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Current peptidomics: Applications, purification, identification, quantification, and functional analysis

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Abstract

Peptidomics is an emerging field branching from proteomics that targets endogenously produced protein fragments. Endogenous peptides are often functional within the body—and can be both beneficial and detrimental. This review covers the use of peptidomics in understanding digestion, and identifying functional peptides and biomarkers. Various techniques for peptide and glycopeptide extraction, both at analytical and preparative scales, and available options for peptide detection with MS are discussed. Current algorithms for peptide sequence determination, and both analytical and computational techniques for quantification are compared. Techniques for statistical analysis, sequence mapping, enzyme prediction, and peptide function, and structure prediction are explored.

Keywords

Functional prediction; MS; Peptidomics; Quantitation; Structure prediction; Systems biology

1 Introduction

Peptidomics, the comprehensive qualitative and quantitative analysis of all peptides in a biological sample [1], is an emerging field derived from proteomics and enabled by modern separation, analytical and computation technologies. The complex biological matrices typically examined in peptidomics experiments require systematic peptide extraction to achieve successful analysis. Peptidomic analysis employs many proteomics techniques but with a different target. Rather than examining a sample for which intact proteins are present, peptidomics examines which endogenous protein fragments are present. This review describes applications of peptidomics and modern approaches for peptide extraction, fractionation, detection, quantification, functional annotation, and structural prediction.

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2 Applications of peptidomics

The applications of peptidomics include comprehensive mapping of food protein digestion, characterizing food-processing related proteolysis, identifying peptide biomarkers of disease, and identifying hormones and other signaling molecules. This section reviews the current range of applications for the field of peptidomics.

2.1 Peptidomics of digestion

Peptidomics is being applied to study how dietary proteins are catabolized in the digestive system and throughout the body. Many proteins and protein fragments (peptides) survive intact throughout the entire digestive process and are excreted in urine and stool [2, 3]. For example, after feeding milk or yogurt to adult humans, casein peptides were identified in the stomach, duodenum, and plasma [4].

Dietary protein digestion has been followed in a number of studies [5–9]. Prior to the emergence of MS-based peptidomic techniques, food protein digestion was examined with techniques such as HPLC followed by Edman sequencing. For example, these techniques were used to show release of opioid peptides in casein-fed miniature [7] and the persistence of functional milk peptides in the plasma of calves [10] and human infants [11] after milk or formula feeding.

Advances in MS have allowed major leaps in the examination of peptides released in digestion. For example, Boutrou et al. [8] employed LC-MS to identify thousands of milk protein-derived peptides in the jejunums of adult humans after casein and whey consumption. Bauchart et al. [9] identified 26 food-derived peptides (some being known antihypertensives) in the duodenums and jejunums of pigs fed beef and trout proteins. Research studies employed peptidomics to reveal that hundreds of peptides are released in gastric digestion of human milk in human infants [5], that milk protein digestion begins within the mammary gland due to milk proteases in humans [12] and bovine [13] and that this initial mammary gland milk degradation varies between healthy and mastitic quarters (teats) of cows [14].

Released protein fragments in the gut are not always beneficial. For example, peptides released from the wheat protein α-gliadin survive digestion to the colon, trigger the opening of the tight junctions between the cells, and lead to inflammation [15]. Likewise, fragments of many other food proteins can elicit allergenic responses.

Already, peptidomic analysis has revealed that different food preparation methods, such as cooking, gelation, fermentation, etc., have major effects on the release of dietary peptides in digestion. For example, MS-based peptidomics revealed that heat-treated caseins released more peptides in a simulated infant digestion than non–heat-treated caseins [15] and that rennet-gelled milks released three times fewer peptides than acid-gelled milks in digestion by miniature pigs [16]. Investigating the interaction between the digestion peptidome and food structure will become increasingly important to understanding health properties of diet.

2.2 Peptidomics of food hydrolysates

Conventional food processing releases peptides from food proteins, and these peptides can be examined with peptidomics. For example, in cheese production, casein proteins are often curdled through the use of exogenous enzymes (typically rennet). Peptide sequencing and MS analysis have revealed the exact sites of rennet cleavage on κ-casein [17], α_{s1}- [18], α_{s2}- [19, 20], and β-casein [20].

Bacteria used in cheese ripening also produce proteases that further degrade the cheeses [21, 22]. Peptidomics data enables examination of the cleavage sites and peptides produced by these bacterial interactions. For example, Miclo et al. [23] employed MS-based peptidomics to identify casein peptides (including many bioactive sequences) released by proteases produced by six *Streptococcus thermophilus* strains used in cheese and yogurt production.

Peptidomic technologies have already been applied to identify hundreds of peptides from several cheeses, including Parmigiano–Reggiano [24], Emmental [25], Gouda [26], and cheddar [27]. Peptidomics reveals that differences in cheese production starting materials, coagulation, and ripening result in major differences in peptides released across cheeses.

The fermentation process of many other foods releases peptides as well (e.g., kefir [28]). Peptidomics can be applied to all food hydrolysates to identify which peptides have been released during production. Once the released fragments are identified, they can be also be examined for potential functional activity.

Allergenic food proteins (e.g., wheat gluten, caseins, β-lactoglobulin) can be hydrolyzed to decrease their allergenicity. Peptidomics can be applied to monitor the extent of degradation to ensure allergenic epitopes are eliminated [29–32].

2.3 Peptidomics for biomarker search

Perhaps the most frequent use of peptidomics thus far has been in search of biomarkers of disease. Peptidomics is appealing for biomarker studies because the knowledge that is generated can present a dynamic view of health status: peptides are created by a complex and fluid interaction of proteases, activators, inhibitors, and protein substrates. A variety of peptide biomarkers have been identified. For example, levels of a fragment of β-amyloid (β-amyloid 1–42) and tau protein in cerebrospinal fluid can predict which patients with mild cognitive impairment will progress to Alzheimer's disease [33]. Combinations of urine peptides have been shown to serve as biomarkers (reviewed in depth in [34]) for diabetic nephropathy [35, 36], chronic kidney disease [37], acute kidney injury [38], acute renal allograft rejection [39,40], prostate cancer [41], and coronary artery disease [42]. The application of peptidomic analysis to identify biomarkers of disease has been thoroughly reviewed in a number of articles [43–45].

Many biological systems (including blood and digestive samples) contain proteases or contain organisms (e.g., bacteria) that can produce proteases. In order to use peptides for biomarkers, postsample collection proteolysis should be eliminated (protease inhibition) or adequately accounted for in these sample types, as discussed by Diamandis [46]. However, some biological samples, such as urine, are more stable and thus do not require additional

treatment to prevent proteolysis [37, 47]. Therefore, these samples can be used without protease inhibitory treatment [48].

2.4 Endogenous peptides as functional units

Besides peptides released from the dietary proteins due to fermentation or digestion, a variety of hormones and other signaling molecules in the body are active endogenously as sequence and structure-specific peptides. For example, the largest class of neuroactive messengers in animals is that of the endogenous peptides called neuropeptides [49]. Neuropeptides act as neurotransmitters, neurohormones, or neuromodulators, and are involved in the regulation of many, if not all, physiological processes in animals [50]. Most neuropeptides are small, ranging from a few to 100 amino acids in length [50].

Small endogenous peptides are essential for most, if not all, physiological processes. Many hormones are endogenous peptides (e.g., insulin, prolactin, oxytocin). These hormone peptides are often modified by disulfide bridges, *N*-terminal pyroglutamination, *N*-terminal acetylation, or *C*-terminal amidation [51,52], which protect the peptide from degradation by amino- and carboxypeptidases [49]. The presence of these modifications can serve as a good predictor of hormone function for novel endogenous peptides [53].

Peptidomic identification of hormones and other peptide signaling molecules has been highly successful. Specific tissues, cells, and even organelles have been isolated and analyzed by MALDI-MS allowing identification of hundreds of hormones and neuropeptides [50, 54, 55].

Short open reading frame-encoded polypeptides (SEPs) have been found through a combination of transcriptomic library building and peptidomics to confirm translation [56]. Ninety SEPs (polypeptides synthesized on the ribosome with <150 amino acids) were found in human cells arising from noncoding ribonucleic acid and multicistronic mRNAs [56]. Many have functions; for example, SEPs as short as 11 amino acids regulate morphogenesis in *Drosophila* [57].

3 Peptide extraction and fractionation

Identification and quantitation of molecules from complex biological matrices using MS typically requires selective enrichment of the compounds of interest. Biological matrices usually contain lipids, salts, proteins, and carbohydrates that decrease the ionization efficiency of the peptides and may cause fouling problems in the LC platforms. The preparative toolbox for peptide extraction is highly diverse and has been guided, in part, by proteomic approaches.

The proteinaceous fraction in biological samples is a continuum that ranges from high-molecular-weight proteins and peptide aggregates [58] to low-molecular-weight peptides. The low-molecular-weight fraction also represents a continuum of sizes, from 1 to 10 KDa [59] to only a few amino acid residues [60]. SEC [61] allows isolation of specific peptide mass ranges; however, this technique is time-consuming. Other simpler techniques, like molecular weight cutoff membrane filtration, do not permit complete separation of a specific

mass range without partial losses of peptides and/or partial contamination from the undesired fractions. However, the simplicity of this approach drives its use in most rapid preparative methodologies for separation of the low-molecular-weight and high-molecular-weight fractions. Filtration can be performed with membranes at various scales—from lab-scale applications [62,63] to large-scale industrial applications [64].

Proteins can also be removed by selective precipitation. Acid addition (e.g., using trichloroacetic acid [12]), the use of different organic solvents [65] or combinations of these precipitation agents [66] are often used for this purpose. However, protein precipitation does not remove proteins as completely as membrane filtration [67], and some peptides may aggregate and be lost in the precipitate [68]. Critical comparisons of protein removal methods for different biological samples can be found in the literature [69, 70].

For many biological samples where proteases are active, it is necessary to prevent any further proteolytic action as soon as the samples are collected. Methods for curtailing protease activity include protein denaturation (e.g., by addition of solvents like acetone [71], addition of acids like trichloroacetic acid [72], microwave irradiation [73]), or the addition of protease inhibitors [74]. Importantly, protease inhibitors should be selected that do not modify the peptide structure and added in concentrations that do not mask the peptide signal in the mass spectrometer [75]. Notably, some samples are more stable (e.g., urine) and require only normal sample storage at −20 °C or −80 °C for preservation [48].

Peptides vary in multiple aspects, such as size, hydrophobicity, and net charge. This physicochemical diversity is increased by PTMs, including oxidation, acetylation, phosphorylation, and glycosylation. As a consequence of this natural diversity, achieving comprehensive isolation of peptides from a complex mixture with a single purification method is challenging, and some physicochemical biases in the purification are expected. To alleviate this problem, multiple peptide purification methods can be used sequentially on the same sample [76], however this strategy increases sample preparation and analysis time and likely limits the reproducibility of the method.

A variety of methods are available for enrichment of specific peptide fractions. These approaches are applied to simplify the complexity of the sample, overcome the dynamic range limitations of the analytical instrument, or to extract a fraction of interest. Several extraction protocols have been developed to purify peptides containing specific amino acid residues including cysteine [77, 78], tryptophan [79], or methionine [80]. A variety of methods are available to isolate or enrich phosphopeptides, all based on the differential interaction of phosphopeptides with metals, including titanium dioxide [81]. Adenosine triphosphate used as the metal carrier was recently shown to provide highly sensitive and selective phosphopeptide extraction [82]. Glycopeptides have been extracted from complex samples by numerous methods. Hydrophilic liquid interaction chromatography-based glycopeptide extractions use different stationary phases, including microcrystalline cellulose [83], zwitterionic materials, [84], and Click chemistry-bound monosaccharides [85]. Glycopeptides can also be extracted with materials based on boronic [77] and hydrazine [86] chemistries. Lectins are also commonly used to enriched glycopeptides containing specific monosaccharides in their glycan moiety [87].

4 Analytical approaches

A wide variety of MS techniques have been employed in peptidomics. Both ESI [5], MALDI [54] and SELDI [88] sources have been successfully applied for peptidomics. MALDI and ESI-based peptidomics approaches have been compared previously [54, 89]. Both LC [5] and CE [36, 90, 91] have been employed for front-end in line separation [36] in peptidomics (reviewed in [5, 54]). Peptidomics has been successfully carried out using a variety of mass spectrometers, including orbitraps [92], quadrupole time-of-flight [5], and micro-TOF [36]. This review does not cover all the traditional MS techniques, as they have been thoroughly described in the following articles ([5,93]). Rather, we examine new technological advances in MS that are applicable to peptidomics.

4.1 Fragmentation approaches

In proteomics, the chances of having peptides with close molecular masses are increased with the complexity of the sample. This factor is especially critical in peptidomics as the number of potential peptide identities can be orders of magnitude larger than in the most complex shotgun-proteomics experiment. Instrument mass resolution is fundamental to reduce the number of possibilities during the peptide identification process and minimize the assignment errors. However, accurate mass alone is often insufficient to identify peptide sequences. Therefore, peptidomics requires instrumentation capable of tandem fragmentation to provide additional information for sequencing.

The most commonly used fragmentation technique for peptidomics is CID. In addition to CID, electron transfer dissociation (ETD) and high-energy CID can fragment peptides for sequence analysis, and each generates slightly different results [92]. Shen et al. [92] showed that a combination of these three approaches provided a more complete profile.

CID alone works poorly for glycopeptides, as it preferentially causes fragmentation of the more labile glycosidic bonds without fragmentation of the peptide backbone necessary for sequence determination [94]. ETD fragmentation is driven by chemical specificity, rather than bond energetics, and preferentially fragments the peptide backbone of glycopeptides, which enables sequence determination. An increasingly common approach for glycopeptide analysis combines ETD and CID to collect information on both the peptide backbone and the glycan [95].

4.2 Direct tissue/cell/organelle MALDI-TOF analysis

Isolated tissues, single cells, and organelles can be affixed to a MALDI target plate mixed with a matrix that facilitates ionization and analyzed directly for peptides (as reviewed in [54,55,89]). Peptides are identifiable even in these complex biological mixtures due to their high ionization efficiencies [54]. This technique can provide fast, sensitive, and accurate identification of peptides [96]. Use of MALDI-TOF–based direct analysis of single cells has led to the discovery of hundreds of neuropeptides [50, 55].

4.3 Imaging mass spectrometry

Imaging mass spectrometry (IMS) allows molecular analysis of tissue samples with spatial resolution. Although IMS is possible with different ionization sources, MALDI is the most widespread [97]. In MALDI-IMS, upon laser impact at specific locations on the target plate, molecules are desorbed from the sample, allowing spatial mapping of compounds. For peptide analysis, sample slices are usually washed to remove lipids and salts [98] and treated with ionic matrices like gentisic acid [99], CHCA derivatives [100], and others specifically developed for IMS applications [101].

Even before the peptidomic concept was defined, IMS was used to identify, with spatial resolution, endogenous peptides present in specific tissue locations [97]. Peptides are obvious targets for IMS because of their high ionization efficiency and convenient mass range. IMS-based peptidomics has been particularly useful in neurobiology. IMS has been used to characterize the neuroendocrine peptidome of crustaceans [102] and mammals [103,104], and to identify peptide biomarkers of neurodegenerative diseases [105, 106].

4.4 Ion mobility mass spectrometry

Ion mobility mass spectrometry (IM-MS) provides millisecond time-scale separation of compounds based on their cross-sectional area and resulting mobility along a drift tubes with subsequent mass detection [107]. IM-MS can be combined with LC to increase the number of separation dimensions. IM-MS can resolve isobaric and isomeric—often indistinguishable by other MS techniques—according to their shape. IM-MS has been used to distinguish between isomeric peptides differing in the amino acid sequence [108,109] and even between peptides with different amino acid stereoisomers (i.e., D versus L) [110]. IM-MS even allows differentiation of specific phosphorylation sites in multiply phosphorylated peptides [111]. The capability of IM-MS to determine the position of PTMs within the peptide sequence has been demonstrated for other modifications such as methylation [112] and glycosylation [113]. This technique has been applied to differentiate between conformations of peptide aggregates in neurodegenerative diseases [114].

5 Quantification

The quantification of minute differences in peptide amounts between samples is one of the most important and challenging tasks in peptidomics. Both labeled (isotopic and isobaric) and label-free quantification can be employed in peptidomics.

5.1 Label-free quantification

Label-free peptide quantification can be performed by extracting peptide signal intensities or by spectral counting. The ion signal intensity approach uses the extracted chromatographic area to compare peptide abundances across samples. Spectral counting totals the number of times a peptide is selected for fragmentation and identified in a data-dependent acquisition experiment. Even though label-free quantitation often is considered less quantitative [115], it has a number of advantages: it requires no additional sample preparation, allows use of smaller sample amounts (as no chemical reaction step is needed), and can be performed with

almost any analytical platform. Label-free approaches have been used to discover new biomarkers for Crohn's disease [116] and kidney dysfunctions [117].

5.2 Isobaric labeling

Isobaric labeling allows for multiplexing many samples in a single analysis, which improves throughput and enables more precise quantification due to the co-ionization of the target peptides [118, 119]. The major isobaric labeling schemes are iTRAQ [118] and Tandem Mass Tags [119]. Though applied extensively in proteomics, these techniques have been applied in only a few peptidomics research studies (e.g., in neuropeptidomics [120]).

5.3 Isotope labeling

Quantification by MS is often performed with isotopically labeled samples. The advantage of isotope labeling over isobaric labeling is that peptide fragmentation is not required to perform the quantification. As the cycle time of automated MS/MS usually does not allow fragmentation of all peptides in a sample, isotope labeling allows, at least initially, the quantification of more peptides. On the other hand, the isotopically labeled spectra are more complex as the number of ion signals increases with the use of isotopes. Several isotopic labeling strategies can be used to quantitate endogenous peptides, including SILAC culture, ICAT, and 4-trimethyl-ammonium-butyryl [121]. The use of SILAC culture has been successfully used in peptidomics to study intracellular proteolytic processes [122].

5.4 Single- and multiple-reaction monitoring

Once peptide sequences have been identified, MRM allows quantification based on detection of specific products formed during the peptide ion dissociation. MRM, typically applied with triple quadrupole instruments, has been extensively used in protein quantification and is highly sensitive and specific. Although MRM is, at least initially, a label-free quantitation methodology, it is usually combined with stable isotope dilution in order to obtain absolute quantitative information. The method has been applied for peptide biomarker quantification of different diseases in serum [123–125] and urine [126, 127] as well as quantification of bioactive peptides in food [60]. A major disadvantage of MRM-based quantification is that peptide sequences must be identified a priori in order to determine transitions to measure.

6 Peptide spectral identification techniques

Bottom-up proteomics employs proteolytic enzymes with high specificity such as trypsin, which allows searching against only peptides matching those specificity patterns. In peptidomic analysis, peptides are cleaved by an array of often-unknown endogenous proteolytic enzymes, which requires searching against all possible peptide fragments, greatly expanding the search space.

Peptide spectral databases [128, 129] are also a ubiquitous tool in bottom-up proteomics but are seldom used for peptidomics. The majority of data in spectral libraries are generated from site-specific enzyme digestion making the data sets difficult to leverage for peptidomics.

6.1 Database searching vs. de novo identification

Database searching matches tandem spectra by comparison to theoretical spectra derived from predicted peptides in a protein library. De novo identification, on the other hand, uses no sequence library—rather, sequences are identified by calculating mass differences between fragments.

Database search engines include X!Tandem [130], Mascot [131], SEQUEST [132], MS-GFDB [133], MS-Fit [134], and OMSSA [135]. To use these programs for peptidomics, however, a “no enzyme” setting is required. De novo sequencing programs include UStags [136], Peaks [137], PepNovo [138], Sherenga [139], DirecTag [140], and MS-Tag [141]. A few recent programs combine both the database and de novo approaches. For example, OpenSea and DirecTag identify de novo–derived sequence tags and align them against the protein database [142]. Peptidomics analysis programs are thoroughly reviewed in [120].

6.2 Searching for PTMs

Most proteomic software adaptable to peptidomic analysis can search for simple PTMs such as phosphorylation, deamidation, and oxidation. However, few software platforms can identify endogenous peptides with more complex modifications such as glycosylation. Several programs successfully identify glycopeptides from tryptic digests or pronase digests of several proteins, but these programs typically cannot address complex biological mixtures of endogenously cleaved glycopeptides as reviewed by Dallas et al. [143]. Progress is being made in this area, however. For example, Byonic allows the combination of CID with ETD to determine glycopeptide sequence and glycan composition [144].

Conventional proteomic spectral matching algorithms are typically incapable of identifying novel peptide modifications. However, several de novo–based algorithms can provide a solution for this problem. Spectral Networks [145] and TagRecon [146] can identify unknown modifications by spectral clustering combined with de novo analysis. Spectral clustering combines groups of tandem spectra identified by peak similarity to create a single consensus spectrum representing the most abundant spectral features shared by all related spectra [147]. De novo analysis is then used to provide deeper feature coverage, and examination of the differences in mass between compounds in a spectral network allows identification of novel PTMs. This approach revealed a large number of diverse modifications in eye lens crystallin [148].

7 Data Analysis

Once endogenous peptides in a system have been identified, techniques for mining the data are essential. Typically, peptidomics researchers need methods to (1) compare peptide quantities between sample groups; (2) visualize the peptide data in the context of the protein sequence; (3) analyze the data for which enzymes released them from the intact protein; (4) predict peptide structure; and (5) predict peptide function.

7.1 Detecting differences

Differences in specific peptide peak areas across sample groups are typically determined with basic statistical tests, such as *t*-tests or ANOVA. Several programs such as XCMS [149] and Agilent Mass Profiler Professional [150] have built in statistical comparisons and can be applied to peptidomics. For many sample types, data normalization is essential before statistical comparisons. For example, urine peptidomics data must be normalized (e.g., to creatinine) to control for large concentration variations due to differences in daily fluid intake [48].

7.2 Site visualization

Mapping where the fragments from a protein derived in relation to the overall sequence can support biological insight into the enzymatic processes occurring in a system. Peptide coverage diagrams [50, 151, 152] allow visualization of peptide–protein relationships by highlighting observed sequence regions or by overlaying lines indicative of coverage. For data sets with extensive and overlapping peptide coverage, these maps become crowded and less informative. A strategy to clarify trends in coverage diagrams is to map peptide spectral intensity to the coverage map. This simultaneously diminishes extraneous near-baseline peaks while promoting visibility of high abundance peptides that may contribute significantly to the biological function. As far as we know, only one program exists for this purpose: PepEx [153]. PepEx was used to map the endogenous peptides in human milk and revealed that the release of peptides was highly specific to regions of the parent protein [153].

7.3 Enzymatic mapping

Peptidomics data can be used to assess which enzymes released the fragments from the protein precursor by evaluating the observed peptides against a proteolytic specificity library. Several programs currently exist for this type of analysis. The online tool EnzymePredictor [152] compares known specificity patterns of a list of common proteases to peptide cleavage sites and calculates the likelihood an enzyme's participation in proteolysis. Another program for enzyme analysis is Proteolytic Enzyme Estimator [13, 153]. This program functions similarly to EnzymePredictor, but uses peptide intensity rather than peptide count in estimation of relative enzyme activity. Proteasix predicts which enzymes were involved in peptide release by comparing peptide sequences around cleavage sites to a cleavage site database containing 3500 human protease–cleavage site combinations [154]. Because proteolytic specificity is often broad, and peptides can be produced with contributions from nonspecific exopeptidases, this informatic approach can only estimate enzyme activity [153]. Ideally, bioinformatic enzyme analysis based on the peptidomics data would be paired with chromogenic substrate assays for each enzyme. In these assays, a short sequence of amino acids specific to a protease's cleavage specificity are added to a solution, and the release of a chromogenic leaving group (e.g., p-nitroanilide) can be measured spectrophotometrically to determine enzyme activity.

7.4 Peptide structure predictions

Predicting peptide three-dimensional structure from the amino acid sequence and PTMs is important in peptidomics research because peptide structure affects function. Several experimental techniques, including x-ray crystallography [155] and NMR [156], can determine peptide structure. As these experiments are expensive and time-consuming, predicting peptide structure with bioinformatics will become increasingly essential for peptidomics.

Programs like Pepstr [157], PEP-FOLD [158, 159], and PepLook [160,161] are designed for peptides and produce better structural predictions for peptides than protein-centric programs. Pepstr [157] provides de novo structural prediction of small peptides. This program combines secondary structure and β-turn predictions, and refines the structure with energy minimization and molecular dynamic simulations [157]. The resulting predicted structures are typically similar to NMR-derived structures. PEP-FOLD [158, 159] performs de novo peptide structure predictions for linear peptides as well as cyclic peptides with disulphide bonds. PepLook [161] performs ab initio peptide structure prediction of linear and cyclic peptides. This algorithm allows for multiple structural results for a single peptide, as peptide structures are less stable than protein structures.

7.5 Peptide function predictions

Functional peptides have a wide range of biological activities. For example, bioactive peptides from milk have actions including antimicrobial, antihypertensive, antithrombotic, and immunomodulatory [162]. Predicting peptide function is challenging because peptides with similar functions might have very different sequences and structures.

One method for predicting peptide function is through a simple homology search against a database of known functional peptide sequences. Several databases of functional peptides exist, including SwePep [163], Erop-Moscow [164], and PeptideDB [165]. Peptides identified in a sample that are highly homologous with library sequences may have similar functions.

8 Conclusions

Peptidomics is an expanding new field with a variety of applications including monitoring digestion, annotating food hydrolysates, characterizing hormone levels and identifying disease biomarkers. Innovations in peptide extraction, detection, and analysis are improving peptidomics throughput, accuracy, and utility.

Peptidomics will continue to advance with faster instrument electronics to facilitate the isolation, fragmentation, and detection of more peptides in less time, as well as more sensitive detectors that will allow the detection of less abundant peptides and, after fragmentation, the detection of less abundant fragment ions.

There is a variety of issues in peptidomic analysis that still need to be addressed. Improved software for identification of peptides with complex modifications is necessary. For example, software that adequately identifies endogenous glycopeptides from complex

biological mixtures remains lacking [143]. Software for peptide functional prediction remains in its infancy, yet will become increasingly important with the large peptide data sets now being produced. Several programs now provide estimates of peptide structure based on sequence, and these can be applied to large peptidomics data sets. However, methods for accurate analytical structure determination (e.g., x-ray crystallography, NMR, and circular dichroism) are typically applied to isolated peptides and have not yet been applied to measure complex peptide mixtures in high-throughput. Strategies for structural determination in-line with LC-MS would be promising.

Traditionally, intact proteins have been considered as the functional units *in vivo*. However, most proteins undergo proteolytic processing such as auto-activation or degradation by enzymes [166]. Therefore, often, protein fragments are produced that can interact directly with the cellular targets, producing a functional effect. Even if a peptide occurs for only a short time before further degradation, it may still transmit a signal [167]. Therefore, mapping even transitory peptides can be important for understanding complex protein/peptide–health interactions. With the advance of peptidomics, we can now monitor peptide release across time and physiological/sub-cellular location to reveal their roles in complex biological interaction networks.

With the recent realization that peptides can have important functions—both beneficial and detrimental—throughout the body, peptidomics will become increasingly important in monitoring how dietary proteins are digested. Food producers will soon need to employ peptidomics to characterize not only what peptides and proteins are in their food products, but also what they become in the digestive tract of the consumer.

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Abbreviations

ETD	electron transfer dissociation
IM-IS	ion mobility mass spectrometry
IMS	imaging mass spectrometry
SEP	short open reading frame-encoded polypeptides

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