Program Final examination on discipline «**Proteomic technologies**

Numerous methods are available to study proteins, sets of proteins, or the whole proteome. In fact, proteins are often studied indirectly, e.g. using computational methods and analyses of genomes.

The proteome term is applied to several different types of biological systems. A **cellular proteome** is the collection of proteins found in a particular cell type under a particular set of environmental conditions such as exposure to hormone stimulation. It can also be useful to consider an organism's **complete proteome**, which can be conceptualized as the complete set of proteins from all of the various cellular proteomes. This is very roughly the protein equivalent of the genome. The term "proteome" has also been used to refer to the collection of proteins in certain sub-cellular biological systems. For example, all of the proteins in a virus can be called a viral proteome.

Module 1 **High-throughput proteomic technologies. Current research methodologies**

Study in Proteins.

Practical significance of study in Proteins

Study and methodology of three-dimensional structure (*3 D* ) of proteins ,

The principle of NMR spectroscopy. Using NMR spectroscopy in study of proteins

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| The procedure for NMR solution structure determination |
| Methods of X-ray crystallography  |
| Procedure using of X-ray crystallography in study of proteins |
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| Mechanisms of phosphorylation |
| Protein kinases |
| Method of detection |
| Major research methods in study of protein post-translational modification (PTM) |
| Significance of phosphorylation in signal transduction cascades and methods to determine them |
| Using of immonodetection in protein phosphorylation |
| Using of immunochemistry in study of proteins |
| The mass spectrometry methods in study of proteins |
| In vitro analysis of protein phosphorylation |
| Quantitative Mass spectrometry |
| Matrix-assisted laser desorption ionization in proteomic analysis |
| Electrospray ionization in proteomic analysis |
| Two-dimensional gel electrophoresis. Using. Stages. Module 2 **Technologies used for post-translational modifications studies**Protein post-translational modification (PTM) |
| Protein methylation as protein post-translational modification |
| Methods in study of protein methylation |
| Phosphoprotein enrichment methodology |
| Significance of acetylation of proteins  |
| Protein acetylation as post-translational regulatory mechanism  |
| Strategies for the detection of acetylated proteins. |
| Methods of confirmation of the presence of acetylation in proteins (radioactive detection or immunodetection techniques)  |
| Mass spectrometry as method to confirm the identity of the protein and to reveal the number of acetylation sites.  |
| In vitro method to separate acetylated proteins 1D, one-dimensional/ 2D, two-dimensional electrophoresis |
| In vitro method to separate acetylated proteins using chromatography- based method  |
| Immunodetection method in study of acetylated proteins using acetyl-lycine specific antibodies |
| Method of determination of intact molecular weight of acetylated proteins using mass spectrometry |
| Western blotting in study of acetylated proteins |
| Immunohistochemical analysis in study of acetylated proteins |
| Mass spectrometric analysis of acetylated proteins |
| Proteins glycosylation, a post-translational modification (PTM).  |
| Functions of proteins glycosylation  |
| Types of protein glycosylation and methods of their determination |
| Methods to detect and analyze glycoproteins |
| Glycan staining or labeling in study of glycosalated proteins |
| Method of glycoprotein purification or enrichment |
| Principles of glycoproteome and glycome analysis by mass spectrometry |
| The general approach of quantitative proteomics. |
| The use of stable isotopes in quantitative proteomics. |
| SILAC, stable isotope labeling with amino acids in cell culture method  |
| Origin and function of ubiquitin-like protein conjugation  |
| Stable isotope labeling in mass spectrometry-based quantitative proteomics |
| The use of stable isotopes in proteomics (Isotope-coded affinity tags (ICATTM) methodology). |
| Enzymatic labeling in quantitative proteomics |
| The AQUATM (Absolute quantification in quantitative proteomics |
| Origin and function of ubiquitin-like protein conjugation |
| Ubiquitination is an enzymatic, protein post-translational modification (PTM) |
| Activation of system ubiquitin  |
| Function and variety of ubiquitin modifications |
| Technology to determine ubiquitylation of a protein |
| Multiple procedures to determine a ubiquitylated protein  |
| Methods to investigate the factors that specifically target the substrate for ubiquitylation |
| Methods to determine the site of ubiquitin conjugation.  |
| The methods to determine ubiquitylation of a protein: |
| Method to determine a ubiquitylated protein in vivo  |
| Method to determine a ubiquitylated protein in vitro |
| Technology to determine the site of ubiquitylation (on a lysine residue or the N‐terminal ‐amino group).  |
| Western blot analysis to identify the ubiquitylated species. |
| Method to preserve the ubiquitylated forms before analysis of them (using an inhibitor of the proteasome for cells)  |
| Western blot analysis in proteomics technology |
| Affinite chromatography in proteomics technology |
| The use of high performance liquid chromatography HLPC in study of proteins  |
| Protein lipidation stages  |
| The principles of high performance liquid chromatography HLPC |
| Regulation of protein trafficking by palmitoylation |
| Regulation by S-Nitrosylation of protein post-translational modification |
| The methylation of proteins, its significance and main approaches to study |
| Methods of determination of arginine-methylated protein complexes |
| MALDI-ToF peptide mass fingerprinting spectra |
| The principles of CNE, clear native electrophoresis  |
| Methods in study of protein-protein inter- interaction ( two-hybrid system) |
| Technology in study of protein lipidation |
| The principles of BNE, blue-native electrophoresis |

Module 3 **Protein detection with antibodies (immunoassays)** The enzyme-linked immunosorbent assay (ELISA) has been used for decades to detect and quantitatively measure proteins in samples. The Western blot can be used for detection and quantification of individual proteins, where in an initial step a complex protein mixture is separated using SDS-PAGE and then the protein of interest is identified using an antibody.

**Hybrid technologies** Examples of these methods are the MSIA (mass spectrometric immunoassay) developed by Randall Nelson in 1995 and the SISCAPA (Stable Isotope Standard Capture with Anti-Peptide Antibodies) method, introduced by Leigh Anderson in 2004.



General schema showing the relationships of the genome, transcriptome, proteome, and metabolome (lipidome).

The teacher,
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