Accepted Manuscript

Mass spectrometry based proteomics as foodomics tool in research and assurance of food quality and safety

Uroš Andjelković, Djuro Josić

Please cite this article as: Andjelković, U., Josić, D., Mass spectrometry based proteomics as foodomics tool in research and assurance of food quality and safety, *Trends in Food Science & Technology* (2018), doi: 10.1016/j.tifs.2018.04.008.

Mass spectrometry based proteomics as foodomics tool in research and assurance of food quality and safety

Uroš Andjelković [a, b, *] and Djuro Josić [b, c, *]

[a] Department of Chemistry - Institute of Chemistry, Technology and Metallurgy, University of Belgrade, 11000 Belgrade, Serbia
[b] Department of Biotechnology, University of Rijeka 51000 Rijeka, Croatia
[c] Warren Alpert Medical School, Brown University, Rhode Island, Providence RI, USA

* Corresponding authors:
Department of Biotechnology, University of Rijeka, Radmile Matejčić 2, 51000 Rijeka, Croatia.
E-mail: djuro.josic@uniri.hr, djuro_josic@brown.edu (D. Josić);
Department of Chemistry - Institute of Chemistry, Technology and Metallurgy, University of Belgrade, Studentski trg 12-16, 11000 Belgrade, Serbia. E-mail: uros@chem.bg.ac.rs (U. Andjelković)

ABSTRACT

Background: As a comprehensive discipline that studies food and nutrition, foodomics requires reliable qualitative and quantitative information about the food proteome component in order to extract new, integrative information from the complex multivariable space of omics. This information is necessary to achieve a higher level of understanding of processes in food science and technology, consequently new functions of food and improved markers of food quality and safety and transform the concept of food safety.

Scope and Approach: We are presenting mass spectrometry (MS) based proteomic approaches that are being utilized in different proteomic studies, not necessarily only in the field of foodomics. Current analytical capabilities of MS-based proteomics together with sample preparation procedures and quantification strategies, and recent technical developments were presented.

Key Findings and Conclusions: MS-based proteomics enables the analysis of different aspects of proteins and provides a variety of approaches for reliable quantification of individual proteins and/or food proteome. This is a complex field and its successful implementation requires a

33 dedicated analyst, thorough design of sample preparation procedure, proper selection of an MS

34 technique and approach, adequate type of mass spectrometer, and both thorough data analysis

35 and validation. Improvements in the technology of mass spectrometery are continuously

36 expanding capabilities of MS-based proteomics.

37

38 *Keywords: foodomics, proteomics, mass spectrometry, food quality, food safety*

39

40 List of abbreviations: AQUA, absolute quantification; CID, collision-induced dissociation; CV,

41 coefficient of variation; DDA, data dependent acquisition; DIA, data independent acquisition;

42 ESI, electrospray ionization; HCD, high energy collision dissociation; HRMS, high-resolution

43 mass spectrometry; ECD, electron capture dissociation; ETD, electron transfer dissociation;

44 iTRAQ, isobaric tag for relative and absolute quantification; LC, liquid chromatography; LFQ,

45 label free quantification; LIT (LTQ), linear ion trap; MALDI, matrix assisted laser desorption

46 ionization; MRM, multiple reaction monitoring; NMR, nuclear magnetic resonance

47 spectroscopy; OT, orbitrap; PPI, protein – protein interactions; PRM, parallel reaction

48 monitoring; PTM, posttranslational modification; QqQ, triple-quadrupole; SILAC, stable isotope

49 labelling with amino acids in cell culture; SRM, selected reaction monitoring; TMT, tandem

50 mass tag; TOF, time of flight;

51

52

53

54

55

56   Highlights:

57   Mass spectrometry based proteomics, as one of the main technologies in foodomics, is presented.

58   MS-based proteomic approaches in food research, quality and safety control are introduced.

59   Improvements in sample preparation for mass spectrometry analyses are described.

60   Critical points for application of MS-based proteomics in food analysis are analysed.

61   Future directions of MS-based proteomics are discussed.

62

63

64

65

66

67

68

69

70

71

72

73

74

75

76

77

78

79

1. Introduction

Contemporary food research provides evidence that food serves not just to fulfil basic dietary needs, but to actively influence a healthy life, playing a pivotal role in both the improvement of its quality, and as natural prevention against a wide range of diseases (D'Alessandro, 2012). Researchers are increasingly aware of the differences among individuals at the genome, proteome and microbiome levels, and the existence of an individual's optimal metabolic space is also becoming evident, which in combination with environmental factors result in an individual's phenotype. Advances in understanding of the molecular basis of disease susceptibility and food quality together with interaction of food with the individual's metabolic space, introduced the concept of personalized nutrition as a part of personalized medicine (Noecker, 2016; Vimaleswaran, 2015).

Analytical approaches based on MS are one of the fastest growing methodologies in food analysis. The application of proteomics in food research, quality control (sensory characteristics, nutritional value, product traceability), authenticity assessment (adulterations, geographic origin, presence of GMO) and safety control (toxins formed from proteins during food processing, bacterial and fungal toxins, allergens, antinutritients, foodborne pathogens, prions, biopesticides, GMO food) of food of animal and plant origin has been extensively reviewed (Agrawal, 2013; Andjelković, 2017; Chassy, 2010; Colgrave, 2017; Cunsolo, 2014; D'Alessandro, 2012; Fasoli, 2015; Martinović, 2016; Piras, 2016; Sauer, 2015; Tedesco, 2014).

Over twenty year old field of MS-based proteomics became one of the main pillars of the group of technologies with the common name "omics". Proteomics methods complement their genomic and transcriptomic counterparts, but also provide additional biological information that is not accessible by genomics and transcriptomics (Mann, 2013; Zubarev, 2013). Moreover, proteomics

4

103 also provide information that is necessary for the understanding metabolome. Proteomics is of

104 special importance for foodomics since from the moment when the genome has lost its active

105 influence, and when food proteins are subjected to different factors that are possibly not

106 genetically regulated, as well as to the food processing conditions. All above listed factors can

107 significantly alternate protein properties and interactions with components in the food matrix.

108 Research of the food proteome and its alterations influenced by plant and animal strains

109 (genetics), different conditions of plant and animal growth, pests, food processing and storage

110 conditions, enable establishment of correlations between the food proteome and quality

111 parameters (nutritional value, sensory characteristics, suitability for processing, safety,

112 sustainable growth, etc.). These correlations will enable tailoring of sensory, nutritional and

113 technological properties of food, personalized diet, and identification of quality and safety

114 control parameters (individual protein or protein networks as quality markers).

115 Future research will explore interactions between many different layers (genome, transcriptome,

116 proteome, peptidome, metabolome, microbiome) of both, food and consumer. Out of this

117 complex picture a way to extract meaningful information from a multilayer interaction network

118 should be paved. It is very important to minimize the increasing risk to become flooded with

119 wrong or biased information. A way of systematic organization of data blocs coming from

120 different sources was presented in order to assess the analytical performance, to improve the

121 interpretability, prevent systematic errors, and finally, unreliable results (Chassy, 2010; Skov,

122 2014).

123 The MS-based proteomics generate a vast amount of data. In order to support research efforts to

124 understand the complexity of food MS-based proteomic data should be collected and submitted

125 to data repositories according to guidelines. This strategy will enable the use of data in a more

126   efficient manner, in order to provide the quality of interpretation and the comparability of

127   analysis. A draft of guidelines was already published for the largest proteomic attempt – The

128   Human Proteome Project (HPP) (Deutsch, 2016). In a broader sense, research of food quality

129   and safety at the level of proteins is the investigation of the interactions between two proteomes:

130   the food proteome and the proteome of a particular consumer. Thus, HPP is the systematic and

131   comprehensive project that provides basis for future complementary proteomic projects or

132   expanding of the HPP to include the topic of food. When combined with the current complexity

133   of MS-based proteomic approaches and in order to be systematically addressed, the impact of the

134   microbiome on the complex food proteome and vice versa, as well as the interactions between

135   the proteomes of the food-microbiome-consumer requires the use of enormous resources in order

136   to be systematically addressed. Hence, the construction of a human proteome and food proteome

137   resource must be a long-term process. The creation of large-scale proteomic methods already

138   paved the way to new types of questions concerning both protein expression and modification

139   profiling. These methods are now poised to address how protein expressions or modifications

140   will change as a function of disease and further in regard to foodomics as a function of factors

141   that influence food quality and safety. Sequencing the genome was perhaps the easiest part, and

142   making sense of the constantly moving and changing picture of the proteome (and later,

143   metabolome) will require a lot of time, effort and creativity (Nilsson, 2010a).

144   In this review available MS-based proteomic technologies, approaches and critical points

145   important for MS-based proteomic experimental design are summarized.

146   **2. MS-based proteomics**

147   Various analytical methods can be used for the investigation of biological systems at the protein

148   level. High degree of proteome complexity and low abundance of many important proteins in the

149 investigated sample require the use of highly specific and sensitive analytical techniques.

150 Availability of genome sequence databases, technical and conceptual advances, as well as

151 advances in bioinformatics, made MS a method of choice for proteome studying (Aebersold,

152 2016). The definition of the proteome changed in line with technical and methodological

153 developments (Ahrens, 2010; Mann, 2013). The complete proteome comprises all expressed

154 proteins in a sample (cells, tissue, or a whole organism), their proteoforms, modification states

155 and organization in macromolecular assemblies, in a given time and space. At the moment it is

156 still not possible to achieve such a vast and in-depth view into the proteome, and it is

157 questionable whether we can achieve it at all, at least with the currently available technologies

158 (Ahrens, 2010). Further technical advances and developments of new bioinformatic tools will

159 certainly open new opportunities to work toward this goal in the near future (Aebersold, 2016).

160 Nevertheless, depending on the question posed, it may not be always necessary to have such a

161 vast and deep view for a given experiment (Michalski, 2011).

162 Mass spectrometry based proteomic techniques are applied to obtain data important for

163 understanding both the structure and function of proteins (Fig.1). Examples of application of

164 MS-based proteomics in research and assurance of food quality and safety are provided in Table

165 1.

2.1. MS-based proteomics in analysis of PTMs

167 More than 300 already known post-translational modifications (www.abrf.org/delta-mass) are

168 one of the sources of proteome complexity (Walsh, 2005). Glycosylation is a highly abundant

169 PTM and about 50% of all proteins are glycosylated. Large numbers of glycan structures (www.

170 glycobase.nibrt.ie) are formed by a combination of relatively small numbers of monosaccharide

171 units. Different physicochemical properties of proteins and glycan components of glycoproteins

7

172 requires different technical and methodological approaches for their analysis by MS (Scott,

173 2011). Glycoproteomics is a sub-group of MS-based proteomic approaches specialized for the

174 analysis of glycoproteins, Fig.1. At the current level of technology, the high complexity of the

175 glycoproteome is hard to elucidate solely by use of glycoproteomic approaches. Consequently, a

176 special group of approaches called glycomics was established, Fig.1-2. Glycomics technologies

177 attempt to define the structure and quantify the complete set of glycans of one cell, tissue or

178 organism (Mechref, 2013). The use of glycomics technologies gives an insight into the enormous

179 capacity of glycans, and the information coding leads us towards understanding of the sugar code

180 in living systems (Gabius, 2015). The growing importance of glycomics in foodomics research

181 also supports the fact that the most important allergens in food belong to the group of

182 glycoproteins (Andjelković, 2017; Leonard, 2005; Li, 2016).

183 The next widely investigated PTM is phosphorylation of hydroxyl amino acids of proteins.

184 Phosphorylated proteins are directed links in signalling networks between upstream kinases and

185 downstream transcription factors altering DNA expression profiles and many other biochemical

186 regulatory mechanisms. Phosphorylation is a dynamic PTM arising in a very short time period,

187 within minutes, as a result of activity of kinases. Moreover, it may also disappear within minutes

188 due to activity of the phosphatases. Physiological importance of this PTM, its vulnerability and

189 available technical level of methods for analysis of protein phosphorylation, especially MS

190 inspired a special proteomic discipline – phosphoproteomics. It strategically uses and further

191 develops selective approaches and bioinformatic tools for the investigation of phosphorylated

192 proteins, their position in interaction networks and the flow of biological signals throughout

193 these networks (Riley, 2016). The response of cells to different stimuli is one of key information

194 about signalling and it can be quantified using phosphoproteomics.

8

195 MS-based proteomic approaches allow for the identification and quantitation of thousands of

196 PTM sites in a single experiment (Doll, 2015). Currently, sensitive and dedicated MS-based

197 proteomic strategies are available only for a few different types of PTM (Doll, 2015; Venne,

198 2014). A comprehensive and simultaneous view of PTMs and PTM sites is important to get

199 insight into the mechanisms of regulation of protein function by multi-PTM interplay (Pejaver,

200 2014; Venne, 2014). Protein conformation can be modulated by PTMs, hence protein turnover,

201 localization, PPI and enzyme activity can be affected.

202 As presented in Table 2, the complexity of the food proteome is additionally increased by the

203 number of non-enzymatic and enzymatic PTMs. Also, PTMs can arise as a result of reactions

204 with food matrix components, additives, microbial enzymes and toxins, or other components

205 emerged during food processing and storage/transport. These modifications are usually not

206 stoichiometric, and are often in low abundance. However, they can have a significant

207 physiological role, whether it is in triggering of food allergies, or other harmful processes such as

208 food poisoning or carcinogenesis. Moreover, they are important for technological properties, as

209 well as for nutritional and sensory food properties. Investigation of enzymatic and non-

210 enzymatic PTMs that have occurred after food processing or storage, showed that PTMs can be

211 used as parameters for food quality control (Agrawal, 2013; Arena, 2017; Paredi, 2012).

212 Consequently, there is a need for the design of enrichment and data analysis strategies for the

213 detection and assessment of these modifications. This fact is very important for experimental

214 design when MS-based proteomics or other high-throughput approaches are used. If not perfectly

215 designed, such kind of investigation can be a source of significant analytical problems and

216 systematic errors. A particular problem in discovery proteomics is data analysis. A large number

217 of possible PTMs of food proteins cannot be easily included in search engines since that requires

218 strong computers and significantly increases analysis time. Simplified strategies cover a few

219 predicted PTMs included as variable modifications, and error-tolerant searches are allowed

220 during a sequence database search. However, once identified and validated as a marker of food

221 quality and safety, a particular PTM can be routinely quantified using a targeted proteomic

222 approach (*vide infra*).

223 **2.2. Conformational proteomics**

224 Conformational changes are essential for biological functions of proteins, the investigation of

225 structural dynamics is necessary to understand their physiological role. A group of proteins

226 known as "intrinsically disordered" are so dynamic that under physiological conditions they are

227 characterized by a complete or an almost complete lack of an ordered structure (Dunker, 2013).

228 Information obtained by MS can significantly complement *in vitro* 3D structure elucidation with

229 X-ray crystallography, electron microscopy, NMR and other spectroscopic methods (Leney,

230 2017; Vandermarliere, 2013). MS-based methods need only a small amount of sample enabling

231 the investigation of naturally occurring structures and PTMs which are complicated for

232 purification and/or expression. Moreover, MS enables the probing of structural transitions of

233 proteins in a complex biological environment on a large scale (Feng, 2014). Mass spectrometry

234 combined with methods such as hydrogen-deuterium exchange (Rand, 2014), limited proteolysis

235 (Feng, 2014), cross-linking (Leitner, 2016; Sinz, 2014) and MS footprinting (chemical surface

236 labelling) can also provide information about surface accessibility of amino acids

237 (Vandermarliere, 2016). This information can be used to characterize protein conformation, as

238 well as the 3D structure of macromolecular protein assemblies and PPI. Consequently, the MS

239 can give an additional contribution to the investigation of macromolecular protein complexes

240 including their composition, stoichiometry, copy number, topology and dynamics (Wohlgemuth,

241 2015).

2.3. Analysis of protein-protein interactions

243 Protein functions can be modulated in different ways, including their expression level, PTMs,

244 metabolites, and PPI. MS-based proteomics offers many different approaches for PPI

245 identification (Smits, 2016). Comprehensive and reproducible information about PPI is necessary

246 to build networks of interacting molecules (genes – their products – proteins – cofactors –

247 messenger molecules – metabolites) as a basis for quantitative/dynamic analyses (Bensimon,

248 2012). Dynamics of these networks that are modulated at different time scale by internal (*e.g.*

249 genomic alterations) and external factors (*e.g.* environmental, food) is believed to determine the

250 phenotype (Aebersold, 2016). Building of interaction networks, their analysis and comparison,

251 fusion, harvesting of information from networks and other sources in order to understand how

252 network capture and process information induce a specific response or phenotype, are complex

253 tasks for bioinformatics scientists (Gligorijević, 2016). Concerning food safety and quality,

254 network includes, as external factors, food processing and storage conditions, as well as the

255 effect of food on its consumer.

2.4. Chemical proteomics

257 Protein quantities do not necessarily represent their activities. A toxic protein whose toxicity is

258 based on its enzyme`s activity, or an enzyme whose activity is of a particular interest for food

259 processing or food value can be present in a denatured or inactivated form. Hence, measuring of

260 total amount will not provide information about quantity of active form. Quantitative information

261 about enzyme active form can be obtained by MS-based proteomics by means of activity-based

11

262 protein profiling (ABPP). Chemical probes are specially designed to contain a reactive group that

263 targets a specific enzyme class by forming an irreversible covalent bond and a reporter group

264 that enables their enrichment and/or detection (Wright, 2016). ABPP are developed for different

265 enzyme classes (Cravatt, 2008), such as proteases (Fonović, 2008), kinases and phosphatases

266 (Ruprecht, 2015), glycosydases, cytochrome P450. Proteins in low abundance that exhibit

267 enzyme activity, can especially be assessed by ABPP (Cravatt, 2008).

268 Chemical probes can be designed based on small molecules derived from natural products or

269 food and used for identification of their interaction partners in proteome (Wright, 2016).

270 Proteomic approaches for the same purpose, but without chemical labelling of small molecule,

271 are also described (Guo, 2017).

272 **3. Sample preparation**

273 Proper sampling, sample preparation and sample handling are seen as among the main problem

274 areas of proteomics (Nilsson, 2010b). The division of labour between those who control

275 sampling and sample preparation and those who work with the MS can result in serious data

276 quality issues, due to the lack of accountability and management of the data generated (Nilsson,

277 2010b) and lack of standardization (Poste, 2011). Protocol should be thoroughly discussed

278 between MS-based proteomics specialists and team members (biologists, chemists, food

279 technologists, nutritionist, clinicians, etc.). Correct sampling requires knowledge of complex

280 structure of the food matrix and the corresponding analytical protocol (Jongenburger, 2015).

281 Sampling methods are not equally useful, and there is no universal method, thus making the

282 choice during experimental design is a critical point (Skold, 2013). After sampling, a proteome

283 can be rapidly modified by released (or activated) proteases, other protein- modifying enzymes

284 (*e.g.* phosphatases) and metabolites (*e.g.* polyphenols, glutathione, organic acids) which are

285 naturally present in analysed food material. Sample preparation itself can be a significant bias of

286 a foodomic method since the accuracy of the experimental data, and both their reproducibility

287 and confidence essentially depend on the accuracy and quality of the clean-up technique.

288 Consequently, sampling, sample handling and sample preparation has to be known and

289 considered during interpretation of MS data (Skold, 2013). MS-based proteomic specialist is the

290 one who must ensure high fidelity of the platform through routinely performing checks and

291 balances (Bittremieux, 2017; Nilsson, 2010b). For this purpose, different standards should be in

292 regular use to control purification efficiency (Gallien, 2014), protease digestion (Lebert, 2015),

293 peptide retention time during LC (Beri, 2015; Escher, 2012), as well as the ionization efficacy.

294 Automated pipelines for quality control of LC-MS/MS are in development (Bereman, 2015a).

295 An overview of techniques in food analysis and sample preparation was recently published

296 (Galloa, 2016).

297 MS-based proteomic techniques can detect about 2000 proteins in 0.1 ug of protein digest.

298 However, detection of more than 9000 proteins requires more than 1 mg ($>5x10^6$ average human

299 cells) of starting material (Mallick, 2010; Zubarev, 2013). Protein extraction is a first step in

300 sample preparation, and it is a great source of variation, its design strongly influencing proteomic

301 results (Dhabaria, 2015). An number of different procedures usually involve physical

302 homogenisation (mechanical force, ultrasound (Kadama, 2015), increased pressure,

303 heating/cooling, etc.), the use of buffers, detergents, chaotropic agents for protein extraction and

304 solubilisation, application of reducing agents, as well as different substances for enzyme

305 inhibition (Bodzon-Kulakowska, 2007). Some of these, alone or in combination with others, can

306 introduce chemical or physical changes of amino acids such as carbamylation or the Maillard

307 reaction (Kollipara, 2013). Sample preparation in foodomics is the topic of a comprehensive

308   overview about sample preparation in foodomics that also includes complex approach by use of

309   different proteolytic enzymes and other methods for protein cleavage before further analysis by

310   LC-MS/MS (Andjelković, 2017).

311   **4. Acquisition of mass spectra in bottom-up proteomics**

312   The term MS-based proteomics is used most often in the context of bottom-up approach (Fig.2).

313   In bottom-up approach proteins are extracted and digested by a sequence-specific protease.

314   Resulting highly complex mixture of peptides is supplied to mass spectrometer in the form,

315   amount and time frame that will enable successful examination using a particular MS technique.

316   Currently, mass spectra can be acquired in three different ways: data dependent, targeted and

317   data independent acquisition (Fig.3).

318   **4.1. Data dependent acquisition**

319   The most common MS-based proteomic approach in food investigation is the shotgun bottom-up

320   approach, also known as "discovery based" (Zhang, 2013), Fig.2. In this approach, an adequately

321   extracted and prepared sample containing a protein mixture is digested to peptides with trypsin

322   or with other site-specific proteases (Switzar, 2013). The obtained peptide mixture is

323   subsequently separated into fractions by LC or other separation techniques, such as capillary

324   electrophoresis. These fractions are either on-line electrosprayed (LC-ESI-MS/MS), or off-line

325   spotted and after addition of proper matrix ionised by MALDI (LC-MALDI-MS/MS), and

326   introduced into the corresponding mass spectrometer. In a further step, the generated ions are

327   scanned (MS scan) and in so-called data dependent acquisition (DDA) mode, usually 3-20 most

328   abundant ("top") ions are selected by predetermined rules (dynamic exclusion, detection

329   window, charge state selection, base line subtraction etc.) in a time dependent manner (Fig.3).

330   Selected ions are then fragmented (CID, HCD, ETD, EThcD) (Frese, 2011). The EThcD

331 fragmentation technique is implemented into the latest hybrid mass spectrometers and

332 substantially improves the level of peptide backbone fragmentation (Frese, 2012). The generated

333 fragments are subsequently analysed by an MS/MS scan. In the final step, the data from MS and

334 MS/MS scans are matched with sequence databases, by means of different algorithms, in order to

335 identify peptides and, subsequently, proteins (Audain, 2017; Ting, 2015). Interpretation of

336 shotgun proteomic data is a complex task that can lead to ambiguities in determining the

337 identities of sample proteins (Nesvizhskii, 2005). Information obtained at the level of peptides

338 has to be analysed in detail in order to make correct conclusions about protein/s (isoforms,

339 proteoforms, sequence redundancy) that contain particular peptide (Nesvizhskii, 2005).

340 **4.1.1. Capacity of shotgun bottom-up approach**

341 MS-based proteomics shows a brilliant development over last 10 years. Seven years ago a

342 standard shotgun LC-MS/MS analysis of a single cell line lysate, over 3-h, elute more than

343 100,000 isotope features, likely representing peptides, and they could be detected with a HRMS

344 scan. However, just 16% of these were targeted by an MS/MS scan and only 9% of them were

345 identified by "top 10" DDA (Michalski, 2011). With a standardized analysis platform, the

346 achieved degree of repeatability and reproducibility was about 70-80% (Tabb, 2010). A higher

347 degree of reproducibility (>90%) with this technology could be achieved by repeating the

348 analysis 7-10x until virtually every peptide has been observed, however, only when results of all

349 subsequent runs have a very high overlap with already collected data (Mitchell, 2010).

350 Application of a longer LC gradient or intensive sample fractionation with subsequent analysis

351 of each fraction could also improve the reproducibility of DDA. However, this strategy requires

352 more time and increases costs (Domon, 2010). Major limiting properties of mass spectrometers

15

353   for the detection of a larger number of peptides in a short time are: sequencing speed (duty

354   cycle), sensitivity, and precursor ion isolation (Michalski, 2011).

355   Five years ago, an advanced commercial instrument under carefully optimized conditions could

356   identify more than 37,000 peptides (belonging to ~5,000 proteins) in a 4-h single dimension LC-

357   MS/MS run (Pirmoradian, 2013). This is about a half of the expressed proteome of an average

358   human cell line. Multidimensional protein identification technology (MudPIT; combination of

359   several separation techniques) (Fournier, 2007) could provide >10,000 proteins, but operational

360   costs, sample preparation and consumption, and working time of LC-MS/MS of more than 24-h

361   were still high (Pirmoradian, 2013). Latest Orbitrap Fusion MS system could analyse ~90% of

362   yeast proteome (~4500 proteins) in 1.5-h of nanoLC work (Hebert, 2014).

363   Mass spectrometers handle a proteome dynamic range of 4-5 orders of magnitude (Domon,

364   2010). However, the proteome dynamic range stretches over at least 6 orders of magnitude,

365   approaching 11 orders of magnitude in the best investigated case of blood plasma (Anderson,

366   2002). A wide dynamic range is one of the most challenging problems in MS-based proteomics

367   and it is still not satisfactory solved. The complexity of analysed peptide mixtures is increased by

368   the proteolytic background coming from ions of peptides that are results of an unspecific tryptic

369   (proteolytic) cleavage. Relative abundance of nonspecific peptide ions is about one order of

370   magnitude lower than the expected abundance of specific ones (Picotti, 2007). Proteolytic

371   digestion increases the dynamic range of signal intensities of peptides for at least one order of

372   magnitude (Zubarev, 2013). This is the intrinsic limitation of the shotgun technique that covers

373   low abundant peptides and impairs their identification (Picotti, 2007). A portion of generated

374   peptides can also be modified during sample preparation (Table 2). These modifications also

375   occur in an undefined fraction of peptides. That means that the modifications are not

376 stoichiometric. Moreover, there are chemical modifications like racemization or isomerization

377 (Table 2) of amino acid side chains that do not change the molecular mass but may influence

378 chromatographic behaviour. All listed modifications are lowering the amount of a particular

379 peptide (or a proteoform) and they also increase the complexity of an analysed mixture. Thus,

380 identification of both low abundant proteins and highly hydrophobic proteins is a complex task

381 that requires a specially designed sample preparation procedure, the choice of an optimal

382 proteomic approach, an optimal LC-MS/MS system and corresponding data analysis (Josić,

383 2007, 2014; Vučković, 2013; Zubarev, 2013).

4.1.2. Future task

385 The exclusive use of trypsin in proteomics could be a reason why our view of the proteome still

386 remains incomplete. For the sake of broadening this view, a parallel analysis with alternative

387 proteases or other cleavage strategies shall be considered in the future. This will enable to access

388 more information rich sequences important for the identification of protein isoforms and

389 proteoforms (Giansanti, 2016; Trevisiol, 2016). Up to date, LysargiNase has been evaluated for

390 its application in shotgun bottom-up proteomics (Tsiatsiani, 2017). It cleaves proteins at the N-

391 terminal side of Arg and Lys. Comparing to the products of tryptic digestion, these peptides

392 contain two protons, following ESI, positioned at the N-terminus. The consequence is a

393 completely different fragmentation pattern that provides additional structural information

394 (Tsiatsiani, 2017). Protection of Lys,\ by a chemical modification before trypsin digestion

395 restricts cleavage to Arg (except when it is followed by Pro) and it could also be an alternative to

396 the conventional trypsin digestion (Golghalyani, 2017). An even better might be achieved by use

397 of protease GingisREX^{TM} that cleaves only the C-terminal side of Arg, regardless of Pro.

398   Stochastic nature of the precursor ion selection in DDA is biased toward the more abundant

399   component in the sample. The consequence is that the changes caused by single nucleotide

400   polymorphisms, mutations, splicing variants, some PTM`s and other protein modifications are

401   mostly inaccessible. Additionally, the low sequencing speeds of mass spectrometers that are

402   applied for analyses cause additional problems with reproducibility of DDA (Domon, 2010;

403   Picotti, 2013). Application of dynamic exclusion in DDA maximizes the number of unique ions

404   to be isolated for fragmentation and MS/MS scan. In the same time, application of dynamic

405   exclusion strongly reduces the probability of isolation of a precursor ion at the apex of its

406   chromatographic elution peak. The repercussion is a negative effect on the quality of the

407   acquired spectra and consequently, on both qualitative and quantitative analysis. Efforts to

408   overcome these problems have led to the development of targeted and directed approaches

409   (Domon, 2010).

410   **4.2. Targeted acquisition**

411   Targeted proteomic approaches ("hypothesis driven proteomic approaches") were developed for

412   accurate and reproducible quantification of any protein or a set of proteins in any biological

413   sample (Picotti, 2013). First targeted approach was based on a MS acquisition technique called

414   selected reaction monitoring (SRM) developed on QqQ mass spectrometers. The first step in a

415   targeted approach is the selection of proteins that are objectives of a particular research

416   (formulation of a hypothesis) and that will be targeted with the MS analysis. For each selected

417   protein, at least one peptide with 2-4 characteristic transitions (pair of m/z values associated with

418   the precursor and one of its fragment ions) is carefully selected for monitoring (Brusniak, 2011;

419   Carr, 2014; Colangelo, 2013). When more than one transition is monitored, SRM is known as

420   multiple reaction monitoring (MRM). If food proteins are analysed, selection of peptides for

421 SRM additionally has to take in consideration modification of proteins presented in Table 2. The

422 total number of targeted peptides that can be reliably quantified is limited by the time available

423 for the transition scan at a particular mass spectrometer, the amount of a particular peptide ion

424 and by the chromatographic elution profile of a peptide. Thus, the total number of proteins that

425 can be reliably quantified in one LC-MS/MS run on QqQ is around 100 (Picotti, 2013).

426 Sensitivity of SRM allows the identification of down to 100 or ~7500 copies per cell in non-

427 fractioned yeast or human proteomes respectively, in a 1-h LC run (Picotti, 2013; Ebhardt, 2012;

428 Picotti, 2009). The limit of detection of SRM can be further improved by sample fractionation or

429 enrichment. This enables the detection of low abundant proteins, isoforms and proteoforms (Liu,

430 2013). Better management of time available for mass spectra acquisition during an LC run can be

431 achieved using scheduled SRM. In this technique the detection window for a particular peptide

432 opens only around its elution time and as a consequence the number of quantified proteins can be

433 increased (Escher, 2012).

434 A **directed MS approach** consists of two distinct experiments. In the first run the sample is

435 analysed in MS mode and peptides are identified by bioinformatics tools. An integrative

436 approach providing any additional information from genome-wide mRNA analysis or

437 metabolome data would be extremely beneficial (Vehmas, 2014). In the second run, only

438 peptides of interest are included in the list of ions which will be selected for fragmentation and

439 MS/MS scan. Consequently, directed MS approach focuses ion selection on non-redundant and

440 information-rich precursor ions. Essentially, with better management of available mass

441 spectrometer time, the duty cycle was directed to the peptides of interest (Schmidt, 2008), and

442 not exclusively to the most intensive ions. Thus, partially removing bias toward more abundant

443 components increases the depth of analysis and the reliability of quantification (Domon, 2010;

444 Schmidt, 2008). Benefits of directed MS in proteomics can only be realized if the sample

445 complexity is high in relation to the duty cycles available, and if the samples are available in

446 amounts that allow multiple LC–MS/MS runs (Schmidt, 2008). This is the case in food research

447 where samples are of high complexity and their amounts are usually not restricted.

448 **4.3. Data independent acquisition**

449 Numerous technical advances, like the development of HRMS, improvements in ion collection,

450 transmission optics and selection, and increase of scan speed, enabled the development of data

451 independent acquisition (DIA) technique. DIA uses a defined window size to systematically, in

452 repeated cycles during a chromatographic run, sample precursor ions from an analysed mass

453 range. All sampled precursor ions are simultaneously fragmented and MS/MS spectra are

454 collected (Bilbao, 2015; Chapman, 2014). The window size in different DIA approaches ranges

455 from a wide window comprising the whole mass range to a very narrow one, down to 0.4 Th. In

456 this way DIA approaches generate very complex MS/MS spectra, especially when wide

457 precursor isolation windows were used. DIA does not provide direct link between the precursor

458 ion and its fragment ions. Hence, the analysis of data acquired with DIA require complex

459 processing strategies as well as software solutions and large informatics resources (Bilbao, 2015;

460 Egertson, 2015; Escher, 2012). Once acquired data with DIA can be later refined and re-mined.

461 Many different DIA approaches are developed and implemented on different mass

462 spectrometers, Table 3. Each of listed approaches has unique characteristics and choosing one

463 over the other involves trade-offs in sensitivity, selectivity and number of samples analysed in

464 certain time frame. Compared to DDA strategies in shotgun proteomics, DIA increased the

465 visibility of low abundant and isobaric peptides, and as a consequence increased the

466 identification of proteins containing these peptides. The dynamic range of DIA spans over 4-6

20

467 orders of magnitude (Aebersold, 2016; Gillet, 2012), up to 8 with CSI-PAcIFIC DIA (Table 3).

468 Selective enrichment and purifications of peptides containing PTMs is a usual strategy for their

469 analysis, (Fig. 2). However, this is not possible for all PTMs and DIA methods could be applied

470 as an alternative.

471 **5. Alternatives to MS-based bottom-up proteomic approach**

472 Alternative and complementary approaches to MS-based bottom-up proteomic approaches are

473 top-down and middle down MS-based approaches.

474 In top-down proteomics (Fig.2), intact proteins or large protein fragments (>15 kDa) are

475 analysed by MS scan to obtain the molecular weight of a particular protein and its proteoforms.

476 The MS scan is performed on an ultra-HRMS, in first line FT-ICR and Orbitrap. However, TOF

477 mass analysers can also be used for certain top-down analyses. Upon MS scan the protein ions

478 are fragmented and fragments are analysed in a consequent MS/MS scan. Fragmentation

479 techniques such as ETD/ECD are of special importance, since they are able to preserve the

480 information about PTM`s. Combination of ECD with CID (EtciD) or HCD (EthcD) can provide

481 high protein sequence coverage that increases the confidence in proteoform identification

482 (Brunner, 2015; Frese, 2012). Infrared multiphoton dissociation or ultraviolet photodissociation

483 exhibited an additional potential to improve *de novo* protein sequencing (Shaw, 2016). Due to

484 the current inefficiency of MS/MS techniques, limit of protein size that can be efficiently

485 sequenced in a time-constraint experiment is around 50 kDa (Laskay, 2013). The MS scan of

486 intact protein contains a large number of highly charged ions which originate from the same

487 molecular species. In the presence of proteoforms and other proteins, isolation of single ion

488 species is a hardly feasible task and the resulting MS/MS spectra are highly convoluted (Laskay,

489 2013). Hence, the top-down approach requires intensive fractionation to obtain less complex

490 protein mixtures (Tran, 2011; Zhang, 2014). The characterization of proteoforms in identified

491 proteoform–spectrum matches still relies mainly on manual annotation (Kou, 2016), but recent

492 technological advances of mass analysers towards ultra-high resolution, as well as new

493 algorithms for data processing, are now making top-down the method of choice when studying

494 complex proteoforms (Kou, 2016; Vyatkina, 2015).

495 Middle down proteomics analyse large peptides with a size of about 7-15 kDa, compared to less

496 than 3 kDa in bottom-up approach, and 3-7 kDa in extended bottom-up proteomics. This strategy

497 combines the advantages of bottom-up and top-down approaches and minimizes their

498 shortcomings (Laskay, 2013). The advantage of longer peptides is their larger chance to contain

499 higher charge, resulting in a more efficient ECD/ETD. The result of increased fragmentation

500 efficiency is the higher sequence coverage. Additionally, larger peptides have a higher chance to

501 contain individual mutations and PTM`s hence it is beneficial for the identification of isoforms

502 and proteoforms. Moreover, the complexity of a peptide mixture is reduced when longer peptides

503 are generated, rendering more time for mass analysers during an LC run, and resulting in a

504 higher resolution for larger number of peptides. Consequently, the analysis of large proteins that

505 still cannot be analysed by top-down approach, as well as the analysis of proteoforms that are

506 difficult to separate, can benefit from the middle down approach (Zhang, 2014). Peptides of

507 average mass >3.4 and >6.3 kDa can be generated by the Sap9 (Srzentić, 2014) and OmpT (Wu,

508 2012) proteases respectively.

509 **6. Quantitative high-throughput proteomics**

510 Mass spectrometry can provide relative quantitative information (a quantitative comparison of

511 proteins between different samples expressed in a fold of change of a particular protein between

512   analysed samples) or an absolute (exact concentration or number of individual protein/s in a

513   given sample). Different technologies and approaches were developed for this purpose, Table 4.

514   The importance of MS-based quantitative proteomics is emphasized with recent studies

515   demonstrating that the identity of cells and tissues seems to be determined primarily by the

516   abundance at which they express their constituent proteins, and perhaps by the manner how the

517   proteins are organized in the proteome, rather than by the presence or absence of certain proteins

518   (Aebersold, 2016).

519   Quantitative changes of the food proteome may be influenced by different factors such as plant

520   or animal strain, genetic engineering, growing conditions, quality of animal food, particular food

521   processing, storage conditions, etc. Consequently, individually or collectively these factors can

522   influence food quality and/or safety (Agrawal, 2013; D'Alessandro, 2012; Piras, 2016; Tedesco,

523   2014).

524   Biological variations of interest in proteomics are often very small. Consequently, the

525   requirements for the precision of quantitative proteomic experiments are very high (Lyutvinskiy,

526   2013). Accurate mass measurement is of a major concern in the development of MS-based

527   proteomics (Aebersold, 2016; Tabb, 2010). MS-based proteomics is an example of a multivariate

528   process with the potential for highly correlated variables as performance declines (Bereman,

529   2015). Major sources of variability are extraction, instrumental variance, instrumental stability

530   and protease digestion (Piehowski, 2013). Every source of variation can be detrimental to the

531   extract of meaningful biological information. Different tools are available to monitor the system

532   suitability and to improve proteomic workflows (Bereman, 2015; Walzer, 2014). The importance

533   of this topic is promoted by HUPO within the proteomic standard initiative specialized quality

534   control working group that has been founded in order to define a community data format and

535 associated controlled vocabulary terms, facilitate data exchange and archiving of MS derived

536 quality control metrics (http://www.psidev.info/groups/quality-control). Quality control methods

537 and standard operating procedures are necessary parts of proteomics, unfortunately still

538 frequently neglected. The evaluation of performance can be achieved by sharing and exchanging

539 results between reference laboratories, by use of common samples, different methodologies and

540 experimental designs (Bereman, 2014; Tabb, 2016). If laboratories deploy different

541 methodologies to analyse the differences between the same two complex samples, then they will

542 assuredly see differences in the gene or protein lists produced by the two technologies (Tabb,

543 2016).

544 Quantitative comparison of proteins from different samples (relative quantification) is mostly

545 performed by two basic technologies (Ong, 2005), Table 4.

546   1. Directly comparing ion abundance between samples while applying different strategies

547 to minimize different mass spectrometer response and differences in sample preparation; this

548 group of approaches are known as label-free quantification (LFQ) (Cox, 2014; Neilson, 2011);

549   2. Upon labelling (metabolic, chemical or enzymatic) of proteins (or peptides) in each of

550 the few analysed samples using unique stable isotopes, samples are mixed and analysed together

551 in the same run; this technology is known as stabile isotope dilution (Ciccimaro, 2010). Most

552 known approaches based on the labelling technology are listed in Table 4.

553 **6.1. Label-free strategies**

554 In label free proteomics, quantification can be performed using different approaches at the MS

555 scan level (area under the curve or signal intensity measurement) or at the MS/MS scan level

556 (spectral counting) (Ahrné, 2013; Arike, 2014; Neilson, 2011). Comparative studies of LFQ

557 approaches demonstrated certain advantages the two former approaches (Ahrné, 2013; Arike,

558 2012; Dowle, 2016).

559 In a label-free quantitative proteomic experiment each sample is prepared and analysed

560 independently. Discrepancy in sample preparation procedure (sampling, sample handling,

561 extraction efficiency, protease digestion efficacy, clean-up efficacy, etc.) is a source of

562 variations. These variations can be reduced by procedure design, training of personnel and

563 application of robotics. Variability of instrument response (*e.g.* variation in the current of ESI

564 during an LC-MS/MS run, ion suppression during ionization, reproducibility of retention times,

565 fluctuation in instrumental sensitivity) can be reduced by use of internal standards (Lyutvinskiy,

566 2013; Piehowski, 2013). In order to standardize sample preparation, the extent of digestion and

567 performance of an LC-MS/MS system, a universal protein standard called DIGESTIF was

568 developed (Lebert, 2015). However, the use of internal standards introduces another level of

569 complexity and increases the costs of the analysis (Lyutvinskiy, 2013). Instrumental response

570 can be corrected by *in silico* post-processing. This significantly improves the accuracy and

571 precision of LFQ (Cox, 2014; Lyutvinskiy, 2013; Tu, 2017). An MS-based proteomic

572 experiment will highly benefit from every step undertaken towards the reduction or correction of

573 the coefficient of variation (CV). Reduction of CV improves efficacy (probability to detect

574 quantitative difference between proteomes) by reducing time and costs of experiments

575 (Lyutvinskiy, 2013). Detailed optimization of parameters for LFQ could provide relative

576 quantification of up to 2900 proteins in 4-h for samples analysed in triplicate (Pirmoradian,

577 2013).

578 **6.2. Label-based strategies**

579 Concerning both precision and accuracy, SILAC is the "golden standard" for relative

580 quantification in discovery proteomics (Lyutvinskiy, 2013; Zhang, 2013). This approach,

581 described in 2002 for *in vitro* non- isobaric metabolic labelling, was subsequently adjusted for

582 many different applications, including *in vivo* labelling of animals and plants, as well as tissue

583 analysis (Ong, 2007; Table 4). In SILAC method, labelled samples are concomitantly analysed

584 by LC-MS/MS and relative quantitative comparison is obtained from the MS scan. High

585 accuracy of this method is a consequence of several facts: mixing of differently labelled samples

586 early in the experimental process, which enables simultaneous sample preparation and LC-

587 MS/MS analysis; the fact that every protein is quantified several times through multiple MS

588 scans and usually (85%) through multiple peptides; and 100% efficiency of metabolic labelling.

589 High costs of an *in vivo* labelling were reduced with the development of spike-in SILAC, while

590 high complexity of tissue proteomes was addressed with the use of "super SILAC", for

591 references see Table 4.

592 When TMT/iTRAQ are used, relative quantitative information is obtained when peptides that are

593 chemically labelled with an isobaric tag upon fragmentation release low m/z reporter ions. These

594 reporter ions are compared in the subsequent MS/MS scan. Peptides labelled with an isobaric tag

595 have the same mass in an MS scan, thus they do not increase the complexity of the MS scan

596 spectrum as it is the case with peptide labelling with non-isobaric tags and SILAC. Comparing to

597 MS-based quantification, a higher dynamic range can be assessed with MS/MS based

598 quantification (Rauniyar, 2014). Currently, the advantage of TMT/iTRAQ over SILAC is

599 multiplexing that allows a simultaneous quantitative analysis of 10 samples (Weekes, 2014).

600 Application of triple-stage MS (MS3) was proposed to eliminate interference in iTRAQ, which

601 comes from near-isobaric ions that are co-isolated and co-fragmented with the selected peptide

602 (Ting, 2011). However, application of MS3 takes a penalty in sensitivity. When the MultiNoch

603 MS3 method developed on the Orbitrap Velos mass spectrometer was applied, the sensitivity of

604 MS3 could be increased 10x without a significant loss of selectivity (McAlister, 2014). Another

605 method that eliminates accuracy and precision problems of TMT/iTRAQ exploits high accuracy

606 and resolution of modern mass spectrometers using complement TMT fragment ion clusters as

607 an alternative to reporter TMT fragment ions (Wühr, 2012).

608 Chemical dimethyl labelling introduces non-isobaric tag to peptides that allow quantification at

609 the level of an MS scan. The main advantages of dimethyl labelling are inexpensive reagents, as

610 well as the labelling procedure that can be easily automated, performed on-line, and applied in a

611 high-throughput manner (Altelaar, 2013). Labelling with different isobaric tandem mass tags or

612 with non-isobaric mass tags is performed at the level of peptides, after protease digestion of a

613 sample. This includes more independent sample preparation steps (that can be significant sources

614 of variability) before mixing differently labelled samples for further simultaneous sample

615 preparation and LC-MS/MS analysis.

616 Additionally, special isobaric tags are developed for cysteine and PTMs, such as carbonyl and

617 glycan modifications (Rauniyar, 2014).

618 Neutron encoding (NeuCode) is a new quantification approach which benefits from ultra-high

619 resolution of FT-ICR and Orbitrap mass analysers that is capable of distinguishing a mDa mass

620 difference in a neutron mass signature of different isotopes (Hebert, 2013). Neutron mass

621 signatures can be encoded in metabolically, chemically or enzymatically introduced tags. Using

622 neutron encoding, the multiplexing capacity of SILAC was increased, currently up to 9-plex in

623 NeuCode SILAC. It combines the accuracy of SILAC with multiplexing capacity of isobaric

624 tagging and does not suffer from the problem of precursor interference which reduces the

625 accuracy of isobaric tagging (Rose, 2013). The number of mechanisms for increasing

626 multiplexing capacity (number of samples which can be simultaneously analysed) of isobaric

627 regents was described (Braun, 2015; Frost, 2015). Results of the latest study evaluating the

628 reproducibility of LFQ and iTRAQ showed an encouraging degree of conformity that suggests a

629 degree of the maturity of proteomic methods (Tabb, 2016).

6.3. Absolute quantification with isotope labelled standards

631 Absolute quantitative information about individual proteins is a prerequisite for modelling

632 studies of biochemical systems (Malmström, 2009), for understanding the complex interplay of

633 the system (food or consumer) components or interplay between components of two systems

634 (food and consumer), as well as for the quality and safety control of food. As in the case of

635 relative quantification, absolute quantification strategies are based on the technology of stable

636 isotope dilution (Brun, 2009; Villanueva, 2014). Essentially, signal intensity of a mass

637 spectrometer is standardized with a known concentration of an isotope labelled reference. This

638 reference can be produced by labelling a standard sample containing a known amount of peptides

639 of interest tagged with isobaric (or non-isobaric) tags. Also, the reference can be an isotope

640 labelled peptide of identical structure as the peptide of interest. The reference isotope labelled

641 peptide can be supplied to the sample using different strategies: AQUA, QconCAT or PSAQ

642 (reference in Table 4).

643 A synthetic isotope labelled peptide can be added into the sample before protease digestion or

644 immediately before LC-MS/MS analysis. This strategy is known as AQUA (absolute

645 quantification). If a subsequent immunoaffinity step is performed, in order to enrich the low

646 abundance peptide of interest, the strategy is known as SISCAPA (Stable Isotope Standards and

28

647   Capture by Anti-Peptide Anti-bodies) (Anderson, 2004). The selection of peptides that will be

648   used as internal standards is important for the success of quantification, and different methods

649   are developed for this purpose (Brusniak, 2011; Eyers, 2011). Peptide standards for AQUA may

650   also contain PTMs if these are of interest for quantification. Quantification accuracy of AQUA

651   strategy may be compromised by incomplete protease digestion of proteins or if pre-fractionation

652   steps are used in sample preparation. Thus, efficiency of digestion has to be monitored, as well

653   as the yield (recovery) after each fractionation step (Gallien, 2014).

654   Quantification concata-mer (QconCAT) strategy uses polypeptide constructs (concatmers)

655   composed of many different isotope labelled peptides. These constructs are biologically

656   synthetized. Concatmers are added to the sample before protease digestion and protease releases

657   isotope labelled peptides. In the same time, these peptides serve as a control for digestion and

658   also as internal standards for quantification. QconCAT enables simultaneous quantification of

659   several proteins and is less cost-intensive than AQUA. However, this method also suffers from

660   the same problem caused by an insufficient protease digestion efficiency and possible low yield

661   during pre-fractionation.

662   In order to provide a reliable absolute quantification, a good internal standard should behave as

663   closely as possible as the analysed protein, following it throughout all sample preparation steps.

664   As a part of the strategy for overcoming problems with accuracy caused by protease digestion

665   efficiency and sample pre-fractionation, protein standard absolute quantification (PSAQ) strategy

666   uses isotope labelled intact proteins. Providing multiple peptide standards for target protein,

667   PSAQ provides also higher efficacy. Production costs of protein standards are limiting factors for

668   a wide application of this strategy. However, cell free systems for protein synthesis now offer a

669   way to reduce them (Madono, 2011). High-throughput system for synthesis of protein standards

670 for quantification of highly hydrophobic transmembrane proteins was also developed (Takemori,

671 2015). Nevertheless, problems with synthesis of protein standards containing particular PTMs

672 are still present.

673 When introduced, S/MRM acquisition technique offered the highest sensitivity, a wide dynamic

674 range, and the high selectivity, highest reproducibility and precision that are necessary for

675 absolute quantification. Quantitative information in SRM is given by the intensity of the

676 fragment ion of targeted transition. Nowadays, main problems with SRM are the number of

677 proteins that can be simultaneously monitored and selectivity due to the resolution of QqQ mass

678 spectrometers. The attempt to increase the number of proteins quantified by SRM requires the

679 sacrifice of some selectivity or sensitivity. The addition of a third stage of mass filtering to MRM

680 with multiple reaction monitoring cubed (MRM^3) method on a hybrid QqQ/LIT mass

681 spectrometer (Fortin, 2009) increased the discrimination of interferences compared to regular

682 S/MRM and limit of quantification. Increased selectivity with MRM^3 has as a consequence a

683 lower number of proteins that can be simultaneously analysed, since a part of the available

684 cycling time was sacrificed to a third stage of mass filtering (Gallien, 2013).

685 In order to increase the number of absolutely quantified proteins, relative and absolute

686 quantification strategies were combined. In one combination, a small group of specially selected

687 proteins was quantified using AQUA SRM. These anchor proteins are used as further calibration

688 points for translating relative abundance measurements into absolute abundance measurements,

689 for a large part of the proteome (Malmström, 2009).

690 Strategies for absolute quantification can be combined with SILAC for absolute quantification of

691 individual proteins in complex mixtures. As a result, "absolute SILAC" (Hanke, 2008) and

692 PrEST SILAC (Zeiler, 2012) were developed.

30

693 Absolute quantification may be performed by use of isobaric and non-isobaric mass tags, if

694 peptide standards are used as one, or more, channels in a multiplexed analysis.

695 High resolution of the hybrid quadruple-Orbitrap mass spectrometer enabled development of an

696 approach called parallel reaction monitoring (PRM) (Gallien, 2013). PRM uses a 2-24 Th wide

697 isolation windows on a quadruple for selecting ions for fragmentation and recording

698 fragmentation products in the Orbitrap mass analyser. High resolution of OT increases selectivity

699 by separating ions of interest from interferences leading to partially improved quantification

700 performance compared to SRM (Gallien, 2014a; Gallien, 2013). By use of internal standards and

701 the on-the-fly adjustment of acquisition parameters, it is possible to organize acquisition time in

702 PRM and quantify 600-1000 peptides in complex samples in ~1-h (Bourmaud, 2016; Gallien,

703 2015).

704 **6.4. Data independent acquisition in quantitative high-throughput proteomics**

705 The problem with precursor ion isolation window width that is required to ensure sufficient

706 sensitivity in mass spectrometers (Michalski, 2011) makes that MS/MS spectra, that are obtained

707 when samples of high complexity analysed, is actually a mixture ("chimera") spectra due to the

708 co-isolation of all ions (originating from co-eluted peptides in the previous LC or capillary

709 electrophoretic separation) falling within the mass isolation windows width (Luethy, 2008). This

710 problem with selectivity may cause difficulties in the following peptide identification since it

711 may increase the complexity of MS/MS spectra (Houel, 2010). Additionally, if fragments of co-

712 isolated ions are similar to the fragments of the selected peptide they will also impair

713 quantification accuracy, and this problem is more pronounced if mass spectrometers with low

714 resolution are used. The level of interference this creates depends on abundances of analysed

715   peptides. Taking in to account these facts, DIA makes an attempt to take advantage of these

716   facts.

717   The DIA provide the possibility to overcome limitations of S/MRM and PRM in absolute

718   quantification. The use of this acquisition technique substantially increases the number of

719   proteins that can be simultaneously quantified; it simplifies the experimental design and provides

720   a flexible post acquisition data analysis. Extraction of quantitative information from data

721   acquired with DIA (DIA fragment ion maps) can be performed with a targeted or an untargeted

722   approach (Egertson, 2015; Li, 2015; Röst, 2014; Ting, 2015; Tsou, 2015).

723   In targeted extraction (peptide-centric matching approach), spectral libraries are used to

724   mine DIA fragment ion maps for constellations of signals that precisely correlate with the known

725   coordinates of a targeted peptide, thus uniquely identifying the peptide in the map. Coordinates

726   that spectral libraries contain are: retention time information and, reference MS/MS spectra with

727   relative intensities of ions (Egertson, 2015; Gillet, 2012; Rosenberger, 2014). Retention time

728   normalization has to be performed for each run according to reference peptides, in order to

729   enable a comparison of the analysed sample and peptide library (Escher, 2012; Parker, 2015;

730   Röst, 2014). This allows for acquired data to be analysed in the same way as by SRM by targeted

731   data extraction of transitions of interest (Egertson, 2015; Gillet, 2012; Parker, 2015). Available

732   software types for peptide-centric matching are Open SWATH, Skyline, Spectronaut, PeakView,

733   and SwathProphet. DIA permits quantification of (at least) as many compounds as those

734   typically identified by regular shotgun proteomics with the accuracy and reproducibility of SRM

735   across many samples (Gillet, 2012). Generation of spectral libraries is a current limitation of the

736   targeted data extraction approach. The problem with coverage and the quality of spectral libraries

737   is particularly pronounced with new food samples and complex food samples containing new

32

738   proteins, their isoforms and proteoforms, especially those proteoforms generated upon food

739   processing. Therefore, targeted data processing of complex food samples is currently restricted

740   and has to be combined with other approaches, such as iterative data mining based on theoretical

741   knowledge to account for previously undetected proteins (Bilbao, 2015; Gillet, 2012). However,

742   once developed, spectral libraries for a particular food sample will be permanently available.

743   In untargeted data extraction (spectrum-centric matching approach), real time correlation,

744   based on the retention time between the MS signal (precursors) and the MS/MS signal

745   (fragments), is performed (Ting, 2015; Tsou, 2015). In that way, the established relationship

746   between the precursor and corresponding fragments enables searching and matching with

747   sequence databases in the same way as DDA spectra. Different software is available for

748   spectrum-centric matching (PLGS, DIA-Umpire, MSPLIT-DIA, Group-DIA).

749   The SWATH MS is a combination of DIA and targeted data analysis, developed on a QqTOF

750   mass spectrometer, which vastly extends the number of proteins that can be quantified in

751   complex sample (Gillet, 2012). The size of the sampling window for precursor ions in SWATH

752   MS is 25(+1) Th. Recently, attempts were made to adjust the sampling window size to the

753   density of precursors across the mass range in order to increase selectivity, depth of coverage and

754   data quality. Using SWATH MS, 2500 proteins of yeast were quantified in a 3-h LC-MS/MS run

755   with reproducibility, precision and accuracy comparable to S/MRM (Selevsek, 2015). The same

756   analysis with S/MRM would take 48-h. The SWATH MS demonstrates high sensitivity (detected

757   >300 proteins more than Western blot) (Selevsek, 2015).

758   **6.5. Selection of quantification strategy, quality control and validation**

759   Many different MS-based quantification approaches were developed. There is no 'one-size-fits-

760   all' proteomic strategy that can be used to address all biological questions (Mallick, 2010).

761 Consequently, an adequate choice of quantitative approach is important for the success of an

762 MS-based proteomic analysis. In order to make the correct choice it is necessary to be well

763 versed in the technology and know the limitations and the advantages of MS-based approaches

764 (Domon, 2010; Mallick, 2010). Moreover, the selection should consider factors such as the type

765 of sample (source and complexity), the number of samples, necessary accuracy and

766 reproducibility, available personnel and equipment, and finally, both the available time and total

767 costs.

768 Quality control is an integral part of high-throughput MS-based proteomic experiments.

769 Inadequate validation or absence of any validation was blamed for wrong conclusions of many

770 high-throughput proteomic studies (Mitchell, 2010), and in combination with problems in

771 reproducibility, that were caused by reckless and incorrect application of this technology, it also

772 was a source of scepticism towards proteomics (Editorial, 2008; Nilsson, 2010b). The main

773 sources of these irregularities are sample preparation, sample handling, data analysis and data

774 evaluation (Nilsson, 2010b). When these tasks are divided between different professions, without

775 consultation and coordination with specialist for MS-based proteomics who best know the

776 limitations and pitfalls of the technology, and who should also take care about all quality control

777 steps and provide practical instructions about data interpretation, they can become a serious

778 source of problems (Bell, 2009; Nilsson, 2010b). Consequently, successful proteomic analysis

779 should be performed in a systematic, accurate and reproducible manner.

780 Antibody-based techniques are standards for the validation of MS-based proteomic experiments

781 (radioimmunoassay, immunoblot, ELISA, immunofluorescence etc.). Moreover, validation can

782 be performed with other methods such as cryo-electron tomography or morphological

783 measurements at the single-cell level (Malmström, 2009). However, high sensitivity and

784 selectivity of SRM make this technique known as "the mass spectrometrist's ELISA"

785 (Aebersold, 2013; Picotti, 2013). The advantage that SRM offers over antibody-based techniques

786 is a fast and cost-effective assay development. The main problems with commercially available

787 antibodies are that they may not work effectively or that they are not available for a particular

788 protein of interest. The complex nature of protein and food matrix modifications may evoke

789 cross-reactivity or reduced affinity in antibody-based techniques. Both can lead to an over- or

790 under-estimation of a particular protein (Koeberl, 2014). Moreover, different protein isoforms, as

791 well as proteoforms cannot be easily distinguished by use of antibodies (Picotti, 2013). MS-

792 based quantification provides a possibility to establish metrological traceability which enables a

793 meaningful comparison of results among laboratories across the globe (Cryar, 2013; Smit, 2014).

794 All of the mentioned advantages of SRM lead to a proposal for the validation of antibody based

795 techniques with SRM (Aebersold, 2013; Nilsson, 2010a) and the SRM approach is now also in

796 use for the validation of orthogonal proteomic approaches (Selevsek, 2015).

797 **7. Recent technical developments important for MS-based proteomics**

798 The quality and reliability of qualitative and quantitative information obtained from a sample in

799 MS-based proteomics, are particularly influenced by the skill of the analyst, the sample

800 preparation procedure, selected MS technique and approach, the type of mass spectrometer used,

801 and data analysis (Mitchell, 2010). MS-based proteomics are still driven by advances in both

802 chromatographic and MS technology (Helm, 2014). Eight years ago, two main properties of

803 mass spectrometers, sequencing speed (cycling time – number of spectra per second (Hz)) and

804 ion current (efficiency of ionisation and ion transmission to detector) were seen as main limiting

805 parameters for the development of shotgun bottom-up proteomics (Michalski, 2011; Mitchell,

806   2010). Since then, different technical improvements tackling these two parameters were

807   commercialized within new LC-MS/MS systems.

808   The mostly employed LC-MS/MS system in shotgun proteomics is nanoESI-LTQ-Orbitrap.

809   During the last five years, the commercial hybrid OT MS has doubled its speed and increased its

810   resolution. Currently, tribrid OT mass spectrometers achieve 20 Hz with ultra-high resolution of

811   500,000 (at 200 m/z) and accuracy better than 1ppm. However, the amount of ions that can be

812   trapped in OT is still a limiting factor for achieving higher dynamic range (Aebersold, 2016).

813   While commercial QqTOF systems can achieve scanning speed of 100 Hz, their resolution is for

814   an order of value lower, with accuracy up to 1ppm. Mass spectra obtained on QqTOF under such

815   high speed usually do not contain a sufficient number of fragment ions to enable productive

816   peptide identification (Helm, 2014; Meier, 2016). However, in recent years, the number of

817   improvements in technology (improved collision cell, orthogonal accelerator scheme, reflectron,

818   and detector) made QqTOF resolution and accuracy compatible with shotgun bottom-up

819   proteomics (Beck, 2015). Both high resolution and high speed are advantageous properties, but

820   at the current technical level, their combination in a single mass spectrometer is still reversely

821   proportional. High resolution and high accuracy are advantageous properties crucial for shotgun,

822   while sequencing speed and the amount of usable ions are very important for targeted

823   proteomics.

824   Development of ion mobility spectrometry (IMS) introduced an additional dimension of

825   separation to the standard m/z scans. IMS separates ion based on their size and shape (size of

826   collisional cross section). Ion separation by IMS is fast (~100Hz) (Helm, 2014). Incorporation of

827   IMS in a QqTOF, after the collision cell, provided MS systems which have an improved duty

828   cycle, therefore, improved sensitivity, up to 10-fold. Consequently, faster data acquisition

829 improved peptide identification and quantification (Distler, 2016; Helm, 2014). Recently, a

830 trapped ion mobility spectrometry (TIMS) device was incorporated in a QqTOF before the first

831 quadruple (Meier, 2016). Synchronization of the quadruple with TIMS enables the accumulation

832 and "elution" of accumulated ions, while quantification capacity is preserved. This can result in a

833 better signal-to-noise ratio and provides additional separation of precursor ions, which minimizes

834 the problem with precursor ion isolation (*vide supra*). The application of method called Parallel

835 accumulation - serial fragmentation on TIMS-QqTOF MS system increased MS/MS scan speed

836 12-20x without losing sensitivity, providing a 10-fold gain in shotgun proteomics (Meier, 2016).

837 **Acknowledgments**

844
845 **8. References**

846 Acunha, T., Ibáñez, C., García-Cañas, V., Simó, C., & Cifuentes, A. (2016). Recent advances in
847 the application of capillary electromigration methods for food analysis and Foodomics.
848 *Electrophoresis, 37*, 111-141.
849 Aebersold, R., & Mann, M. (2016). Mass-spectrometric exploration of proteome structure and
850 function. *Nature, 537*, 347-355.
851 Aebersold, R., Burlingame, A. L., & Bradshaw, R. A. (2013). Western blots versus selected
852 reaction monitoring assays: time to turn the tables? . *Molecular & Cellular Proteomics,
853 12*, 2381-2382.
854 Agrawal, G. K., Timperio, A. M., Zolla, L., Bansal, V., Shukla, R., & Rakwal, R. (2013).
855 Biomarker discovery and applications for foods and beverages: Proteomics to
856 nanoproteomics. *Journal of Proteomics, 93*, 74-92.

857 Ahrens, C. H., Brunner, E., Qeli, E., Basler, K., & Aebersold, R. (2010). Generating and
858   navigating proteome maps using mass spectrometry. *Nature Reviews Molecular Cell*
859   *Biology, 11*, 789-801.
860 Ahrné, E., Molzahn, L., Glatter, T., & Schmidt, A. (2013). Critical assessment of proteome-wide
861   label-free absolute abundance estimation strategies. *Proteomics, 13*, 2567-2578.
862 Altelaar, A. F., Frese, C. K., Preisinger, C., Hennrich, M. L., Schram, A. W., Timmers, H. T.,
863   Heck, A. J., & Mohammed, S. (2013). Benchmarking stable isotope labeling based
864   quantitative proteomics. *Journal of Proteomics, 88*, 14-26.
865 Anderson, L. C., DeHart, C. J., Kaiser, N. K., Fellers, R. T., Smith, D. F., Greer, J. B., LeDuc, R.
866   D., Blakney, G. T., Thomas, P. M., Kelleher, N. L., & Hendrickson, C. L. (2017).
867   Identification and Characterization of Human Proteoforms by Top-Down LC-21 Tesla
868   FT-ICR Mass Spectrometry. *Journal of Proteome Research, 16*, 1087-1096.
869 Anderson, N. L., & Anderson, N. G. (2002). The human plasma proteome: history, character,
870   and diagnostic prospects. *Molecular & Cellular Proteomics, 1*, 845-867.
871 Anderson, N. L., Anderson, N. G., Haines, L. R., Hardie, D. B., Olafson, R. W., & Pearson, T.
872   W. (2004). Mass Spectrometric Quantitation of Peptides and Proteins Using Stable
873   Isotope Standards and Capture by Anti-Peptide Antibodies (SISCAPA). *Journal of*
874   *Proteome Research, 3*, 235-244.
875 Andjelković, U., Gavrović-Jankulović, M., Martinović, T., & Josić, D. (2017). Omics methods as
876   a tool for investigation of food allergies. *Trends in Analytical Chemistry, 96,* 107-115.
877 Andjelković, U., Tufegdžić, S., & Popović, M. (2017a). Use of monolithic supports for high☐
878   throughput protein and peptide separation in proteomics. *Electrophoresis, 38,* 2851-2869.
879 Arena, S., Renzone, G., D'Ambrosio, C., Salzano, A. M., & Scaloni, A. (2017). Dairy products
880   and the Maillard reaction: A promising future for extensive food characterization by
881   integrated proteomics studies. *Food Chemistry, 219*, 477-489.
882 Arike, L., & Peil, L. (2014). Spectral Counting Label-Free Proteomics. *Methods in Molecular*
883   *Biology, 1156*, 213-222.
884 Arike, L., Valgepea, K., Peil, L., Nahku, R., Adamberg, K., & Vilu, R. (2012). Comparison and
885   applications of label-free absolute proteome quantification methods on Escherichia coli.
886   *Journal of Proteomics, 75*, 5437-5448.
887 Audain, E., Uszkoreit, J., Sachsenberg, T., Pfeuffer, J., Liang, X., Hermjakob, H., Sanchez, A.,
888   Eisenacher, M., Reinert, K., Tabb, D. L., Kohlbacher, O., & Perez-Riverol, Y. (2017). In-
889   depth analysis of protein inference algorithms using multiple search engines and well-
890   defined metrics. *Journal of Proteomics, 150*, 170- 182.
891 Beck, S., Michalski, A., Raether, O., Lubeck, M., Kaspar, S., Goedecke, N., Baessmann, C.,
892   Hornburg, D., Meier, F., Paron, I., Kulak, N. A., Cox, J., & Mann, M. (2015). The Impact
893   II, a Very High-Resolution Quadrupole Time-of-Flight Instrument (QTOF) for Deep
894   Shotgun Proteomics. *Molecular & Cellular Proteomics, 14*, 2014-2029.
895 Bell, A. W., Deutsch, E. W., Au, C. E., Kearney, R. E., Beavis, R., Sechi, S., Nilsson, T.,
896   Bergeron, J. J., & HUPO Test Sample Working Group. (2009). A HUPO test sample
897   study reveals common problems in mass spectrometry-based proteomics. *Nature*
898   *Methods, 6*, 423-430.
899 Bensimon, A., Heck, A. J. R., & Aebersold, R. (2012). Mass Spectrometry-Based Proteomics
900   and Network Biology. *Annual Review of Biochemistry, 81*, 379-405.
901 Bereman, M. S. (2015). Tools for monitoring system suitability in LC MS/MS centric proteomic
902   experiments. *Proteomics, 15*, 891-902.

903 Bereman, M. S., Beri, J., Sharma, V., Nathe, C., Eckels, J., MacLean, B., & MacCoss, M. J.
904   (2015a). An Automated Pipeline to Monitor System Performance in Liquid
905   Chromatography-Tandem Mass Spectrometry Proteomic Experiments. *Journal of*
906   *Proteome Research, 15*, 4763-4769.
907 Bereman, M. S., Johnson, R., Bollinger, J., Boss, Y., Shulman, N., MacLean, B., Hoofnagle, A.
908   N., & MacCoss, M. J. (2014). Implementation of statistical process control for proteomic
909   experiments via LC MS/MS. *Journal of The American Society for Mass Spectrometry,*
910   *25*, 581-587.
911 Beri, J., Rosenblatt, M. M., Strauss, E., Urh, M., & Bereman, M. S. (2015). Reagent for
912   Evaluating Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS)
913   Performance in Bottom-Up Proteomic Experiments. *Analytical Chemistry, 87*, 11635-
914   11640.
915 Bilbao, A., Varesio, E., Luban, J., Strambio-De-Castillia, C., Hopfgartner, G., Müller, M., &
916   Lisacek, F. (2015). Processing strategies and software solutions for data-independent
917   acquisition in mass spectrometry. *Proteomics, 15*, 964-980.
918 Bittremieux, W., Tabb, D. L., Impens, F., Staes, A., Timmerman, E., Martens, L., & Laukens K.
919   (2017). Quality control in mass spectrometry-based proteomics. *Mass Spectrometry*
920   *Reviews, doi: 10.1002/mas.21544*
921 Blonder, J., Hale, M. L., Chang, K. C., Yu, L. R., Lucas, D. A., Conrads, T. P., Zhou, M.,
922   Popoff, M. R., Issaq, H. J., Stiles, B. G., & Veenstra, T. D. (2005). Quantitative profiling
923   of detergent-resistant membrane proteome of iota-B toxin induced vero cells. *Journal of*
924   *Proteome Research, 4*, 523-531.
925 Bodzon-Kulakowska, A., Bierczynska-Krzysik, A., Dylag, T., Drabik, A., Suder, P., Noga, M.,
926   Jarzebinska, J., & Silberring, J. (2007). Methods for sample preparation in proteomic
927   research. *Journal of Chromatography B, 849*, 1-31.
928 Bourmaud, A., Gallien, S., & Domon, B. (2016). Parallel reaction monitoring using quadrupole-
929   Orbitrap mass spectrometer: Principle and applications. *Proteomics, 16*, 2146-2159.
930 Braun, C. R., Bird, G. H., Wühr, M., Erickson, B. K., Rad, R., Walensky, L. D., Gygi, S. P., &
931   Haas, W. (2015). Generation of multiple reporter ions from a single isobaric reagent
932   increases multiplexing capacity for quantitative proteomics. *Analytical Chemistry, 87*,
933   9855-9863.
934 Brgles, M., Clifton, J. G., Walsh, R., Huang, F., Ručević, M., Cao, L., Hixson D., Müller, E., &
935   Josić, D. (2011). Selectivity of monolithic supports under overloading conditions and
936   their use for separation of human plasma and isolation of low abundance proteins.
937   *Journal of Chromatography A, 1218*, 2389-2395.
938 Brun, V., Masselon, C., Garin, J., & Dupuis, A. (2009). Isotope dilution strategies for absolute
939   quantitative proteomics. *Journal of Proteomics, 72*, 740-749.
940 Brunner, A. M., Lossl, P., Liu, F., Huguet, R., Mullen, C., Yamashita, M., Zabrouskov, V.,
941   Makarov, A., Altelaar, A. F. M., & Heck, A. J. R. (2015). Benchmarking multiple
942   fragmentation methods on an Orbitrap Fusion for top-down phospho-proteoform
943   characterization. *Analytical Chemistry, 87*, 4152-4158.
944 Brusniak, M. Y. K., Kwok, S. T., Christiansen, M., Campbell, L., Reiter, L., Picotti, P.,
945   Kusebauch, U., Ramos, H., Deutsch, E. W., Chen, J., Moritz, R. L., & Aebersold, R.
946   (2011). ATAQS: A computational software tool for high throughput transition
947   optimization and validation for selected reaction monitoring mass spectrometry. *BMC*
948   *Bioinformatics, 12*, 78-93.

949 Cacciola, F., Donato, P., Sciarrone, D., Dugo, P., & Mondello L. (2017). Comprehensive Liquid
950   Chromatography and Other Liquid-Based Comprehensive Techniques Coupled to Mass
951   Spectrometry in Food Analysis. *Analytical Chemistry, 89*, 414-429.
952 Cao, L., Clifton, J. G., Reutter, W., & Josić, D. (2013). Mass Spectrometry-Based Analysis of
953   Rat Liver and Hepatocellular Carcinoma Morris Hepatoma 7777 Plasma Membrane
954   Proteome. *Analytical Chemistry, 85*, 8112-8120.
955 Carr, S. A., Abbatiello, S. E., Ackermann, B. L., Borchers, C., Domon, B., Deutsch, E. W.,
956   Grant, R. P., Hoofnagle, A. N., Uumlttenhain, R., Koomen, J. M., Liebler, D. C., Liu, T.,
957   Maclean, B., Mani, D. R., Mansfield, E., Neubert, H., Paulovich, A. G., Reiter, L., Vitek,
958   O., Aebersold, R., Anderson, L., Bethem, R., Blonder, J., Boja, E., Botelho, J., Boyne,
959   M., Bradshaw, R. A., Burlingame, A. L., Chan, D., Keshishian, H., Kuhn, E., Kinsinger,
960   C., Lee, J., Lee, S. W., Moritz, R., Oses-Prieto, J., Rifai, N., Ritchie, J., Rodriguez, H.,
961   Srinivas, P. R., Townsend, R. R., Van Eyk, J., Whiteley, G., Wiita, A., & Weintraub, S.
962   (2014). Targeted Peptide Measurements in Biology and Medicine: Best Practices for
963   Mass Spectrometry-based Assay Development Using a Fit-for-Purpose Approach.
964   *Molecular & Cellular Proteomics, 13*, 907-917.
965 Castro-Puyana, M., Mendiola, J. A., & Ibañez, E. (2013). Strategies for a cleaner new scientific
966   discipline of green foodomics. *Trends in Analytical Chemistry, 52*, 23-35.
967 Chao, T. C., & Hansmeier, N. (2013). Microfluidic devices for high-throughput proteome
968   Analyses. *Proteomics, 13*, 467-479.
969 Chapman, J. D., Goodlett, D. R., & Masselon, C. D. (2014). Multiplexed and data-independent
970   tandem mass spectrometry for global proteome profiling. *Mass Spectrometry Reviews,*
971   *33*, 452-470.
972 Chassy, B. M. (2010). Can -omics inform a food safety assessment? *Regulatory Toxicology and*
973   *Pharmacology, 58*, S62-S70.
974 Chevalier, F. (2010). Highlights on the capacities of "Gel-based" proteomics. *Proteome Science,*
975   *8*, 23.
976 Ciccimaro, E., & Blair, I. A. (2010). Stable-isotope dilution LC-MS for quantitative biomarker
977   analysis. *Bioanalysis, 2*, 311-341.
978 Colangelo, C. M., Chung, L., Bruce, C., & Cheung, K. H. (2013). Review of software tools for
979   design and analysis of large scale MRM proteomic datasets. *Methods, 61*, 287-298.
980 Colgrave, M. (2017). *Proteomics in Food Science: From Farm to Fork*: Academic Press.
981 Cox, J., Hein, M. Y., Luber, C. A., Paron, I., Nagaraj, N., & Mann, M. (2014). Accurate
982   Proteome-wide Label-free Quantification by Delayed Normalization and Maximal
983   Peptide Ratio Extraction, Termed MaxLFQ. *Molecular & Cellular Proteomics, 13*, 2513-
984   2526.
985 Cravatt, B. F., Wright, A. T., & Kozarich, J. W. (2008). Activity-Based Protein Profiling: From
986   Enzyme Chemistry to Proteomic Chemistry. *Annual Review of Biochemistry, 77*, 383-
987   414.
988 Cryar, A., Pritchard, C., Burkitt, W., Walker, M., O'Connor, G., Burns, D., Thorburn, Q., &
989   Quaglia M. (2013). Towards Absolute Quantification of Allergenic Proteins in Food-
990   Lysozyme in Wine as a Model System for Metrologically Traceable Mass Spectrometric
991   Methods and Certified Reference Materials. *Journal of AOAC International, 96*, 1350-
992   1361.
993 Cunsolo, V., Muccilli, V., Saletti, R., & Foti, S. (2014). Mass spectrometry in food proteomics: a
994   tutorial. *Journal of Mass Spectrometry, 49*, 768-784.

995 D'Alessandro, A., & Zolla, L. (2012). We Are What We Eat: Food Safety and Proteomics.
996   *Journal of Proteome Research, 11*, 26-36.
997 D'Alessandro, A., & Zolla, L. (2013). Foodomics to investigate meat tenderness. *Trends in*
998   *Analytical Chemistry, 52*, 47-53.
999 Deutsch, E. W., Overall, C. M., Van Eyk, J. E., Baker, M. S., Paik, Y. K., Weintraub, S. T.,
1000   Lane, L., Martens, L., Vandenbrouck, Y., Kusebauch, U., Hancock, W. S., Hermjakob,
1001   H., Aebersold, R., Moritz, R. L., & Omenn, G. S. (2016). Human Proteome Project Mass
1002   Spectrometry Data Interpretation Guidelines 2.1. *Journal of Proteome Research, 15*,
1003   3961-3970.
1004 Dhabaria, A., Cifani, P., Reed, C., Steen, H., & Kentsis, A. A. (2015). High-Efficiency Cellular
1005   Extraction System for Biological Proteomics. *Journal of Proteome Research, 14*, 3403-
1006   3408.
1007 Di Palma, S., Hennrich, M. L., Heck, A. J. R., & Mohammed, S. (2012). Recent advances in
1008   peptide separation by multidimensional liquid chromatography for proteome analysis.
1009   *Journal of Proteomics, 75*, 3791-3813.
1010 Distler, U., Kuharev, J., Navarro, P., & Tenzer, S. (2016). Label-free quantification in ion
1011   mobility-enhanced data-independent acquisition proteomics. *Nature Protocols, 11*, 795-
1012   812.
1013 Doll, S., & Burlingame, A. L. (2015). Mass spectrometry-based detection and assignment of
1014   protein posttranslational modifications. *ACS Chemical Biology, 10*, 63-71.
1015 Domon, B., & Aebersold, R. (2010). Options and considerations when selecting a quantitative
1016   proteomics strategy. *Nature Biotechnology, 28*, 710-721.
1017 Dowle, A., A., Wilson, J., & Thomas, J. R. (2016). Comparing the Diagnostic Classification
1018   Accuracy of iTRAQ, Peak-Area, Spectral-Counting, and emPAI Methods for Relative
1019   Quantification in Expression Proteomics. *Journal of Proteome Research, 15*, 3550-3562.
1020 Dunker, A. K., Babu, M. M., Barbar, E., Blackledge, M., Bondos, S. E., Dosztanyi, Z., Dyson,
1021   H. J., Forman-Kay, J., Fuxreiter, M., Gsponer, J., Han, K. H., Jones, D. T., Longhi, S.,
1022   Metallo, S. J., Nishikawa, K., Nussinov, R., Obradovic, Z., Pappu, R. V., Rost, B.,
1023   Selenko, P., Subramaniam, V., Sussman, J. L., Tompa, P. & Uversky, V. N. (2013).
1024   What's in a name? Why these proteins are intrinsically disordered. *Intrinsically*
1025   *Disordered Proteins, 1*, e24157.
1026 Ebhardt, H. A., Sabido, E., Huttenhain, R., Collins, B., & Aebersold, R. (2012). Range of protein
1027   detection by selected/multiple reaction monitoring mass spectrometry in an
1028   unfractionated human cell culture lysate. *Proteomics, 12*, 1185-1193.
1029 Editorial. (2008). The big ome. It's time to make the case for proteins. *Nature, 452*, 913-914.
1030 Egertson, J. D., MacLean, B., Johnson, R., Xuan, Y., & MacCoss, M. J. (2015). Multiplexed
1031   peptide analysis using data-independent acquisition and Skyline. *Nature Protocols, 10*,
1032   887-903.
1033 Escher, C., Reiter, L., MacLean, B., Ossola, R., Herzog, F., Chilton, J., MacCoss, M. J., &
1034   Rinner, O. (2012). Using iRT, a normalized retention time for more targeted
1035   measurement of peptides. *Proteomics, 12*, 1111-1121.
1036 Eyers, C. E., Lawless, C., Wedge, D. C., Lau, K. W., Gaskell, S. J., & Hubbard, S. J. (2011).
1037   CONSeQuence: Prediction of Reference Peptides for Absolute Quantitative Proteomics
1038   Using Consensus Machine Learning Approaches. *Molecular & Cellular Proteomics, 10*,
1039   M110.003384.

1040 Fasoli, E., & Righetti, P. G. (2015). Proteomics of Fruits and Beverages. *Current Opinion in*
1041 *Food Science, 4*, 76-85.
1042 Feng, X., Liu, B. F., Li, J., & Liu, X. (2015). Advances in Coupling Microfluidic Chips to Mass
1043 Spectrometry. *Mass Spectrometry Reviews, 34*, 535-557.
1044 Feng, Y., De Franceschi, G., Kahraman, A., Soste, M., Melnik, A., Boersema, P. J., Polverino de
1045 Laureto, P., Nikolaev, Y., Oliveira, A. P., & Picotti, P. (2014). Global analysis of protein
1046 structural changes in complex proteomes. *Nature Biotechnology, 32*, 1036-1044.
1047 Fonović, M., & Bogyo, M. (2008). Activity Based Probes as a tool for Functional Proteomic
1048 Analysis of Proteases. *Expert Review of Proteomics, 5*, 721-730.
1049 Fonslow, B. R., Stein, B. D., Webb, K. J., Xu, T., Choi, J., Park, S. K., & Yates III, J. R. (2013).
1050 Digestion and depletion of abundant proteins improves proteomic coverage. *Nature*
1051 *Methods, 10*, 54-56.
1052 Fonslow, B. R., Stein, B. D., Webb, K. J., Xu, T., Choi, J., Park, S. K., & Yates III, J. R. (2014).
1053 Addendum: Digestion and depletion of abundant proteins improves proteomic coverage.
1054 *Nature Methods, 11*, 347-348.
1055 Fortin, T., Salvador, A., Charrier, J. P., Lenz, C., Bettsworth, F., Lacoux, X., Choquet-
1056 Kastylevsky, G., & Lemoine, J. (2009). Multiple reaction monitoring cubed for protein
1057 quantification at the low nanogram/milliliter level in nondepleted human serum.
1058 *Analytical Chemistry, 81*, 9343-9352.
1059 Fournier, M. L., Gilmore, J. M., Martin-Brown, S. A., & Washburn, M. P. (2007).
1060 Multidimensional Separations-Based Shotgun Proteomics. *Chemical Reviews, 107*,
1061 3654–3686.
1062 Frese, C. K., Altelaar, A. F., Hennrich, M. L., Nolting, D., Zeller, M., Griep-Raming, J., Heck,
1063 A. J. R., & Mohammed, S. (2011). Improved Peptide Identification by Targeted
1064 Fragmentation Using CID, HCD and ETD on an LTQ-Orbitrap Velos. *Journal of*
1065 *Proteome Research, 10*, 2377-2388.
1066 Frese, C. K., Altelaar, A. F., van den Toorn, H., Nolting, D., Griep-Raming, J., Heck, A. J., &
1067 Mohammed, S. (2012). Toward Full Peptide Sequence Coverage by Dual Fragmentation
1068 Combining Electron-Transfer and Higher-Energy Collision Dissociation Tandem Mass
1069 Spectrometry. *Analytical Chemistry, 84*, 9668-9673.
1070 Frost, D. C., Greer, T., & Li, L. (2015). High-Resolution Enabled 12-Plex DiLeu Isobaric Tags
1071 for Quantitative Proteomics. *Analytical Chemistry, 87*, 1646-1654.
1072 Gabius, H. J. (2015). The magic of the sugar code. *Trends in Biochemical Sciences, 40*, 341.
1073 Gallien, S., Bourmaud, A., & Domon, B. (2014). A Simple Protocol To Routinely Assess the
1074 Uniformity of Proteomics Analyses. *Journal of Proteome Research, 13*, 2688-2695.
1075 Gallien, S., Bourmaud, A., Kim, S. Y., & Domon, B. (2014a). Technical considerations for
1076 large-scale parallel reaction monitoring analysis. *Journal of Proteomics, 100*, 147-159.
1077 Gallien, S., Duriez, E., Demeure, K., & Domon, B. (2013). Selectivity of LC-MS/MS analysis:
1078 Implication for proteomics experiments. *Journal of Proteomics, 81*, 148-158.
1079 Gallien, S., Kim, S. Y., & Domon, B. (2015). Large-Scale Targeted Proteomics Using Internal
1080 Standard Triggered-Parallel Reaction Monitoring (IS-PRM). *Molecular & Cellular*
1081 *Proteomics, 14*, 1630-1644.
1082 Galloa, M., & Ferranti, P. (2016). The evolution of analytical chemistry methods in foodomics.
1083 *Journal of Chromatography A, 1428*, 3-15.

1084 Gavasso, S., Gullaksen, S. E., Skavland, J., & Gjertsen, B. T. (2016). Single-cell proteomics:
1085   potential implications for cancer diagnostics. *Expert Review of Molecular Diagnostics,*
1086   *16*, 579-589.
1087 Giansanti, P., Tsiatsiani, L., Low, T. Y., & Heck, A. J. (2016). Six alternative proteases for mass
1088   spectrometry-based proteomics beyond trypsin. *Nature Protocols, 11*, 993-1006.
1089 Gillet, L. C., Navarro, P., Tate, S., Rost, H., Selevsek, N., Reiter, L., Ron Bonner, R., &
1090   Aebersold, R. (2012). Targeted Data Extraction of the MS/MS Spectra Generated by
1091   Data-independent Acquisition: A New Concept for Consistent and Accurate Proteome
1092   Analysis. *Molecular & Cellular Proteomics, doi: 10.1074/mcp.O111.016717.*
1093 Gligorijević, V., Malod-Dognin, N., & Pržulj, N. (2016). Integrative methods for analyzing big
1094   data in precision medicine. *Proteomics, 16*, 741-758.
1095 Golghalyani, V., Neupärtl, M., Wittig, I., Bahr, U., & Karas, M. (2017). ArgC-Like Digestion:
1096   Complementary or Alternative to Tryptic Digestion? . *Journal of Proteome Research, 16*,
1097   978-987.
1098 Guo, H., Peng, H., & Emili, A., (2017). Mass spectrometry methods to study protein metabolite
1099   interactions. *Expert Opinion on Drug Discovery*, doi:10.1080/17460441.2017.1378178
1100 Hakhverdyan, Z., Domanski, M., Hough, L. E., Oroskar, A. A., Oroskar, A. R., Keegan. S.,
1101   Dilworth, D. J., Molloy, K. R., Sherman, V., Aitchison, J. D., Fenyö, D., Chait, B. T.,
1102   Jensen, T. H., Rout, M. P., & LaCava, J. (2015). Rapid, optimized interactomic
1103   screening. *Nature Methods, 12*, 553-560.
1104 Hanke, S., Besir, H., Oesterhelt, D., & Mann, M. (2008). Absolute SILAC for Accurate
1105   Quantitation of Proteins in Complex Mixtures Down to the Attomole Level. *Journal of*
1106   *Proteome Research, 7*, 1118-1130.
1107 Heath, J. R., Ribas, A., & Mischel, P. S. (2016). Single-cell analysis tools for drug discovery and
1108   development. *Nature Reviews, 15*, 204-216.
1109 Hebert, A. S., Merrill, A. E., Bailey, D. J., Still, A. J., Westphall, M. S., Strieter, E. R.,
1110   Pagliarini, D. J., & Coon, J. J. (2013). Neutron-encoded mass signatures for multiplexed
1111   proteome quantification. *Nature Methods, 10*, 332-334.
1112 Hebert, A. S., Richards, A. L., Bailey, D. J., Ulbrich, A., Coughlin, E. E., Westphall, M. S., &
1113   Coon, J. J. (2014). The One Hour Yeast Proteome. *Molecular & Cellular Proteomics, 13*,
1114   339-347.
1115 Helm, D., Vissers, J. P., Hughes, C. J., Hahne, H., Ruprecht, B., Pachl, F., Grzyb, A.,
1116   Richardson, K., Wildgoose, J., Maier, S. K., Marx, H., Wilhelm, M., Becher, I., Lemeer,
1117   S., Bantscheff, M., Langridge, J. I., & Kuster, B. (2014). Ion Mobility Tandem Mass
1118   Spectrometry Enhances Performance of Bottom-up Proteomics. *Molecular & Cellular*
1119   *Proteomics, 13*, 3709-3715.
1120 Hirabayashi, J., Yamada, M., Kuno, A., & Tateno, H. (2013). Lectin microarrays: concept,
1121   principle and applications. *Chemical Society Reviews, 42*, 4443-4458.
1122 Houel, S., Abernathy, R., Renganathan, K., Meyer-Arendt, K., Ahn, N. G., & Old, W. M. (2010).
1123   Quantifying the impact of chimera MS/MS spectra on peptide identification in large-scale
1124   proteomics studies. *Journal of Proteome Research, 9*, 4152-4160.
1125 Hu, H., Khatri, K., & Zaia, J. (2016). Algorithms and design strategies towards automated
1126   glycoproteomics analysis. *Mass Spectrometry Reviews, doi: 10.1002/mas.21487.*
1127 Hughes, C. S., Foehr, S., Garfield, D. A., Furlong, E. E., Steinmetz, L. M., & Krijgsveld, J.
1128   (2014). Ultrasensitive proteome analysis using paramagnetic bead technology. *Molecular*
1129   *Systems Biology, 10*, 757.

1130 Jongenburger, I., den Besten, H.M.W., & Zwietering, M.H. (2015). Statistical Aspects of Food
1131   Safety Sampling. *The Annual Review of Food Science and Technology, 6*, 20.21-20.25.
1132 Josić, D. (2014). Strategies for Complete Proteomic Analysis of Hydrophobic Proteins in
1133   Complex Biological Samples - Hyde-and Seek. *Journal of Data Mining in Genomics and*
1134   *Proteomics, doi:10.4172/2153-0602.1000e111*.
1135 Josić, D., & Clifton, J. (2007). Mammalian plasma membrane proteomics. *Proteomics, 7*, 3010-
1136   3029.
1137 Kadama, S. U., Tiwarib, B. K., Álvarezb, C., & O'Donnell, C. P. (2015). Ultrasound for the
1138   extraction, identification and delivery of food proteins and bioactive Peptides. *Trends in*
1139   *Food Science & Technology, 46*, 60-67.
1140 Koeberl, M., Clarke, D., & Lopata, A. L. (2014). Next Generation of Food Allergen
1141   Quantification Using Mass Spectrometric Systems. *Journal of Proteome Research, 13*,
1142   3499–3509.
1143 Kolarich, D., Jensen, P. H., Altmann, F., Packer, N. H. (2012). Determination of site-specific
1144   glycan heterogeneity on glycoproteins. *Nature Protocols, 7*, 1285-1298.
1145 Kollipara, L., & Zahedi, R. P. (2013). Protein carbamylation: in vivo modification or in vitro
1146   artefact? . *Proteomics, 13*, 941-944.
1147 Kou, Q., Zhu, B., Wu, S., Ansong, C., Tolić, N., Paša-Tolić, L., & Liu, X. (2016).
1148   Characterization of Proteoforms with Unknown Post-translational Modifications Using
1149   the MIScore. *Journal of Proteome Research, 15*, 2422-2432.
1150 Kulak, N. A., Pichler, G., Paron, I., Nagaraj, N., & Mann, M. (2014). Minimal, encapsulated
1151   proteomic-sample processing applied to copy-number estimation in eukaryotic cells.
1152   *Nature Metods, 11*, 319-324.
1153 Laskay, U. A., Lobas, A. A., Srzentić, K., Gorshkov, M. V., & Tsybin, Y. O. (2013). Proteome
1154   Digestion Specificity Analysis for Rational Design of Extended Bottom-up and Middle-
1155   down Proteomics Experiments. *Journal of Proteome Research, 12*, 5558-5569.
1156 Lebert, D., Louwagie, M., Goetze, S., Picard, G., Ossola, R., Duquesne, C., Basler, K., Ferro,
1157   M., Rinner, O., Aebersold, R., Garin, J., Mouz, N., Brunner, E., & Brun, V. (2015).
1158   DIGESTIF: A Universal Quality Standard for the Control of Bottom-Up Proteomics
1159   Experiments. Research. *Journal of Proteome Research, 14*, 787-803.
1160 Leitner, A., Faini, M., Stengel, F., & Aebersold, R. (2016). Crosslinking and Mass Spectrometry:
1161   An Integrated Technology to Understand the Structure and Function of Molecular
1162   Machines. *Trends in Biochemical Sciences, 41*, 20-32.
1163 Leney, A. C., & Heck, A. J. R. (2017). Native Mass Spectrometry: What is in the Name? .
1164   *Journal of The American Society for Mass Spectrometry, 28*, 5-13.
1165 Leonard, R., Petersen, B. O., Himly, M., Kaar, W., Wopfner, N., Kolarich, D., van Ree, R.,
1166   Ebner, C., Duus, J. O., Ferreira, F., & Altmann, F. (2005). Two novel types of O-glycans
1167   on the mugwort pollen allergen art v 1 and their role in antibody binding. *Journal of*
1168   *Biological Chemistry, 280*, 7932-7940.
1169 Li, L., Wang, C., Qiang, S., Zhao, J., Song, S., Jin, W., Wang, B., Zhang, Y., Huang, L., &
1170   Wang, Z. (2016). Mass Spectrometric Analysis of N-Glycoforms of Soybean Allergenic
1171   Glycoproteins Separated by SDS-PAGE. *Journal of Agricultural and Food Chemistry,*
1172   *64*, 7367-7376.
1173 Li, Y., Zhong, C. Q., Xu, X., Cai, S., Wu, X., Zhang, Y., Chen, J., Shi, J., Lin, S., & Han, J.
1174   (2015). Group-DIA: analyzing multiple data-independent acquisition mass spectrometry
1175   data files. *Nature Methods, 12*, 1105-1106.

1176 Liu, X., Jin, Z., O'Brien, R., Bathon, J., Dietz, H.C. Grote, E., & Van Eyk, J. E. (2013).
1177   Constrained selected reaction monitoring: Quantification of selected post-translational
1178   modifications and protein isoforms. *Methods, 61*, 304-312.
1179 Luethy, R., Kessner, D. E., Katz, J. E., McLean, B., Grothe, R., Kani, K., Faca, V., Pitteri, S.,
1180   Hanash, S., Agus, D. B., & Mallick, P. (2008). Precursor-ion mass reestimation improves
1181   peptide identification on hybrid instruments. *Journal of Proteome Research, 7*, 4031-
1182   4039.
1183 Lyutvinskiy, Y., Yang, H., Rutishauser, D., & Zubarev, R. A. (2013). In silico instrumental
1184   response correction improves precision of label-free proteomics and accuracy of
1185   proteomics-based predictive models. . *Molecular & Cellular Proteomics, 12*, 2324-2331.
1186 Madono, M., Sawasaki, T., Morishita, R., & Endo, Y. (2011). Wheat germ cell-free protein
1187   production system for post-genomic research. *Nature Biotechnology, 28*, 211-217.
1188 Mallick, P., & Kuster, B. (2010). Proteomics: a pragmatic perspective. *Nature Biotechnology,*
1189   *28*, 695-709.
1190 Malmström, J., Beck, M., Schmidt, A., Lange, V., Deutsch, E. W., & Aebersold, R. (2009).
1191   Proteome-wide cellular protein concentrations of the human pathogen Leptospira
1192   interrogans. *Nature, 480*, 762-765.
1193 Mann, M., Kulak, N. A., Nagaraj, N., & Cox, J. (2013). The Coming Age of Complete,
1194   Accurate, and Ubiquitous Proteomes. *Molecular Cell, 49*, 583-590.
1195 Martens, L., & Hermjakob, H. (2007). Proteomics data validation: why all must provide data.
1196   *Molecular BioSystem, 3*, 518-522.
1197 Martinović, T., Andjelković, U., Šrajer-Gajdošik, M., Rešetar, D., & Josić, D. (2016). Foodborne
1198   pathogens and their toxins. *Journal of Proteomics, 147*, 226-235.
1199 McAlister, G. C., Nusinow, D. P., Jedrychowski, M. P., Wühr, M., Huttlin, E. L., Erickson, B.
1200   K., Rad, R., Haas, W., Gygi, S. P. (2014). MultiNotch MS3 Enables Accurate, Sensitive,
1201   and Multiplexed Detection of Differential Expression across Cancer Cell Line Proteomes.
1202   *Analytical Chemistry, 86*, 7150-7158.
1203 Mechref, Y., Hu, Y., Desantos-Garcia, J. L., Hussein, A., & Tang, H. (2013). Quantitative
1204   Glycomics Strategies. *Molecular & Cellular Proteomics, 12*, 874-884.
1205 Meier, F., Beck, S., Grassl, N., Lubeck, M., Park, M. A., Raether, O., & Mann, M. (2016).
1206   Parallel Accumulation–Serial Fragmentation (PASEF): Multiplying Sequencing Speed
1207   and Sensitivity by Synchronized Scans in a Trapped Ion Mobility Device. *Journal of*
1208   *Proteome Research, 15*, 5378-5387.
1209 Michalski, A., Cox, J., & Mann, M. (2011). More than 100,000 Detectable Peptide Species Elute
1210   in Single Shotgun Proteomics Runs but the Majority is Inaccessible to Data-Dependent
1211   LC–MS/MS. *Journal of Proteome Research, 10*, 1785-1793.
1212 Mitchell, P. (2010). Proteomics retrenches. *Nature Biotechnology, 28*, 665-670.
1213 Neilson, K. A., Ali, N. A., Muralidharan, S., Mirzaei, M., Mariani, M., Assadourian, G., Lee, A.,
1214   van Sluyter, S. C., & Haynes, P. A. (2011). Less label, more free: approaches in label-
1215   free quantitative mass spectrometry. *Proteomics, 11*, 535-553.
1216 Nesvizhskii, A. I., & Aebersold, R. (2005). Interpretation of Shotgun Proteomic Data. The
1217   protein inference problem. *Molecular & Cellular Proteomics, 4*, 1419-1440.
1218 Nilsson, T., Mann, M., Aebersold, R., Yates III, J. R., Bairoch, A., & Bergeron, J. J. M. (2010a).
1219   Mass spectrometry in high-throughput proteomics: ready for the big time. *Nature*
1220   *Methods, 9*, 681-685.

45

1221 Nilsson, T., Mann, M., Aebersold, R., Yates III, J. R., Bairoch, A., & Bergeron, J. J. M. (2010b).
1222   Mass spectrometry in high-throughput proteomics: ready for the big time. . *Nature*
1223   *Methods, 7*, 681-685.
1224 Noecker, C., & Borenstein, E. . (2016). Getting personal about nutrition. *Trends in Molecular*
1225   *Medicine, 22*, 83-85.
1226 Ong, S. E., & Mann, M. (2005). Mass spectrometry-based proteomics turns quantitative. *Nature*
1227   *Chemical Biology, 1*, 252-262.
1228 Ong, S. E., & Mann, M. (2007). Stable Isotope Labeling by Amino Acids in Cell Culture for
1229   Quantitative Proteomics. *Methods in Molecular Biology, 359*, 37-52.
1230 Paredi, G., Raboni, S., Bendixen, E., de Almeida, A. M., & Mozzarelli, A. (2012). "Muscle to
1231   meat" molecular events and technological transformations: The proteomics insight.
1232   *Journal of Proteomics, 75*, 4275-4289.
1233 Parker, S. J., Rost, H., Rosenberger, G., Collins, B. C., Malmström, L., Amodei, D.,
1234   Venkatraman, V., Raedschelders, K., Van Eyk, J. E., & Aebersold, R. (2015).
1235   Identification of a Set of Conserved Eukaryotic Internal Retention Time Standards for
1236   Data-independent Acquisition Mass Spectrometry. *Molecular & Cellular Proteomics, 14*,
1237   2800-2813.
1238 Pejaver, V., Hsu, W. L., Xin, F., Keith Dunker, A., Uversky, V. N., & Radivojac, P. (2014). The
1239   structural and functional signatures of proteins that undergo multiple events of post-
1240   translational modification. *Protein Science, 23*, 1077-1093.
1241 Picotti, P., Aebersold, R., & Domon, B. (2007). The implications of proteolytic background for
1242   shotgun proteomics. *Molecular & Cellular Proteomics, 6*, 1589-1598.
1243 Picotti, P., Bodenmiller, B., & Aebersold, R. (2013). Proteomics meets the scientific method.
1244   *Nature Methods, 10*, 24-27.
1245 Picotti, P., Bodenmiller, B., Mueller, L. N., Domon, B., & Aebersold, R. (2009). Full dynamic
1246   range proteome analysis of S. cerevisiae by targeted proteomics. *Cell, 138*, 795-806.
1247 Piehowski, P. D., Petyuk, V. A., Orton, D. J., Xie, F., Moore, R. J., Ramirez-Restrepo, M.,
1248   Engel, A., Lieberman, A. P., Albin, R. L., Camp, D. G., Smith, R. D., & Myers, A. J.
1249   (2013). Sources of Technical Variability in Quantitative LC-MS Proteomics: Human
1250   Brain Tissue Sample Analysis. *Journal of Proteome Research, 12*, 2128-2137.
1251 Pinto, G., Radulović, M., & Godovac-Zimmermann, J. (2016). Spatial perspectives in the redox
1252   code-mass spectrometric proteomics studies of moonlighting proteins. *Mass*
1253   *Spectrometry Reviews, doi: 10.1002/mas.21508*.
1254 Piras, C., Roncada, P., Rodrigues, P. M., Bonizzi, L., & Soggiu, A. (2016). Proteomics in food:
1255   Quality, safety, microbes, and allergens. *Proteomics, 16*, 799-815.
1256 Pirmoradian, M., Budamgunta, H., Chingin, K., Zhang, B., Astorga-Wells, J., & Zubarev, R. A.
1257   (2013). Rapid and Deep Human Proteome Analysis by Single-dimension Shotgun
1258   Proteomics. *Molecular & Cellular Proteomics, 12*, 3330-3338.
1259 Pirmoradian, M., Zhang, B., Chingin, K., Astorga-Wells, J., & Zubarev, R. A. (2014).
1260   Membrane-Assisted Isoelectric Focusing Device As a Micropreparative Fractionator for
1261   Two-Dimensional Shotgun Proteomics. *Analytical Chemistry, 86*, 5728-5732.
1262 Poste, G. (2011). Bring on the biomarkers. *Nature, 469*, 156-157.
1263 Rabilloud, T. (2009). Membrane proteins and proteomics: love is possible, but so difficult.
1264   *Electrophoresis, 30*, S174-S180.

1265   Rand, K. D., Zehl, M., & Jørgensen, T. J. D. (2014). Measuring the hydrogen/deuterium
1266   exchange of proteins at high spatial resolution by mass spectrometry: overcoming gas-
1267   phase hydrogen/deuterium scrambling. *Accounts of Chemical Research, 47*, 3018–3027.
1268   Rauniyar, N., & YatesIII, J. R. (2014). Isobaric Labeling-Based Relative Quantification in
1269   Shotgun Proteomics. *Journal of Proteome Research, 13*, 5293-5309.
1270   Righetti, P. G., Candiano, G., Citterio, A., & Boschetti, E. (2015). Combinatorial Peptide Ligand
1271   Libraries as a "Trojan Horse" in Deep Discovery Proteomics. *Analytical Chemistry, 87*,
1272   293-305.
1273   Righetti, P. G., Fasoli, E., D`Amato, A., & Boschetti, E. (2014). The "Dark Side" of Food Stuff
1274   Proteomics: The CPLL-Marshals Investigate. *Foods, 3*, 217-237.
1275   Riley, N. M., & Coon, J. J. (2016). Phosphoproteomics in the Age of Rapid and Deep Proteome
1276   Profiling. *Analytical Chemistry, 88*, 74-94.
1277   Rose, C. M., Merrill, A. E., Bailey, D. J., Hebert, A. S., Westphall, M. S., & Coon, J. J. (2013).
1278   Neutron Encoded Labeling for Peptide Identification. *Analytical Chemistry, 85*, 5129-
1279   5137.
1280   Rosenberger, G., Koh, C. C., Guo, T., Röst, H. L., Kouvonen, P., Collins, B. C., Heusel, M., Liu,
1281   Y., Caron, E., Vichalkovski, A., Faini, M., Schubert, O. T., Faridi, P., Ebhardt, H. A.,
1282   Matondo, M., Lam, H., Bader, S. L., Campbell, D. S., Deutsch, E. W., Moritz R. L., Tate,
1283   S., & Aebersold, R. (2014). A repository of assays to quantify 10,000 human proteins by
1284   SWATH-MS. *Scientific Data, 1*, 140031.
1285   Röst, H. L., Rosenberger, G., Navarro, P., Gillet, L., Miladinović, S. M., Schubert, O. T.,
1286   Wolski, W., Collins, B. C., Malmström, J., Malmström, L., & Aebersold, R. (2014).
1287   OpenSWATH enables automated, targeted analysis of data-independent acquisition MS
1288   data. *Nature Biotechnology, 32*, 219-223.
1289   Ručević, M., Clifton, J. G., Huang, F., Li, X., Callanan, H., Hixson, D. C., & Josić, D. (2006).
1290   Use of short monolithic columns for isolation of low abundance membrane proteins.
1291   *Journal of Chromatography A, 1123*, 199-204.
1292   Ruprecht, B., Zecha, J., Heinzlmeir, S., Médard, G., Lemeer, S., & Kuster, B. (2015). Evaluation
1293   of kinase activity profiling using chemical proteomics. *ACS Chemical Biology, 10*, 2743-
1294   2752.
1295   Sauer, S., & Luge, T. (2015). Nutriproteomics: Facts, concepts, and perspectives. *Proteomics,*
1296   *15*, 997-1013.
1297   Schmidt, A., Gehlenborg, N., Bodenmiller, B., Mueller, L. N., Campbell, D., Mueller, M.,
1298   Aebersold, R., & Domon, B. (2008). An integrated, directed mass spectrometric approach
1299   for in-depth characterization of complex peptide mixtures. *Molecular & Cellular*
1300   *Proteomics, 7*, 2138-2150.
1301   Scott, N. E., Parker, B. L., Connolly, A. M., Jana Paulech, J., Edwards, A. V. G., Crossett, B.,
1302   Falconer, L., Kolarich, D., Djordjevic, S. P., Højrup, P., Packer, N. H., Larsen, M. R., &
1303   Cordwell S. J. (2011). Simultaneous Glycan-Peptide Characterization Using Hydrophilic
1304   Interaction Chromatography and Parallel Fragmentation by CID, Higher Energy
1305   Collisional Dissociation, and Electron Transfer Dissociation MS Applied to the N-Linked
1306   Glycoproteome of Campylobacter Jejuni. *Molecular & Cellular Proteomics, 10*,
1307   M000031-MCP000201.
1308   Selevsek, N., Chang, C. Y., Gillet, L. C., Navarro, P., Bernhardt, O. M., Reiter, L., Cheng, L. Y.,
1309   Vitek, O., & Aebersold, R. (2015). Reproducible and Consistent Quantification of the

Saccharomyces cerevisiae Proteome by SWATH-mass spectrometry. *Molecular & Cellular Proteomics, 14*, 739-749.

Shaw, J. B., Robinson, E. W., & Paša-Tolić, L. (2016). Vacuum Ultraviolet Photodissociation and Fourier Transform-Ion Cyclotron Resonance (FT-ICR) Mass Spectrometry: Revisited. *Analytical Chemistry, 88*, 3019-3023.

Sinz, A. (2014). The advancment of chemical cross-linking and mass spectrometry for structural proteomics: from single proteins to protein interaction networks. *Expert Review of Proteomics, 16*, 1-11.

Skold, K., Alm, H., & Scholz, B. (2013). The Impact of Biosampling Procedures on Molecular Data Interpretation. *Molecular & Cellular Proteomics*, 12, 1489–1501.

Skov, T., Honoré, A. H., Jensen, H. M., Naes, T., & Engelsen, S. B. (2014). Chemometrics in foodomics: Handling data structures from multiple analytical platforms. *Trends in Analytical Chemistry, 60*, 71-79.

Smit, N. P., Romijn F. P., van den Broek, I., Drijfhout J. W., Haex, M., van der Laarse, A., van der Burgt, Y. E., & Cobbaert, C. M. . (2014). Metrological traceability in mass spectrometry-based targeted protein quantitation: A proof-of-principle study for serum apolipoproteins A-I and B100. *Journal of Proteomics, 109*, 143-161.

Smith, L. M., Kelleher, N. L., & The Consortium for Top Down Proteomics. (2013). Proteoform: a single term describing protein complexity. *Nature Mathods, 10*, 186-187.

Smits, A. H., & Vermeulen, M. (2016). Characterizing Protein-Protein Interactions Using Mass Spectrometry: Challenges and Opportunities. *Trends in Biotechnology, 34*, 825-834.

Srajer-Gajdošik, M., Kovač, S., Malatesti, N., Müller, E., & Josić, D. (2013). Ion-Exchange Sample Displacement Chromatography as a Method for Fast and Simple Isolation of Low- and High-Abundance Proteins from Complex Biological Mixtures. *Food Technology and Biotechnology, 52*, 58-63.

Srzentić, K., Fornelli, L., Laskay, U. A., Monod, M., Beck, A., Ayoub, D., & Tsybin, Y. O. (2014). Advantages of Extended Bottom-Up Proteomics Using Sap9 for Analysis of Monoclonal Antibodies. *Analytical Chemistry, 86*, 9945-9953.

Switzar, L., Giera, M., & Niessen, W. M. A. (2013). Protein Digestion: An Overview of the Available Techniques and Recent Developments. *Journal of Proteome Research, 12*, 1067-1077.

Tabb, D. L., Vega-Montoto, L., Rudnick, P. A., Variyath, A. M., Ham, A. J. L., Bunk, D. M., Kilpatrick, L. E., Billheimer, D. D., Blackman, R. K., Cardasis, H. L., Carr, S. A., Clauser, K. R., Jaffe, J. D., Kowalski, K. A., Neubert, T. A., Regnier, F. E., Schilling, B., Tegeler, T. J., Wang, M., Wang, P., Whiteaker, J. R., Zimmerman, L. J., Fisher, S. J., Gibson, B. W., Kinsinger, C. R., Mesri, M., Rodriguez, H., Stein, S. E., Tempst, P., Paulovich, A. G., Liebler, D. C., & Spiegelman, C. (2010). Repeatability and Reproducibility in Proteomic Identifications by Liquid Chromatography-Tandem Mass Spectrometry. *Journal of Proteome Research, 9*, 761-776.

Tabb, D. L., Wang, X., Carr, S. A., Clauser, K. R., Mertins, P., Chambers, M. C., Holman, J. D., Wang, J., Zhang, B., Zimmerman, L. J., Chen, X., Gunawardena, H. P., Davies, S. R., Ellis, M. J., Li, S., Townsend, R.R., Boja E. S., Ketchum, K. A., Kinsinger, C. R., Mesri, M., Rodriguez, H., Liu, T., Kim, S., McDermott, J. E., Payne, S. H., Petyuk, V. A., Rodland, K. D., Smith, R. D., Yang, F., Chan, D. W., Zhang, B., Zhang, H., Zhang, Z., Zhou, J. Y., & Liebler, D. C. (2016). Reproducibility of Differential Proteomic

Technologies in CPTAC Fractionated Xenografts. *Journal of Proteome Research, 15*, 691-706.

Takemori, N., Takemori, A., Matsuoka, K., Morishita, R., Matsushita, N., Aoshima, M., Takeda, H., Sawasaki, T., Endo, Y., & Higashiyama, S. (2015). High-throughput synthesis of stable isotopelabeled transmembrane proteins for targeted transmembrane proteomics using a wheat germ cell-free protein synthesis system. *Molecular Biosystems, 11*, 361-365.

Tedesco, S., Mullen, W., Cristobal, S., (2014). High-Throughput Proteomics: A New Tool for Quality and Safety in Fishery Products. *Current Protein and Peptide Science, 15*, 118-133.

Ting, L., Rad, R., Gygi S. P., & Haas, W. (2011). MS3 eliminates ratio distortion in isobaric multiplexed quantitative proteomics. *Nature Methods, 8*, 937-940.

Ting, Y. S., Egertson, J. D., Payne, S. H., Kim, S., MacLean, B., Käll, L., Aebersold, R., Smith, R. D., Noble, W. S., & MacCoss, M. J. (2015). Peptide-Centric Proteome Analysis: An Alternative Strategy for the Analysis of Tandem Mass Spectrometry Data. *Molecular & Cellular Proteomics, 14*, 2301-2307.

Tran, J. C., & Doucette, A. A. (2009). Multiplexed size separation of intact proteins in solution phase for mass spectrometry. *Analytical Chemistry, 81*, 6201-6209.

Tran, J. C., Zamdborg, L., Ahlf, D. R., Lee, J. E., Catherman, A. D., Durbin, K. R., Tipton, J. D., Vellaichamy, A., Kell, J. F., Li, M., Wu, C., Sweet, S. M., Early, B. P., Siuti, N., LeDuc, R. D., Compton, P. D., Thomas, P. M., & Kelleher, N. L. (2011). Mapping intact protein isoforms in discovery mode using top-down proteomics. *Nature, 480*, 254-258.

Trevisiol, S., Ayoub, D., Lesur, A., Ancheva, L., Gallien, S., & Domon, B. (2016). The use of proteases complementary to trypsin to probe isoforms and modifications. *Proteomics, 16*, 715-728.

Tsiatsiani, L., Giansanti, P., Scheltema, R. A., van den Toorn, H. W. P., Overall, C. M., Altelaar, A. F., & Heck, A. J. R. (2017). Opposite ETD/HCD fragmentation characteristics of proteolytic K/R(X)n and (X)nK/R peptides provide benefits for peptide sequencing in proteomics and phosphoproteomics. *Journal of Proteome Research, 16*, 852-861.

Tsou, C. C., Avtonomov, D., Larsen, B., Tucholska, M., Choi, H., Gingras, A. C., & Nesvizhskii, AI. (2015). DIA-Umpire: comprehensive computational framework for data-independent acquisition proteomics. *Nature Methods, 12*, 258-264.

Tu, C., Shen, S., Sheng, Q., Shyr, Y., & Qu, J. (2017). A peptide-retrieval strategy enables significant improvement of quantitative performance without compromising confidence of identification. *Journal of Proteomics, 152*, 276-282.

Vandermarliere, E., & Martens, L. (2013). Protein structure as a means to triage proposed PTM sites. *Proteomics, 13*, 1028-1035.

Vandermarliere, E., Stes, E., Gevaert, K., Martens, L. (2016). Resolution of protein structure by mass spectrometry. *Mass Spectrometry Reviews, 35*, 653-665.

Vehmas, A. P., Muth-Pawlak, D., Huhtinen, K., Saloniemi-Heinonen, T., Jaakkola, K., Laajala, T. D., Kaprio, H., Suvitie, P. A., Aittokallio, T., Siitari, H., Perheentupa, A., Poutanen, M., & Corthals, G. L. (2014). Ovarian Endometriosis Signatures Established through Discovery and Directed Mass Spectrometry Analysis. *Journal of Proteome Research, 13*, 4983-4994.

Venne, A. S., Kollipara, L., & Zahedi, R. P. (2014). The next level of complexity: crosstalk of posttranslational modifications. *Proteomics, 14*, 513-524.

Villanueva, J., Carrascal, M., & Abian, J. (2014). Isotope dilution mass spectrometry for absolute quantification in proteomics: Concepts and strategies. *Journal of Proteomics, 96*, 184-199.

Vimaleswaran, K. S., Roy, C. I. L., & Claus, S. P. (2015). Foodomics for personalized nutrition: how far are we? . *Current Opinion in Food Science, 4*, 129-135.

Vučković, D., Dagley, L. F., Purcell, A. W., & Emili, A. (2013). Membrane proteomics by high performance liquid chromatography-tandem mass spectrometry: Analytical approaches and challenges. *Proteomics, 13*, 404-423.

Vyatkina, K., Wu, S., Dekker, L. J. M., VanDuijn, M. M., Liu, X., Tolić, N., Dvorkin, M., Alexandrova, S., Luider, T. M., Paša-Tolić, L., & Pevzner, P. A. (2015). De Novo Sequencing of Peptides from Top-Down Tandem Mass Spectra. *Journal of Proteome Research, 14*, 4450-4462.

Walsh, C. T., Garneau-Tsodikova, S., & Gatto, G. J. (2005). Protein Posttranslational Modifications: The Chemistry of Proteome Diversifications. *Angewandte Chemie International Editions, 44*, 7342-7372.

Walzer, M., Pernas, L. E., Nasso, S., Bittremieux, W., Nahnsen, S., Kelchtermans, P., Pichler, P., van den Toorn, H. W., Staes, A., Vandenbussche, J., Mazanek, M., Taus, T., Scheltema, R. A., Kelstrup, C. D., Gatto, L., van Breukelen, B., Aiche, S., Valkenborg, D., Laukens, K., Lilley, K. S., Olsen, J. V., Heck, A. J., Mechtler, K., Aebersold, R., Gevaert, K., Vizcaíno, J. A., Hermjakob, H., Kohlbacher, O., Martens, L. (2014). qcML: an exchange format for quality control metrics from mass spectrometry experiments. . *Molecular & Cellular Proteomics, 13*, 1905-1913.

Weekes, M. P., Tomasec, P., Huttlin, E. L., Fielding, C. A., Nusinow, D., Stanton, R. J., Wang, E. C., Aicheler, R., Murrell, I., Wilkinson, G. W., Lehner, P. J., & Gygi, S. P. (2014). Quantitative Temporal Viromics: An Approach to Investigate Host-Pathogen Interaction. *Cell, 157*, 1460-1472.

Wiśniewski, J. R. (2016). Quantitative Evaluation of Filter Aided Sample Preparation (FASP) and Multienzyme Digestion FASP Protocols. *Analytical Chemistry, 88*, 5438-5443.

Wohlgemuth, I., Lenz, C., & Urlaub, H. (2015). Studying macromolecular complex stoichiometries by peptide-based mass spectrometry. *Proteomics, 15*, 862-879.

Wright, M. H., & Sieber, S. A. (2016). Chemical proteomics approaches for identifying the cellular targets of natural products. *Natural Product Reports, 33*, 681-708.

Wu, C., Duan, J., Liu, T., Smith, R. D., & Qian, W. J. (2016). Contributions of Immunoaffinity Chromatography to Deep Proteome Profiling of Human Biofluids. *Journal of Chromatography B, 1021*, 57-68.

Wu, C., Tran, J. C., Zamdborg, L., Durbin, K. R., Li, M., Ahlf, D. R., Early, B. P., Thomas, P. M., Sweedler, J. V., & Kelleher, N. L. (2012). A protease for 'middle-down' proteomics. *Nature Methods, 9*, 822-824.

Wühr, M., Haas, W., McAlister, G. C., Peshkin, L., Rad, R., Kirschner, M. W., & Gygi, S. P. (2012). Accurate Multiplexed Proteomics at the MS2 Level Using the Complement Reporter Ion Cluster. *Analytical Chemistry, 84*, 9214-92221.

Yang, M., Nelson, R., & Ros, A. (2016). Toward Analysis of Proteins in Single Cells: A Quantitative Approach Employing Isobaric Tags with MALDI Mass Spectrometry Realized with a Microfluidic Platform. *Analytical Chemistry, 88*, 6672-6679.

Ye, M., Pan, Y., Cheng, K., & Zou, H. (2014). Protein digestion priority is independent of protein abundances. *Nature Methods, 11*, 220-222.

1447 Zeiler, M., Straube, W. L., Lundberg, E., Uhlen, M., & Mann, M. (2012). A Protein Epitope
1448   Signature Tag (PrEST) Library Allows SILAC-based Absolute Quantification and
1449   Multiplexed Determination of Protein Copy Numbers in Cell Lines. *Molecular &*
1450   *Cellular Proteomics, 11*, O111.009613.
1451 Zhang, Y., Fonslow, B. R., Shan, B., Baek, M. C., & Yates III, J. Y. (2013). Protein Analysis by
1452   Shotgun/Bottom-up Proteomics. *Chemical Reviews, 113*, 2343-2394.
1453 Zhang, Z., Si Wu, S., Stenoien, D. L., & Paša-Tolić, L. (2014). High-Throughput Proteomics.
1454   *Annual Review of Analytical Chemistry, 7*, 427-454.
1455 Zubarev, R. A. (2013). The challenge of the proteome dynamic range and its implications for in-
1456   depth proteomics. *Proteomics, 13*, 723-726.

1457

1458

1459

1460 **Figure captions**

1461 Fig. 1. Contribution of proteomics to foodomics.

1462 Fig. 2. Current MS-based proteomic workflow, from the first step – material sampling to the last

1463 step – data analysis. Different sample preparation techniques can be combined with different

1464 MS-based proteomic approaches, as it is depicted by arrows, in order to design a method for a

1465 particular analytical problem.

1466 Fig. 3. Techniques for acquisition of mass spectra in high-throughput bottom-up proteomics.

Table 1

A) Examples of application of MS-based proteomics in food research and assurance of food quality and safety

MS-based proteomic app.		Application in foodomics	Reference
High-throughput proteomics	Bottom-up	Identification of orange proteome	Lerma-García, M. J., D'Amato, A., Simó-Alfonso, E. F., Righetti, P. G., & Fasoli, E. (2016). Orange proteomic fingerprinting: From fruit to commercial juices. *Food Chemistry, 196*, 739-749.
		Identification and comparison of proteomes of milk whey from different animals	Yang, Y., Bu, D., Zhao, X., Sun, P., Wang, J., & Zhou, L. (2013). Proteomic Analysis of Cow, Yak, Bu□alo, Goat and Camel Milk Whey Proteins: Quantitative Di□erential Expression Patterns. *Journal of Proteome Research, 12*, 1660–1667.
	Top-down Middle-down	Characterization of allergenic 2S albumin and its proteoforms in Hazelnut cultivars	Korte, R., Happe, J., Brümmer, I., & Brockmeyer, J. (2017). Structural Characterization of the Allergenic 2S Albumin Cor a 14: Comparing Proteoform Patterns across Hazelnut Cultivars. *Journal of Proteome Research, 16*, 988-998.
Identification of primary structure of protein and proteoforms		Characterization of Mustard 2S albumin allergens	Hummel, M., Wigger, T., & Brockmeyer, J. (2015). Characterization of Mustard 2S Albumin Allergens by Bottom-up, Middle-down, and Top-down Proteomics: A Consensus Set of Isoforms of Sin a 1. *Journal of Proteome Research, 14*, 1547–1556.
		Identification of structural changes of milk Gal d 1 allergen upon lipid peroxidation	Nikolić, J., Nešić, A., Čavić, M., Đorđević, N., Anđelković, U., Atanasković-Marković, M., Drakulić, B., & Gavrović-Jankulović, M. (2017). Effect of malondialdehyde on the ovalbumin structure and its interactions with T84 epithelial cells. *Biochimica et Biophysica Acta (BBA) - General Subjects, 1861*, 126-134.
PTM	Glysco-proteomics	Characterization of glycoproteome of wheat flour albumins and its potential effect on wheat beer quality	Dedvisitsakul, P., Jacobsen, S., Svensson, B., Bunkenborg, J., Finnie, C., & Hägglund, P. (2014). Glycopeptide Enrichment Using a Combination of ZIC-HILIC and Cotton Wool for Exploring the Glycoproteome of Wheat Flour Albumins. *Journal of Proteome Research, 13*, 2696-2703.
	Phospho-proteomics	Influence of phosphoproteome changes on meet quality	Huang, H., Larsen, M. R., Palmisano, G., Dai, J., & Lametsch, R. (2014). Quantitative phosphoproteomic analysis of porcine muscle within 24 h postmortem. *Journal of Proteomics, 106*, 125-139.
Conformational proteomics		Investigation of correlation between	Nyemb, K., Jardin, J., Causeur, D., Guérin-Dubiard, C., Dupont, D., Rutherfur, S. M., & Nau, F. (2014). Investigating the impact of

	aggregate morphology and digestibility of ovalbumin	ovalbumin aggregate morphology on in vitro ovalbumin digestion using label-free quantitative peptidomics and multivariate data analysis. *Food Research International, 63*, 192-202.	
Protein – protein interactions		Identification of 14-3-3 proteins interaction partners in rice proteome	Zhang, Z., Zhang, Y., Zhao, H., Huang, F., Zhang, Z., Lin, W. (2017). The important functionality of 14-3-3 isoforms in rice roots revealed by affinity chromatography. *Journal of Proteomics, 158*, 20-30.
Chemical proteomics		Identification of curcumin interaction partners in human proteome	Abegg, D., Frei, R., Cerato, L., Prasad Hari, D., Wang, C., Waser, J., & Adibekian, A. (2015). Proteome-Wide Profiling of Targets of Cysteine reactive Small Molecules by Using Ethynyl Benziodoxolone Reagents. *Angewandte Chemie Int. Ed. 54*, 10852–10857.

B) Examples of different acquisition modes

Data dependent acquisition	"Top 8"	Identification of allergens and glycation products in thermally processed peanut	Hebling, C. M., McFarland, M. A., Callahan, J. H., & Ross, M. M. (2013). Global Proteomic Screening of Protein Allergens and Advanced Glycation Endproducts in Thermally Processed Peanuts. *Journal of Agricultural and Food Chemistry, 61*, 5638-5648.
	"Top 20"	Identification of beer proteome	Grochalová, M., Konečná, H., Stejskal, K., Potěšil, D., Fridrichová, D., Srbová, E., Ornerová, K., & Zdráhal, Z. (2017). Deep coverage of the beer proteome. *Journal of Proteomics, 162*, 119-124.
Targeted acquisition	SRM / MRM	Detection of wheat contamination in foods	Colgrave, M. L., Goswami, H., Byrne, K., Blundell, M., Howitt, C. A., & Tanner, G. J. (2015). Proteomic Profiling of 16 Cereal Grains and the Application of Targeted Proteomics To Detect Wheat Contamination. *Journal of Proteome Research, 14*, 2659–2668.
		Identification of banned processed animal proteins in meat and bone meal	Marbaix, H., Budinger, D., Dieu, M., Fumière, O., Gillard, N., Delahaut, P., Mauro, S., & Raes, M. (2016). Identification of Proteins and Peptide Biomarkers for Detecting Banned Processed Animal Proteins (PAPs) in Meat and Bone Meal by Mass Spectrometry. *Journal of Agricultural and Food Chemistry, 64*, 2405-2414.
	PRM	Rapid detection of parasite (Anisakids) in fishery products	Carrera, M., Gallardo, J. M., Pascual, S., González, Á. F., & Medina, I. (2016). Protein biomarker discovery and fast monitoring for the identification and detection of Anisakids by parallel reaction monitoring (PRM) mass spectrometry. *Journal of Proteomics, 142*, 130-137.
Data independent acquisition	SWATH	Quantification of barley gluten in selectively bred barley lines	Colgrave, M. L., Byrne, K., Blundell, M., Heidelberger, S., Lane, C. S., Tanner, G. J., & Howitt, C. A. (2016). Comparing Multiple Reaction Monitoring and Sequential Window Acquisition of All Theoretical Mass Spectra for the Relative Quantification of Barley Gluten in Selectively Bred Barley Lines. *Analytical Chemistry, 88*, 9127–9135.
	WiSIM	Identification of tomato	Martin, L. B., Sherwood, R. W., Nicklay, J. J., Yang, Y., Muratore-Schroeder, T. L., Anderson, E. T., Thannhauser, T. W., Rose, J. K., &

		fruit proteins regulated by transcription factor CD2	Zhang, S. (2016). Application of wide selected-ion monitoring data-independent acquisition to identify tomato fruit proteins regulated by the CUTIN DEFICIENT2 transcription factor. *Proteomics, 16*, 2081-2094.

C) Examples of different quantification approaches and strategies

LFQ	MS signal intensity measurement	Characterization of muscle tissue from farmed and wild fish	Chiozzi, R. Z., Capriotti, A. L., Cavaliere, C., La Barbera, G., Montone, C. M., Piovesana, S., & Laganà, A. (2018). Label-Free Shotgun Proteomics Approach to Characterize Muscle Tissue from Farmed and Wild European Sea Bass (Dicentrarchus labrax). *Food Analytical Methods, 11*, 292-301.
		Quantification of proteins that influence meet quality	Gallego, M., Mora, L., Aristoy, M. C., & Toldrá, F. (2016). The use of label-free mass spectrometry for relative quantification of sarcoplasmic proteins during the processing of dry-cured ham. *Food Chemistry, 196*, 437-444.
	MS/MS spectral counting	Authentication of processed meet products	Montowska, M., & Fornal, E. (2017). Label-free quantification of meat proteins for evaluation of species composition of processed meat products. *Food Chemistry, 237*, 1092-1100.
Metabolic labeling based relative quant.	SILAC	Studying of mechanisms of resveratrol action in cell	Alayev, A., Doubleday, P. F., Berger, S. M., Ballif, B. A., & Holz, M. K. (2014). Phosphoproteomics Reveals Resveratrol-Dependent Inhibition of Akt/mTORC1/S6K1 Signaling. *Journal of Proteome Research, 13*, 5734-5742.
	In vivo SILAC (SILAM)	Studying interaction between host and microbiome important for pre- or prebiotic treatment	Oberbach, A., Haange, S. B., Schlichting, N., Heinrich, M., Lehmann, S., Till, H., Hugenholtz, F., Kullnick, Y., Smidt, H., Frank, K., Seifert, J., Jehmlich, N., & von Bergen, M. (2017). Metabolic in Vivo Labeling Highlights Differences of Metabolically Active Microbes from the Mucosal Gastrointestinal Microbiome between High-Fat and Normal Chow Diet. *Journal of Proteome Research, 16*, 1593-1604.
Chemical labeling based relative quant.	TMT / iTRAQ	Quantification of changes in proteome during fruiting process in *F.velutipes*	Liu, J. Y., Chang, M. C., Meng, J. L., Feng, C. P., Zhao, H., & Zhang, M. L. (2017). Comparative Proteome Reveals Metabolic Changes during the Fruiting Process in Flammulina velutipes. *Journal of Agricultural and Food Chemistry, 65*, 5091-5100.
	Dimethyl labeling	Characterization of muscle tissue from farmed and wild fish	Piovesana, S., Capriotti, A. L., Caruso, G., Cavaliere, C., La Barbera, G., Chiozzi, R. Z. & Lagana, A. (2016). Labeling and label free shotgun proteomics approaches to characterize muscle tissue from farmed and wild gilthead sea bream (Sparus aurata). *Journal of Chromatography A, 1428*, 193-201.
Enzym. labeling based	Proteolytic labeling with ^{18}O	Quantification of bovine and porcine gelatin	Sha, X. M., Tu, Z. C., Wang, H., Huang, T., Duan, D. L., He, N., Li, D. J., & Xiao, H. (2014). Gelatin Quantification by Oxygen-18 Labeling and Liquid Chromatography–High-Resolution Mass Spectrometry.

relative quant.			*Journal of Agricultural and Food Chemistry, 62*, 11840–11853.
Absolute quant.	AQUA	Quantification of Pru av 2 allergen in sweet cherry and other food	Ippoushi, K., Sasanuma, M., Oike, H., Kobori, M., & Maeda Yamamoto, M. (2016). Absolute quantification of Pru av 2 in sweet cherry fruit by liquid chromatography/tandem mass spectrometry with the use of a stable isotope-labelled peptide. *Food Chemistry, 204*, 129-134.
		Microfluidic – MS system for quantification of peanut allergens in complex food matrices	Sayers, R. L., Gethings, L. A., Lee, V., Balasundaram, A., Johnson, P. E., Marsh, J. A., Wallace, A., Brown, H., Rogers, A., Langridge, J. I., & Mills, E. N. C. (2018). Microfluidic separation coupled to mass spectrometry for quantification of peanut allergens in a complex food matrix. *Journal of Proteome Research, 17*, 647-655.
	QconCAT	Quantification of proteins in spore coat of food contaminant *B.cereus*	Stelder, S. K., Benito de Moya, C., Hoefsloot, H. C. J., de Koning, L. J., Brul, S., & de Koster, C. G. (2018). Stoichiometry, Absolute Abundance, and Localization of Proteins in the Bacillus cereus Spore Coat Insoluble Fraction Determined Using a QconCAT Approach. *Journal of Proteome Research, 17*, 903-917.
	PSAQ	Quantification of milk allergens in baked food samples	Newsome, G. A., & Scholl, P. F. (2013). Quantification of Allergenic Bovine Milk αS1-Casein in Baked Goods Using an Intact 15N-Labeled Protein Internal Standard. *Journal of Agricultural and Food Chemistry, 61*, 5659-5668.

Table 2.

Some examples of physical and chemical changes, introduced during food processing, food storage and sample preparation, of particular concern for MS based proteomic identification and quantification of food proteins or their proteoforms

- denaturation
- aggregation
- reduction of protein solubility in water due to structural changes of food matrix
- physical separation and removal of proteins
- unspecific and partial hydrolysis
- partial deglycosylation
- phosphorylation and dephosphorylation
- degradation of other PTMs
- activation or inactivation of enzymes
- reduction of disulfide bonds, or their formation
- formylation, methylation, acetylation (N-terminal amino acid, Lys)
- chemical reactions between proteins and different constituents of food matrix
   - modification of proteins (on: Cys, His, Lys, Met, Phe, Trp, Tyr) with reactive molecular species (reactive oxygen species, reactive nitrogen species, reactive carbonyl species, reactive sulphur species)
     - carbonylation (Arg, Lys, Pro, Thr)
     - oxidation (most frequently of Met, Cys, Phe, His, Pro, Trp, Tyr)
     - hydroxylation (Val, Phe, Trp, Leu)
     - nitration (Trp, Phe, His, Tyr)
     - nitrozylation (Tyr,)
   - modification of proteins (on: Cys, His, Lys, Arg, Gln, Asn) by lipoxidation products
   - glycation of proteins (usually on Lys) with reducing sugars (Maillard reaction)
   - formation of acrylamide from Asn and its subsequent interaction with proteins
- crosslinking (oligomerization and polymerization)
- isomerization and racemization (Asp->isoAsp, L-Pro->D-Pro and other amino acids)
- degradation of amino acids (most frequently deamidation of Asn->Asp and Gln-> Glu)
- carbamylation by urea (N-terminal amino acid)
- formation of dehydro and cross-linked amino acids such as dehydroalanine, methyldehydroalanine, beta-aminoalanine, lysinoalanine, ornithinoalanine, histidinoalanine, phenylethylaminoalanine, lanthionine, and methyl-lanthionine

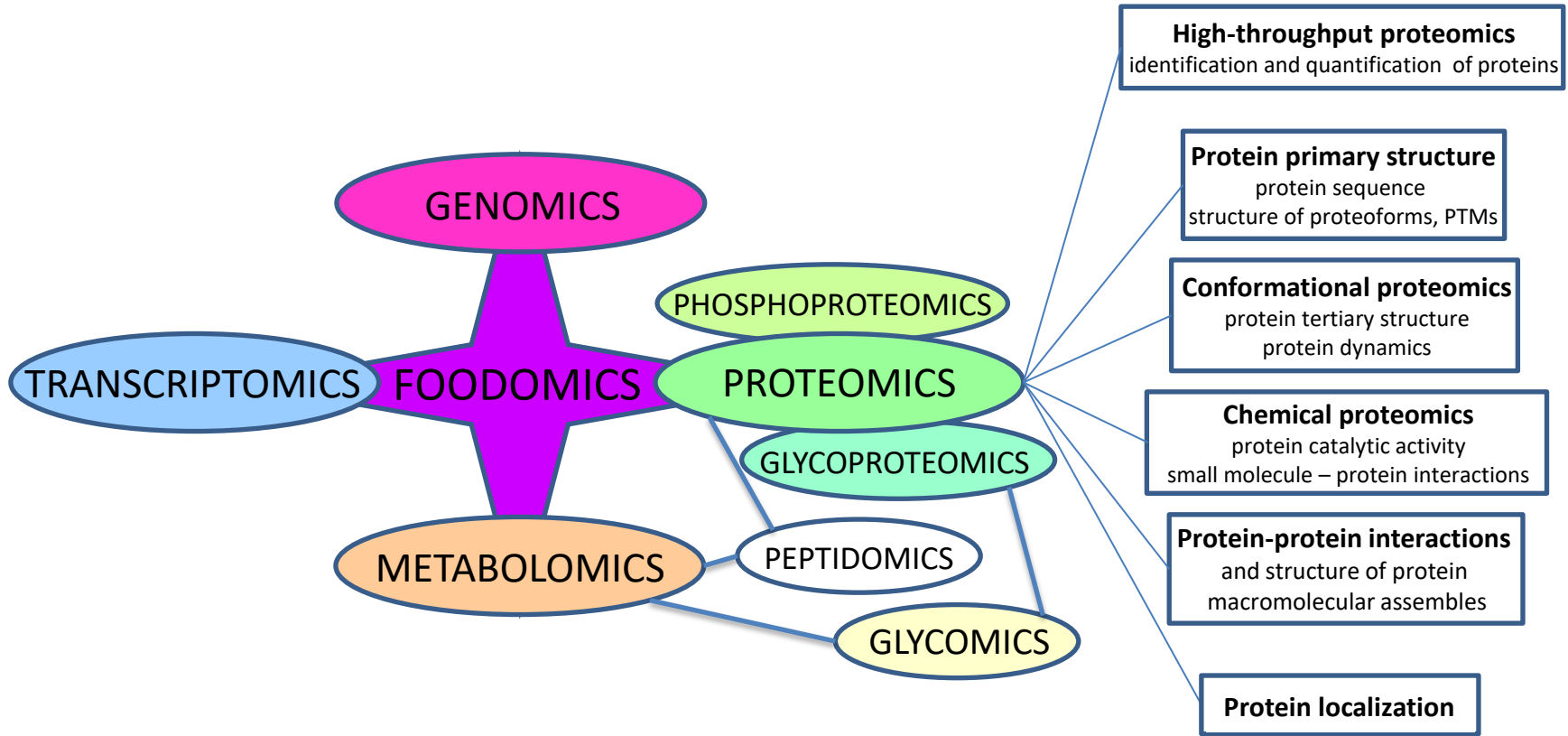Table. 3.
Different data independent acquisition (DIA) setups

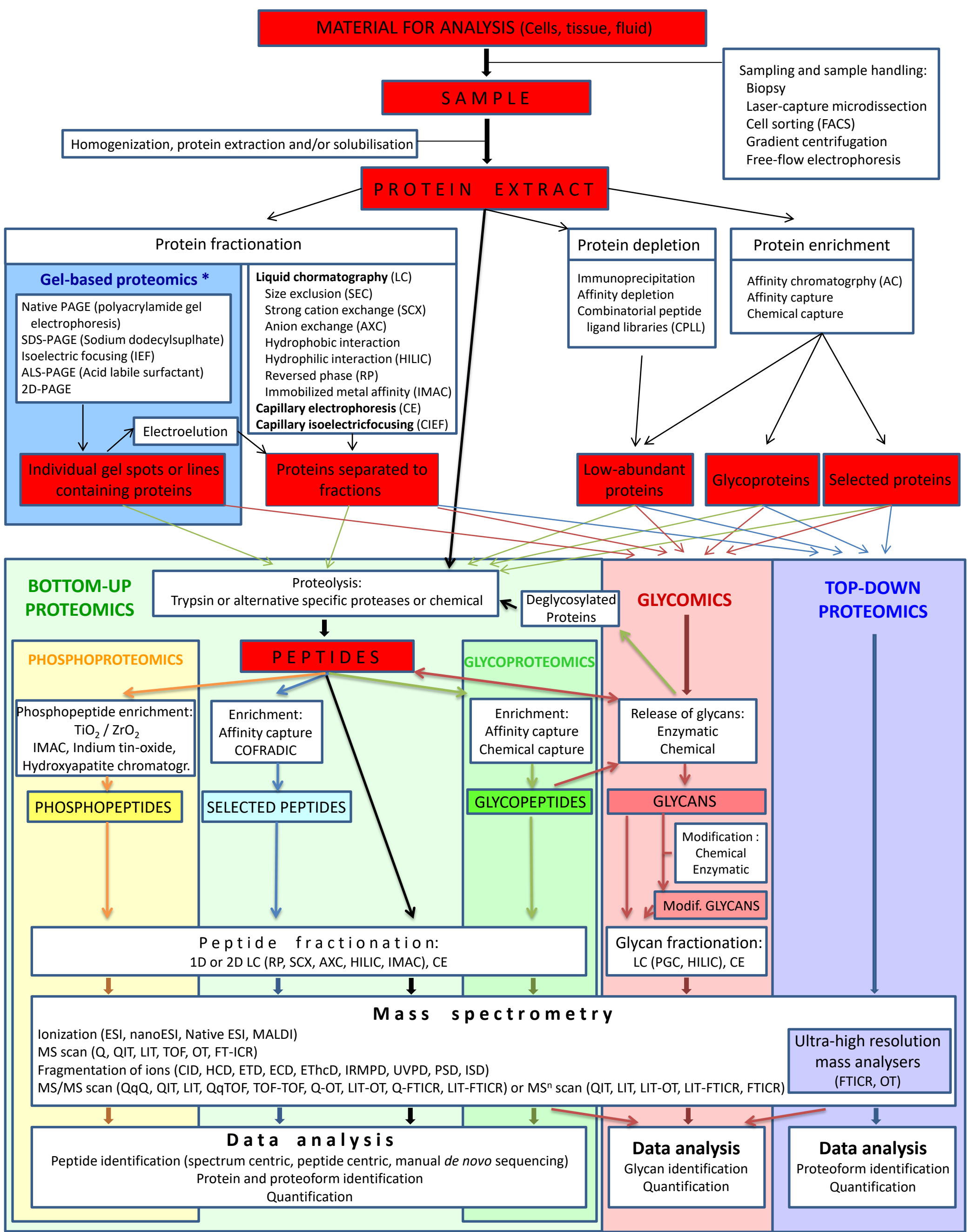| DIA setup	MS system	Reference
Multiplexed MS/MS	ESI-FT-ICR	Masselon, C., Anderson, G. A., Harkewicz, R., Bruce, J. E., Paša-Tolić, L., & Smith, R. D. (2000). Accurate Mass Multiplexed Tandem Mass Spectrometry for High-Throughput Polypeptide Identification from Mixtures. *Analytical Chemistry*, 72, 1918-1924.
Shotgun CID *(Shotgun collision-induced dissociation)*	μLC-μESI-TOF μLC-μESI -QIT μLC-μESI -QqTOF	Purvine, S., Eppel, J. T., Yi, E. C., & Goodlett, D. R. (2003). Shotgun collision-induced dissociation of peptides using a time of flight mass analyzer. *Proteomics*, 3, 847-850.
Original DIA	μLC-μESI -LTQ	Venable, J. D., Dong, M. Q., Wohlschlegel, J., Dillin, A., & Yates III, J. R. (2004). Automated approach for quantitative analysis of complex peptide mixtures from tandem mass spectra. *Nature Methods*, 1, 39-45.
MS^E	μLC-μESI-High resolution QqTOF	Silva, J. C., Denny, R., Dorschel, C. A., Gorenstein, M., Kass, I. J., Li, G. Z., McKenna, T., Nold, M. J., Richardson, K., Young, P., & Geromanos, S. (2005). Quantitative proteomic analysis by accurate mass retention time pairs. *Analytical chemistry*, 77, 2187–2200.
p^{2}CID *(parallel collision-induced-dissociation)*	ESI-QqTOF	Ramos, A. A., Yang, H., Rosen, L. E., & Yao, X. D. (2006). Tandem parallel fragmentation of peptides for mass spectrometry. *Analytical Chemistry*, 78, 6391–6397.
AIF *(All ion fragmentation)*	nLC-nESI-Q-OT (Exactive)	Geiger, T., Cox, J., & Mann, M. (2010). Proteomics on an Orbitrap benchtop mass spectrometer using all-ion fragmentation. *Molecular & Cellular Proteomics*, 9, 2252–2261.
XDIA	ESI-LTQ-OT	Carvalho, P. C., Han, X., Xu, T., Cociorva, D., da Gloria Carvalho, M., Barbosa, V. C., & Yates, III, J. R. (2010). XDIA: improving on the label-free data-independent analysis. *Bioinformatics*, 26, 847–848.
PaCIFIC *(Precursor acquisition independent from ion count)*	nLC-nESI-LTQ nLC-nESI-LTQ-OT (XL)	Panchaud, A., Jung, S., Shaffer, S. A., Aitchison, J. D., & Goodlett, D. R. (2011). Faster, quantitative, and accurate precursor acquisition independent from ion count. *Analytical chemistry*, 83, 2250–2257.
MXS *(multiplexing strategy DIA)*	nLC-nESI-Q-OT (Exactive)	Egertson, J. E., Kuehn, A., Merrihew, G. E., Bateman, N. W., MacLean, B. X., Ting, Y. S., Canterbury, J. D., Marsh, D. M., Kellmann, M., Zabrouskov, V., Wu, C. C., & MacCoss, M. J. (2011). Multiplexed MS/MS for improved data-independent acquisition. *Nature Methods*, 10, 744-746.

FT-ARM *(Fourier transformation all ion monitoring)*	nLC-nESI-LTQ-OT nLC-nESI-LTQ-FTICR	Weisbrod, C. R., Eng, J. K., Hoopmann, M. R., Baker, T., & Bruce, J. E. (2012). Accurate peptide fragment mass analysis: Multiplexed peptide identification and quantification. *Journal of Proteome Research*, 11, 1621-1632.
SWATH-MS *(sequential windowed acquisition of all theoretical fragment ion mass spectra)*	nLC-ESI-High resolution QqTOF	Gillet, L. C., Navarro, P., Tate, S., Rost, H., Selevsek, N., Reiter, L., Ron Bonner, R., & Aebersold, R. (2012). Targeted Data Extraction of the MS/MS Spectra Generated by Data-independent Acquisition: A New Concept for Consistent and Accurate Proteome Analysis. *Molecular & Cellular Proteomics*, doi: 10.1074/mcp.O111.016717.
HDMS[E] *(High definition MS*[E]*)*	nLC-nESI-Q-TWIMS-TOF	Shliaha, P. V., Bond, N. J., Gatto, L., & Lilley, K. S. (2013). Effects of Traveling Wave Ion Mobility Separation on Data Independent Acquisition in Proteomics Studies. *Journal of Proteome Research*, 12, 2323-2339.
UDMS[E] *(Ultra-high definition MS*[E]*)*	nLC-nESI-Q-TWIMS-TOF	Distler, U., Kuharev, J., Navarro, P., Levin, Y., Schild, H., & Tenzer, S. (2014). Drift time-specific collision energies enable deep-coverage data-independent acquisition proteomics. *Nature Methods*, 11, 167-170.
WiSIMDIA *(wide selected-ion monitoring DIA)*	nLC-nESI-Q-LIT-OT (Fusion, Fusion Lumos)	Kiyonami, R., Patel, B., Senko, M., Zabrouskov, V., Egertson, J., Ting, S., MacCoss, M., Rogers, J., & Hühmer, A. F. R. (2014). Large Scale Targeted Protein Quantification Using WiSIM-DIA on an Orbitrap Fusion Tribrid Mass Spectrometer. Thermo Scientific Application Note 600.
CSI PaCIFIC *(captive spray ionization PaCIFIC)*	nLC-nESI-CSI-LTQ-OT	Chapman, J. D., Edgar, J. S., Goodlett, D. R., & Ah Goo, Y. (2016). Use of captive spray ionization to increase throughput of the data-independent acquisition technique PAcIFIC. *Rapid Communications in Mass Spectrometry*, 30, 1101-1107.

Table 4. MS-based proteomic approaches and strategies for quantification of individual proteins and proteome.

LFQ (Label free quantification)		MS scan level	Area under the curve	Ahrné, E., Molzahn, L., Glatter, T., & Schmidt, A. (2013). Critical assessment of proteome-wide label-free absolute abundance estimation strategies. *Proteomics, 13*, 2567-2578.
			Signal intensity measurement	Neilson, K. A., Ali, N. A., Muralidharan, S., Mirzaei, M., Mariani, M., Assadourian, G., Lee, A., van Sluyter, S. C., & Haynes, P. A. (2011). Less label, more free: approaches in label-free quantitative mass spectrometry. *Proteomics, 11*, 535-553.
		MS/MS scan level	Spectral counting	Dowle, A., A., Wilson, J., & Thomas, J. R. (2016). Comparing the Diagnostic Classification Accuracy of iTRAQ, Peak-Area, Spectral-Counting, and emPAI Methods for Relative Quantification in Expression Proteomics. *Journal of Proteome Research, 15*, 3550-3562.
				Arike, L., & Peil, L. (2014). Spectral Counting Label-Free Proteomics. *Methods in Molecular Biology, 1156*, 213-222.
Relative quantification	**Label-based quant.**	Metabolic	SILAC (Stable isotope labeling with amino acids in cell culture)	Ong, S. E., Blagoev, B., Kratchmarova, I., Kristensen, D. B., Steen, H., Pandey, A., & Mann, M. (2002). Stable isotope labeling by amino acids in cell culture, SILAC, as a simple and accurate approach to expression proteomics. *Molecular & Cellular Proteomics, 1*, 376-386.
				Mann, M. (2014). Fifteen Years of Stable Isotope Labeling by Amino Acids in Cell Culture (SILAC). *Methods in Molecular Biology, 1188*, 1-7.
			In vivo SILAC (SILAM)	Krüger, M., Moser, M., Ussar, S., Thievessen, I., Luber, C. A., Forner, F., Schmidt, S., Zanivan, S., Fässler, R., & Mann, M. (2008). SILAC mouse for quantitative proteomics uncovers kindlin-3 as an essential factor for red blood cell function. *Cell, 134*, 353-364.
				Zanivan, S., Meves, A., Behrendt, K., Schoof, E. M., Neilson, L. J., Cox, J., Tang, H. R., Kalna, G., van Ree, J. H., van Deursen, J. M., Trempus, C. S., Machesky, L. M., Linding, R., Wickström, S. A., Fässler, R., & Mann, M. (2013). In Vivo SILAC-Based Proteomics Reveals Phosphoproteome Changes during Mouse Skin Carcinogenesis. *Cell Reports, 3*, 552-566.
			Plant SILAC	Lewandowska, D., ten Have, S., Hodge, K., Tillemans, V., Lamond, A. I., & Brown, J. W. S. (2013). Plant SILAC: Stable-Isotope Labelling with Amino Acids of Arabidopsis Seedlings for Quantitative Proteomics. *PLOS One, doi: 10.1371/journal.pone.0072207*
				Matthes, A., Köhl, K., & Schulze, W. X. (2014). SILAC and Alternatives in Studying Cellular Proteomes of Plants. *Methods in Molecular Biology, 1188*, 65-83.
			Super SILAC	Geiger, T., Cox, J., Ostasiewicz, P., Wisniewski, J. R., & Mann, M. (2010). Super-SILACmix for quantitative proteomics of human tumor tissue. *Nature Methods, 7*, 383-385.

				Reference
				Shenoy, A., & Geiger, T. (2015). Super-SILAC: current trends and future perspectives. *Expert Review of Proteomics, 12*, 13-19.
		Spike-in SILAC		Geiger, T., Wisniewski, J. R., Cox, J., Zanivan, S., Kruger, M., Ishihama, Y., & Mann, M. (2011). Use of stable isotope labeling by amino acids in cell culture as a spike-in standard in quantitative proteomics. *Nature Protocols, 6*, 147-157.
		Isobaric	TMT / iTRAQ	Christoforou, A., & Lilley, K. S. (2011). Taming the isobaric tagging elephant in the room in quantitative proteomics. *Nature Methods, 8*, 911-913.
			Other	Rauniyar, N., & YatesIII, J. R. (2014). Isobaric Labeling-Based Relative Quantification in Shotgun Proteomics. *Journal of Proteome Research, 13*, 5293-5309.
	Chemical	Non-isobaric	Dimethyl labeling	Boersema, P. J., Raijmakers, R., Lemeer, S., Mohammed, S., & Heck, A. J. R. (2009). Multiplex peptide stable isotope dimethyl labeling for quantitative proteomics. *Nature Protocols, 4*, 484-494. Lau, H. T., Suh, H. W., Golkowski, M., & Ong, S. E. (2014). Comparing SILAC- and Stable Isotope Dimethyl-Labeling Approaches for Quantitative Proteomics. *Journal of Proteome Research, 13*, 4164-4174.
			mTRAQ	DeSouza, L. V., Taylor, A. M., Li, W., Minkoff, M. S., Romaschin, A. D., Colgan, T. J., & Siu, K. W. (2008). Multiple Reaction Monitoring of mTRAQ-Labeled Peptides Enables Absolute Quantification of Endogenous Levels of a Potential Cancer Marker in Cancerous and Normal Endometrial Tissues. *Journal of Proteome Research, 7*, 3525-3534. Oppermann, F. S., Klammer, M., Bobe, C., Cox, J., Schaab, C., Tebbe, A., & Daub, H. (2013). Comparison of SILAC and mTRAQ Quantification for Phosphoproteomics on a Quadrupole Orbitrap Mass Spectrometer. *Journal of Proteome Research, 12*, 4089-4100.
	Enzymatic	Proteolytic labeling with ^{18}O		Hajkova, D., Sekhar Rao, K. C., & Miyagi, M. (2011). Recent Technological Developments in Proteolytic ^{18}O Labeling. *Current Proteomics, 8*, 39-46.
Absolute quantification	Spike-in of isotope-labeled standards	AQUA (Absolute quantification)		Gerber, S. A., Rush, J., Stemman, O., Kirschner, M. W., & Gygi, S. P. (2003). Absolute quantification of proteins and phosphoproteins from cell lysates by tandem MS. *Proceedings of the National Academy of Sciences, 100*, 6940-6945.
		QconCAT (Quantification concata-mer)		Beynon, R. J., Doherty, M. K., Pratt, J. M., & Gaskell, S. J. (2005). Multiplexed absolute quantification in proteomics using artificial QCAT proteins of concatenated signature peptides. *Nature Methods, 2*, 587-589.
		PSAQ (Protein standard for absolute quantification)		Brun, V., Dupuis, A., Adrait, A., Marcellin, M., Thomas, D., Court, M., Vandenesch, F., & Garin, J. (2007). Isotope-labeled protein standards: toward absolute quantitative proteomics. *Molecular & Cellular Proteomics, 6*, 2139-2149.

MATERIAL FOR ANALYSIS (Cells, tissue, fluid)

S A M P L E

Sampling and sample handling:
Biopsy
Laser-capture microdissection
Cell sorting (FACS)
Gradient centrifugation
Free-flow electrophoresis

Homogenization, protein extraction and/or solubilisation

P R O T E I N   E X T R A C T

Protein fractionation

Gel-based proteomics *

Native PAGE (polyacrylamide gel electrophoresis)
SDS-PAGE (Sodium dodecylsuplhate)
Isoelectric focusing (IEF)
ALS-PAGE (Acid labile surfactant)
2D-PAGE

Liquid chormatography (LC)
Size exclusion (SEC)
Strong cation exchange (SCX)
Anion exchange (AXC)
Hydrophobic interaction
Hydrophilic interaction (HILIC)
Reversed phase (RP)
Immobilized metal affinity (IMAC)
Capillary electrophoresis (CE)
Capillary isoelectricfocusing (CIEF)

Electroelution

Individual gel spots or lines containing proteins

Proteins separated to fractions

Protein depletion
Immunoprecipitation
Affinity depletion
Combinatorial peptide ligand libraries (CPLL)

Protein enrichment
Affinity chromatogrphy (AC)
Affinity capture
Chemical capture

Low-abundant proteins

Glycoproteins

Selected proteins

BOTTOM-UP PROTEOMICS

Proteolysis:
Trypsin or alternative specific proteases or chemical

Deglycosylated Proteins

GLYCOMICS

TOP-DOWN PROTEOMICS

PHOSPHOPROTEOMICS

P E P T I D E S

GLYCOPROTEOMICS

Phosphopeptide enrichment:
TiO_2 / ZrO_2
IMAC, Indium tin-oxide,
Hydroxyapatite chromatogr.

Enrichment:
Affinity capture
COFRADIC

Enrichment:
Affinity capture
Chemical capture

Release of glycans:
Enzymatic
Chemical

PHOSPHOPEPTIDES

SELECTED PEPTIDES

GLYCOPEPTIDES

GLYCANS

Modification :
Chemical
Enzymatic

Modif. GLYCANS

Peptide   fractionation:
1D or 2D LC (RP, SCX, AXC, HILIC, IMAC), CE

Glycan fractionation:
LC (PGC, HILIC), CE

Mass   spectrometry

Ionization (ESI, nanoESI, Native ESI, MALDI)
MS scan (Q, QIT, LIT, TOF, OT, FT-ICR)
Fragmentation of ions (CID, HCD, ETD, ECD, EThcD, IRMPD, UVPD, PSD, ISD)
MS/MS scan (QqQ, QIT, LIT, QqTOF, TOF-TOF, Q-OT, LIT-OT, Q-FTICR, LIT-FTICR) or MS^n scan (QIT, LIT, LIT-OT, LIT-FTICR, FTICR)

Ultra-high resolution mass analysers (FTICR, OT)

D a t a   a n a l y s i s
Peptide identification (spectrum centric, peptide centric, manual *de novo* sequencing)
Protein and proteoform identification
Quantification

Data analysis
Glycan identification
Quantification

Data analysis
Proteoform identification
Quantification

Highlights:

Mass spectrometry based proteomics, as one of the four main sources of data in foodomics, are presented.

MS-based proteomic approaches applicable in food research, quality and safety control are described.

Improvements in sample preparation and in the technology of mass spectrometers are presented.

Critical points for application of MS-based proteomics in food analysis are described.

Continuously growing capabilities of MS-based proteomics and future directions are discussed.