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FORENSIC PROTEOMICS

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Proteins are naturally occurring highly complex substances that consist of amino acid residues joined by a peptide bond. Protein is the major component of all biological evidence, where it can be used to identify body fluids and tissues, as well as convey genetic information in the form of single amino acid polymorphism. Proteomics is the study of proteomes (i.e, the total proteins of a given sample such as cultured cells, tissues, or an organism). It is a powerful approach to studying biological systems.

Compared with immunological methods, proteomics may reduce time and overall costs. Forensic Proteomics directly analyzes proteins in blood cells, clothing fibers, medication, etc, for the applications like microbial characterization, protein toxin detection, and forensic fluid analysis.

PROTEOMICS METHODS

Proteomics methods can be separated into top-down, middle-down, and bottom-up approaches.

In top-down proteomics, intact proteins are extracted from samples that are directly separated and analyzed by LC-MS/MS (Liquid Chromatography-Mass Spectrometry). This approach is widely used in sports anti-doping to identify banned proteins or peptides. In the bottom-up approach, proteins are digested into thousands of peptides such as trypsin and LysC. In the middle-down approach, protein digestion is carried out but the real aim is to yield large peptides. The

bottom-up method is the most used method of the three approaches. The steps of the approach are shown here:

i. Sample preparation: includes sample pre-treatment, protein extraction, proteolytic digestion, and peptide purification

- Sampling and sample pre-treatment: Samples can be collected from human bodies (if there is) and crime scenes. The techniques include wipes, dry swabs, aspirating needles, air vacuums, and filters. After collection, for short-term storage and -20 degree or -80 degree celsius for long-term storage. Pre-treatment methods are selected depending on the sample type, if the sample-containing proteins are exosomes, serum, plasma saliva, etc they don't require lysis pre-treatment, instead an amount of buffer is added to favor enzymatic digestion steps. If the samples are cells, a lysis step is applied to break the cell membrane.
- Protein extraction: They are extracted by simultaneous removal of contaminants. Protein precipitation is the common method in which proteins are precipitated by organic solvents and their mixtures with acids or sodium deoxycholate. After precipitation, the protein pellets are collected and washed with pre-chilled acetone ahead of proteolytic digestion.
- Proteolytic digestion: Methods that are widely used are in-gel, in-solution, on- bead, and filter-aided sample preparation (FASP). In in-solution digestion, protein pellets are mixed with 8M urea, which can increase protein stability and denature protein structures, these are reduced and then alkylated. Then proteins are digested with enzymes. The resultant peptides have the preferred size for MS Sequencing.

- Peptide purification: pellet-purification is conducted using reversed-phase solid-phase extraction (SPE). The eluted peptide from the extraction is subjected to lyophilization or vacuum drying.
- Sample fractionation: It is the rare step that can reduce the complexity of pellets before LC-MS/MS Analysis. It is a 3D fractionation required to achieve a proteome profile.

ii. Data acquisition: Depending on data acquisition types, there is targeted and untargeted proteomics, where the untargeted aims to collect data in large numbers and is used to profile the proteome of the sample, the targeted only detects and quantifies a small number of peptides. It is carried out in a triple-quadrupole mass spectrometer.

iii. Data analysis: Database search is one method where the theoretical spectra are generated from peptide sequences via silico-digestion. Another approach is MS/MS spectra library search. The next method is de novo peptide identification, which uses spectra to determine sequences without databases.

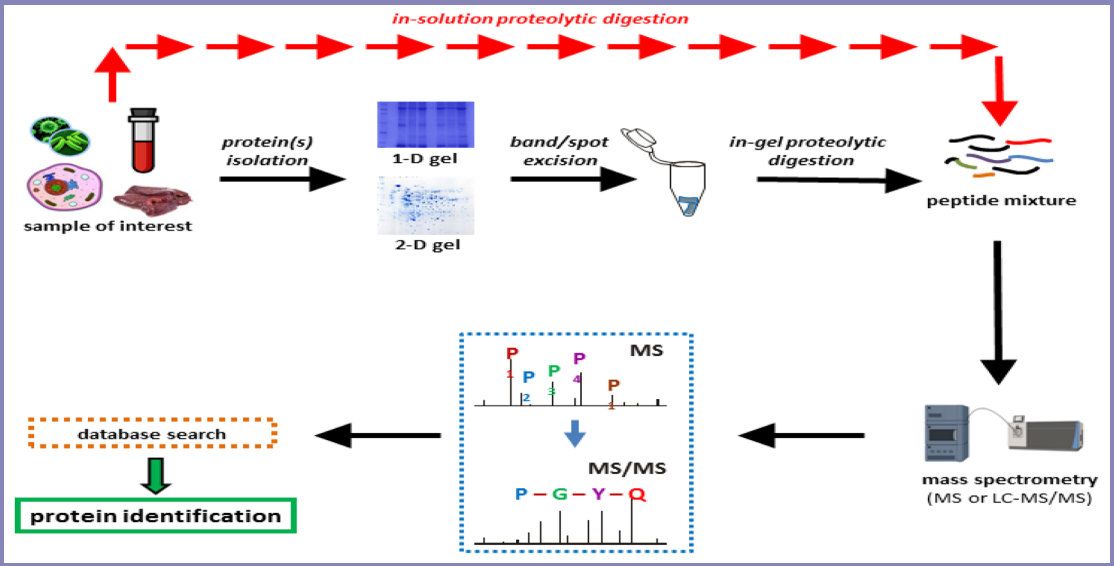

Figure: Processes of the bottom-up approach
(https://images.app.goo.gl/U1WizdGSR5sJ2XCh7)

APPLICATIONS OF FORENSIC PROTEOMICS

- Hair proteome: Human hair proteins are extracted by the shotgun-proteomics approach, which demonstrates a large extractability and variety of hair proteins after detergent extraction.
- Bone proteome: This can be used to study biomarkers and therapeutic procedures in osteoporosis, bone marrow aging linked to genetic changes in the proteome, and also about bone cancer.
- Organ identification: The study found highly discriminating proteins in different organs. The proteomics analysis of the tissue can be further investigated for future applications.
- Brain and cerebrospinal fluid (CSF): CSF is used to determine the time of death by comparing the proteome profiles of antemortem and postmortem CSF, which is performed by 2D gel electrophoresis and MS.

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