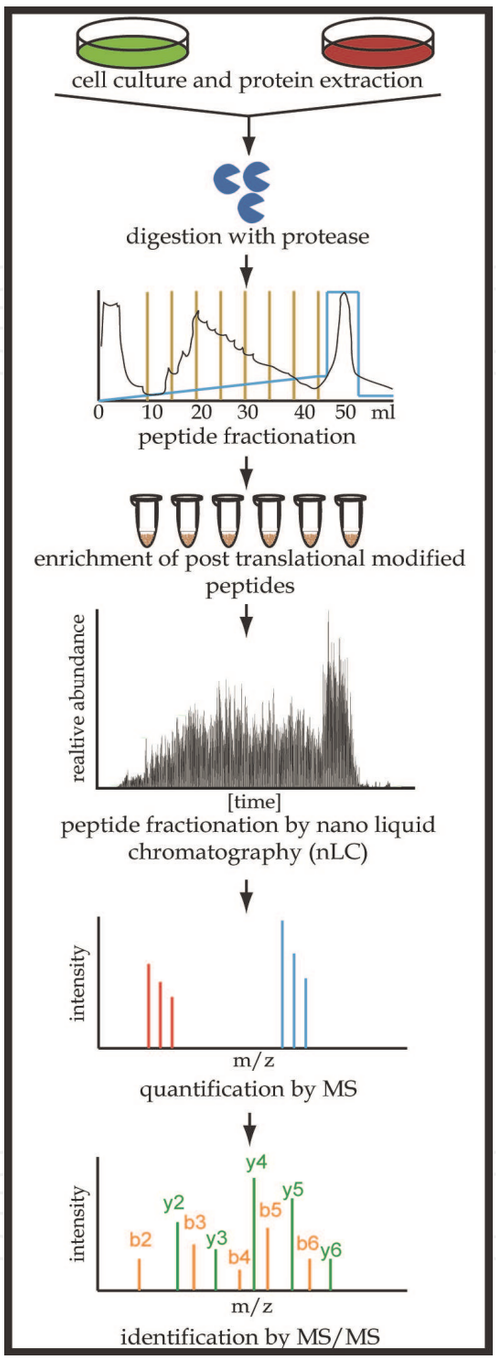
their unmodified counterparts. Thus, N-glycosylated proteins are enriched for analysis by affinity purification using lectins (Bunkenborg et al., 2004) or by chemical linkage of the sugar moiety to a solid phase (Zhang et al., 2003).
After enrichment, samples are treated with a global deglycosylating enzyme leading to the deamidation of the asparagine residue to aspartic acid. The resulting mass increase of 0.9848 Da can be detected in the precursor scan as well as in the peptide fragments (Kuster and Mann, 1999). Previously, N-glycosylated peptides were identified with low resolution of the peptide precursor mass. A recent study, using high resolution MS instead, mapped roughly 6,400 N-glycosylation sites in different murine cell lines quantitatively (Zielinska et al., 2010).

2.2.3 Acetylation and methylation

Other common PTMs are acetylation or methylation of lysines and arginines. These are reversible PTMs that change the charge of the amino acid thereby possibly regulating protein function. The most prominent example is histone acetylation or methylation, which regulates transcription. Acetylated or methylated peptides are relatively low abundant and specific enrichment is required, similar as in the case of phosphorylation analysis. Enrichment with antibodies which recognize the modified amino acid is generally performed prior to analysis by MS. A recent study identified an unexpected large number of acetylation sites. By using high resolution MS in combination with SILAC, 3600 acetylation sites were identified on 1750 proteins. Due to the low abundance of acetylated peptides, Choudhary et al used isoelectric focusing after enrichment of acetylated peptides to further reduce sample complexity (Choudhary et al., 2009). A previous study revealed an unexpected role of acetylation in mitochondrial function (Kim et al., 2006). A method to measure methylation quantitatively is heavy methyl- SILAC. In this approach, heavy methionine serves as the sole donor for the methyl group. The mass shift of the peptide containing the heavy versus light methyl groups is detected by high resolution mass spectrometry (Ong et al., 2004).

2.2.4 Ubiquitination

Ubiquitination of proteins is another PTM amenable to MS based proteomics. The protein ubiquitin is crosslinked to lysine residues on target proteins. Ubiquitination plays an important role in proteasomal degradation and endocytosis of plasma membrane proteins. Since ubiquitin is a protein, it can be tagged for subsequent affinity enrichment. HIS tagged versions of ubiquitin can be used to isolate ubiquitinated proteins by affinity purification, leading in one example to the identification of 110 ubiquitination sites on 72 proteins in *Saccharomyces cerevisiae* (Peng et al., 2003). Instead of isolating ubiquitinated proteins, a site specific antibody that detects the diGly motif of ubiquitinated peptides, yielded 374 diglycine modified lysines on 236 ubiquitinated proteins from HEK293 cells (Xu et al., 2010). One problem analyzing ubiquitinated proteins are their very complex fragmentation spectra. In addition it is difficult to distinguish by MS analysis modification by ubiquitin or by other ubiquitin-like molecules, such as interferon-induced 17kDA protein (ISG15) that leave the same diGly tag after digestion. Furthermore, it is not possible to distinguish if a protein is mono- or polyubiquitinated by LC MS/MS since digestion of the proteins with a protease is necessary and cleaves polyubiquitin chains. For a representative analysis of post translational modified proteins see Figure 1.



Cells are grown (label free or SILAC) and lysed for protein extraction. Extracted proteins are digested with an endoproteinase (Trypsin; LysC) and resulting peptides are fractionated (strong cation exachange (SCX) or strong anion exchange (SAX) chromatography). Peptides are enriched by their PTMs (specific antibodies, IMAC, TiO_2, lectin affinity). Enriched peptides are fractionated by nano-flow LC and directly injected by electrospray ionization into the MS. Relative abundances of peptides are quantified by the first, full-scan MS event (MS). Peptide identification and determination of the PTM is achieved by fragmentation (CID, HCD, ETD, ECD) in the second stage of MS (MS/MS).

Fig. 1. Workflow for quantitative proteomics of post translational modified proteins.

3. Spatial analysis of the cellular proteome

3.1 MS-based proteomics approaches to map protein composition of organelles

Each organelle is composed of a specific set of proteins that contribute to its function and that allow for communication with the rest of the cell. The content of organelles is highly dynamic and includes resident as well as transient proteins coming from and going to other compartments in the cell. Traditionally, to identify protein localization, cell biologists employ protein tagging by fluorescent labels or subcellular fractionation of cells in combination with detection of proteins by immunolabeling. These techniques have been successfully applied over many years and were adapted to high throughput studies, for example to tag nearly the complete proteome of budding (Huh et al., 2003) or of fission yeast (Matsuyama et al., 2006). However, technical limitations (e.g., absence of homologous recombination in many systems) and the enormous effort required for these approaches impede its application to more complex systems, such as mammalian cells. Therefore, organelle proteomics based on LS-MS was developed into a complementary approach that has made valuable contributions to the elucidation of organelles' inventory in many systems. In this approach, organelles or parts thereof, including protein complexes, are purified biochemically and analyzed by MS-based techniques.

3.1.1 MS based proteomics of purified organelle

For few compartments, such as nucleus or mitochondrion, that are rather easy to isolate with high purity, MS-based proteomics has yielded protein inventories (Andersen et al., 2002; Taylor et al., 2003). These and all other organellar proteomics experiments usually start with cell disruption under mild conditions designed to maintain organelle integrity. Organelles are then purified from crude cell extracts by differential centrifugations and are then processed for MS analysis. In this scheme, separation of organelles depends on their sedimentation velocity, a function of their size and density. Generally purification is improved by combining velocity sedimentation centrifugation with density gradient centrifugation (Michelsen and von Hagen, 2009; Wiederhold et al., 2010). Alternatively, higher purity is obtained by affinity purification using e.g., an antibody directed against a surface protein of the organelle, free flow electrophoresis (Islinger et al., 2010) or by modification of organelles density. An example for the latter is the report of phagosomes or endosomes inventories isolated after flotation gradient. In these cases, latex beads of different diameters can be internalized either by phagocytosis (Duclos and Desjardins, 2011; Jutras et al., 2008) or endocytosis (Duclos et al., 2011) and the resulting organelles are purified by a single step flotation gradient centrifugation before analysis by MS.

For some organelles, no efficient purification schemes exist based solemnly on differences of density or sedimentation. Therefore, in some instances, chemical modification is used to facilitate the purification. For example, cell surface modification by cell impermeable chemicals is commonly used to modify plasma membrane proteins from the extracellular environment. In such strategy, plasma membrane proteins are covalently linked to biotin, which in turn is used as an affinity tag for subsequent purification on a column carrying streptavidin. Cell surface modification relying on different chemical properties of proteins such as the reactivity of primary amines (N-termini of proteins and side chains of lysines, (Elia, 2008; Scheurer et al., 2005), thiols (Laragione et al., 2003) or of carbohydrates (Teckchandani et al., 2009) have been developed to analyze the proteome of plasma membranes in normal (Zhao et al., 2004) and in pathological conditions (Hubbard et al.,

2011; Yang et al., 2011) or to identify the dynamic proteome of the plasma membrane (Christiano et al., 2010).

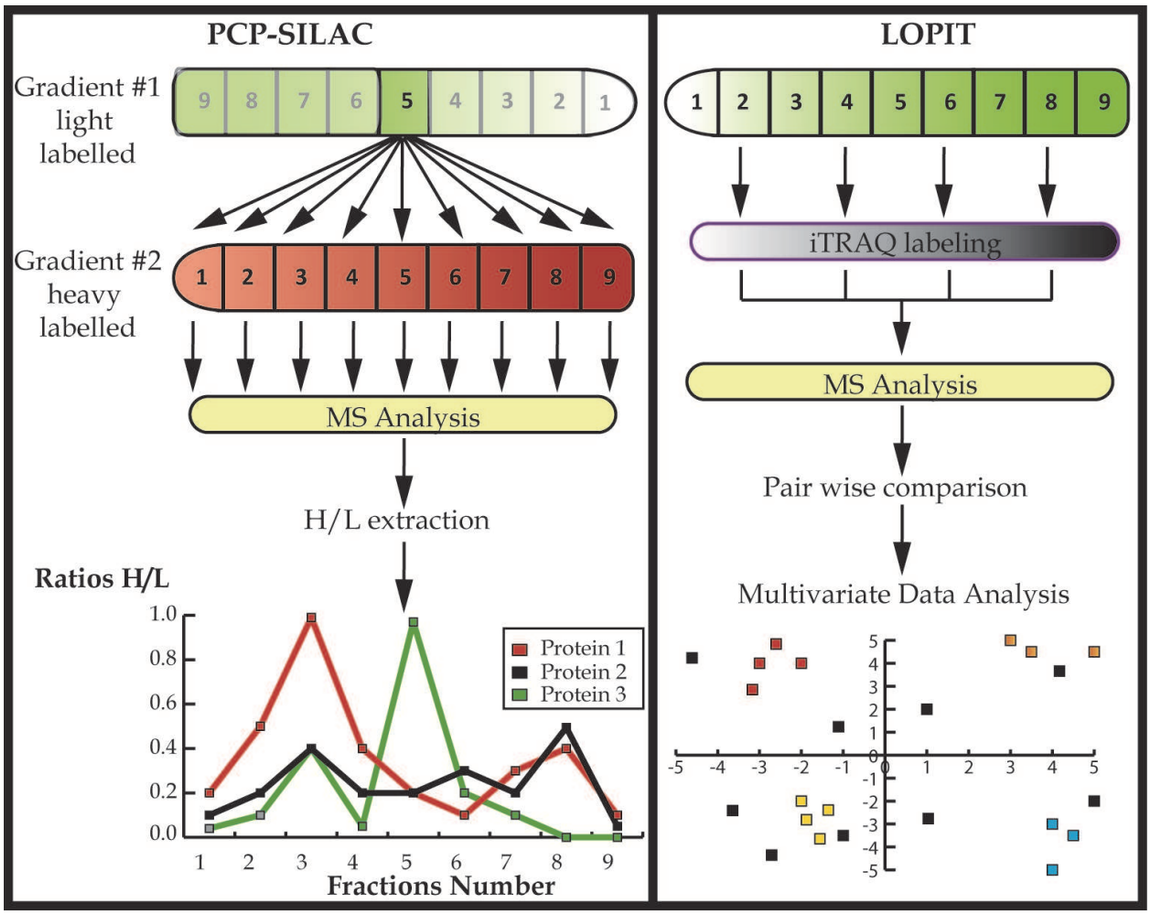
3.1.2 Differential proteomics

Despite the remarkable success of these simple organelle proteomics approaches in some cases, formidable technical challenges to obtain pure organelles remain. Particularly in combination with highly sensitive mass spectrometers, simple purification schemes often result in the identification of large numbers of proteins. It is difficult to determine which ones among them are *bona fide* constituents of the target organelle and which ones are contaminants. One solution to overcome this limitation is the application of differential proteomics to subtract the proteins in the fraction in which the target organelle is found from a closely resembling fraction containing the most prominent contaminants. For this approach to work, the target organelle of interest should be enriched to the highest possible degree in one fraction, but be absent completely from the control fraction. This control sample could be as simple as a crude cell extract devoid of the target organelle, or better, reflect a similar purification in the absence of the organelle. After analysis of both samples, proteins that have been exclusively identified in the fraction enriched in the target organelle are considered as genuine proteins. In this approach, the accuracy of the obtained results strongly depends on the quality of two different MS analyses, as missing a protein in the control will lead its assignment to the target organelle. This point is critical considering the variability of analysis inherent to many MS approaches. For example, identification of a particular protein depends on different properties of the protein (such as abundance in the sample, ability of its peptide to be ionized in the mass spectrometer), as well as the MS methodology used. Failure to detect a protein does not necessarily mean it is absent from the sample. In differential proteomics approaches, this may lead to false negative or positive results, if a protein is randomly not detected in either the organelle enriched or the control fraction, respectively. Moreover, as sensitivity of MS analysis is improved and hence the number of identified proteins in each sample increases, the lists derived from each of the two fractions will more and more overlap, since proteins are likely to differ only in abundance and not strictly be absent from one sample. Thus, a large set of false negative proteins of the target organelle may be subtracted due to their identification in both samples.

3.1.3 Quantitative organellar MS-based proteomics

Quantitative proteomics is particularly useful in cases where sufficient enrichment for a specific organelle cannot be achieved. For example, organelles of the secretory pathway are similar to each other in their physical properties, with their content dynamically exchanging between them. Methods that only catalogue proteins in a sample cannot provide information on the dynamic behavior of proteins and are prone to detect contamination from co-purifying organelles. Intensive efforts have focused on sample preparation prior to MS. Successful strategies combine gradient profiling and quantitative MS-based proteomics. Such strategies rely on partial separation and distribution of subcellular compartments along density gradients. In **p**rotein **c**orrelation **p**rofiling (PCP) protein abundance profiles along the gradient are obtained by MS analysis and matched to profiles of known organelle markers. In this strategy, the accuracy of the quantification is a critical parameter to obtain reliable mapping of the organelle constituents. To date MS-based quantitative PCP analyses

have been performed using label free (Andersen et al., 2003) and SILAC quantitation (Dengjel et al., 2010). SILAC-PCP allows determination of accurate profiles. In that case, two independent gradients are obtained from two differently labeled cells. A fraction enriched for the organelle of interest is isolated from a gradient and spiked into each fraction of the other gradient (with a ratio 1 to 1). Profiles based on SILAC ratios can be extracted from each fraction of the gradient. Genuine proteins from the organelle of interest will show the highest ratio (close to one) in the organelle fraction, but have lower fractions elsewhere in the profile. In contrast, contaminants have higher ratios in other fractions that represent the organelles that they mostly purify with (Figure 2). Such approaches rely on the reproducibility of the gradient separation. An alternate gradient-based approach, **l**ocalization of **p**roteins by **i**sotope **t**agging (LOPIT) has been successfully applied to plant



PCP-SILAC: A light labeled (L) fraction enriched in a target organelle by gradient centrifugation is spiked into each fraction of a gradient of heavy (H) labeled cells Then, each combined fraction is analyzed independently. H/L ratios are extracted for proteins in all fractions and then abundance profiles are estimated. LOPIT: Specific organelle enriched fractions from a gradient are separated and labeled separately with different iTRAQ reagents. Fractions are pooled and processed into peptides. Then, the fraction is analyzed in a single MS run. Ratios from pair wise analyses are extracted and submitted to multivariate data analyses to identify genuine proteins.

Fig. 2. Gradient Profiling based organelle proteomics.

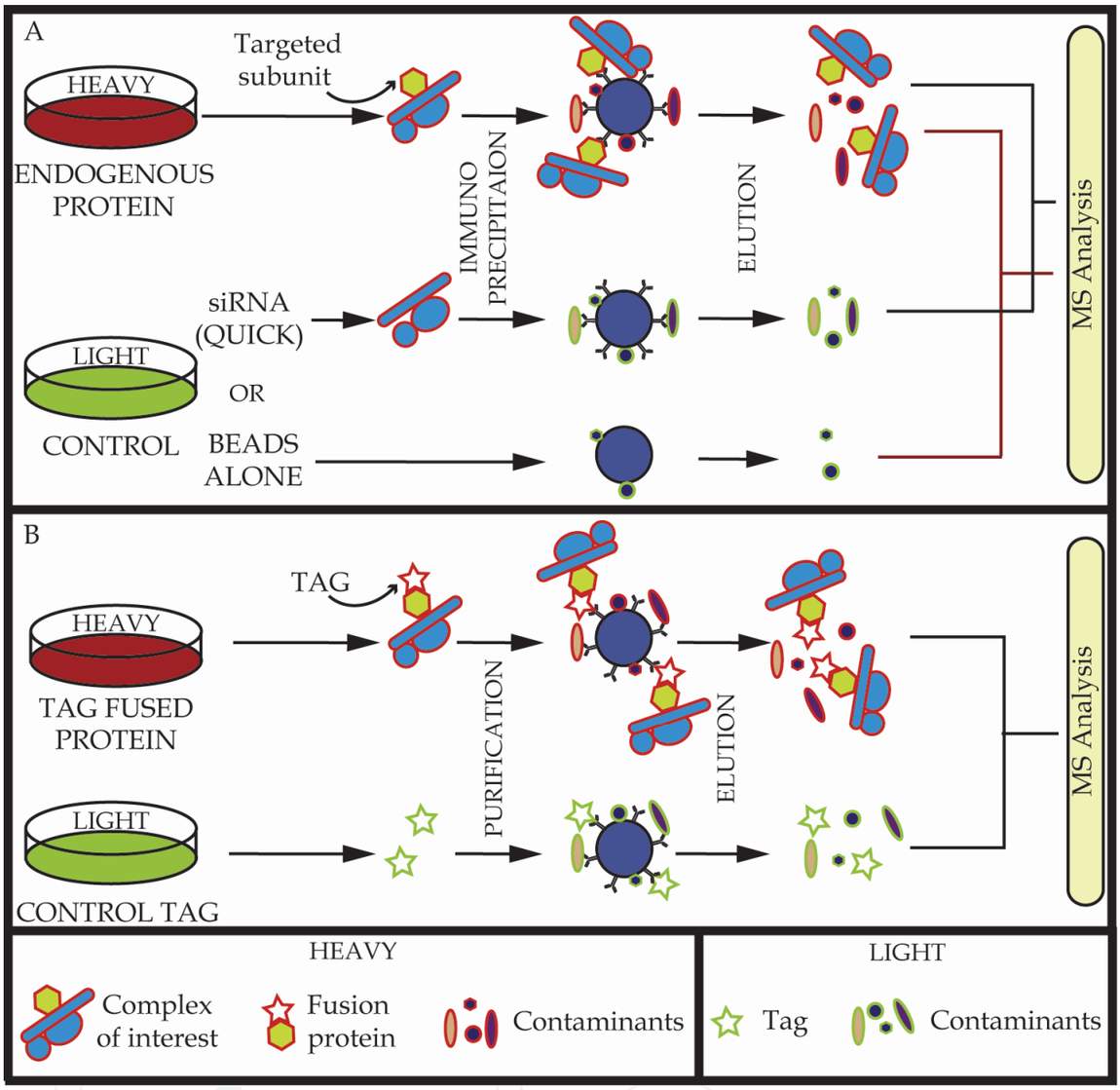
organelles (Dunkley et al., 2004; Lilley and Dunkley, 2008). In that case, several organelle-enriched fractions from one gradient are separately labeled with different iTRAQ reagents and pooled. Ratios derived from pair-wise comparisons are analyzed by multivariate data analyses to assign organelle localization to the proteins identified. (Figure 2)

3.2 Protein complexes

Proteins interact with each other to form complexes that contribute to cellular functions. Therefore, identifying protein interactions is of critical importance to unravel mechanisms underlying cellular functions. To identify complexes, **a**ffinity **p**urification-based **MS** (AP-MS) is the method of choice: Prior to MS analysis, protein complexes are purified with affinity matrices that bind to one subunit of the complex. The confident identification of true binders to the bait protein relies on the comparison with a good control fraction of the affinity purification. The emergence of affinity tags and robust antibodies greatly contributed to the success of AP-MS.

The use of affinity tags is particularly attractive because it allows standardizing procedures that can be applicable to any system or protein. To date, a wide variety of tags has been used in AP-MS ranging from small peptides to proteins of several kDa. Fluorescent tags, such as **g**reen **f**luorescent **p**rotein (GFP), are common because they can also be used for live cell imaging. Furthermore, GFP purification allows recovery of most of the GFP-fused protein from a complex mixture in a single step. In this approach, proteins co-purifying with the bait are compared to a control condition where only the GFP protein is expressed (Figure 3 B). Nevertheless, tag insertion might affect protein functions or prevent/trigger interactions with other proteins. Moreover, expression levels of fusion protein can be significantly different compared to their native counterparts and can affect binding capacities. Therefore, when efficient and specific antibodies are available, it is preferable to rely on purification of the native protein subunits to purify complexes. In that case, the control fraction can be obtained by incubating lysates with the beads alone (Trinkle-Mulcahy et al., 2008) or by depleting the protein of interest by RNA interference (**qu**antitative **i**mmunoprecipitation **c**ombined with **k**nockdown, QUICK) (Selbach and Mann, 2006) (Figure 3 A). Recently, Hubner et al. (Hubner et al., 2010) described **qu**antitative **b**acterial artificial chromosome **i**nter**ac**tomics (QUBIC), using tagged proteins expressed under endogenous control in mammalian cells. Identification of interacting partners is achieved by robust and efficient affinity purification based on the GFP tag (Hubner and Mann, 2011; Vermeulen et al., 2010). However, most protein-protein interactions are dynamic and are hardly assessed by single AP-MS experiments. Depending on internal or external cues protein binding specificities can be modulated, e.g., by post-translational modification. This can result in their release from or recruitment to specific protein complexes thus potentially affecting their function(s) or their localization.

At steady state, different forms of a protein are present in the cell which can hinder the discrimination among its interactors. A solution to this problem can be to use exogenous baits corresponding to a specific state of the protein of interest or of a peptide to identify interactions specific to a particular state. Different strategies have been developed for the identification of genuine interactors with high confidence. Generally, single step purifications are associated with a large number of contaminants. Lower background signal is achieved using more stringent purification, for instance by sequential purification using two affinity tags (**t**andem **a**ffinity **p**urification; TAP). In TAP, two tags separated by a



A) Immunoprecipitation of endogenous proteins. Complexes from heavy (red) and light (green, control) labeled cells are purified using antibody-conjugated beads that target a specific subunit. Then the fractions are pooled before sample preparation and subsequent MS analysis. Control fraction can be obtained by using a cell lysate devoid of the target subunit by siRNA (QUICK) or by incubating a total lysate with the beads alone. B) One step purification of tagged proteins. A tagged subunit is expressed in heavy labeled cells (red). Using affinity columns, protein complexes are affinity purified using the tag and then eluted before being pooled with the control light fraction (green). After sample preparation the combined fraction is analyzed by MS. The control light fraction is obtained by expressing the tag alone in the cells.

Fig. 3. Affinity purification based MS (AP-MS).

cleavage site are fused to the protein of interest. In a first step the complex is purified with the fusion protein using the first tag, then the protein of interest (along with its interactors) is released from the affinity matrix by specific cleavage of the tag before performing a second round of purification with the remainder of the tag. Different combinations of tags

have been combined and TAP strategies have been extensively used for AP-MS purification in many model systems (Gavin et al., 2006; Krogan et al., 2006). However, one drawback is the loss of weak interactors during stringent purification. A compromise is achieved by inserting a cleavage site between a tag and the protein of interest thus reducing the number of treatments and decreasing significantly the number of non-specific binders (Aguilar et al., 2010). In case of weak interactions, complexes can be fixed by treating with cross-linking chemicals either in live cells (Stingl et al., 2008; Yong et al., 2010) or cell lysates (Sinz, 2010).

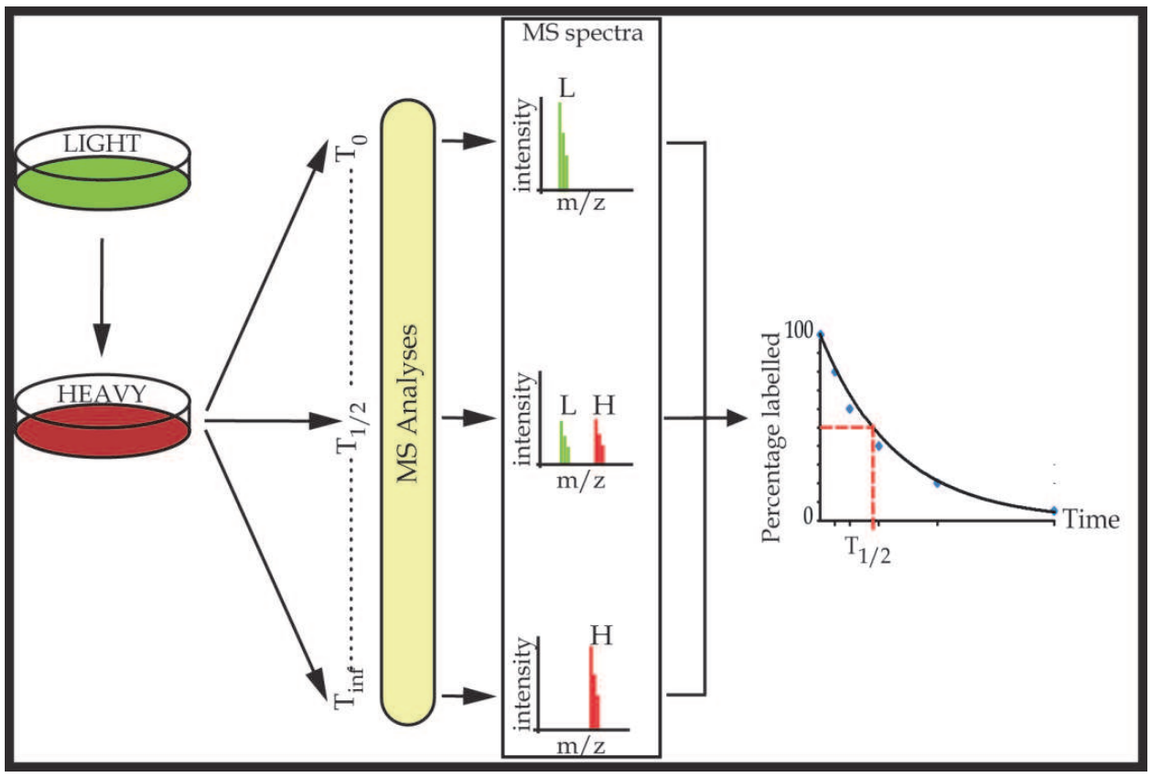
In general, quantitation greatly helps to discriminate false positive from true interactors. SILAC quantitation is more accurate and has advantages for protein complexes that are difficult to purify and where enrichment over background is smaller. As in all SILAC experiments, two differently labeled samples, here a control and a sample enriched in the target complex, are mixed and relative abundances of protein ratios are extracted from the MS spectra. In case of AP-MS, background proteins have a ratio of one as they bind as efficiently in both conditions. In contrast, true interactors have a high ratio as they are preferable purified from the labeled sample enriched in the complex of interest. Samples can be mixed either before (**p**urification **a**fter **m**ixing, PAM, (Wang and Huang, 2008)) or after purification (**m**ixed **a**fter **p**urification, MAP). The main drawback of PAM strategies is that dynamic interactions result in equilibrium between the light and heavy form during the purification, thus decreasing the observed ratio even for true interactors.

4. Temporal analysis of proteomes

4.1 Quantification of protein turnover by pulsed SILAC

Protein turnover and protein degradation are critical for numerous biological processes, including the cell cycle, signal transduction and apoptosis. Organelle proteomes change over time and can be quickly adapted to respond to changing conditions. Therefore, unraveling mechanisms that determine protein abundances is important for understanding organelle dynamics and regulation.

In general, quantitative approaches described before can be employed to gather snapshots of protein repertoires over time or in different conditions. However, such approaches quantify abundance changes of a given protein but fail to address what are the mechanisms underlying protein dynamics (decrease/increase turnover). Pulse-chase isotope tracer-based methods have been the method of choice to study protein degradation for decades. In such approaches, cells or animals are first metabolically pulse-labeled with radioisotope tracers (most commonly ^{3}H, ^{15}N, ^{35}S are used). After a chase period concomitant with the beginning of the experiment, loss of the radiolabel from the protein of interest is followed by scintillation or autoradiography as a readout of protein degradation. Such classical pulse chase experiments have been successfully translated to protein turnover analyses by MS. In MS-based turnover analyses, cells are only pulsed. For cells in culture, the most common approach is a modification of SILAC. Instead of combining two differently labeled cell populations, cells are pulsed with stable isotope containing amino acids (pSILAC). The replacement of amino-acids in proteins is followed by MS analysis over time to extract turnover rates. Similarly to results from a standard SILAC experiment, pSILAC yields two forms of the same peptide. At the time point corresponding to the half-life ($t_{1/2}$) of a specific protein, the intensities of the light and heavy peaks for a peptide pair derived from this protein are equal (Figure 4).



Light labeled cells (green) are pulsed with heavy amino acid containing medium (red). Cells are harvested at different time points, lysed, processed into peptides and analyzed independently. Ratios H/L are derived from intensities of heavy (H, red) and light (L, green) peptide peaks in the MS spectra. Note, at protein half life ($T_{1/2}$) intensity of light and heavy peptides are equal in the mass spectra. Then loss of light label can be calculated over time and turnover rates are extracted from non-linear curve fitting.

Fig. 4. pSILAC to analyze protein turnover.

To date, pSILAC has been mostly applied to cells in culture: *Saccharomyces cerevisiae* (Pratt et al., 2002), *Streptomyces coelicolor* (Jayapal et al., 2010) and human cells (Doherty et al., 2009; Schwanhäusser et al., 2011; Zee et al., 2010). Schwanhäusser et al. measured protein abundances and turnover of more than 5,000 proteins in HeLa cells. Combination of pSILAC with metabolic pulse labeling of mRNA in the same cells enabled comparison of proteins and mRNAs turnover at an unprecedented depth.

At steady state, protein levels are constant. Therefore synthesis and degradation rates are equal. This feature is critical when comparing proteome turnover, but this assumption may not always be fulfilled when comparing different conditions. An alternative approach overcoming this limitation is to follow synthesis and degradation independently from each other. To follow regulation of protein synthesis during changes of cellular iron, Schwannäusser et al. combined control (non-treated) and iron-treated HeLa cells that have been differently SILAC pulsed (Schwanhäusser et al., 2009). At the beginning of the experiment, cells in both conditions were identically light labeled (L). Concomitantly with iron treatment, cells were differently pulsed with medium (M) or heavy (H) amino-acids. Then cell lysates were combined and analyzed by MS. The relative abundance of newly synthesized proteins in both conditions was then extracted from intensities of the M and H peaks in the MS spectrum. As expected, most of the 1311 proteins identified in this study do not present

specific synthesis regulation upon iron treatment (ratio M/H of 1). However proteins such as ferritins show up to 13 fold synthesis upregulation compared to normal conditions.

4.2 Analysis of cell signaling as an example of dynamic PTM analysis

Unbiased, quantitative proteomics enables studying signaling networks in a time resolved manner. Protein abundance is often the output of complete signaling cascades. MS can detect PTM changes as an early response to system perturbations, as well as the changed protein abundance as the output of a signaling cascade.

Combination of phosphoproteomics with quantitative approaches such as SILAC enables the temporal analysis of signaling networks. In these experiments, cells are labeled in different SILAC states and stimulated for different periods of time. Applying this methodology, Olsen et al. quantified 6,600 phosphorylation sites in HeLa cells at 5 different time points after stimulation with **e**pidermal **g**rowth **f**actor (EGF) (Olsen et al., 2006). These experiments revealed insights into the early response of cells to EGF stimulation. Interestingly, this study showed that different phosphorylation sites on the same protein can react with completely different timing to a stimulus. Such results are easiest obtained by MS because it allows for detection of phosphorylation changes at particular amino acids of the protein sequence. Effects at different sites can otherwise easily be missed with classical methods that detect total phosphorylation of a protein. Other studies used a combination of SILAC labeling and different drug treatments to analyze the proteome and phosphoproteome of HeLa cells over the complete cell cycle (Olsen et al., 2010). A similar study in *Saccharomyces cerevisiae* used a mutant of the cell cycle kinase Cdk1 that allows inhibition of the kinase with an ATP analogue (Holt et al., 2009). Another recent phosphoproteome revealed great insights into the early differentiation of embryonic stem cells after stimulation with a diacylglycerol analogue. This study yielded roughly 20,000 phosphosites with almost 50% of them responding to the stimulus (Rigbolt et al., 2011). These experiments help to dissect complex signaling networks since proteins are clustered into certain groups according to their response. In addition, the high resolution datasets serve as a great resource for the scientific community and provide data for further analyses to generate models for signaling networks.

5. Conclusion

Quantitative mass spectrometry based proteomics emerged over the last few years as a crucial technique for cell biology and biochemistry research. The exciting developments in this field discussed in this chapter have provided unexpected aspects of organelle dynamics, protein turnover and PTMs. Future developments in methodology, computation and technical developments will make these technologies accessible for a larger group of scientists. This should help to generate more high quality datasets which will serve as a reference for the larger scientific community. In addition, integration of proteomics data with data from other system-wide approaches, such as genetic screens or transcriptome analysis, will help to understand complex biological processes.

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7. References

Aebersold, R., and M. Mann. 2003. Mass spectrometry-based proteomics. *Nature*. 422:198-207.

Aguilar, P.S., F. Fröhlich, M. Rehman, M. Shales, I. Ulitsky, A. Olivera-Couto, H. Braberg, R. Shamir, P. Walter, M. Mann, C.S. Ejsing, N.J. Krogan, and T.C. Walther. 2010. A plasma-membrane E-MAP reveals links of the eisosome with sphingolipid metabolism and endosomal trafficking. *Nature structural & molecular biology*. 17:901-8.

Andersen, J.S., C.E. Lyon, A.H. Fox, A.K.L. Leung, Y.W. Lam, H. Steen, M. Mann, and A.I. Lamond. 2002. Directed proteomic analysis of the human nucleolus. *Current biology: CB*. 12:1-11.

Andersen, J.S., C.J. Wilkinson, and T. Mayor. 2003. Proteomic characterization of the human centrosome by protein correlation profiling. *Nature*. 426.

Beausoleil, S.A., J. Villen, S.A. Gerber, J. Rush, and S.P. Gygi. 2006. A probability-based approach for high-throughput protein phosphorylation analysis and site localization. *Nat Biotechnol*. 24:1285-92.

Bunkenborg, J., B.J. Pilch, A.V. Podtelejnikov, and J.R. Wisniewski. 2004. Screening for N-glycosylated proteins by liquid chromatography mass spectrometry. *Proteomics*. 4:454-65.

Choudhary, C., C. Kumar, F. Gnad, M.L. Nielsen, M. Rehman, T.C. Walther, J.V. Olsen, and M. Mann. 2009. Lysine acetylation targets protein complexes and co-regulates major cellular functions. *Science*. 325:834-40.

Choudhary, C., and M. Mann. 2010. Decoding signalling networks by mass spectrometry-based proteomics. *Nat Rev Mol Cell Biol*. 11:427-39.

Christiano, R., M. Amessou, G. Shi, M. Azoulay, A. Blanpain, H. Drobecq, O. Melnyk, J.-C. Florent, and L. Johannes. 2010. Chemistry-based protein modification strategy for endocytic pathway analysis. *Biology of the cell* 102:351-9.

Corthals, G.L., R. Aebersold, and D.R. Goodlett. 2005. Identification of phosphorylation sites using microimmobilized metal affinity chromatography. *Methods Enzymol*. 405:66-81.

de Godoy, L.M., J.V. Olsen, J. Cox, M.L. Nielsen, N.C. Hubner, F. Frohlich, T.C. Walther, and M. Mann. 2008. Comprehensive mass-spectrometry-based proteome quantification of haploid versus diploid yeast. *Nature*. 455:1251-4.

Dengjel, J., L. Jakobsen, and J.S. Andersen. 2010. Organelle proteomics by label-free and SILAC-labeled protein correlation profiling. *Methods in Molecular Biology*. 658:255-265.

Doherty, M.K., D.E. Hammond, M.J. Clague, S.J. Gaskell, and R.J. Beynon. 2009. Turnover of the human proteome: determination of protein intracellular stability by dynamic SILAC. *Journal of proteome research*. 8:104-12.

Duclos, S., G. Clavarino, G. Rousserie, G. Goyette, J. Boulais, V. Camossetto, E. Gatti, S. LaBoissière, P. Pierre, and M. Desjardins. 2011. The endosomal proteome of macrophage and dendritic cells. *Proteomics*. 11:854-64.

Duclos, S., and M. Desjardins. 2011. Gel-Free Proteomics. *Methods in Molecular Biology*. 753:117-128.

Dunkley, T.P.J., R. Watson, J.L. Griffin, P. Dupree, and K.S. Lilley. 2004. Localization of organelle proteins by isotope tagging (LOPIT). *Molecular & cellular proteomics : MCP*. 3:1128-34.

Elia, G. 2008. Biotinylation reagents for the study of cell surface proteins. *Proteomics*. 8:4012-24.

Gavin, A.-C., P. Aloy, P. Grandi, R. Krause, M. Boesche, M. Marzioch, C. Rau, L.J. Jensen, S. Bastuck, B. Dümpelfeld, A. Edelmann, M.-A. Heurtier, V. Hoffman, C. Hoefert, K. Klein, M. Hudak, A.-M. Michon, M. Schelder, M. Schirle, M. Remor, T. Rudi, S. Hooper, A. Bauer, T. Bouwmeester, G. Casari, G. Drewes, G. Neubauer, J.M. Rick, B. Kuster, P. Bork, R.B. Russell, and G. Superti-Furga. 2006. Proteome survey reveals modularity of the yeast cell machinery. *Nature*. 440:631-6.

Geiger, T., J. Cox, P. Ostasiewicz, J.R. Wisniewski, and M. Mann. 2010. Super-SILAC mix for quantitative proteomics of human tumor tissue. *Nat Methods*. 7:383-5.

Gnad, F., J. Gunawardena, and M. Mann. 2011. PHOSIDA 2011: the posttranslational modification database. *Nucleic Acids Res*. 39:D253-60.

Holt, L.J., B.B. Tuch, J. Villen, A.D. Johnson, S.P. Gygi, and D.O. Morgan. 2009. Global analysis of Cdk1 substrate phosphorylation sites provides insights into evolution. *Science*. 325:1682-6.

Hubbard, S.C., M. Boyce, C.T. McVaugh, D.M. Peehl, and C.R. Bertozzi. 2011. Cell surface glycoproteomic analysis of prostate cancer-derived PC-3 cells. *Bioorganic & medicinal chemistry letters*. 21:4945-4950.

Hubner, N.C., A.W. Bird, J. Cox, B. Splettstoesser, P. Bandilla, I. Poser, A. Hyman, and M. Mann. 2010. Quantitative proteomics combined with BAC TransgeneOmics reveals in vivo protein interactions. *The Journal of cell biology*. 189:739-54.

Hubner, N.C., and M. Mann. 2011. Extracting gene function from protein-protein interactions using Quantitative BAC InteraCtomics (QUBIC). *Methods (San Diego, Calif.)*. 53:453-9.

Huh, W.-K., J.V. Falvo, L.C. Gerke, A.S. Carroll, R.W. Howson, J.S. Weissman, and E.K. O'Shea. 2003. Global analysis of protein localization in budding yeast. *Nature*. 425:686-91.

Islinger, M., K.W. Li, M. Loos, S. Liebler, S. Angermu, C. Eckerskorn, G. Weber, A. Abdolzade, and A. Vo. 2010. Peroxisomes from the Heavy Mitochondrial Fraction : Isolation by Zonal Free Flow Electrophoresis and Quantitative Mass Spectrometrical Characterization research articles. *Journal of Proteome Research*: 113-124.

Jaffe, J.D., H. Keshishian, B. Chang, T.A. Addona, M.A. Gillette, and S.A. Carr. 2008. Accurate inclusion mass screening: a bridge from unbiased discovery to targeted assay development for biomarker verification. *Mol Cell Proteomics*. 7:1952-62.

Jayapal, K.P., S. Sui, R.J. Philp, Y.-J. Kok, M.G.S. Yap, T.J. Griffin, and W.-S. Hu. 2010. Multitagging proteomic strategy to estimate protein turnover rates in dynamic systems. *Journal of proteome research*. 9:2087-97.

Jin, W.H., J. Dai, H. Zhou, Q.C. Xia, H.F. Zou, and R. Zeng. 2004. Phosphoproteome analysis of mouse liver using immobilized metal affinity purification and linear ion trap mass spectrometry. *Rapid Commun Mass Spectrom*. 18:2169-76.

Jutras, I., M. Houde, N. Currier, J. Boulais, S. Duclos, S. LaBoissière, E. Bonneil, P. Kearney, P. Thibault, E. Paramithiotis, P. Hugo, and M. Desjardins. 2008. Modulation of the phagosome proteome by interferon-gamma. *Molecular & cellular proteomics : MCP*. 7:697-715.

Kim, S.C., R. Sprung, Y. Chen, Y. Xu, H. Ball, J. Pei, T. Cheng, Y. Kho, H. Xiao, L. Xiao, N.V. Grishin, M. White, X.J. Yang, and Y. Zhao. 2006. Substrate and functional diversity of lysine acetylation revealed by a proteomics survey. *Mol Cell*. 23:607-18.

Kitteringham, N.R., R.E. Jenkins, C.S. Lane, V.L. Elliott, and B.K. Park. 2009. Multiple reaction monitoring for quantitative biomarker analysis in proteomics and metabolomics. *J Chromatogr B Analyt Technol Biomed Life Sci*. 877:1229-39.

Krogan, N.J., G. Cagney, H. Yu, G. Zhong, X. Guo, A. Ignatchenko, J. Li, S. Pu, N. Datta, A.P. Tikuisis, T. Punna, J.M. Peregrín-Alvarez, M. Shales, X. Zhang, M. Davey, M.D. Robinson, A. Paccanaro, J.E. Bray, A. Sheung, B. Beattie, D.P. Richards, V. Canadien, A. Lalev, F. Mena, P. Wong, A. Starostine, M.M. Canete, J. Vlasblom, S. Wu, C. Orsi, S.R. Collins, S. Chandran, R. Haw, J.J. Rilstone, K. Gandi, N.J. Thompson, G. Musso, P. St Onge, S. Ghanny, M.H.Y. Lam, G. Butland, A.M. Altaf-Ul, S. Kanaya, A. Shilatifard, E. O'Shea, J.S. Weissman, C.J. Ingles, T.R. Hughes, J. Parkinson, M. Gerstein, S.J. Wodak, A. Emili, and J.F. Greenblatt. 2006. Global landscape of protein complexes in the yeast Saccharomyces cerevisiae. *Nature*. 440:637-43.

Kruger, M., M. Moser, S. Ussar, I. Thievessen, C.A. Luber, F. Forner, S. Schmidt, S. Zanivan, R. Fassler, and M. Mann. 2008. SILAC mouse for quantitative proteomics uncovers kindlin-3 as an essential factor for red blood cell function. *Cell*. 134:353-64.

Kuster, B., and M. Mann. 1999. 18O-labeling of N-glycosylation sites to improve the identification of gel-separated glycoproteins using peptide mass mapping and database searching. *Anal Chem*. 71:1431-40.

Laragione, T., V. Bonetto, F. Casoni, T. Massignan, G. Bianchi, E. Gianazza, and P. Ghezzi. 2003. Redox regulation of surface protein thiols: identification of integrin alpha-4 as a molecular target by using redox proteomics. *Proceedings of the National Academy of Sciences of the United States of America*. 100:14737-41.

Larsen, M.R., T.E. Thingholm, O.N. Jensen, P. Roepstorff, and T.J. Jorgensen. 2005. Highly selective enrichment of phosphorylated peptides from peptide mixtures using titanium dioxide microcolumns. *Mol Cell Proteomics*. 4:873-86.

Lilley, K.S., and T.P.J. Dunkley. 2008. Determination of genuine residents of plant endomembrane organelles using isotope tagging and multivariate statistics. *Methods in molecular biology (Clifton, N.J.)*. 432:373-87.

Malmstrom, J., M. Beck, A. Schmidt, V. Lange, E.W. Deutsch, and R. Aebersold. 2009. Proteome-wide cellular protein concentrations of the human pathogen Leptospira interrogans. *Nature*. 460:762-5.

Matsuyama, A., R. Arai, Y. Yashiroda, A. Shirai, A. Kamata, S. Sekido, Y. Kobayashi, A. Hashimoto, M. Hamamoto, Y. Hiraoka, S. Horinouchi, and M. Yoshida. 2006. ORFeome cloning and global analysis of protein localization in the fission yeast Schizosaccharomyces pombe. *Nature biotechnology*. 24:841-7.

Michalski, A., E. Damoc, J.P. Hauschild, O. Lange, A. Wieghaus, A. Makarov, N. Nagaraj, J. Cox, M. Mann, and S. Horning. 2011. Mass Spectrometry-based Proteomics Using Q Exactive, a High-performance Benchtop Quadrupole Orbitrap Mass Spectrometer. *Mol Cell Proteomics*. 10:M111 011015.

Michelsen, U., and J. von Hagen. 2009. Isolation of subcellular organelles and structures. *Methods in enzymology*. 463:305-28.

Monetti, M., N. Nagaraj, K. Sharma, and M. Mann. 2011. Large-scale phosphosite quantification in tissues by a spike-in SILAC method. *Nat Methods*. 8:655-8.

Mueller, L.N., M.Y. Brusniak, D.R. Mani, and R. Aebersold. 2008. An assessment of software solutions for the analysis of mass spectrometry based quantitative proteomics data. *J Proteome Res*. 7:51-61.

Muszynska, G., G. Dobrowolska, A. Medin, P. Ekman, and J.O. Porath. 1992. Model studies on iron(III) ion affinity chromatography. II. Interaction of immobilized iron(III) ions with phosphorylated amino acids, peptides and proteins. *J Chromatogr*. 604:19-28.

Nagaraj, N., R.C. D'Souza, J. Cox, J.V. Olsen, and M. Mann. 2010. Feasibility of large-scale phosphoproteomics with higher energy collisional dissociation fragmentation. *J Proteome Res*. 9:6786-94.

Nielsen, M.L., M. Vermeulen, T. Bonaldi, J. Cox, L. Moroder, and M. Mann. 2008. Iodoacetamide-induced artifact mimics ubiquitination in mass spectrometry. *Nat Methods*. 5:459-60.

Olsen, J.V., B. Blagoev, F. Gnad, B. Macek, C. Kumar, P. Mortensen, and M. Mann. 2006. Global, in vivo, and site-specific phosphorylation dynamics in signaling networks. *Cell*. 127:635-48.

Olsen, J.V., M. Vermeulen, A. Santamaria, C. Kumar, M.L. Miller, L.J. Jensen, F. Gnad, J. Cox, T.S. Jensen, E.A. Nigg, S. Brunak, and M. Mann. 2010. Quantitative phosphoproteomics reveals widespread full phosphorylation site occupancy during mitosis. *Sci Signal*. 3:ra3.

Ong, S.E., B. Blagoev, I. Kratchmarova, D.B. Kristensen, H. Steen, A. Pandey, and M. Mann. 2002. Stable isotope labeling by amino acids in cell culture, SILAC, as a simple and accurate approach to expression proteomics. *Mol Cell Proteomics*. 1:376-86.

Ong, S.E., G. Mittler, and M. Mann. 2004. Identifying and quantifying in vivo methylation sites by heavy methyl SILAC. *Nat Methods*. 1:119-26.

Ow, S.Y., M. Salim, J. Noirel, C. Evans, I. Rehman, and P.C. Wright. 2009. iTRAQ underestimation in simple and complex mixtures: "the good, the bad and the ugly". *J Proteome Res*. 8:5347-55.

Peng, J., D. Schwartz, J.E. Elias, C.C. Thoreen, D. Cheng, G. Marsischky, J. Roelofs, D. Finley, and S.P. Gygi. 2003. A proteomics approach to understanding protein ubiquitination. *Nat Biotechnol*. 21:921-6.

Pinkse, M.W.H., P.M. Uitto, M.J. Hilhorst, B. Ooms, and A.J.R. Heck. 2004. Selective Isolation at the Femtomole Level of Phosphopeptides from Proteolytic Digests Using 2D-NanoLC-ESI-MS/MS and Titanium Oxide Precolumns. *Analytical Chemistry*. 76:3935-3943.

Pratt, J.M., J. Petty, I. Riba-garcia, D.H.L. Robertson, S.J. Gaskell, S.G. Oliver, and R.J. Beynon. 2002. Dynamics of Protein Turnover , a Missing Dimension in Proteomics *. *Molecular & Cellular Proteomics*:579-591.

Rigbolt, K.T., T.A. Prokhorova, V. Akimov, J. Henningsen, P.T. Johansen, I. Kratchmarova, M. Kassem, M. Mann, J.V. Olsen, and B. Blagoev. 2011. System-wide temporal characterization of the proteome and phosphoproteome of human embryonic stem cell differentiation. *Sci Signal*. 4:rs3.

Ross, P.L., Y.N. Huang, J.N. Marchese, B. Williamson, K. Parker, S. Hattan, N. Khainovski, S. Pillai, S. Dey, S. Daniels, S. Purkayastha, P. Juhasz, S. Martin, M. Bartlet-Jones, F. He, A. Jacobson, and D.J. Pappin. 2004. Multiplexed protein quantitation in

Saccharomyces cerevisiae using amine-reactive isobaric tagging reagents. *Mol Cell Proteomics*. 3:1154-69.

Scheurer, S.B., C. Roesli, D. Neri, and G. Elia. 2005. A comparison of different biotinylation reagents, tryptic digestion procedures, and mass spectrometric techniques for 2-D peptide mapping of membrane proteins. *Proteomics*. 5:3035-9.

Schmidt, A., M. Claassen, and R. Aebersold. 2009. Directed mass spectrometry: towards hypothesis-driven proteomics. *Curr Opin Chem Biol*. 13:510-7.

Schroeder, M.J., J. Shabanowitz, J.C. Schwartz, D.F. Hunt, and J.J. Coon. 2004. A neutral loss activation method for improved phosphopeptide sequence analysis by quadrupole ion trap mass spectrometry. *Anal Chem*. 76:3590-8.

Schwanhäusser, B., D. Busse, N. Li, G. Dittmar, J. Schuchhardt, J. Wolf, W. Chen, and M. Selbach. 2011. Global quantification of mammalian gene expression control. *Nature*. 473:337-42.

Schwanhäusser, B., M. Gossen, G. Dittmar, and M. Selbach. 2009. Global analysis of cellular protein translation by pulsed SILAC. *Proteomics*. 9:205-9.

Selbach, M., and M. Mann. 2006. Protein interaction screening by quantitative immunoprecipitation combined with. *Nature Methods*. 3:981-983.

Sinz, A. 2010. Investigation of protein-protein interactions in living cells by chemical crosslinking and mass spectrometry. *Analytical and bioanalytical chemistry*. 397:3433-40.

Soufi, B., C.D. Kelstrup, G. Stoehr, F. Frohlich, T.C. Walther, and J.V. Olsen. 2009. Global analysis of the yeast osmotic stress response by quantitative proteomics. *Mol Biosyst*. 5:1337-46.

Stingl, K., K. Schauer, C. Ecobichon, A. Labigne, P. Lenormand, J.-C. Rousselle, A. Namane, and H. de Reuse. 2008. In vivo interactome of Helicobacter pylori urease revealed by tandem affinity purification. *Molecular & cellular proteomics : MCP*. 7:2429-41.

Syka, J.E., J.J. Coon, M.J. Schroeder, J. Shabanowitz, and D.F. Hunt. 2004. Peptide and protein sequence analysis by electron transfer dissociation mass spectrometry. *Proc Natl Acad Sci U S A*. 101:9528-33.

Taylor, S.W., E. Fahy, B. Zhang, G.M. Glenn, D.E. Warnock, S. Wiley, A.N. Murphy, S.P. Gaucher, R.a. Capaldi, B.W. Gibson, and S.S. Ghosh. 2003. Characterization of the human heart mitochondrial proteome. *Nature biotechnology*. 21:281-6.

Teckchandani, A., N. Toida, J. Goodchild, C. Henderson, J. Watts, B. Wollscheid, and J.a. Cooper. 2009. Quantitative proteomics identifies a Dab2/integrin module regulating cell migration. *The Journal of cell biology*. 186:99-111.

Tholey, A., J. Reed, and W.D. Lehmann. 1999. Electrospray tandem mass spectrometric studies of phosphopeptides and phosphopeptide analogues. *J Mass Spectrom*. 34:117-23.

Trinkle-Mulcahy, L., S. Boulon, Y.W. Lam, R. Urcia, F.-M. Boisvert, F. Vandermoere, N.a. Morrice, S. Swift, U. Rothbauer, H. Leonhardt, and A. Lamond. 2008. Identifying specific protein interaction partners using quantitative mass spectrometry and bead proteomes. *The Journal of cell biology*. 183:223-39.

Varki, A. 2009. Essentials of glycobiology. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. xxix, 784 p. pp.

Vermeulen, M., H.C. Eberl, F. Matarese, H. Marks, S. Denissov, F. Butter, K.K. Lee, J.V. Olsen, A.A. Hyman, H.G. Stunnenberg, and M. Mann. 2010. Quantitative

interaction proteomics and genome-wide profiling of epigenetic histone marks and their readers. *Cell*. 142:967-80.

Villen, J., and S.P. Gygi. 2008. The SCX/IMAC enrichment approach for global phosphorylation analysis by mass spectrometry. *Nat Protoc*. 3:1630-8.

Walther, T.C., and M. Mann. 2010. Mass spectrometry–based proteomics in cell biology. *Cell*.

Wang, X., and L. Huang. 2008. Identifying dynamic interactors of protein complexes by quantitative mass spectrometry. *Molecular & cellular proteomics : MCP*. 7:46-57.

Wiederhold, E., L.M. Veenhoff, B. Poolman, and D.J. Slotboom. 2010. Proteomics of Saccharomyces cerevisiae Organelles. *Molecular & cellular proteomics : MCP*. 9:431-45.

Wolf-Yadlin, A., S. Hautaniemi, D.A. Lauffenburger, and F.M. White. 2007. Multiple reaction monitoring for robust quantitative proteomic analysis of cellular signaling networks. *Proc Natl Acad Sci U S A*. 104:5860-5.

Wong, J.W., and G. Cagney. 2010. An overview of label-free quantitation methods in proteomics by mass spectrometry. *Methods Mol Biol*. 604:273-83.

Xu, G., J.S. Paige, and S.R. Jaffrey. 2010. Global analysis of lysine ubiquitination by ubiquitin remnant immunoaffinity profiling. *Nature biotechnology*. 28:868-73.

Yang, L., J.O. Nyalwidhe, S. Guo, R.R. Drake, and O.J. Semmes. 2011. Targeted Identification of Metastasis-associated Cell-surface Sialoglycoproteins in Prostate Cancer. *Molecular & cellular proteomics : MCP*. 10:M110.007294.

Yong, J., M. Kasim, J.L. Bachorik, L. Wan, and G. Dreyfuss. 2010. Gemin5 delivers snRNA precursors to the SMN complex for snRNP biogenesis. *Molecular cell*. 38:551-62.

Zee, B.M., R.S. Levin, P.A. Dimaggio, and B.A. Garcia. 2010. Global turnover of histone post-translational modifications and variants in human cells. *Epigenetics & Chromatin*. 3:22.

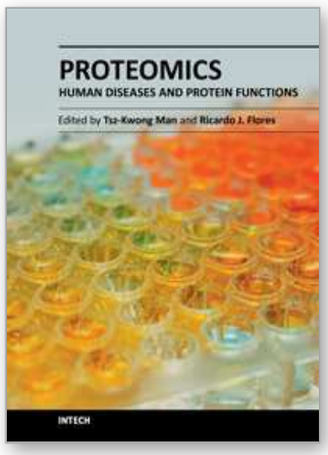
Zhang, H., X.J. Li, D.B. Martin, and R. Aebersold. 2003. Identification and quantification of N-linked glycoproteins using hydrazide chemistry, stable isotope labeling and mass spectrometry. *Nat Biotechnol*. 21:660-6.

Zhang, Y., M. Askenazi, J. Jiang, C.J. Luckey, J.D. Griffin, and J.A. Marto. 2010. A robust error model for iTRAQ quantification reveals divergent signaling between oncogenic FLT3 mutants in acute myeloid leukemia. *Mol Cell Proteomics*. 9:780-90.

Zhao, Y., W. Zhang, Y. Kho, and Y. Zhao. 2004. Proteomic analysis of integral plasma membrane proteins. *Analytical chemistry*. 76:1817-23.

Zielinska, D.F., F. Gnad, J.R. Wisniewski, and M. Mann. 2010. Precision mapping of an in vivo N-glycoproteome reveals rigid topological and sequence constraints. *Cell*. 141:897-907.

Zubarev, R.A., D.M. Horn, E.K. Fridriksson, N.L. Kelleher, N.A. Kruger, M.A. Lewis, B.K. Carpenter, and F.W. McLafferty. 2000. Electron capture dissociation for structural characterization of multiply charged protein cations. *Anal Chem*. 72:563-73.



Proteomics - Human Diseases and Protein Functions
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Biomedical research has entered a new era of characterizing a disease or a protein on a global scale. In the post-genomic era, Proteomics now plays an increasingly important role in dissecting molecular functions of proteins and discovering biomarkers in human diseases. Mass spectrometry, two-dimensional gel electrophoresis, and high-density antibody and protein arrays are some of the most commonly used methods in the Proteomics field. This book covers four important and diverse areas of current proteomic research: Proteomic Discovery of Disease Biomarkers, Proteomic Analysis of Protein Functions, Proteomic Approaches to Dissecting Disease Processes, and Organelles and Secretome Proteomics. We believe that clinicians, students and laboratory researchers who are interested in Proteomics and its applications in the biomedical field will find this book useful and enlightening. The use of proteomic methods in studying proteins in various human diseases has become an essential part of biomedical research.

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