We are IntechOpen, the world's leading publisher of Open Access books
Built by scientists, for scientists

6,900
Open access books available

185,000
International authors and editors

200M
Downloads

154
Countries delivered to

Our authors are among the

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

BOOK CITATION INDEX
CLARIVATE ANALYTICS
INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

A Critical Review of Trypsin Digestion for LC-MS Based Proteomics

Hanne Kolsrud Hustoft, Helle Malerod, Steven Ray Wilson,
Leon Reubsaet, Elsa Lundanes and Tyge Greibrokk
University of Oslo
Norway

1. Introduction

Proteomics is defined as the large-scale study of proteins in particular for their structures and functions (Anderson and Anderson 1998), and investigations of proteins have become very important since they are the main components of the physiological metabolic pathways in eukaryotic cells. Proteomics increasingly plays an important role in areas like protein interaction studies, biomarker discovery, cancer prevention, drug treatment and disease screening medical diagnostics (Capelo et al. 2009).

Proteomics can be performed either in a comprehensive or "shotgun" mode, where proteins are identified in complex mixtures, or as "targeted proteomics" where "selective reaction monitoring" (SRM) is used to choose in advance the proteins to observe, and then measuring them accurately, by optimizing the sample preparation as well as the LC-MS method in accordance to the specific proteins (Mitchell 2010).

Whether "MS-based shotgun proteomics" has accomplished anything at all regarding clinically useful results was recently addressed by Peter Mitchell in a feature article (Mitchell 2010), and he states that the field needs to make a further step or even change direction. Referring to discussions with among others John Yates and Matthias Mann, Mitchell addresses the failure in the search for biomarkers as indicators of disease, the difficulties of protein arrays, the uncertainty of quantification in "shotgun proteomics" (due to among others the efficiency of ionization in the mass spectrometers), database shortcomings, the problems of detecting post translational modifications (PTMs), and finally the huge disappointment in the area of drug discovery. The field points in the direction of targeted proteomics, but targeted proteomics will not be the solution to all our questions and comprehensive proteomics will still be needed. In order to get as much information, with as high quality as possible, from a biological sample, both the sample preparation and the final LC-MS analyses need to be optimized.

The most important step in the sample preparation for proteomics is the conversion of proteins to peptides and in most cases trypsin is used as enzyme. Trypsin is a protease that specifically cleaves the proteins creating peptides both in the preferred mass range for MS sequencing and with a basic residue at the carboxyl terminus of the peptide, producing information-rich, easily interpretable peptide fragmentation mass spectra. Some other proteases can be used as well, such as Lys-C, which is active in more harsh conditions with 8 M urea, and give larger fragments than trypsin. Asp-N and Glu-C are also highly sequence-

specific proteases, but less active than the previously mentioned. Other less sequence-specific proteases are generally avoided since they create complex mixtures of peptides, difficult to interpret (Steen and Mann 2004). During a chromatographic separation of a complex mixture of peptides derived from a tryptic digestion, thousands of mass spectra are produced and sophisticated software is necessary to find matching proteins to the peptides identified. In complex proteomic samples, protein identification is performed by searching databases with search engines like Mascot, Sequest or Phenyx (IS 2011).

Protein identification traditionally follows two different workflows depending on the approach (Figure 1). In the gel electrophoresis-based approach the proteins are separated in one or two dimensions (1D/2D) on a gel and enzymatic digestion is performed in-gel, which is a time-consuming and tedious process (López-Ferrer et al. 2006). In the gel-free or in-solution based approach, the proteins or peptides, or both, are separated chromatographically using on-line LC systems and the proteins are digested in-solution
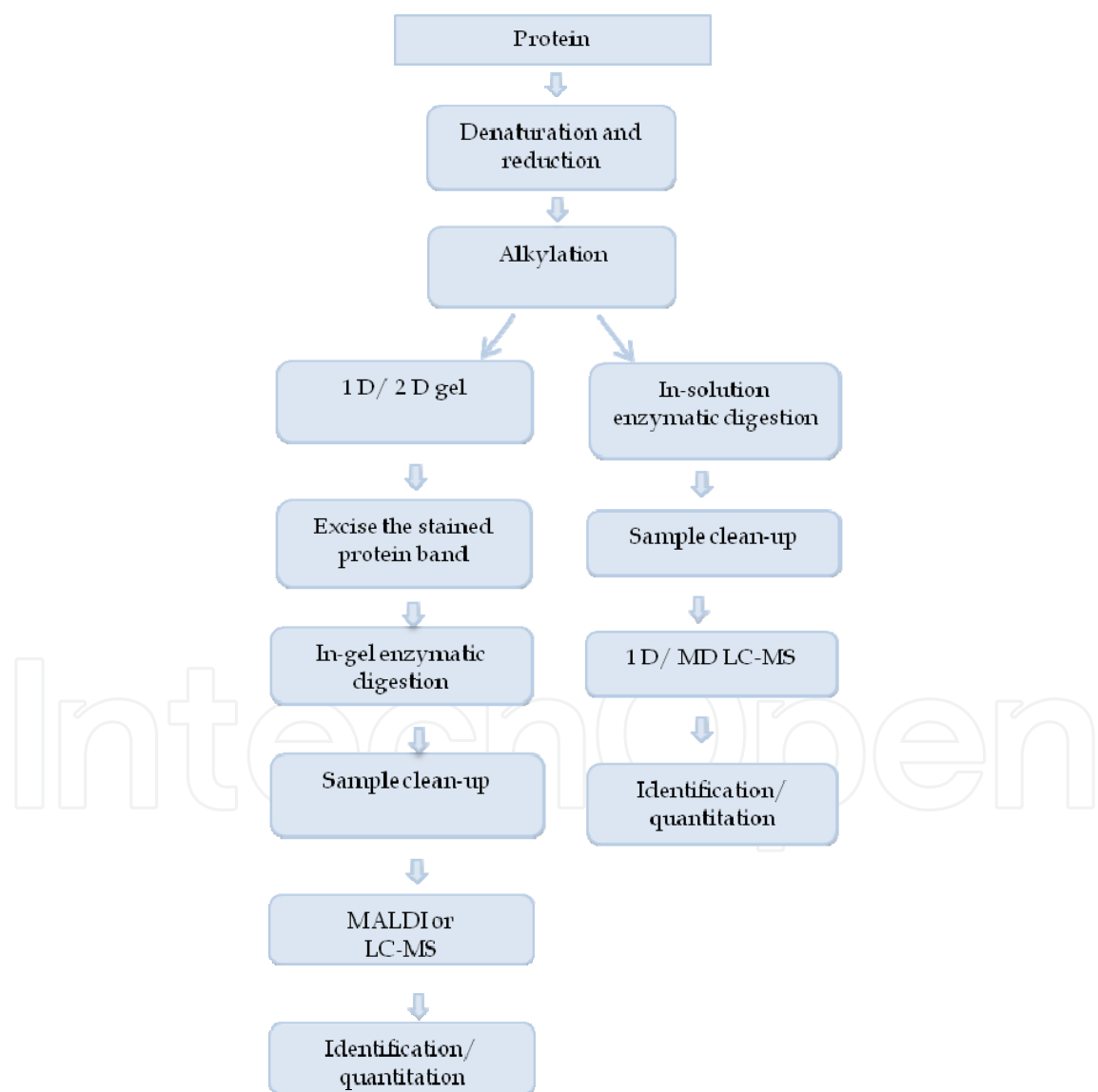

Fig. 1. Workflows of in-gel (left) and in-solution (right) digestion and subsequent LC-MS analysis of a protein sample.

(Capelo et al. 2009). The in-solution based approach tends to be the simplest in terms of sample handling and speed, but on the other hand it requires sophisticated LC-MS instrumentation which again requires constant maintenance.

The digestion step is the most time consuming step in the sample preparation workflow and different techniques to accelerate this procedure have been developed. Comparing these techniques, including some of our own experiments, the question of how we can evaluate the digestion efficiency materialized. The amino acid sequence coverage (SQ %) is often used as a measure for both the completeness of the protein digestion and the detection efficiency of the various tryptic peptides, and is a common way in proteomics to define the digestion rate (Xu et al. 2010). However, SQ % might be a misleading parameter to use, as different mass spectrometers and different search parameters in subsequent data analysis may reveal various SQ %. In addition it is of principal importance to relate SQ % to the degree of miss – cleavage peptides used to calculate this value: a high SQ % calculated from tryptic peptides without missed cleavages indicated a more complete digest than the same high SQ % calculated from tryptic peptides with many missed cleavages.

To get some information of the digestion efficiency, as a check before performing the data analysis, the possible presence of intact protein in the total ion chromatogram (TIC) may be used. However, this method can only be used for proteins small enough to be detected by the MS, such as cytochrome-C (cyt-C) (unless you have a MALDI MS available). On the other hand, evaluating the digestion rate this way, using an easily digested protein such as cyt-C, will give a good indication of the efficiency of the method; if an intact protein peak from cyt-C is detected in the chromatogram, then the digestion can be considered insufficient. Other non-protein reagents that are cleaved by trypsin might also be used as an internal standard when performing tryptic digestion of a complex sample, to have control over the digestion efficiency.

For quantitation of proteins it is necessary to find relevant indicators of their abundance in the mass spectrometer output. Several ways of protein quantitation have been suggested and they can be divided into two main categories; the isotope based and the label free methods. Two papers which give good overviews over the different labeling methods have been published recently (Capelo et al. 2010; Vaudel et al. 2010). In brief; the main modern strategies for isotopic labeling are divided into metabolic labeling at cell growth called SILAC, chemical labeling at protein level, called iCAT, enzymatic labeling at peptide level, after protein digestion like iTRAQ and labeling during protein digestion, such as [18]O labeling (Capelo et al. 2010). SILAC can only be used for samples which are produced using labeled amino acids, while the other methods can be used for all types of protein samples. Thiede *et al.* have recently introduced a promising new labeling method with relative or absolute quantification for identification and quantification of two differentially labelled states using MS/MS spectra, and which is called isobaric peptide termini labeling (IPTL) (Thiede and Koehler 2010). The method involve digestion of the protein samples and cross-wise labeling of N- and C-terminal ends of the obtained peptides, like the principle in [18]O labeling (Thiede and Koehler 2010).

The digestion efficiency in comprehensive proteomics is as important as the digestion repeatability in targeted proteomics. Everyone working in this field should strive to have control over these parameters during the sample preparation in proteomics, producing correctly identified proteins and reliable results. The focus in this review is on the in-solution based protocols in comprehensive proteomics, with emphasis on in-solution tryptic digestion and alternative methods to speed up the digestion, and also on how to evaluate the digestion efficiency of the used method.

2. Factors influencing proteolytic results

An issue that is little discussed in the literature of proteomics is the sample handling prior to the protein digestion. Some mention the need for enrichment, or elimination of interfering substances (López-Ferrer et al. 2006), but few focus on the steps prior to the enzymatic digestion of the protein fractions.

2.1 Protein concentration

A proper digestion procedure starts with the measurement of the protein content of the sample. This is necessary to determine for, among others, the needed amount of reduction and alkylation reagents, as well as the amount of enzyme in in-solution digestion. Quantifying the protein content of a sample separated on a gel is often relatively easy. In this case, guidelines of intensity of the stained gel-band can be used as a "semi-quantitative" measurement. The amount of the total protein content of gel-free samples can be measured with standard procedures like the NanoDrop (detection down to 10-15 µg/ml, using 2 µl sample) (NanoDrop 2011), the Bradford assay (detection down to 2.5 µg/ml, using 150 µl sample) (Bradford 1976) and the BCA assay (detection down to 20 µg/ml, using 25 µl sample) (Smith et al. 1985).

2.2 Keratin contamination

Avoiding keratin contamination, which is a problem common in both 1D or 2D gel and in-solution methods, but mostly in the gel-based analysis (Bell et al. 2009), is important. Keratins are naturally occurring structural proteins and appear more often in the sample as interference from the environment rather than from natural abundance. Fingerprints, hair, dead skin flakes, wool clothing, dust and latex gloves are common sources of contaminating keratins (Greenebaum 2011). If keratins are present at concentration levels greater than that of the protein of interest, their abundance will overwhelm the analytical capacity of the LC-MS system and obscure the protein of interest. This is particularly problematic when performing data dependent mass spectrometry, as the peptides from the more abundant keratins will be selected for tandem-MS analysis, providing little or no information about the actual proteins of interest. However, at low concentration levels, compared to the protein of interest, keratins are not a problem at all (Greenebaum 2011).

2.3 Detergents

Detergents are often used for total solubilisation of cells and tissues in biochemical studies, and sodium dodecyl sulphate (SDS) is often the choice. However, even at low concentrations, detergents can give rise to problems both concerning enzymatic digestion and in the subsequent LC-MS analysis. Hence it is most often necessary to deplete the detergents prior to the steps in the analytical method hampered by the detergent, or to find alternative ways to lyse the cells which are more compatible with the downstream steps in the analysis. This problem will be further discussed in section 5.2.2.

3. From proteins to peptides

3.1 Denaturation, reduction and alkylation

Prior to in-solution protein digestion the proteins in most samples need to be denatured, reduced and alkylated, using various reagents, for the proteolytic enzyme to be able to

efficiently cleave the peptide chains of the proteins. A sample preparation workflow is presented in Figure 2 together with different suggested procedures to accelerate the tryptic digestion of proteins to peptides. These methods are presented in section 5.

Procedure	Intended effect	Experiment
Protein quantitation	To add correct amount of reagents	NanoDrop, Bradford assay, BCA assay
Denaturation and reduction	Reduces diculfide binds to avoid renaturation	1+50 DTT:protein, 15 min 95 °C
Alkylation	Alkylation of SH groups, avoid renaturation	1+10 IAM:protein, 15 min in the dark
Enzymatic digestion	Cleavage of proteins to peptides	1+20 E+S, pH 8.5, 37 °C overnight
Stop enzymatic digestion	To avoid autodigestion	Addition of 2.5 % formic acid (FA)
Desalting and enrichment	Clean-up and enrichment of peptides	C18 ZipTip procedure

Speed up →

Immobilized trypsin acceleration
-Immobilized reactors
-Microspin columns
-FASP filters

Temperature related acceleration
-Heating
-Infrared radiation
-Microwave radiation

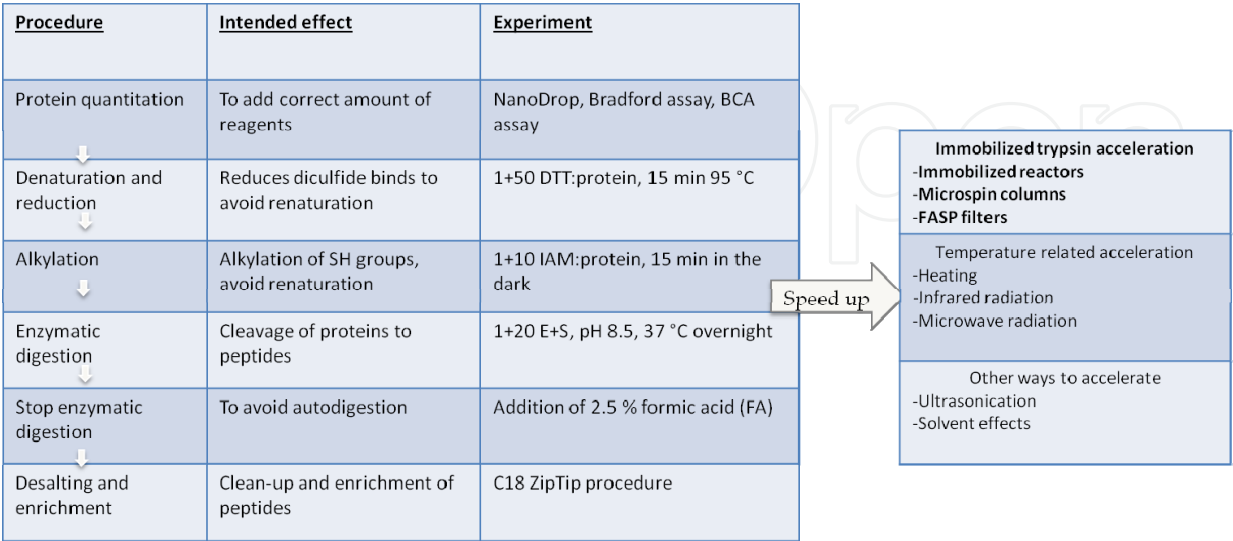Other ways to accelerate
-Ultrasonication
-Solvent effects

Fig. 2. Procedure, intended effect and experimental conditions of a classical workflow for in-solution based sample preparation approaches in proteomics. To the very right different accelerating digestion techniques are presented.

In a study by Proc *et al*. the denaturation process of human plasma proteins was examined applying 14 different combinations of heat, solvents, chaotropic agents and surfactants for their effectiveness to improve tryptic digestion (Proc et al. 2010). The experiment was performed by quantifying the production of proteolytic tryptic peptides from 45 moderate-to-high-abundance plasma proteins which were grouped into rapidly digested proteins, moderately digested proteins and proteins resistant to digestion. Proc *et al*. did not find an "optimal" digestion method for all 45 proteins, but the denaturation procedure with the surfactant sodium deoxycholate (DOC), which is more compatible with MS than SDS, together with a digestion time of 9 hours, was found to be the most promising protocol for all proteins (Proc et al. 2010).

Denaturation and reduction can often be carried out simultaneously by a combination of heat and a reagent, like 1,4-dithiothreitol (DTT) (Choudhary et al. 2002), β-mercaptoethanol (Sundqvist et al. 2007) or tris(2-carboxyethyl)phosphine (Hale et al. 2004). Most used is DTT, which is a strong reducing agent, that reduce the disulfide bonds and prevent inter and intra-molecular disulfide formation between cysteines in the protein. By combining denaturation and reduction, renaturation of the proteins due to reduction of the disulfide bonds can be avoided (see Figure 3). Renaturation can be a problem using heat solely as the denaturation agent (Strader et al. 2005; Capelo et al. 2009).

Following protein denaturation and reduction, alkylation of cysteine is necessary to further reduce the potential renaturation (Figure 3), and the most commonly used agents for alkylation of protein samples prior to digestion are iodoacetamide (IAM) and iodoacetic acid (IAA) (López-Ferrer et al. 2006; Vukovic et al. 2008).
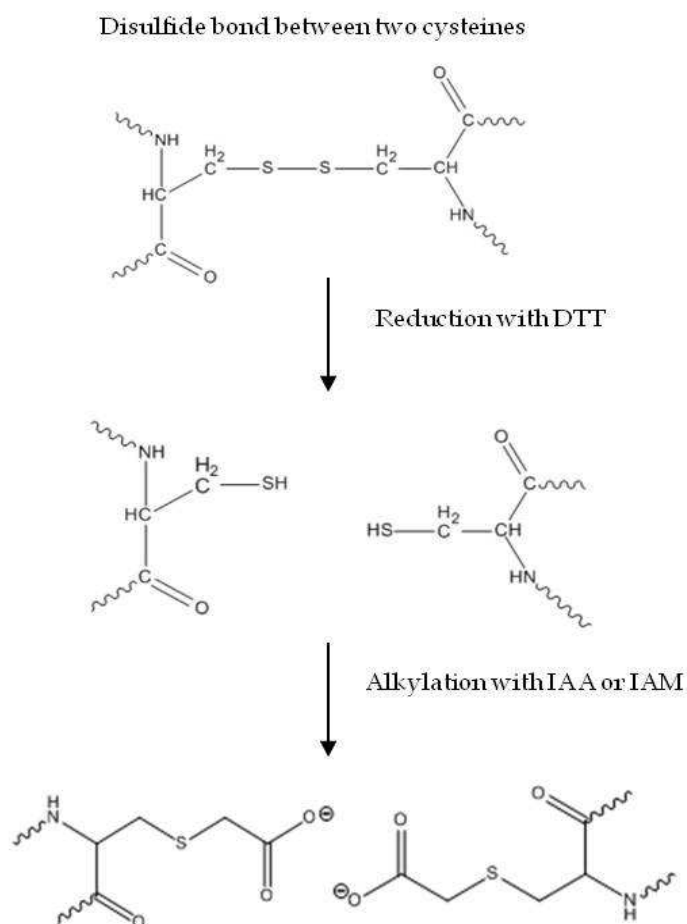
Fig. 3. The reduction and alkylation process: The breaking of disulfide bonds in proteins. Reduction by DTT to form cysteine residues must be followed by further modification of the reactive –SH groups (to prevent reformation of the disulfide bond) by acetylation by, in this case iodoacetic acid (adapted from (Nelson and Cox 2008)).

3.2 Trypsin digestion

Protein digestion with proteases is one of the key sample-preparation steps in proteomics, followed by LC-MS. As already mentioned, trypsin is the most commonly used protease for this purpose since it has a well defined specificity; it hydrolyzes only the peptide bonds in which the carbonyl group is followed either by an arginine (Arg) or lysine (Lys) residue, with the exception when Lys and Arg are N-linked to Aspartic acid (Asp). The cleavage will not occur if proline is positioned on the carboxyl side of Lys and Arg. Since trypsin is a protein it may digest itself in a process called autolysis. However, Ca^{2+}, naturally present in most samples, binds at the Ca^{2+}-binding loop in trypsin and prevents autolysis (Nord et al. 1956). With the modified trypsin presently used in most laboratories, autolysis is additionally reduced. Still addition of 1 mM $CaCl_2$ is recommended in the digestion medium, but not always absolutely necessary, when the contribution of Ca^{2+} from natural sources is low (Minnesota 2011). Tryptic digestion is performed at an optimal pH in the range 7.5-8.5 (Worthington 2011), and commonly at 37 °C for in-solution digestion. Thus prior to the addition of trypsin, a buffer is added (usually 50 mM triethyl ammonium bicarbonate (tABC) or 12.5 mM ammonium bicarbonate (ABC) buffer (López-Ferrer et al. 2006) to provide an optimal pH for the enzymatic cleavage. A 2-amino-2-hydroxymethyl-

propane-1,3-diol (Tris) buffer may also be used for this purpose, but it should be taken into consideration that the Tris buffer is incompatible with the down stream MS analysis, such as MALDI and ESI-MS, and needs to be depleted through solid phase extraction (SPE) or ZipTips prior to such (Shieh et al. 2005; Sigma-Aldrich 2011).

Information about the enzyme to protein ratio needed for digestion of a protein sample is crucial to ensure an enzyme amount sufficient to perform the digestion, but not too high resulting in autolysis products from the trypsin used. Recent experiments indicate that a sufficient ratio of enzyme to substrate (E+S) is 1+20 (Hustoft et al. 2011). For targeted proteomics it may be beneficial to perform a pilot study on the necessary digestion time for the type of sample to be analyzed, to obtain an optimal digestion efficiency of the sample. For more comprehensive proteomics a longer digestion time, up to 9 hours is recommended to ensure the best overall digestion efficiency, as described by Proc *et al.* (Proc et al. 2010). Thus dealing with these long digestion times, an overnight digestion is often more convenient, starting with the post digestion sample preparation steps the following day.

Proteins may act differently in different environments and less effective digestions have been observed when model proteins were digested in a mixture as compared to being digested separately (Hustoft et al. 2011). One reason for these observations could be increased competition for the trypsin cleavage sites, when more proteins are digested together.

As mentioned in the introduction one of the main issues regarding digestion is how to measure the digestion efficiency of a method for a given complex sample of proteins. Examples from the literature show that different groups use various measures for the efficiency of their digestive method, where amino acid SQ % is the most common. However, based on our experience, we question whether the SQ % can serve as a reliable measurement of digestion efficiency, or not? Using a relatively high concentration of 250 ng/ ml of each of the model proteins, no significant difference in SQ % could be seen with a 5 min digestion versus an overnight digestion. Thus another measure for the digestion efficiency had to be evaluated. Since cyt-C was one of the model proteins, undigested intact protein could be detected by the Ion-Trap MS being used. The area of the intact protein peak decreased with increasing digestion time, - indicating better trypsination efficiency. The size of the intact protein peak could hence in some cases be used to compare the efficiency of digestion methods (Hustoft et al. 2011). When exploring the potential of microwave oven accelerated digestion of a mixture of proteins, different temperatures were examined both for the microwave oven and the Thermoshaker control samples. A decrease in the intact protein peak of cyt-C was detected indicating better efficiency at higher temperatures, in both cases. However, the peak area of four distinct tryptic peptides from cyt-C revealed that the decrease in cyt-C peak area was caused by denaturation of the sample as a function of higher temperatures, and not because of increased digestion. Hence, the area of the undigested protein peak is not necessarily a good measure for the digestion efficiency. Another way to describe the digestion efficiency is through the yield of peptides, used to study the effect of temperature, enzyme concentration, digestion time and surface area of the gel pieces in in-gel proteomics (Havliš et al. 2003).

4. Sample handling post digestion

4.1 Clean-up and enrichment of digests

Prior to LC-MS analysis, the digests must be purified to remove e.g. buffers and salts added during the sample preparation. This is most often carried out with ZipTips, which

concentrate and purifies the samples for sensitive downstream analysis (Capelo et al. 2009). A C18 ZipTip is a 10 μl pipette tip with a 0.6 or 0.2 μl bed of C18 silica based medium fixed at its tip, used for single-step desalting, enrichment, and purification. Such ZipTips can be used for purification of, for instance peptides, proteins and nucleic acids. Purifying tryptic peptides with the C18 ZipTip results in high recovery, but noteworthy is that the capacity of the C18 ZipTips is limited; however, up to 10 μg digested protein could be loaded without losses (Hustoft et al. 2011). Another possible disadvantage of the C18 ZipTip procedure is the loss of small hydrophilic peptides which may be lost due to washing with an aqueous mobile phase containing 0.1 % trifluoroacetic acid (TFA) (Hustoft et al. 2011). Still, when the sample must be purified prior to LC-MS analysis the ZipTips are convenient to use because they are easy to handle and commercially available at a reasonable price, producing good recoveries.

5. Accelerating the protein digestion

An efficient proteolytic digestion, which is important to correctly identify proteins in comprehensive proteomics and to obtain low detection limits in targeted proteomics, requires the generation of peptides in a minimal amount of time. Conventional methods often involve up to 12-16 hours of incubation, but digestion times up to 24 hours are reported, due to protein heterogeneity in samples (López-Ferrer et al. 2006). Alternative methods have therefore been introduced in order to speed up the digestion method. Capelo *et al.* report eight ways to speed up the protein identification workflow (Capelo et al. 2009); heating, microspin columns, ultrasonic energy, high pressure, infrared (IR) energy, microwave energy, alternating electric fields and microreactors where the trypsin is immobilized on a solid support. The pros and cons of these methods were assembled in a table, including citations or validations of the methods from other research groups (Capelo et al. 2009). Capleo *et al.* found that heating, ultrasonication, microwave energy and microreactors (immobilized trypsin) are used in most applications, and recommend that the systems with microspin columns, high pressures, alternating electric fields and IR energy need to be further validated. In a recent study (Hustoft et al. 2011) we have evaluated some of these techniques; IR energy, microwave energy, solvent effects as well as a newly developed filter aided sample preparation (FASP) technique to perform both depletion of detergents like SDS and tryptic digestion of proteins on the same filter device. The different methods are grouped into "temperature related accelerated digestion", "immobilized trypsin accelerated digestion" and "other ways to accelerate digestion" in the following. The terminology used gives an indication of the acceleration method for enzymatic digestion.

5.1 Temperature related accelerated digestion
5.1.1 Heating
Enzymes perform best at a given temperature and for in-solution tryptic digestion, 37 °C has been suggested as the optimal temperature (Havliš et al. 2003), and is the temperature most commonly used both for overnight in-gel and in-solution based tryptic digestion. Havlis *et al.* showed that reductive methylation of trypsin decreases autolysis and shifts the optimum of its catalytic activity to 50-60 °C, with enzymatic digestion of bovine serum albumin (BSA) 12 times faster than in-solution at 37 °C, using the yield of peptides as a parameter of the digestion efficiency (Havliš et al. 2003). From time to time some approaches have been introduced regarding the use of elevated temperatures for trypsin digestion (Capelo et al. 2009), but no new papers have been published recently.

5.1.2 Ultrasonic assisted digestion

Among the different ways to speed up protein digestion, ultrasonic energy has been considered the most promising method of the techniques requiring specialized equipment (Capelo et al. 2009). Three different commercial devices are used for ultrasonication in laboratories today. The most available is the ultrasonic bath, but for the purpose of accelerating tryptic digestion this is not sufficiently powerful to shorten the digestion times (Capelo et al. 2009). Regardless of this, Li *et al.* claimed that an ultrasound bath-assisted method gave successful in-solution proteolysis of three model proteins; BSA, cyt-C and myoglobin, revealing higher SQ % than conventional overnight incubation at 37 °C (Li et al. 2010) . However, the experimental set up and type of samples used should be considered carefully. It would probably be more correct to compare the ultrasonic bath method to 37 °C incubation without the ultrasonic bath, using proteins denatured, reduced and alkylated in the same fashion. Sonoreactors and ultrasonic probes are more effective, revealing a higher number of peptides and thus better SQ %, giving digestion in seconds as shown in the direct ultrasonic assisted enzymatic digestion of the soybean proteins (Domínguez-Vega et al. 2010). Carreira *et al.* proposed in another study a methodology that uses ultrasonic energy to speed up the protein digestion and throughput of ^{18}O labeling for protein quantification and peptide mass mapping through mass spectrometry based techniques (Carreira et al. 2010). This is a promising technique to accelerate the trypsin digestion of proteins, thus requiring specialized equipment, as mentioned in the start of this section.

5.1.3 Infrared radiation assisted digestion

In 2008 Wang *et al.* introduced a system where infrared (IR) energy was used to speed up the rate of trypsin digestion of proteins (Wang et al. 2008). The type of instrumentation used is presented in Figure 4, and the infrared light contributed, according to the authors, to shorter digestion times by increasing the excitation of the molecules and thus increasing the interaction between trypsin and the peptide bonds in the molecule.
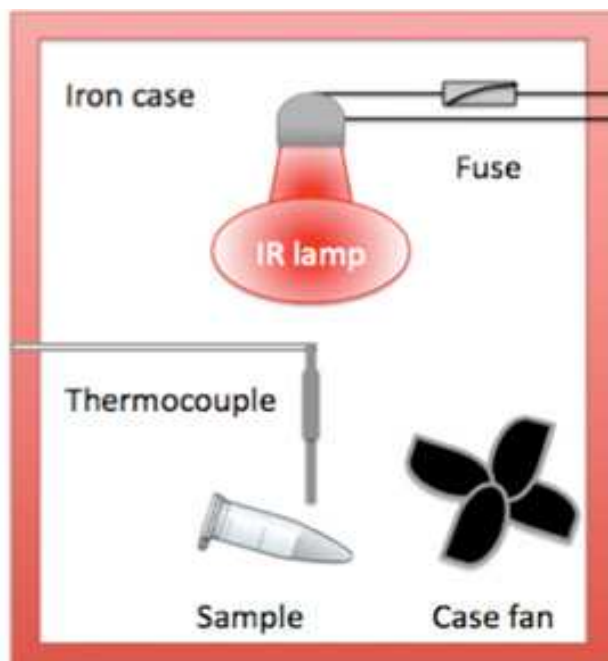

Fig. 4. Schematic diagram of the IR-assisted proteolysis system, adapted from (Wang et al. 2008).

In their first study IR assisted digestion was carried out for 5 min with trypsin in-solution and revealed almost a 100 % increase in SQ % of BSA and a 20 % increase in the SQ % of myoglobin compared to conventional trypsin digestion for 12 h at 37 °C. The method was considered repeatable when examined with a series of eight digestions giving myoglobin SQs of 90 % for all. Wang *et al*. later used the same system to study the digestion by another commonly used protease, α -chymotrypsin, which typically needs in-solution digestion times of 12-24 h (Wang et al. 2008). Using IR radiation the digestion time was reduced to 5 min for the digestion of BSA and cyt-C with SQs of 41 and 75 %, respectively. When the IR contribution was eliminated, the SQs were reduced to 11 and 56 % for BSA and cyt-C, respectively. For comparison the 12 h digestion at 37 °C yielded SQs comparable to those of 5 min IR radiation (37 % (BSA) and 75 % (cyt-C)). The same system was further examined three times in the years 2008-2009 (Bao et al. 2008; Wang et al. 2008; Bao et al. 2009) for the digestion of proteins on-plate MALDI-TOF-MS for in-gel proteolysis and one approach using trypsin-immobilized silica microspheres for peptide mapping. In 2010 another technique called photo thermal heating was introduced by Chen *et al*. A near infrared (NIR) diode laser was used to increase the reaction temperature during tryptic digestion on a Glass@AuNP slide, in a short period of time. The technique was used for four different proteins without the need for reduction and alkylation. The sequence coverages were in the range 43-95 % compared to 28-75 % with 12 h incubation at 37°C (Chen et al. 2010). Unfortunately no comparison of trypsin digestion efficiency with and without the NIR source was undertaken. We have found that, proteins can be digested in an IR oven, but compared to the traditional digestion procedure using 37 °C, there are no indications that the IR method has improved digestion efficiency for the commonly employed amount of proteins, at digestion times from 5 minutes up to 5 hours (Hustoft et al. 2011).

5.1.4 Microwave assisted digestion

Microwave assisted tryptic digestion was introduced in 2002 by Pramanik *et al*. as a tool to speed up the proteolytic cleavage of proteins (Pramanik et al. 2002). Other enzymes, as the endoproteinase Glu-C, has been reported to be inactivated by microwave induced denaturation, but trypsin digestion is accelerated according to the authors (Lill et al. 2007). In an attempt to investigate the acceleration of enzymatic cleavage, trypsin digestion with unmodified trypsin was performed at different microwave temperature settings, 37, 45 and 55 °C. The temperatures in the sample were found to be significantly higher than their microwave settings, and the authors emphasized that it was important to note the elevation of the reaction temperature which greatly enhanced the digestion reaction (Pramanik et al. 2002). Whether microwave accelerated digestion is a convenient way of heating, or whether the microwaves have a non-thermal positive effect on the digestion reaction, can be questioned. In a review on microwave-assisted proteomics, Lill *et al*. addressed the "heating principle" and stated that the kinetics in the microwave assisted incubation are different from the water bath incubation in that proteolysis was greatly enhanced when mediated by microwave radiation and that tightly folded proteins benefit the most from the microwave-assisted proteolysis (Lill et al. 2007). Two papers by Lin *et al*. (Lin et al. 2007; Lin et al. 2008) and one by Hahn *et al*. (Hahn et al. 2009) showed acceleration of digestion through a combination of immobilized trypsin and microwave radiation, when the digestion efficiency was measured as SQ % of different model proteins. In a short communication by Reddy *et al*. various solvents, temperatures and different enzyme: substrate (E+S) ratios were

compared to see how they affected protein digestion under conventional heating and microwave-assisted digestion. Digestion efficiencies were referred to as the ratio of the abundance of the most abundant peptide product to that of this peptide plus the undigested protein. Optimal conditions were found to be microwave-assisted irradiation at 60 °C for 30 min in a 50 mM Tris buffer with a of 1:5 or 1:25 (Reddy et al. 2010). It should be noted that this method is incompatible with subsequent MS analysis when Tris is used as a buffer, without a buffer exchange. To make sure that no denaturation of the trypsin occurs at 60 °C, modified enzyme should be used.

The microwave approach has also been used in some recent papers (Hasan et al. 2010; Liu et al. 2010), for effective enrichment of phosphopeptides and [18]O labeling. High sensitivity and SQ % of phosphopeptides were obtained and explained by absorption of microwave radiation by accelerated activation of trypsin for efficient digestion of the phosphoproteins (Hasan et al. 2010). The microwave assisted [18]O labeling resulted in peptide mixtures with [18]O incorporation in less than 15 min with a low rate of back exchange (Liu et al. 2010). We have evaluated microwave assisted protein digestion using both a specialized temperature controlled microwave oven and a domestic microwave oven. No differences in SQ % (or area of intact protein peak of cyt-C) were found for microwave and temperature assisted protein digestion for four model proteins. As previously suggested, microwave irradiation seems to have no advantage over normal temperature induced digestion, within our experimental framework (Hustoft et al. 2011).

5.2 Immobilized trypsin accelerated digestion
5.2.1 Microreactors

The immobilization of enzymes onto solid materials can be traced back to the 1950s according to Ma *et. al* (Ma et al. 2009), and in the last decades numerous immobilization methods have been developed. Proteolytic enzymes can be covalently bonded or physically adsorbed onto different carriers, such as inorganic silica materials, and organic materials that display a great variability and good biocompatibility like polystyrene divinylbenzenes (PS-DVB), polyacrylamides and methacrylates (Ma et al. 2009). These types of reactors appear to have a promising future, and constitute the most used accelerating digestion techniques the last couple of years. Immobilized microreactors have a high enzymatic turnover rate, low reagent consumption, less contribution of enzyme autolysis and the possibility to be coupled on-line to nanoLC-MALDI or nanoLC-ESI (Capelo et al. 2009; Ma et al. 2009). In a review by Monzo *et al.* from 2009 the most important proteolytic enzyme-immobilization processes are summarized with emphasis on trypsin immobilized micro- and nanoreactors (Monzo et al. 2009). Another review on immobilized enzymatic reactors was published by Ma *et al*. in 2009 (Ma et al. 2009). Different inorganic and organic carriers for particle based, monolithic, open tubular capillaries and membranes with immobilized enzymes were included and the authors predicted that immobilized enzyme reactors might be one of the key points to combine the top-down and bottom-up strategies in the field of proteomics. Still, some characteristics like higher mechanical strength, larger surface area, lower backpressure, higher enzyme loading capacity and better biocompatibility, are needed.

In 2009 three papers concerning immobilized enzyme microreactors and LC-MS/MS were published (Krenkova et al. 2009; Yamaguchi et al. 2009; Yuan et al. 2009). Yuan *et al*. presented an integrated protein analysis platform based on column switch recycling size exclusion chromatography (SEC), a microenzymatic reactor and μLC-ESI-MS/MS. The

system combines conventional SEC separation of intact proteins with on-line protein digestion on an immobilized enzymatic reactor (IMER) of conventional size and subsequent separation of peptides on a 300 μm (inner diameter) ID C18 column using ESI-MS/MS for identification (Yuan et al. 2009). The system requires large sample amounts and needs to be evaluated with real samples in order to be classified as a promising tool in proteomic studies. Monolithic enzymatic microreactors have been applied to digest, among others, immunoglobulin G at room temperature in only 6 minutes with reduced nonspecific adsorption of proteins and peptides to the stationary phase, as shown by Krenkova *et al.* (Krenkova et al. 2009). The SQ % was used as a measure for the digestion efficiency. Another microreactor was introduced by Yamaguchi *et al.*, using a PTFE microtube (500 μm ID, 13 cm length) with covalent binding of the enzyme. This tube was used to digest cyt-C and BSA, where the proteins (denatured in guanidine-HCl) were pumped into the immobilized microreactor and the tryptic peptides were subsequently purified on a C18 cartridge prior to LC or MS analysis. Yamaguchi *et al.* claimed that BSA could be digested without any reduction and alkylation procedures. Immobilized assisted digestion for 5.2 min at 30°C was compared with in-solution digestion of denatured BSA for 15 h at 37°C, however producing a rather low SQ % in both cases, 12 and 8 %, respectively (Yamaguchi et al. 2009). The authors claim that the low SQ % obtained was due to the stabilized tertiary structure by the 16 disulphide bonds that BSA contains, and probably better results would be obtained if reduction and alkylation of BSA had been performed in advance.

Xu *et al.* demonstrated a microporous reactor where polystyrene sulfonate and trypsin were adsorbed to a nylon membrane, to make a syringe based system for protein digestion. They used SQ % as the parameter for digestion efficiency claiming that the sequence coverage is a function of both the completeness of the protein digestion and the detection efficiency for the various tryptic peptides (Xu et al. 2010). The system showed improved SQ % of 84 % for BSA in only 6.4 seconds residence time compared to in-solution digestion for 16 h, and more promising cleavage in the presence of small amounts of SDS (Xu et al. 2010). Recently a critical overview of some highly efficient immobilized enzyme reactors termed IMERs, were presented (Ma et al.). This paper includes some newly developed IMERs and systems for protein-expression profiling, IMERs for characterization of proteins with PTMs and IMERs for protein quantification.

There are some drawbacks associated with the use of microreactors, like for instance the costs of the commercially available products of immobilized reactors. Self-fabrication requires adequate tools and experience in immobilization on different supports with enzymes. Automation is also still not easily achieved. However, as previously mentioned, on-going research can be expected to improve the techniques.

5.2.2 From microspin columns to filter-aided sample preparation

Commercial microspin columns or so called trypsin spin columns, where trypsin is immobilized at a high density on a solid support, - has been introduced by among others Sigma-Aldrich. It has been claimed that they reduce digestion times of proteins to 15 min, compared to conventional digestion times of 12 h, and give little autolysis fragments. However, the total microspin column method has been found to be both labor intensive and complex. The disappearance of these columns have been predicted because they do not give any apparent advantage over other types of immobilized trypsin which are commercially available and can be prepared in any lab (Capelo et al. 2009). This prediction can also be

supported by the fact that Sigma-Aldrich's trypsin columns are no longer available. A kit intended for ^{18}O labeling called Prolytica ^{18}O labeling kit from Stratagene, based on trypsin immobilized spin columns, is also now out of production. Promega additionally had one product available, called "Immobilized trypsin", where, with the use of the spin column format, digested peptides could easily be separated from the immobilized trypsin, reducing enzyme interference during analysis (Wiśniewski et al. 2010). This product is also now withdrawn because of low demand.

Wisniewski *et al.* presented a "Universal sample preparation method for proteome analysis" based on a Filter-Aided Sample Preparation (FASP) (Wisniewski et al. 2009). The enzyme is not directly immobilized onto the ultrafiltration device, but the device acts as a "proteomic reactor" for detergent removal, buffer exchange, chemical modification and protein digestion, where trypsin is added in a dissolved form to the filter (Figure 5). Lately four other papers have been published based on this method, and it seems promising for both membrane proteins, brain phosphoproteins and the N-glycoproteins (Wiśniewski et al. 2009; Ostasiewicz et al. 2010; Wiśniewski et al. 2010; Zielinska et al. 2010).

Detergent removal
Buffer exchange
Chemical modification
Protein digestion
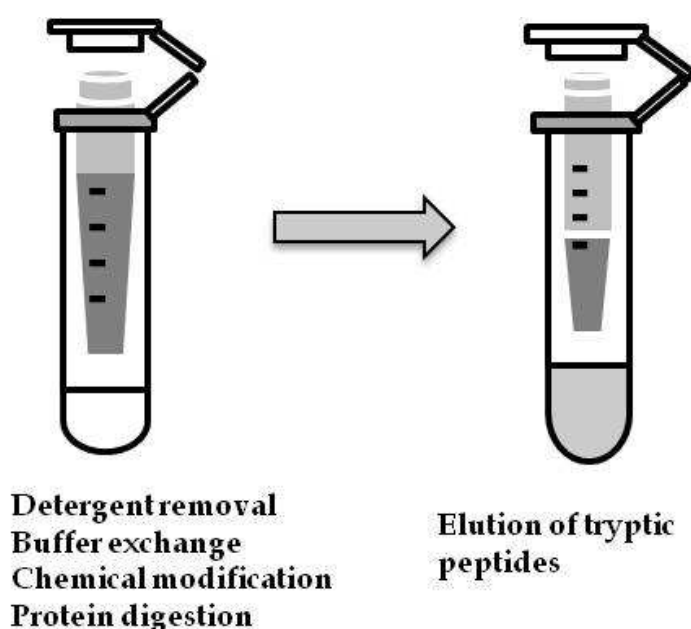
Elution of tryptic peptides

Fig. 5. The Amicon Ultra-0.5 mL Centrifugal Filters used in the FASP procedure. Adapted from, (Millipore 2011).

We, however, found that the filter device was not able to deplete all SDS, and this can lead to problems with the subsequent LC-MS analysis (Hustoft et al. 2011). The FASP procedure was found rather time consuming (using up to 3.5 h prior to the trypsin digestion) and the recommended 1:100 enzyme to protein ratio was not found satisfactory in our laboratory. Recently the FASP method was made commercially available through a FASP™ Protein Digestion Kit, from Protein Discovery. In this protocol the time of the centrifugation steps has been decreased, still it takes more than 2 hours to complete the protocol prior to 4-18 hours of trypsin digestion. Since the method has been made commercially available through a kit, and found to be convenient (Ostasiewicz et al. 2010; Wiśniewski et al. 2010; Zielinska et al. 2010; Hustoft et al. 2011) this method of trypsin digestion can be recommended when e.g. working with in-solution digestion of samples solubilised in detergents like SDS.

5.3 Solvent effects

The enzyme activity can also be improved in organic solvents as reported by Gupta and Roy (Gupta and Roy 2004). This was additionally shown by Strader *et al*. (Strader et al. 2005) who used an organic-aqueous system for digestion, containing 80 % acetonitrile (ACN), and which consistently provided the most complete digestion of microgram to nanogram quantities of proteins, by producing more peptide identifications at a shorter time (only 1 h compared to overnight). In a following paper Hervey *et al*. compared five different in-solution digestion protocols revealing that by adding 80 % ACN to the digestion solution the sequence coverages were as good as or in some cases better than using solvents with lower ACN % or chaotropes in the digests (Hervey IV et al. 2007). Addition of ACN to the digestion medium can cause (partial) denaturation of proteins and thus better accessibility to the cleavage sites of the protein. ACN can also improve digestion efficiency and enhance the solubility as well as elution of tryptic digests from e.g. a trypsin immobilized column (Tran et al. 2008). For the digestion of cyt-C, BSA, lysozyme and α-lactalbumin, addition of organic solvent up to 80 % did not increase the digestion efficiency regarding the area of the intact cyt-C peak or increased the sequence coverage (Hustoft et al. 2011). When more than 40 % ACN was used in combination with the tABC buffer, protein precipitation was seen. A solution to this problem is to use a buffer system with Tris-HCl/$CaCl_2$ when amounts of 40 % organic solvent or more are added to the sample solution (Hustoft et al. 2011). But, as before mentioned the Tris buffer is incompatible with the subsequent MS analysis and needs to be depleted prior to such.

6. Conclusions and recommended trypsin digestion procedure for LC-MS based proteomics

As has been pointed out, for some of the accelerating techniques used for tryptic digestion there is a need for more validation. We have thoroughly evaluated four of these techniques (Hustoft et al. 2011) finding no clear increase in the digestion efficiency (measured as SQ % or intact protein peak of cyt-C) of four model proteins when using IR energy, microwave energy, aqueous-organic solvent systems or FASP filters. What is of importance when comparing novel methods to established ones, is to include control experiments where the same treatment is used but without the accelerating factor for the control. When the digestion efficiency is measured based on amino acid sequence coverage, results have been found to be strongly dependent on the LC-MS data quality of the analyzed samples. Hence more replicates are strongly recommended for correct evaluation of the methods. The MS instrument available is of importance for examining the digestion efficiency and also the choice of model proteins are crucial because of their different response to tryptic digestion. Working with conventional shotgun (bottom-up) proteomic techniques the overall digestion efficiency is more intricate to study than when working with targeted proteomics. In targeted proteomics much more information can be found about the proteins to be determined, e.g. whether they have cysteines and need to be reduced and alkylated prior to enzymatic digestion. The literature can be searched in order to find relevant information and even established methods used for the targeted proteins, and selected reaction monitoring (SRM) can be used for targeted quantitative proteomics.

It should be kept in mind however, that many different variants of key words denoting the same method or process are used in the literature. One example is the method of trypsin digestion where different papers were found depending on which key word was entered
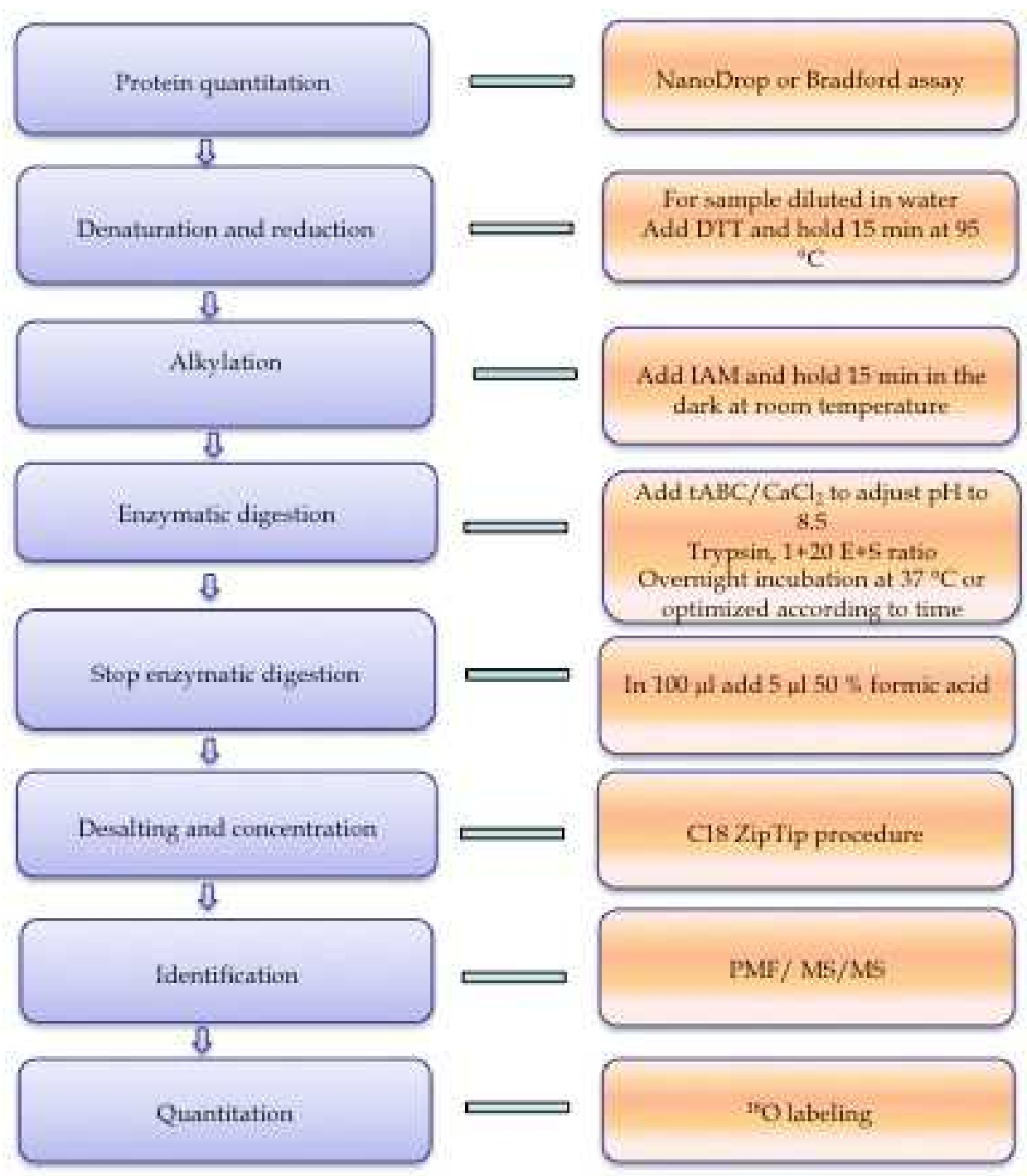
Fig. 6. A recommended procedure for in-solution based sample preparation and protein identification. PMF refers to peptide mass fingerprinting. (PFM).

into the search field of, in this case, SciFinder®: *Proteolysis, protein digestion, trypsin digestion, tryptic digestion, enzyme reaction, enzymatic digestion* or *enzymatic cleavage*, all produced hits for papers concerning trypsin/tryptic digestion (as we have chosen to use). Rounding up with Mitchell, he refers to a test sample study done in 2009, where 27 labs were included in reproducibility testing of standardized samples of 20 known proteins each containing one or more unique tryptic peptide. Only seven of the 27 labs reported the 20 proteins correctly, and only one identified all the proteolytic peptides (Bell et al. 2009). When they collected and analyzed the raw MS data from the labs, they found that all proteins and most peptides had been detected in all labs, but just not been interpreted correctly, indicating that it was the human element that failed. Due to the difficulties in correctly identifying the proteins in comprehensive proteomics the future of the field of proteomics will probably be more directed against targeted proteomics. However, as mentioned in the introduction, not every proteomic problem can be solved through targeted proteomics and it will still be a need for comprehensive analyses of complex samples.

Reviewing and in-house experiments of some of the suggested accelerating methods for trypsin digestion did not provide us with a better procedure for speeding up the sample preparation step in in-solution based proteomics, with the possible exception of ultrasonication. A complete recommended sample preparation procedure for newcomers in the field is presented in Figure 6, partially based on some of the conclusions from our investigations. The recommended procedure gives a robust and effective sample preparation guideline to comprehensive in-solution trypsin digestion of complex protein samples in proteomics. This procedure is more or less business as usual, since none of the suggested accelerating procedures revealed faster and more efficient digestion of proteins, than the inexpensive overnight in-solution digestion at 37 °C.

7. References

Anderson, N. and N. Anderson (1998). "Proteome and proteomics: new technologies, new concepts, and new words." *Electrophoresis* 19(11): 1853-1861.

Bao, H., et al. (2008). "Efficient In-Gel Proteolysis Accelerated by Infrared Radiation for Protein Identification." *Journal of Proteome Research* 7(12): 5339-5344.

Bao, H., et al. (2009). "Infrared-assisted proteolysis using trypsin-immobilized silica microspheres for peptide mapping." *PROTEOMICS* 9(4): 1114-1117.

Bell, A. W., et al. (2009). "A HUPO test sample study reveals common problems in mass spectrometry-based proteomics." *Nat Meth* 6(6): 423-430.

Bradford, M. M. (1976). "A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding." *Analytical Biochemistry* 72(1-2): 248-254.

Capelo, J., et al. (2010). "Latest developments in sample treatment for 18O-isotopic labeling for proteomics mass spectrometry-based approaches: A critical review." *Talanta* 80(4): 1476-1486.

Capelo, J. L., et al. (2009). "Overview on modern approaches to speed up protein identification workflows relying on enzymatic cleavage and mass spectrometry-based techniques." *Analytica Chimica Acta* 650(2): 151-159.

Carreira, R. J., et al. (2010). "Indirect ultrasonication for protein quantification and peptide mass mapping through mass spectrometry-based techniques." *Talanta* 82(2): 587-593.

Chen, J.-Y., et al. (2010). "Multilayer gold nanoparticle-assisted protein tryptic digestion in solution and in gel under photothermal heating." *Analytical and Bioanalytical Chemistry*: 1-9.

Choudhary, G., et al. (2002). "Multiple Enzymatic Digestion for Enhanced Sequence Coverage of Proteins in Complex Proteomic Mixtures Using Capillary LC with Ion Trap MS/MS." *Journal of Proteome Research* 2(1): 59-67.

Domínguez-Vega, E., et al. (2010). "First approach based on direct ultrasonic assisted enzymatic digestion and capillary-high performance liquid chromatography for the peptide mapping of soybean proteins." *Journal of Chromatography A* 1217(42): 6443-6448.

Greenebaum, C. C. (2011). From http://www.umgcc.org/research/proteomics_services.htm.

Gupta, M. N. and I. Roy (2004). "Enzymes in organic media." *European Journal of Biochemistry* 271(13): 2575-2583.

Hahn, H. W., et al. (2009). "Ultrafast Microwave-Assisted In-Tip Digestion of Proteins." *Journal of Proteome Research* 8(9): 4225-4230.

Hale, J. E., et al. (2004). "A simplified procedure for the reduction and alkylation of cysteine residues in proteins prior to proteolytic digestion and mass spectral analysis." *Analytical Biochemistry* 333(1): 174-181.

Hasan, N., et al. (2010). "Two-step on-particle ionization/enrichment via a washing- and separation-free approach: multifunctional TiO_2 nanoparticles as desalting, accelerating, and affinity probes for microwave-assisted tryptic digestion of phosphoproteins in ESI-MS and MALDI-MS: comparison with microscale TiO_2" *Analytical and Bioanalytical Chemistry* 396(8): 2909-2919.

Havliš, J., et al. (2003). "Fast-Response Proteomics by Accelerated In-Gel Digestion of Proteins." *Anal. Chem.* 75(6): 1300-1306.

Hervey IV, W., et al. (2007). "Comparison of digestion protocols for microgram quantities of enriched protein samples." *J. Proteome Res* 6(8): 3054-3061.

Hustoft, H. K., et al. (2011). "Critical assessment of accelerating trypsination methods." *J. Pharmaceut. Biomed. Anal.* 10.1016/j.jpba.2011.08.013.

IS, M. S. R. (2011). from http://ionsource.com/.

Krenkova, J., et al. (2009). "Highly Efficient Enzyme Reactors Containing Trypsin and Endoproteinase LysC Immobilized on Porous Polymer Monolith Coupled to MS Suitable for Analysis of Antibodies." *Anal. Chem.* 81(5): 2004-2012.

Li, Y.-P., et al. (2010). "Ultrasound-assisted urea-free chemical denaturants combined with thermal denaturation to accelerate enzymatic proteolysis." *Fenxi Huaxue* 38(Copyright (C) 2010 American Chemical Society (ACS). All Rights Reserved.): 663-667.

Lill, J. R., et al. (2007). "Microwave-assisted proteomics." *Mass Spectrometry Reviews* 26(5): 657-671.

Lin, S., et al. (2007). "Development of microwave-assisted protein digestion based on trypsin-immobilized magnetic microspheres for highly efficient proteolysis followed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry analysis." *Rapid Communications in Mass Spectrometry* 21(23): 3910-3918.

Lin, S., et al. (2008). "Fast and Efficient Proteolysis by Microwave-Assisted Protein Digestion Using Trypsin-Immobilized Magnetic Silica Microspheres." *Anal. Chem.* 80(10): 3655-3665.

Liu, N., et al. (2010). "Microwave-Assisted 18O-Labeling of Proteins Catalyzed by Formic Acid." *Anal. Chem.* 82(21): 9122-9126.

López-Ferrer, D., et al. (2006). "Sample treatment for protein identification by mass spectrometry-based techniques." *TrAC Trends in Analytical Chemistry* 25(10): 996-1005.

Ma, J., et al. "Immobilized enzyme reactors in proteomics." *TrAC Trends in Analytical Chemistry* In Press, Accepted Manuscript.

Ma, J., et al. (2009). "Recent advances in immobilized enzymatic reactors and their applications in proteome analysis." *Analytica Chimica Acta* 632(1): 1-8.

Millipore. (2011). from http://www.millipore.com.

Minnesota, U. (2011). from http://www.cbs.umn.edu/msp/protocols/insolution.shtml.

Mitchell, P. (2010). "Proteomics retrenches." *Nat Biotech* 28(7): 665-670.

Monzo, A., et al. (2009). "Proteolytic enzyme-immobilization techniques for MS-based protein analysis." *TrAC Trends in Analytical Chemistry* 28(7): 854-864.

NanoDrop (2011).

Nelson, D. N. and M. M. Cox, Eds. (2008). *Lehninger: Principles of Biochemistry*, Freeman and Company.

Nord, F. F., et al. (1956). "On the mechanism of enzyme action. LXI. The self digestion of trypsin, calcium-trypsin and acetyltrypsin." *Archives of Biochemistry and Biophysics* 65(1): 120-131.

Ostasiewicz, P., et al. (2010). "Proteome, Phosphoproteome, and N-Glycoproteome Are Quantitatively Preserved in Formalin-Fixed Paraffin-Embedded Tissue and Analyzable by High-Resolution Mass Spectrometry." *Journal of Proteome Research* 9(7): 3688-3700.

Pramanik, B. N., et al. (2002). "Microwave-enhanced enzyme reaction for protein mapping by mass spectrometry: A new approach to protein digestion in minutes." *Protein Science* 11(11): 2676-2687.

Proc, J. L., et al. (2010). "A Quantitative Study of the Effects of Chaotropic Agents, Surfactants, and Solvents on the Digestion Efficiency of Human Plasma Proteins by Trypsin." *Journal of Proteome Research* 9(10): 5422-5437.

Reddy, P. M., et al. (2010). "Digestion Completeness of Microwave-Assisted and Conventional Trypsin-Catalyzed Reactions." *Journal of the American Society for Mass Spectrometry* 21(3): 421-424.

Shieh, I. F., et al. (2005). "Eliminating the Interferences from TRIS Buffer and SDS in Protein Analysis by Fused-Droplet Electrospray Ionization Mass Spectrometry." *Journal of Proteome Research* 4(2): 606-612.

Sigma-Aldrich. (2011). from
    http://www.sigmaaldrich.com/etc/medialib/docs/Sigma/Bulletin/t6567bul.Par.
    0001.File.tmp/t6567bul.pdf.
Smith, P. K., et al. (1985). "Measurement of protein using bicinchoninic acid." *Analytical Biochemistry* 150(1): 76-85.
Steen, H. and M. Mann (2004). "The abc's (and xyz's) of peptide sequencing." *Nat Rev Mol Cell Biol* 5(9): 699-711.
Strader, M. B., et al. (2005). "Efficient and Specific Trypsin Digestion of Microgram to Nanogram Quantities of Proteins in Organic−Aqueous Solvent Systems." *Anal. Chem.* 78(1): 125-134.
Sundqvist, G., et al. (2007). "A general, robust method for the quality control of intact proteins using LC-ESI-MS." *Journal of Chromatography B* 852(1-2): 188-194.
Thiede, B. and C. Koehler (2010). Mass spectrometry-based quantitative proteomics using isobaric peptide termini labeling, Universitetet i Oslo: 47pp.
Tran, B. Q., et al. (2008). "On-Line multitasking analytical proteomics: How to separate, reduce, alkylate and digest whole proteins in an on-Line multidimensional chromatography system coupled to MS." *J. Sep. Sci.* 31(16-17): 2913-2923.
Vaudel, M., et al. (2010). "Peptide and protein quantification: A map of the minefield." *PROTEOMICS* 10(4): 650-670.
Vukovic, J., et al. (2008). "Improving off-line accelerated tryptic digestion:: Towards fast-lane proteolysis of complex biological samples." *Journal of Chromatography A* 1195(1-2): 34-43.
Wang, S., et al. (2008). "Infrared-Assisted On-Plate Proteolysis for MALDI-TOF-MS Peptide Mapping." *Anal. Chem.* 80(14): 5640-5647.
Wang, S., et al. (2008). "Efficient Chymotryptic Proteolysis Enhanced by Infrared Radiation for Peptide Mapping." *Journal of Proteome Research* 7(11): 5049-5054.
Wang, S., et al. (2008). "Infrared-assisted tryptic proteolysis for peptide mapping." *PROTEOMICS* 8(13): 2579-2582.
Wisniewski, J., et al. (2009). "Universal sample preparation method for proteome analysis." *Nat Meth* 6(5): 359-362.
Wiśniewski, J. R., et al. (2010). "Brain Phosphoproteome Obtained by a FASP-Based Method Reveals Plasma Membrane Protein Topology." *Journal of Proteome Research* 9(6): 3280-3289.
Wiśniewski, J. R., et al. (2009). "Combination of FASP and StageTip-Based Fractionation Allows In-Depth Analysis of the Hippocampal Membrane Proteome." *Journal of Proteome Research* 8(12): 5674-5678.
Worthington, B. C. (2011). from http://www.worthington-biochem.com/try/default.html.
Xu, F., et al. (2010). "Facile Trypsin Immobilization in Polymeric Membranes for Rapid, Efficient Protein Digestion." *Anal. Chem.*: null-null.
Yamaguchi, H., et al. (2009). "Rapid and efficient proteolysis for proteomic analysis by protease-immobilized microreactor." *Electrophoresis* 30(18): 3257-3264.
Yuan, H., et al. (2009). "Integrated protein analysis platform based on column switch recycling size exclusion chromatography, microenzymatic reactor and [mu]RPLC-ESI-MS/MS." *Journal of Chromatography A* 1216(44): 7478-7482.

Zielinska, D. F., et al. (2010). "Precision Mapping of an In Vivo N-Glycoproteome Reveals Rigid Topological and Sequence Constraints." *Cell* 141(5): 897-907.
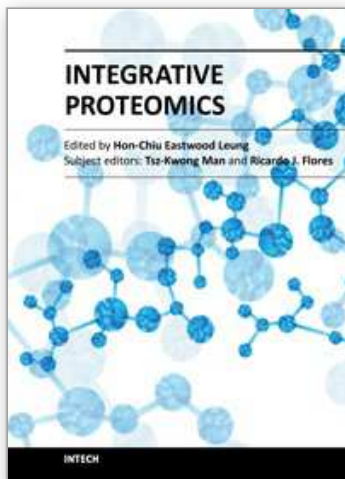
Integrative Proteomics

Edited by Dr. Hon-Chiu Leung

Proteomics was thought to be a natural extension after the field of genomics has deposited significant amount of data. However, simply taking a straight verbatim approach to catalog all proteins in all tissues of different organisms is not viable. Researchers may need to focus on the perspectives of proteomics that are essential to the functional outcome of the cells. In Integrative Proteomics, expert researchers contribute both historical perspectives, new developments in sample preparation, gel-based and non-gel-based protein separation and identification using mass spectrometry. Substantial chapters are describing studies of the sub-proteomes such as phosphoproteome or glycoproteomes which are directly related to functional outcomes of the cells. Structural proteomics related to pharmaceutics development is also a perspective of the essence. Bioinformatics tools that can mine proteomics data and lead to pathway analyses become an integral part of proteomics. Integrative proteomics covers both look-backs and look-outs of proteomics. It is an ideal reference for students, new researchers, and experienced scientists who want to get an overview or insights into new development of the proteomics field.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Hanne Kolsrud Hustoft, Helle Malerod, Steven Ray Wilson, Leon Reubsaet, Elsa Lundanes and Tyge Greibrokk (2012). A Critical Review of Trypsin Digestion for LC-MS Based Proteomics, Integrative Proteomics, Dr. Hon-Chiu Leung (Ed.), ISBN: 978-953-51-0070-6, InTech, Available from: http://www.intechopen.com/books/integrative-proteomics/a-critical-review-of-trypsin-digestion-for-lc-ms-based-proteomics

INTECH
open science | open minds