International Union of
Pure and Applied Chemistry

Terminology of bioanalytical methods

Journal:	*Pure and Applied Chemistry*
Manuscript ID	PAC-REC-16-11-20
Manuscript Type:	Recommendation
Date Submitted by the Author:	21-Nov-2016
Complete List of Authors:	Labuda, Jan; Slovak University of Technology, Department of Analytical Chemistry Bowater, Richard; University of East Anglia, School of Biological Sciences Fojta, Miroslav; Akademie ved Ceské Republiky, Institute of Biophysics Gauglitz, Günter; Universität Tübingen, Institut für Physikal. und Theoretische Chem. Glatz, Zdeněk ; Masarykova Univerzita, Department of Biochemistry and Central European Institute of Technology Hapala, Ivan ; Slovak Academy of Sciences, Institute of Animal Biochemistry and Genetics Havliš, Jan; Masarykova Univerzita, National Centre for Biomolecular Research and Central European Institute of Technology Kilar, Ferenc ; Pecsi Tudomanyegyetem, Medical School Kilar, Aniko; Pecsi Tudomanyegyetem, Medical School Malinovská, Lenka ; Masarykova Univerzita, Central European Institute of Technology Sirén, Heli; Helsingin Yliopisto, Department of Chemistry Skládal, Petr ; Masarykova Univerzita, Department of Biochemistry and Central European Institute of Technology Torta, Federico ; National University of Singapore, Center for Life Sciences Valachovič, Martin; Slovak Academy of Sciences, Institute of Animal Biochemistry and Genetics Wimmerová, Michaela ; Masarykova Univerzita, Department of Biochemistry Zdráhal, Zbyněk ; Masarykova Univerzita, National Centre for Biomolecular Research and Central European Institute of Technology Hibbert, David; University of New South Wales, School of Chemistry
Keywords:	bioanalytical samples, enzymatic methods, immunoanalytical methods, genomics, nucleic acid analysis, proteomics, metabolomics, glycomics, lipidomics, biomolecule interaction studies
Author-Supplied Keywords:	

Terminology of bioanalytical methods (IUPAC Recommendations 201x)

Ján Labuda[1], Richard P. Bowater[2], Miroslav Fojta[3], Günter Gauglitz[4], Zdeněk Glatz[5], Ivan Hapala[6], Jan Havliš[5], Ferenc Kilar[7], Aniko Kilar[7], Lenka Malinovská[5], Heli M. M. Sirén[8], Petr Skládal[5], Federico Torta[9], Martin Valachovič[6], Michaela Wimmerová[5], Zbyněk Zdráhal[5], D Brynn Hibbert[10]

[1]Slovak University of Technology in Bratislava, Institute of Analytical Chemistry, 81237 Bratislava, Slovakia, [2]University of East Anglia, School of Biological Sciences, Norwich Research Park, Norwich NR4 7TJ, United Kingdom, [3]Academy of Sciences of the Czech Republic, Institute of Biophysics, 612 65 Brno, Czech Republic, [4]Eberhard-Karls University, Institute for Fysical and Theoretical Chemistry, 72076 Tübingen, Germany, [5]Masaryk University, Faculty of Science and Central European Institute of Technology, 625 00, Brno, Czech Republic, [6]Slovak Academy of Sciences, Institute of Animal Biochemistry and Genetics, 90028 Ivanka pri Dunaji, Slovakia, [7]University of Pécs, Medical School, Institute of Bioanalytics, 7624 Pécs, Hungary, [8]University of Helsinki, Department of Chemistry, 00014 University of Helsinki, Finland, [9]National University of Singapore, Center for Life Sciences, Singapore 117456, [10]School of Chemistry, UNSW Australia, Sydney, NSW 2052, Australia

Abstract: Recommendations are given concerning the terminology of methods of bioanalytical chemistry. With respect to dynamic development particularly in the analysis and investigation of biomacromolecules, terms related to bioanalytical samples, enzymatic methods, immunoanalytical methods, methods used in genomics and nucleic acid analysis, proteomics, metabolomics, glycomics, lipidomics, and biomolecules interaction studies are introduced.

Keywords: bioanalytical samples, enzymatic methods, immunoanalytical methods, genomics, nucleic acid analysis, proteomics, metabolomics, glycomics, lipidomics, biomolecule interaction studies.

This work was started under project 2011-047-1-500: Recent advances in bioanalytical chemistry: characterization and classification, with membership of Jan Labuda, Günter Gauglitz, Pier Giorgio Righetti, Frantisek Foret, Frank-Michael Matysik, Boris Mizaikoff, and D. Brynn Hibbert.

CONTENTS

1 PREFACE

The recommendations contained in this document complete the terminology of bioanalytical chemistry in general and analysis of biomacromolecules in particular. The previous IUPAC recommendation covers mostly the analytical terminology related to body fluids, enzymology, and immunology [1]. Selected terms related to the bioanalysis are included within the recommendations and reports devoted to the unit "katal" [2], biotechnology [3], clinical chemistry[4], toxicology [5, 6], medicinal chemistry [7, 8], proteomics [9], and electrochemical biosensors [10, 11] and physical organic chemistry[12]. Definitions of some terms have been updated here with respect to new reports and considerations and number of new terms has been introduced particularly on the topics of "–omics", DNA analysis and studies of the interactions between biomolecules.

These recommendations will become part of a chapter in the revised Orange Book (Compendium of Terminology in Analytical Chemistry, 4th edition).

2 INTRODUCTION

Bioanalytical chemistry is a branch of science that represents large and dynamic areas of research and practical analytical activities from human health screening (medical, clinical, food and pharmaceutical analysis) to forensic analysis and from basic biochemistry studies to special apparatus development.

Bioanalytical chemistry and biochemical analysis often require special methods for sampling, sample preparation and handling biomolecules like isolation, purification, separation, and fragmentation. Special attention is paid to apparatus and techniques with immobilized bioreceptors (biosensors, bioarrays). The methods of analysis and identification of biomacromolecules are radically different from analysing relatively small organic molecules. Most of "classical" analytical methods are not, or only to a limited extent, suitable for the analysis of biomacromolecules.

There is no complete vocabulary on this topic. With respect to terminologic broadness of the methods used in bioanalysis, the approach taken here is to offer complete and informative definitions specific for the bioanalysis, however, whithout coverage of definitions utilized generally in branches of analytical chemistry such as separation methods, mass spectrometry and others also widely used in the bionalysis.
Note that "Source: [xx]" indicates a copy of a previously published definition; "Source: [xx] with minor changes" means that the intent of the existing definition is unchanged but that wording or grammar has been updated; "Source: Adapted from [xx]" indicates that changes tyo the definition have been made.

2.1 bioanalytical chemistry

Sub-field of analytical chemistry dealing with analysis of *biomolecules*.

Note: Bioanalytical chemistry covers identification (identity, sequence), characterization (properties such as, polarity and charge, structure, folding,

intermolecular interactions,), quantification and monitoring (stability, dynamics, fragmentation, degradation, metabolism, and others).

Source: [13]

2.2 biomolecule

Molecule of biological origin.

Note 1: Among typical biomolecules representing subjects of bioanalytical chemistry are amino acids, peptides, polypeptides, proteins, nucleotides, nucleic acids, glyco-compounds, lipids, antibodies, haptenes, receptors, and also biomimetics.

Note 2: Modern bioanalytical chemistry shifts its attention to the analysis and investigation of *biomacromolecules* such as nucleic acids, proteins, and others.

2.3 biomacromolecule

biopolymer

Biomolecule of high relative molecular mass.

Note 1: Macromolecule of biological origin possessing biological functions, the structure of which essentially comprises the multiple repetition of units derived, actually or conceptually, from molecules of low relative molecular mass.

Note 2: If a part or the whole of the molecule has a high relative molecular mass and essentially comprises the multiple repetitions of units derived, actually or conceptually, from molecules of low relative molecular mass, it may be described as either macromolecular or polymeric, or by polymer used adjectivally.

Note 3: In many cases, especially for synthetic polymers, a molecule can be regarded as having a high relative molecular mass if the addition or removal of one or a few of the units has a negligible effect on the molecular properties. This statement fails in the case of certain macromolecules for which the structural and/or functional properties may be critically dependent on fine details of the molecular structure.

Note 4: Non-natural biomacromolecule-like synthetic molecules such as poly(lactic acid) and nucleic acid mimics (such as *peptide nucleic acid* or locked nucleic acid) are also used in bioanalysis.

Examples: Protein, nucleic acid, glycan, lipid.

Source: [14]

3 BIOANALYTICAL SAMPLES

3.1 amniotic fluid

Fluid that surrounds the foetus in the amniotic sac.

Note: A specimen is obtained by a technique called amniocentesis in which a long needle is inserted into the amniotic sac through the abdominal wall through which fluid is withdrawn.

Source: [1]

3.2 blood

Fluid that circulates through the heart, arteries, capillaries, and veins.

Note: Blood is composed of *plasma*, the fluid portion, and *blood cells*, the particles suspended in the plasma.

Source: [1]

3.3 blood cell
hematocyte
hemocyte
haematopoietic cell

Any cellular element of the blood including erythrocytes, leucocytes, and platelets.

Note: A blood cell is produced by hematopoiesis and normally found in blood. In mammals, these fall into three general categories: red blood cells – erythrocytes, white blood cells – leukocytes, and platelets – thrombocytes.

Source: [1]

3.4 blood cell count

Number of red *blood cells* and white *blood cells* per unit volume in a specimen of venous *blood*.

Source: [1]

3.5 blood group

Red cell phenotypes classified by their antigenic structural characteristics, which are under the control of various allelic genes.

Note: The cell membrane properties that provide the specific antigenicity of the blood groups are called agglutinogens as they agglutinate or clump in the presence of their specific antibody.

Example: O, A, B

Source: [1]

3.6 body fluids

Liquids originating from the bodies of living or dead humans, animals and other organisms.

Note: They include fluids that are excreted or secreted from the body.

3.7 cerebrospinal fluid (CSF)

Clear, colourless fluid that fills spaces within and around the central nervous system.

Note: CSF originates from *plasma* by a biological ultrafiltration process. Specimens are obtained by a lumbar puncture (i.e., a spinal tap).

Source: [1]

3.8 cord blood

Blood contained in the vessels of the umbilical cord at the time of birth.

Note: Cord blood contains stem cells.

Source: [1]

3.9 lymph

Yellowish, slightly basic fluid circulating through the lymphatic system.

Note: Lymph is similar to *plasma* and contains *white blood cells*.

Source: [1]

3.10 occult blood

Blood present in such small amounts that its presence can be ascertained only by chemical analysis or by spectroscopic or microscopic examination; particularly the blood found in stools.

Source: [1]

3.11 peripheral blood

Blood obtained from parts of the body that are located at some distance from the heart.

Example: Blood drawn from the earlobe, fingertip, or heel pad.

Source: [1]

3.12 plasma

Clear, yellowish fluid that accounts for about 55 % of the total volume of *blood*.

Note 1: Plasma is obtained by centrifuging whole blood with anticoagulant addition.

Note 2: Plasma from which fibrinogen and related coagulation proteins have been removed is called *serum*.

Source: [1]

3.13 protein-free filtrate

Sample of *blood*, *serum*, or *plasma* from which all *proteins* have been removed by chemical or physical denaturation, dialysis, ultrafiltration, or solvent extraction.

Source: [1]

3.14 saliva

Clear, viscous secretion from the parotid, submaxillary, sublingual, and smaller mucous glands in the cavity of the mouth.

Note: Saliva contains many important substances, including electrolytes, mucus, antibacterial compounds and various enzymes.

Source: [1]

3.15 serum

Clear, yellowish fluid that separates from *blood* when it is allowed to clot. It closely resembles *plasma* except for the absence of some coagulation factors.

Note: Serum contains hydrophilic proteins and electrolytes, *antibodies*, *antigens*, hormones, and exogenous substances.

Source: [1]

3.16 skin

Soft outer covering of vertebrates. In humans, it is the largest organ of the intergumentary system.

3.17 sperm

Seminal fluid containing male reproductive cells.

3.18 sweat

Fluid excreted by the sweat glands in the skin of mammals during perspiration.

3.19 tears

Clear salty liquid that is secreted by the lachrymal gland of the eye to lubricate the surface between the eyeball and eyelid to wash away irritants.

3.20 tissue

Group of biological cells that perform a specific function.

Note: A cellular organizational level intermediate between cells and a complete organ.

3.21 urine

Fluid containing metabolic products that is excreted by the kidneys, stored in the bladder, and normally discharged by way of the urethra.

Source: [1]

4 ANALYSIS OF BIOMOLECULES

4.1 General Terms

4.1.1 assay

Set of operations for the identification of a component or measurement of a quantity in analytical chemistry.

Note 1: Assay is an historical term now largely obsolete as a synonym for the metrological terms examination or measurement but still used in compound terms e.g. *immunoassay*, *bioassay*.

Source: [15] with minor changes.

4.1.2 assay kit

Set of components (reagents, solutions, and other necessary materials) and procedural instructions needed to perform an *assay*.

Source: [15] with minor changes.

4.1.3 bioassay

Assay in which an effect on an organism, tissue, cell, enzyme or receptor is compared with the effect of a reference material.

Note 1: The analyte may be of biological origin (e.g., vitamin, hormone, plant growth factor, antibiotic drug, enzyme).

Source: Adapted from [3, 7]

4.1.4 bioinformatics

Discipline encompassing the development and utilisation of computational tools to store, analyse, and interpret biological data.

Note: Among the most important bioinformatics tools belong specialised databases with genetic and protein sequences accompanied with related data (biological function, cell localisation, *etc*.), search, alignment and sorting algorithms or graphical modelling.

Source: [8]

4.1.5 biological recognition element
biochemical receptor
biocomponent, at a biosensor

Component of biological origin that translates information from the phyisco-chemical domain into an *indication* [VIM 4.1] with a defined *selectivity* [VIM 4.13].

Note 1: The main purpose of the biological recognition element is to provide a *biosensor* or other systems with a high degree of selectivity for the kind of quantity to be measured.

Note 2: Typical biological recognition elements include biocatalysts, immune receptors, nucleic acids, and others. Biologically-derived (biomimetic) recognition elements such as oligopeptides, oligosaccharides, peptide nucleic acids, nucleic acid aptamers, peptide aptamers, imprints, are also of great interest.

Note 3: A biocatalytic recognition element is based on a biocatalytic reaction. Three types of biocatalyst are commonly used: (a) Enzyme (mono-or multi-enzyme): the most common and well-developed recognition system. (b) Whole cells (micro-organisms, such as bacteria, fungi, eukaryotic cells or yeast) or cell organelles or particles (mitochondria, cell walls). (c) Tissue (plant or animal tissue slice).

Note 4: Biocomplexing or bioaffinity recognition element is based on the binding interaction of the analyte with macromolecules or organized molecular assemblies that have either been isolated from their original biological environment or engineered.

Source: [10].

4.1.6 biomarker
marker

Molecule specifically related to a particular state of a biological system, used to provide information about an organ function, disease or other aspect of health.

Note 1: Biomarkers are traceable substances that are either introduced into an organism or formed in the body.

Note 2: The molecule signals an event or condition in a biological system or sample and giving a measure of exposure, effect, or susceptibility.

Examples: Protein or peptide, nucleic acid, metabolite, hormones, *etc.*

Source: Adapted from [8].

4.1.7 biopolymer
See *biomacromolecule*

4.1.8 biosensor

Measuring instrument [VIM 3.1] requiring no additional reagents providing *selective* quantitative analytical information using a *biological recognition element*.

Note 1: The biological recognition system mediated by isolated *enzymes*, immunosystems, tissues, organelles, whole cells or others which translates information from the biochemical domain, usually an analyte concentration, into a chemical or physical (electrical, thermal or optical) output signal with a defined sensitivity.

Note 2: As a biosensor is a self-contained integrated *receptor* - *transducer* device, it should be clearly distinguished from an analytical system which incorporates additional separation steps, such as high performance liquid chromatography (HPLC), or additional hardware and/or sample processing such as specific reagent introduction, e.g. flow injection analysis (FIA). Thus, a biosensor should be a reagentless analytical device, although the presence of ambient cosubstrates, such as water for hydrolases or oxygen for oxidoreductases, may be required for the analyte determination. HPLC or FIA system may incorporate a biosensor as a detecting device.

Note 3: It should be necessary to distinguish strictly between biosensor (e.g. nucleic acid-based biosensors) and biosensing or *bioassay* (e.g. nucleic acid sensing).

Note 4: Variation of a biosensor response depending on method of preparation and operational conditions is called biosensor stability. Storage stability and working stability are typically distinguished.

Source: Adapted from [3, 10]

4.1.9 enzymatic decomposition

Decomposition of organic materials (e.g. starch, sugars, proteins, lignin, hemicelluloses, etc.) with specific *enzymes* or microbes in which the enzyme converts a high molecular mass compound into lower molecular mass species.

Example: Enzyme-catalyzed hydrolytic degradation of biopolymers.

Source: [16]

4.1.10 Hansch analysis

Investigation of the quantitative relationship between biological and physicochemical activity of compounds and global parameters representing hydrophobic, electric, steric, or/and other effects using multiple regression correlation.

Note: It belongs to regression analysis for quantitative structure-activity relationship (QSAR).

Source: [7]

4.1.11 hydrolases

Enzymes that catalyse the cleavage of C–O, C–N, C–C and other bonds by reactions involving water.

See *enzymatic decomposition*

Source: Adapted from [3] page 156

4.1.12 immobilization (in bioassay and biosensors)

A procedure or state when the *biological recognition element* is fixed in a thin layer (usually a single layer) at the *transducer*.

Source: Adapted from [10] and [3] page 156

4.1.13 label
tracer

Species covalently bound to a *biomolecule* and used for its detection.

Note: The analytical signal is either a measurable property of the *label* or the signal is produced by the *label*.

Example: In *enzyme immunoassay* (EIA) the label is an enzyme, in *fluorescence immunoassay* (FIA) the label is a fluorescent chemical; in *radioimmunoassay* (RIA) the label is a radionuclide.

4.1.14 ligand (in bioanalysis)

Ion, group or molecule that binds to a specific *receptor*.

Example: Ion or molecule like hormone that binds to a biomolecular target to elicit, block, or attenuate a biological response, or to produce a specific analytical signal.

Source: [12] page 1136, [8].

4.1.15 lysis

Breaking down of a cell.

Note 1: Lysis may be caused by physical, (bio)chemical or biological means.

Note 2: A fluid containing the contents of lysed cells is called a "lysate".

Source: Adapted from [3] page 159

4.1.16 microarray

Two-dimensional arrangement of a device for analysis of sub-microlitre sample volume.

Note 1: This screening format is a direct offshoot of genomic microarray technologies and makes use of ultra-low-volume miniaturization provided by nanodispensing technologies.

Note 2: A collection of molecules spatially addressed on a surface within features that have micrometer dimensions. Planar device (flat plane) used for off-line determination and identification of biocompounds.

Example: As a multiplex lab-on-a-chip it can be used as an analytical device to measure the mRNA abundance (gene expression) of thousands of genes in one experiment binding the imobilized *DNA* fragments.

Source: Adapted from [8].

4.1.17 receptor

Molecular structure in or on a cell that specifically recognizes and binds to a compound and acts as a physiological signal transducer, or mediator of an effect.

Note: A receptor can also be immobilized onto a surface used in *immunoassays*, *biosensors*, and microchip separation devices.

Source: [17]

4.1.18 response time (of biosensor)

Time interval after introduction of the sample at which the biosensor signal is measured.

Note 1: Steady-state response time is time necessary to reach 90% of the steady-state response.

Note 2: Transient response time is time for the first derivative of the output signal R to reach its maximum value $(dR/dt)_{max}$ following the addition of *analyte*.

Source: Adapted from [10].

4.1.19 sample quality control

Test of purity of sample that will be subjected to detailed biochemical analysis.

Note: *Microelectrophoresis* belongs to methods used for sample quality testing.

Example: DNA or protein analysis.

4.1.20 sequence

The order of neighbouring *amino carboxylic acids* in a *protein* or of the *nucleotides* in *DNA* or *RNA*.

Source: [18] page 1296

4.1.21 sequencing

Analytical procedures for the determination of the order of monomer subunits (primary structure) in a linear polymer (such as *amino carboxylic acids* in a polypeptide chain or *nucleotides* in a *DNA* or an *RNA* molecule).

Source: [3] page 165

4.1.22 steady-state response time (of biosensor)

See *response time (of biosensor)*

4.1.23 transducer, biosensor

Part of *biosensor* that converts a detected physical or chemical change at the level of the *biological recognition element* into an observable (usually electronic) signal.

Example: Electrode at voltammetric/amperometric or potentiometric mode, optical fiber, etc.

Source: Adapted from [10].

4.1.24 transient response time (of biosensor)

See *response time (of biosensor)*

4.2 Enzymatic Methods

4.2.1 allostery

allosteric regulation

Phenomenon whereby the conformation of an *enzyme* or other *protein* is altered by combination, at a site other than the substrate-binding site, with a small molecule, referred to as an effector, which results in either increased or decreased activity by the enzyme.

Source: [1]

4.2.2 apoenzyme

Protein part of an *enzyme* without the *cofactor* necessary for catalysis.

Note: The cofactors can be a metal ion (e.g. Cu^{2+}, Cu^{+}, Co^{2+}, Fe^{3+}, Fe^{2+}, Zn^{2+}, Mn^{2+}), an organic molecule (coenzyme or prosthetic group), or a combination of both.

Source: [1]

4.2.3 catalytic activity (in enzymology)

enzyme activity

enzyme catalytic activity

Property of an *enzyme* as the biocatalyst expressed as the rate of the catalyzed conversion of a specified chemical reaction produced in a specified assay system.

Note 1: The SI unit of *enzyme activity* is katal, the amount of enzyme activity that converts one mole of substrate per second under specified reaction conditions. "katal", kat = 1 mol s^{-1}.

Note 2: International Unit (U) was defined as the amount of enzyme that catalyzes the conversion of one micromole of substrate per minute under the specified conditions of the assay method (1 U = 1 μmol min^{-1} ≈ 16.67 nkat). This unit of enzyme activity was originally proposed by the International Union of Biochemistry in 1964 and it unit is no longer recommended because the term does not indicate what physical quantity it refers to, and because the minute is not the SI unit of time.

Note 3: Derivatives of *enzyme activity*, a, lead to the following pairs of kind-of-quantity and coherent SI unit:
specific enzyme activity, $a_s = a/m$ with the unit katal per kilogram, kat kg^{-1}
molar catalytic activity, $a_m = a/n_e$, equal to the turnover number with the unit katal per mole of enzyme, kat $mol^{-1} = s^{-1}$.

Source: Adapted from [1, 2], [4] page 972

4.2.4 catalytic activity concentration
catalytic concentration

Catalytic activity divided by the volume of the system.

Note 1: In clinical chemistry the component is usually an *enzyme*.
Note 2: The term catalytic concentration is accepted for use in clinical chemistry.
Note 3: Use of the term level as a synonym for concentration is not favoured.
Note 4: In describing a quantity, concentration must be clearly differentiated from amount.

Source: Adapted from [1], [4] page 964

4.2.5 catalytic activity content

Catalytic activity divided by the mass of the system.

Note 1: The term catalytic content is accepted for use in clinical chemistry.

Source: [4] page 964

4.2.6 catalytic activity fraction

Quotient of the *catalytic activity* of a particular form of an *enzyme*, such as isozyme and the catalytic activity of all the isozymes of the same enzyme in the system.

Note 1: The term catalytic fraction is accepted for use in clinical chemistry.
Note 2: The definition also applies to other multiple forms of an enzyme that are not isoenzymes.

Source: [4] page 964

4.2.7 coenzyme

Organic molecule that is required by a certain *enzyme* to carry out catalysis.

Note 1: This low-relative-molecular-mass and active group of an enzyme is an intermediate carrier of chemically active groups, specific atoms, and/or electrons. A coenzyme binds with its associated protein (*apoenzyme*) to form the active enzyme (*holoenzyme*).
Note 2: Coenzymes may be dissociable or covalently bound (in the latter case they are called prosthetic groups).
Example: The most common is NADH.

Source: Adapted from [1]

4.2.8 denaturation of protein

Partial or total alteration of the structure of a *protein* without change in covalent structure by the action of certain physical procedures (heating, agitation) or chemical agents (alcohols, tensides, foaming agents).

Note 1: Denaturation is the result of the disruption of tertiary bonding, which causes the opening of the folded structure of a protein and the loss of characteristic physiologic, enzymatic, or physicochemical properties.
Note 2: Denaturation can be either reversible or irreversible.

Source: Adapted from [1]

4.2.9 Eadie-Hofstee plot

See *Lineweaver-Burk plot*

4.2.10 enzyme

Macromolecule, usually a *protein* that functions as a *catalyst*.

Note: In general, an enzyme catalyzes only one reaction type (reaction selectivity) and operates on only one type of *substrate* (substrate selectivity). Substrate molecules are transformed at the same site (region selectivity), and often only one enatiomer of a chiral substrate is transformed.

Source: Adapted from [1] page 2593, [7], [8].

4.2.11 holoenzyme

Active enzyme consisting of the *apoenzyme* and *coenzyme* (cofactor).

Source: [1]

4.2.12 inhibitor

Substance that diminishes the rate of a chemical reaction.

Note: The process is called inhibition.

Source: Adapted from [1]

4.2.13 isoenzymes

Related *enzymes* catalyzing the same reaction but having different molecular structure.

Note: Isoenzymes are characterized by different physical, biochemical, or immunological properties.

Source: [1]

4.2.14 Lineweaver-Burk plot

Plot of the reciprocal of velocity of an enzyme-catalyzed reaction (ordinate) versus the reciprocal of substrate concentration (abscissa).

Note 1: The plot is used to graphically define the maximum velocity of an enzyme-catalyzed reaction and the Michaelis constant (*see Michaelis-Menten kinetics*) for the enzyme.

Note 2: Eadie-Hofstee plot is an alternative using a linear plot of velocity versus the ratio of velocity and substrate concentration.

Source: Adapted from [1].

See *Michaelis-Menten kinetics*

4.2.15 Michaelis-Menten kinetics

The relationship between the rate of an *enzyme-catalyzed reaction v* and the substrate concentration [S] in the form

$v = V[S]/(K_m+[S])$

where V and K_m are constants at a given temperature and a given enzyme concentration.

Note 1: The term 'hyperbolic kinetics' is also sometimes used because a plot of v against [S] has the form of a rectangular hyperbola through the origin with asymptotes $v = V$ and $[S] = -K_m$. This term, and others that imply the use of

particular kinds of plot, should be used with care to avoid ambiguity, as they can be misleading if used out of context.

Note 2: The quantity $k_0[E]_0$ is given by the symbol of V and its name is the *limiting rate*. It is particularly useful, when k_0 cannot be calculated because the total catalytic-centre concentration is unknown, as in studies of enzymes of unknown purity, sub-unit structure and molecular mass. The symbol V_{max} and the names maximum rate and maximum velocity are also in widespread use, although under normal circumstances, in general, there is no finite substrate concentration at which $v = V$. Hence, there is no maximum in the mathematical sense. The form V_{max} is convenient in speech as it avoids the need for a cumbersome distinction between "capital V" and "lower case v".
The constant K_m is known as the *Michaelis constant* for substrate S; the alternative name Michaelis concentration may also be used and has the advantage of emphasizing that the quantity concerned has the dimensions of a concentration and is not, in general, an equilibrium constant.
The Michaelis constant (or Michaelis concentration) is the substrate concentration at which $v = V/2$, and its usual unit is mol dm^{-3}, which may be written as mol l^{-1}. The term Michaelis constant and the symbol K_m should not be used when Michaelis-Menten kinetics are not obeyed.

Source: Adapted from [1]

4.2.16 molar catalytic activity
See *catalytic activity, in enzymology*

4.2.17 specific enzyme activity
See *catalytic activity, in enzymology*

4.3 Immunoanalytical Methods

4.3.1 adjuvant

Material introduced to an animal immunized with an *antigen* to augment its immunogenicity.

Source: [1]

4.3.2 affinity (in immunology)
binding affinity

Strength of association between a single *antibody* and a single *epitope* of *antigen*.

Note 1: Affinity of the immunochemical reaction:
Antibody + Antigen = Antibody-Antigen
is characterized by the affinity (binding, association) constant
K_A = [Antibody-Antigen]/[Antibody][Antigen]

Note 2: The average binding constant reflects a population of *antibody* molecules (polyclonal antibody).

Source: Adapted from [1]

4.3.3 agglutination

Immunospecific aggregation of particulate biological matter or synthetic particles.

Immunochemical specific reaction leading to the aggregation of particulate biological matter or synthetic particles.

Note 1: The biological matter can be bacteria, erythrocytes, or other cells, and the synthetic particles can be polymer beads coated with *antigens* or *antibodies*.

Note 2: Primarily, such aggregation is usually dependent on surface reactions mediated either by antigens or by antibodies. Physically or chemically they are attached to the particulate surfaces; agglutination of clumping of the particles follows as a secondary immune reaction.

Source: Adapted from [1] and [3] page 145

4.3.4 agglutination inhibition

A type of *agglutination*, in which particulate and soluble *antigen* compete for soluble *antibody*.

Note: Typically, it is soluble *antigen* in the test medium, which reacts first with the soluble antibody, inhibiting agglutination of indicator particles. With viral hemagglutination inhibition *assays*, host *antibodies* resulting from a specific infection are the most common forms of agglutination inhibition assays. In this case, viral-specific antibodies block the sites on the virus that agglutinate erythrocytes.

Source: [1]

4.3.5 antibody (Ab)

Protein produced by the immune system of an organism in response to exposure to a foreign molecule (*antigen*).

Note 1: These *proteins* are *immunoglobulins* and bind by means of specific binding sites to a specific antigenic determinant.

Note 2: An antibody molecule is, by definition, monospecific but might also be "idiospecific," "heterospecific," "polyspecific," or of "unwanted specificity." It cannot be "nonspecific" except in the sense of nonimmunochemical binding.

Source: Adapted from [1] and [3] page 146

4.3.6 antigen (Ag)

Substance that stimulates the immune system to produce a set of specific *antibodies* in a suitable host, and will combine with such generated antibodies through its antibody-binding sites (*antigenic determinants, epitopes*).

Source: Adapted from [1] and [3] page 146

4.3.7 antigenic determinant
epitope

Part of the structure of an *antigen* molecule that is responsible for specific interaction with *antibody* molecules evoked by the same or a similar *antigen*.

Source: [1]

4.3.8 antiserum

A *serum* containing *antibodies*.

Source: [1]

4.3.9 avidity

Net *affinity* of all binding sites of all *antibodies* in the *antiserum* under specified physicochemical reaction conditions.

Source: Adapted from [1]

4.3.10 blocking

Coating of an immunospecific surface (*serum*, *tissues*, cells, *biopolymers*) to form an active cover with selectivity or inert layer.

Note: Blocking is a pre-treatment method with blocking agents (e.g. monovalent Fab fragment, mono or divalent antibodies, organic ligands, metals).

4.3.11 binding capacity

Amount of a *ligand* that binds to a *receptor* expressed in operational units.

Note 1: Operational units are in contrast to the quantitative mass units of the *affinity* constant.

Note 2: Binding capacity is determined by saturating the receptor and then measuring the amount of complex forming ligand. Parameters like pH and ionic strength influence the capacity value. It will be changed depending on the experimental conditions.

Source: Adapted from [1]

4.3.12 bound/free ratio (in immunoassay) (B/F)

Amount ratio of bound to free-labelled analyte.

Source: Adapted from [1]

4.3.13 carrier protein

(1) *Protein* to which a specific *ligand* or *hapten* is conjugated.

(2) Unlabelled *protein* introduced into an *assay* at relatively high concentrations, which distributes in a fractionation process in the same manner as a labelled protein analyte, which is present in very low concentrations.

(3) *Protein* added to prevent nonspecific interaction of reagents with surfaces, sample components, and each other.

Source: [1]

4.3.14 complement (in immunology)

Array of serum *proteins* (some of which are *enzymes*) that become sequentially activated after the first member of the series is activated by either *antigen-antibody* complexes or microbial products.

Source: [1]

4.3.15 conjugate

Material produced by attaching two or more substances together.

Note: Conjugates of *antibody* with fluorochromes, radioactive isotopes, or enzymes are often used in immunoassays.

Source: [1]

4.3.16 cross reactivity (in immunology)

Reaction of an *antibody* with an *antigen* other than that which elicited the formation due to the presence of related *antigenic determinants*.

Note: Cross reactivity is due to non-*selectivity* of the antibody.

Source: Adapted from [1]

4.3.17 enzyme conjugate

Material or compound that forms a covalently-bonded *conjugate* with an *enzyme*.

Source: Adapted from [1]

4.3.18 epitope

See *antigenic determinant*

4.3.19 hapten

Low molecular weight molecule that contains an *antigenic determinant* but which is not itself antigenic unless combined with an antigenic carrier.

Note 1: The carrier may be one that also does not elicit an immune response by itself. It interacts with specific antibody-combining sites of an *antibody* molecule, but is not immunogenic by itself.

Source: Adapted from [1], [7]

4.3.20 hemagglutination

Agglutination reactions in which the particles used are *erythrocytes*.

Note: Hemagglutination may be either direct, in which erythrocyte antigens are reactants, or indirect (passive) for coated *antigen* or, in the case of reverse (passive) assays, coated *antibody*. One of the most common uses of hemagglutination is to quantitate the number of hemagglutinating viruses (cf. influenza) or their soluble hemagglutinating surface subunits. It can also be used for blood typing.

4.3.21 immune response

Selective reaction of the body to substances that are foreign to it, or that the immune system identifies as foreign, shown by the production of *antibodies* and antibody-bearing cells or by a cell-mediated hypersensitivity reaction.

Source: [5] page 2058

4.3.22 immunoassay

Ligand-binding *assay* that uses a specific *antigen* or *antibody* capable of binding to the analyte.

Note 1: The antibody can be linked to a radiosotope (radioimmunoassay, RIA), or to an enzyme which catalyses an easily monitored reaction (enzyme immunoassay, enzyme-linked immunosorbent assay, ELISA), or to a highly fluorescent compound by which the location of an antigen can be visualized (immunofluorescene, fluorescence immunoassay, chemiluminescent immunoassay).

Type of immunoassay	Ligand/label	Physical/chemical phenomenon observed
radioimmunoassay, RIA, immunoradiometric assay	radioactive nuclide	radioactivity
enzyme immunoassay, EIA, and	enzyme like horseradish peroxidase,	catalytic activity of a specific enzyme conjugate
enzyme-linked immunosorbent assay, ELISA (heterogeneous EIA when antigen or antibody is firmly attached to a solid support)	enzyme like horseradish peroxidase	catalytic activity of a specific enzyme conjugate
fluorescence immunoassay	fluorescent compound	fluorescence
chemiluminescent immunoassay	chemiluminescent system	chemiluminiscence
light-scattering immunoassay	none	changes in turbidity (*turbidimetry*) or light scattering (*nephelometry*)

Note 2: In general, immunoassays fall into two broad categories, competitive and non-competitive. In a competitive assay, analyte (antigen) in a sample competes with a constant amount of labelled analyte for a limited amount of antibody. For instance, radioligand assay as a type of radioimmunoassay in which unlabelled and radioactive-labelled molecules of the same analyte compete for a limited number of binding sites on a specific binding protein (an *antibody*, transport protein, hormone receptor in radioreceptor assay, or any other cell associated receptor or tissue componen).

A non-competitive immunoassay involves the capture of all the analyte in the sample by excess antibody usually immobilized on a solid phase.

Note 3: Sandwich immunoassay is immunoassay using chemical or immunochemical binding of *analyte* to a solid phase and the immunochemical binding of a second (typically labelled) reagent to the analyte. This is one of the most common assays in clinical practice.

Examples: Fluorescent excitation transfer immunoassay, fluorescence polarization immunoassay, solid-phase "dipstick" immunoassay, solid-phase microbead fluorescence immunoassay, substrate-labelled fluorescence immunoassay, fluorescence immunoassays using e.g. internal reflectance spectroscopy.

Source: Adapted from [1], [3] page 157, [15] page 2520 and [19] page 93

4.3.23 immunoblotting

Technique that uses *antibodies* (or other specific ligands in related techniques) to identify target *proteins* among a number of unrelated protein species.

Note: The techniques allow the detection of proteins from complex mixtures separated by gel chromatography and then transferred to a membrane. After

incubation of the attached product from membrane with labeled antibody, the target proteins become visible.

4.3.24 immunochemical specificity
specificity

The extent, to which the *immunoassay* responds only to (all subsets of) a specific analyte and not to other substances of similar structure that are present in the sample.

Source**:** Adapted from [5] page 2058

4.3.25 immunochemistry

Study of biochemical and molecular aspects of immunology, especially the nature of *antibodies*, *antigens*, and their interactions.

Source**:** Adapted from [5] page 2058

4.3.26 immunocomplex

Product of an *antigen-antibody* reaction that may also contain components of the complement system.

Note 1: Formation of immunocomplex is characterized by the binding constant

Source**:** Adapted from [5] page 2058
See: affinity

4.3.27 immunogen

Substance that elicits a cellular immune response and/or *antibody* production (cf. *antigen*).

Source: [1], [3] page 157

4.3.28 immunoglobulin (Ig)

Antibody which is a large Y-shape *protein* produced by plasma cells.

Note 1: As glycoprotein is found in serum or other body fluids and possesses antibody activity.
Note 2: An individual Ig molecule is built up from two light (L) and two heavy (H) polypeptide chains linked together by disulfide bonds. Igs are divided into five classes based on antigenic and structural differences in the H chains. It is used by the immune system to identify and neutralize foreign objects such as bacteria and viruses. It is a protein of the globulin-type structure which is found in serum or in other body fluids that possesses antibody activity.
Note 3: It denotes also family of closely related glycoproteins.

Source: Adapted from [1], [3] page 157 and [5] page 2058

4.3.29 immunoglobulin class

Classification of *immunoglobulins* based on antigenic and structural differences of the heavy peptide chain.

Note 1: There are five classes: IgG, IgA, IgM, IgD, and IgE.
Note 2: Immunoglobulin subclass (isotype) represents the subdivision of an *immunoglobulin class* based on structural and antigenic differences in the heavy peptide chain. Four human IgG subclasses and two IgA subclasses

have currently been recognized; IgM subclasses have been postulated; IgD and IgE subclasses are unknown.

Source: Adapted from [1]

4.3.30 immunoprecipitation

Isolation and concentration of an *antigen* by forming a precipitate with an *antibody*.

Note: Immunoprecipitation requires that the antibody be coupled to a solid substrate at some point in the procedure.

Source: Adapted from [1]

4.3.31 immunosensor

Biosensor where one of the immunointeracting partners is immobilized on the surface of a physico-chemical *transducer*.

Note: The generated signal either directly reflects the amount of formed *immunocomplex*, or corresponds to the amount of *label*.

4.3.32 indirect agglutination
passive agglutination

Agglutination in which *antigen* is first immobilized artificially onto the particulate surfaces, either by physical adsorption or by chemical and/or immunochemical linkers.

Note: Antigen-loaded particles can be used to detect the presence of the corresponding specific agglutinins in sample. Agglutination results by cross-linking of the antigen-bearing particles onto an extensive antigen-antibody lattice (i.e., in detectable agglutination of the particles).

4.3.33 lateral flow test

Migration of the sample zone driven by capillary forces in a porous carrier strip.

Note 1: During migration the dissolved tracer becomes distributed between capture and control zones with immobilized *antibodies*.

Note 2: Amount of the label compound in both zones depends on the concentration of the compound in the studied sample.

Example: Most common test is the one for pregnancy.

4.3.34 monoclonal

Arising from a single clone of cells.

Note 1: In the case of immunoglobulin ‘monoclonal’ refers to its origin.

Note 2: Usually, the monoclonal *antibody* is of a single immunoglobulin class containing only one light chain type of either the K or L variety. It also refers to all antibody molecules having identical physical-chemical characteristics and antibody specificity. Monoclonal antibodies have very restricted structural diversity and they are homogeneous compared with polyclonal antibodies.

Source: Adapted from [1].

4.3.35 monospecificity

Immunoreactivity of an antiserum with its designated antigen

Note: In practice, true monospecificity to naturally occurring antigens does not occur in antisera produced by the immunization of the intact animal. An attempt is made to reduce the level of unwanted specificities below that which will interfere with the intended use of a particular immunochemical test.

Example: Reaction of an antiserum with antihuman IgG.

Source: [1]

4.3.36 polyclonal

Arising from multiple, different clones.

Note: Polyclonal *antibodies* (pAbs) are antibodies that are secreted by different B cell lineages within the body. They are a collection of immunoglobulin molecules that react against a specific antigen, each identifying a different epitope.

Example: A typical antiserum obtained from a conventional immunization is polyclonal.

Source: Adapted from [1]

4.3.37 reverse passive agglutination

Modification of the indirect agglutination assay, where the particles are coated with *antibody*.

Note: These antibody laden particles become probes for detecting specific antigens in the sample material. Presence of the relevant *antigen* will result in *agglutination* of particles.

4.3.38 sandwich immunoassay
two-site, noncompetitive immunoassay

Immunoassay using chemical or immunochemical binding of *analyte* to a solid phase carying *antibody* and the immunochemical binding of a second (labelled) reagent to the analyte.

Note: This is one of the most common assays in clinical practice.

Source: Adapted from [1]

4.4 Genomics and Nucleic Acid Analysis

4.4.1 amplification of DNA

Increasing the number of copies of a specific *DNA sequence*.

4.4.2 aptamer

See *nucleic acid aptamer*

4.4.3 base pair (bp)

Pair of two *nucleobases* consisting of one purine and one pyrimidine base, held together by hydrogen bonds.

Note: In *DNA*, guanine typically pairs with cytosine and adenine typically pairs with thymine. In *RNA* adenine typically pairs with uracil.

4.4.4 base sequence

See *nucleotide sequence*

4.4.5 base sequence analysis

Method for determining the *nucleotide sequence*.

4.4.6 basic local alignment search tool (BLAST)

Computer algorithm that finds regions of local similarity between DNA or protein sequences.

4.4.7 chromosome

Organized, defined structure of *DNA* and *proteins* found in cells that in its *nucleotide sequence* bears the linear array of genes.

4.4.8 clone

Exact copy of biological material.

Example: The biological material can be *DNA* segment, whole cell, complete organism.

4.4.9 cloning

Specialized *DNA* technology for production of multiple, exact copies of a single gene or another segment of DNA.

4.4.10 cloning vector

DNA molecule into which another DNA fragment of appropriate size can be integrated without loss of its capacity for self-replication.

4.4.11 complementary DNA (cDNA)

Artificial *DNA* that is synthesized in the laboratory from a messenger RNA template.

4.4.12 complementary sequence

Nucleotide sequence that can form a double-stranded structure with another *DNA sequence* by following base-pairing (see *base pair*) rules.

4.4.13 complete genome sequence

High-quality, low error, gap-free *DNA sequence* of an entire *genome* of an organism.

4.4.14 damage to DNA
damage

Alteration in the *DNA* chemical structure resulting from interactions with physical or chemical agents occurring in the environment, generated in the organisms as by-products of metabolism or used as therapeutics.

Note 1: The main types of DNA damage include interruptions of the sugar-phosphate backbone (strand breaks), release of bases due to hydrolysis of N-glycosidic bonds (resulting in abasic sites), inter- and intra-strand crosslinks (such as pyrimidine dimers) and a variety of nucleobase lesions (adducts) resulting from reactions of DNA with a broad range of oxidants, alkylating compounds and other agents.

Note 2: Terms "(product of) DNA damage" (lesion, adduct) and "mutation" should not be intermingled. Mutations refer to hereditable changes in DNA sequence—substitutions, deletions, or insertions of (one or more) standard base pair(s) and are not synonyms to "base mismatches". Importantly, DNA damage and its repair can promote the occurrence of mutations.

Source: [11]

4.4.15 deletion

Loss of part (one or more base pairs) of *DNA*.

4.4.16 deoxyribonucleotide

See *nucleotide*

4.4.17 deoxyribose

Type of pentose sugar that is incorporated in the structure of *DNA*.

4.4.18 deoxyribonucleic acid (DNA)

High molecular weight linear polymer composed of *nucleotides* containing *deoxyribose* and linked by phosphodiester bonds. DNA contains the genetic information of organisms.

Note: DNA is a polyanionic biopolymer consisting of chains of nucleotides linked with phosphate bridges (phosphodiester bonds) at the 3‘ and 5‘ positions of neighboring sugar (2-deoxyribose) units (single stranded, ssDNA). Complementary base pairing results in the specific association of two polynucleotide chains that wind around a common helical axis to form a *double helix* (double stranded DNA, dsDNA). Supercoiled DNA represents a contortion of circular DNA into more complex, conformations in which the DNA helices fold around each other. DNA supercoiling is important to assist with DNA packaging within all cells.

Source: Adapted from [3]

4.4.19 DNA fragmentation

Breaking and/or separation of large *DNA* molecules into smaller pieces (fragments).

Note 1: DNA fragmentation is usually achieved via cleavage with restriction *endonucleases* at specific sites or non-specifically by e.g., sonication.

Note 2: DNA fragmentation is also a consequence of DNA *damage*.

4.4.20 DNA fragmentation index (DFI)

Fraction of *DNA* having fragmented DNA.

Example: Results of sperm DNA damage are generally reported as the percentage of sperm having fragmented DNA, the so called sperm DNA fragmentation index.

4.4.21 DNA integrity index

Ratio of longer fragments to shorter *DNA*.

Note 1: Integrity index may be clinically useful used as surrogate for detection of tumors.

Note 2: DNA strand integrity is measured by *quantitative PCR* (QPCR) using a real-time system.

4.4.22 DNA mismatch

Defect in the double stranded *DNA* structure in which the DNA *double helix* contains one (or more) non-standard *base pairs*.

Note: One mismatch is known as a single mismatch. Multiple mismatches are called multi-base mismatches.

Example: Guanine paired with thymine instead of cytosine, or adenine with cytosine instead of thymine.

4.4.23 DNA profiling

Identification of individuals by matching characteristics of their *genome DNA sequence*.

Note 1: *DNA* profiles are at a lower resolution than a full *genome DNA sequence*, but this is adequate for forensic purposes such as in parental testing and criminal investigations.

Note 2: Characteristics used include the frequency (or size) of tandem repeats in the *genome*.

4.4.24 DNA replication

Synthesis of new *DNA* strands using existing DNA as a template.

4.4.25 DNA sequencing

Determination of nucleotide sequence.

Note: Next-generation sequencing refers to non-Sanger-based high-throughput DNA sequencing technologies where millions of DNA strands can be sequenced in parallel.

4.4.26 double helix

The twisted shape of two complementary *DNA* strands.

Note 1: The complementary chains are held together by hydrogen bonds between pairs of the complementary *nucleobases*. The helical conformation is promoted by several relatively weak interactions, including hydrophobic (stacking) ones between the bases.

Note 2: The double helix is most frequent DNA structure formed of two complementary DNA strands. It can also be formed of two complementary RNA strands, one DNA and one RNA strand as well of any of them with synthetic nucleic acids analogues like locked nucleic acid, LNA, or *peptide nucleic acid*, PNA.

4.4.27 endonuclease

Enzyme that cleaves nucleic acids by hydrolysing phosphodiester bonds between nucleotides within a nucleic acid molecule.

4.4.28 exogenous DNA

DNA molecule of foreign origin that has been introduced into an organism.

4.4.29 exonuclease

Enzyme that cleaves nucleotides from free ends of a linear nucleic acid molecule.

4.4.30 gene

Molecular unit of heredity of a living organism that encodes a specific feature, usually a *protein*.

Note 1: The gene includes, however, regions preceding and following the coding region (leader and trailer) as well as (in eukarvotes) intervening sequences (introns) between individual coding segments (exons).

Note 2: Functionally, the gene is defined by the cis-trans test that determines whether independent mutations of the same phenotwe occur within a single gene or in several genes involved in the same function.

Source: [3]

4.4.31 gene expression

Process for converting a gene's coded information into molecules present and operating in the cell.

4.4.32 genome

Complete set of chromosomal and extrachromosomal *genes* of an organism, a cell, an organelle or a virus; the complete *DNA* component of an organism.

Source: [3] page 155 and [7]

4.4.33 genomics

Complex and comprehensive study of *genome* structure and function.

Note 1: Also specifically refers to the science of using DNA- and RNA-based technologies to demonstrate alterations in gene expression.

Note 2: Functional genomics represents a branch of methods to study the sequencing data for description of the gene functions and interactions.

Source: Adapted from [17] page 1053 and [8].

4.4.34 genotype

Genetic constitution of an organism as revealed by genetic or molecular analysis.

Source: [3] page 155

4.4.35 insertion

Incorporation of a *nucleotide sequence* of DNA into a *genome*.

4.4.36 multi-base mismatch

See *DNA mismatch*

4.4.37 mutagen

Agent that causes a permanent genetic change in a cell.

Source: [7]

4.4.38 mutation

Alterations in the genomic *nucleotide sequence*, such as substitutions of single *base pairs*, insertions or deletions of base pairs, or longer i stretches.

Source: [11]

4.4.39 Northern blot

Technique involving nucleic acids *hybridization* used to study *gene expression* at the level of RNA.

Note: The Northern blot approach is opposed to *Southern* (and other) blots.

4.4.40 nucleic acid

Large biomolecule composed of *nucleotide* subunits.

Note: The major organic matter of the nuclei of biological cell. It can be hydrolysed into certain *pyrimidine* or *purine* bases (usually adenine, cytosine, guanine, thymine, and uracil), d-ribose or 2-deoxy-d-ribose and phosphoric acid.

Examples: Ribonucleic acid and deoxyribonucleic acid.

Source: Adapted from [3], [20] page 1352 and [7].

4.4.41 nucleic acid aptamers

Single-stranded (ss) *oligonucleotides* (mainly *DNA* or *RNA*) originating from in vitro selection, which, starting from random sequence libraries, optimize the nucleic acids for high affinity binding to a given target.

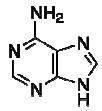
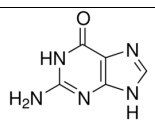
Note 1: In vitro selection is an iterative method mainly known as Systematic Evolution of Ligands by EXponential enrichment (SELEX).

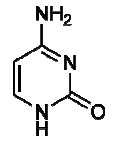
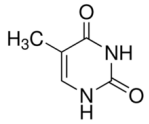
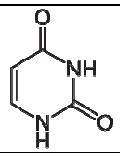
Source: [11]

4.4.42 nucleobase

Purine or *pyrimidine* nitrogenous base occurring in *DNA* or *RNA*.

Example: Purine and pyrimidine bases of ribo- and deoxyribo- *nucleotides*.

Purine bases	
Adenine (A)	
Guanine (G)	
Pyrimidine bases	

Cytosine (C)	
Thymine) (T)	
Uracil (U)[a)]	

a) Uracil is normally found in RNA but not in DNA.

4.4.43 nucleobase lesion

Chemical modification of nucleobase.

Example: Product of nucleobase oxidation, alkylation, etc.

4.4.44 nucleoside

Compound in which a *purine* or *pyrimidine* base is bound via a N-atom to C-1 replacing the hydroxy group of either 2-deoxy-~-ribose or D-ribose, but without any phosphate groups attached to the sugar.

Note: The common nucleosides in biological systems are adenosine, guanosine, cytidine, and uridine (which contain ribose) and deoxyadenosine, deoxyguanosine, deoxycytidine and thymidine (which contain deoxyribose).

Source: Adapted from [20] page 1352, and [7].

4.4.45 nucleotide

Monomer subunit of DNA or RNA consisting of a nitrogenous *nucleobase* (*purine* or *pyrimidine*) linked to a pentose sugar (2-deoxyribose or D-ribose) to which at least one phosphate group is attached.

Note 1: Compounds formally obtained by esterification of the 3- or 5-hydroxy group of nucleosides with phosphoric acid.

Note 2: Nucleotides are the monomers of nucleic acids.

Note 3: Nucleotides can be obtained from nucleic acids by *hydrolytic cleavage*.

Source: Adapted from [3] and [7].

4.4.46 nucleotide sequence

Order of *nucleotides* in a *DNA* or *RNA* molecule.

4.4.47 oligonucleotide

Short nucleic acid polymer, typically consisting up to 50 bases resulting from a linear sequence of *nucleotides*.

Source: [7].

4.4.48 **open reading frame (ORF)**

Sequence of *DNA* or *RNA nucleotides* located between the start-code sequence (initiation codon) and the stop-code sequence (termination codon), which is transcribed and translated to generate a *protein*.

4.4.49 peptide nucleic acid (PNA)

Synthetic *DNA* mimic, which contains 2-aminoethylglycine linkages instead of the negatively charged phosphodiesteric backbone of oligonucleotides.

Note: The PNA probes are particularly convenient for the detection of single-base *mismatches* (*point mutations*, SNPs) because the stability of DNA-PNA duplexes is strongly influenced by a single-base *mismatch*.

Source: [11]

4.4.50 plasmid

Autonomously replicating extra-chromosomal circular *DNA* molecule.

Source: Adapted from [3]

4.4.51 polymerase chain reaction (PCR)

Laboratory technique for rapid amplification and pre-determination of regions of double-stranded *DNA* using DNA *polymerase.*

See *quantitative PCR*, *real-time PCR*

Source: [18] page 1293

4.4.52 polymerase, DNA or RNA

Enzyme that catalyzes the synthesis of nucleic acids on preexisting *nucleic acid* templates.

4.4.53 primer

Short preexisting polynucleotide chain attached to a complementary stretch in template *DNA*, to which new deoxyribonucleotides can be added by *DNA polymerase*.

4.4.54 probe, in genomics

Single-stranded DNA or RNA molecule used to determine the *nucleotide sequence*.

Note: Typically a probe is either immobilized at a surface to capture the complentary target sequence from a sample (*capture probe*) or labeled (radioactively, immunologically, by fluorescent, electroactive or other species to produce a specific signal upon hybridization – *signalling or reporter probe*).

4.4.55 purine

Nitrogen-containing, double-ring, basic compound that occurs in nucleic acids.

See *nucleobase*

4.4.56 pyrimidine

Nitrogen-containing, single-ring, basic compound that occurs in nucleic acids.

See *nucleobase*

4.4.57 quantitative PCR (qPCR)

Polymerase chain reaction to quantify target nucleotide sequences of interest.

4.4.58 real-time PCR

Polymerase chain reaction where data are collected and monitored in real-time as the reaction proceeds.

Note: Digital PCR is a new alternate method to conventional real-time PCR and *quantitative PCR* for absolute quantification and rare allele detection. Digital PCR works by partitioning a sample of DNA or cDNA into many individual, parallel PCR reactions; some of these reactions contain the target molecule (positive) while others do not (negative). A single molecule can be amplified a million-fold or more.

4.4.59 recombinant DNA

DNA molecules joined together using *recombinant DNA technology*.

4.4.60 recombinant DNA technology

Procedure used to join together different *DNA* segments (sequences).

Note: Recombinant DNA technology can include integration of DNA into the genome of a cell.

4.4.61 recombination

Process by which genetic material is broken and joined to other genetic material, which can leading to rearrangement of genetic sequences.

4.4.62 replication

Process by which *DNA polymerase* generates two identical copies of *DNA* from one original DNA molecule.

Note: Different processes of replication have been identified, though cells typically use semi-conservative replication in which each strand of the original DNA molecule serves as a template for the production of a new strand containing a complementary *DNA sequence*.

4.4.63 restriction enzyme
restriction endonuclease

Protein that recognizes specific, short *nucleotide sequences* (restriction site) and hydrolyzes the DNA backbone at the sequences, generating one (or more) fragments from the DNA molecule.

4.4.64 restriction fragment length polymorphism (RFLP)

Variation between individuals in DNA fragment sizes cut by specific *restriction enzymes*.

4.4.65 reverse transcriptase

Enzyme used to form a complementary DNA sequence (cDNA) from a RNA template.

4.4.66 ribonucleotide
See nucleotide

4.4.67 ribosomal RNA (rRNA)

Single-stranded molecule found in the catalytic core of the ribosome.

4.4.68 ribosomes

Small cellular components performing protein synthesis, composed of specialized ribosomal RNA and protein.

4.4.69 ribonucleic acid (RNA)

High molecular weight linear polymers composed of *nucleotides* containing ribose as the sugar and linked by phosphodiester bonds.

Note 1: RNA molecules play important roles in protein synthesis and other chemical activities of the cell.

Note 2: Classes of RNA molecules include messenger RNA (mRNA), transfer RNA (tRNA), ribosomal RNA (rRNA), and other types of non-coding RNAs with diverse regulatory functions.

See *nucleic acid*

4.4.70 Sanger sequencing

Method used for determining *nucleotide sequence* of DNA using dideoxy-nucleotide termination.

4.4.71 Shotgun method

Method used for determining the order of bases in long DNA using sequencing of DNA broken up randomly into numerous small segments.

4.4.72 single mismatch
See *DNA mismatch*

4.4.73 single nucleotide polymorphisms (SNPs)
point mutations

Variant of *nucleotide sequence* of DNA in which the *purine* or *pyrimidine* base of a single *nucleotide* is replaced by another such base.

Note 1: SNP is the most common type of change in DNA.

Note 2: SNPs occur normally throughout a human DNA once in every 300 nucleotides on average, which means there are roughly 10 million SNPs in any typical human genome. They can act as biological (diagnostic) markers.

4.4.74 Southern blot

Technique based on DNA *hybridization* used for the detection of a DNA fragment of specific *nucleotide sequence*.

4.4.75 strand break

Interruption of the sugar-phosphate backbone of a polynucleotide chain.

4.4.76 tandem repeat sequences

Multiple consecutive copies of the same *nucleotide sequence* on a *chromosome*.

Note: Tandem repeat sequences can be used as markers in physical mapping e.g. by *DNA profiling*.

4.4.77 telomerase

Enzyme that directs the replication of *telomeres*.

4.4.78 telomere

End cap of a *chromosome* involved in the replication and stability of linear *DNA* molecules.

4.4.79 yeast artificial chromosome (YAC)

Vector used to clone large *DNA* fragments.

See cloning vector

General reference:
[21] page 592, [22-26]

4.5 Proteomics

4.5.1 alternative splicing

Process occurring during gene expression, which allows the generation of multiple protein molecules from one gene. It generally involves processing of mRNA via elimination of introns.

Source: [21] page 69

4.5.2 bottom-up proteomics

Approach to identify *proteins*, characterise their amino acid sequences or post-translational modifications by sequential combination of *proteolysis* (*enzymatic proteolysis* and *chemical proteolysis*) prior to mass spectrometric analysis of the resulted *proteolytic peptides*.

Note: In shotgun proteomics the proteins in a mixture are digested, usually by a specific enzyme, and the resulting peptides are separated by liquid chromatography and identified by tandem mass spectrometry.

Source: [27], [28], [31] page 1526.

See *top-down proteomics*

4.5.3 C-terminal analysis

Chemical method for C-terminal sequencing of proteins and peptides starting at their C-terminus.

Source: [29] page 344, [30] page 103. See *C-terminus*

4.5.4 C-terminus

The end of an amino acid chain (*protein* or polypeptide), terminated by a carboxylic group.

Source: [30] page 71

4.5.5 collision-induced dissociation (CID) (in proteomics)

Ion fragmentation technique in mass spectrometry driven proteomics used to generate peptide fragment ions from proteins in peptide sequencing by tandem mass spectrometry.

Source: [31] page 1531

4.5.6 database searching (in proteomics)

Bioinformatics analysis of proteomic mass spectrometric data used in order to identify protein or its post-translational modification. It results in a list of candidates presented mostly in order of decreasing statistical significance of the hit.

4.5.7 difference gel electrophoresis (DIGE) (in proteomics)

Mode of *two-dimensional gel electrophoresis* used to study protein expression regulation by differential staining of multiple gels, representing different states of a *proteome*, preferably with fluorescent dyes (e.g. Cy3, Cy5, Cy2).

Note: The resulting stained gels are analysed using 2D image analysis.

Source: [32].

4.5.8 differential proteomics

See *expression proteomics*

4.5.9 disulphide bond cleavage

Chemical reduction of protein disulphide bonds.

Note: Reductants, such as tributylphosphine (TBP), 2-mercaptoethanol (BME) or dithiothreitol (DTT) are used.

Source: [30] page 101

4.5.10 Edman degradation

Method of N-terminal analysis in which the N-terminal amino acid residue is labelled and cleaved from the peptide without affecting other peptide bonds in the peptide chain.

Source: [33].

4.5.11 electron-transfer dissociation (ETD) (in proteomics)

Ion fragmentation technique in mass spectrometry driven proteomics based on transfer of low energy electrons to the molecules selected for fragmentation.

Note: ETD is particularly effective for analysis of post-translational modification.

Source: [31] page 1541

4.5.12 electron-transfer / higher-energy collision dissociation (EthcD) (in proteomics)

Ion fragmentation technique in mass spectrometry driven proteomics, which is a combination of electron-transfer dissociation (ETD) and higher-energy collision dissociation (HCD) in orbitrap instruments.

Note: The dual fragmentation allows generation of rich MS/MS spectra for peptide sequencing and PTM analysis.

Source: [34]

4.5.13 electrospray ionisation (ESI) (in proteomics)

Soft ionisation technique in mass spectrometry driven proteomics.

Note: Electrospray ionisation is optimal to ionise liquid eluates from separation methods as well as for direct introduction of liquid samples to fragmentation in MS. Ionization is made by positive or negative mode depending on the ability of the molecules to form cations or anions. During the ionisation process a *biomacromolecule* obtains a number of charges roughly proportional to its molecular mass. Also, non-covalent protein complexes may be ionized with ESI.

Source: [31] page 1541

4.5.14 expression proteomics

Field of study in proteomics, which deals with determining changes of the protein expression and regulation in response to different stimuli, internal or external. It includes also analysis of post-translational modifications (e.g. phosphorylation, glycosylation, *etc.*), their identification and localisation.

Source: [35]

4.5.15 functional proteomics

Field of study in *proteomics* studying protein interactions on different levels: protein-protein interactions (*interactomics*), formation of protein complexes, and interaction of proteins with other *biomolecules*.

Source: [36]

4.5.16 gel electrophoresis (1D PAGE) (in proteomics)

Separation technique used to separate or fractionate proteins and larger peptides according to their relative molecular mass using electric field in polyacrylamide gel (PAGE).

Note 1: 1D PAGE has two basic modes: denaturing (or SDS) PAGE to estimate molecular masses and a native PAGE to separate proteins as their native structures.

Source: [21] page 592

4.5.17 higher-energy collision dissociation (HCD) (in proteomics)

Ion fragmentation technique in mass spectrometry driven proteomics which is used mostly at orbitrap instruments. It does not have low mass cut-off of collision-induced dissociation (CID), and therefore it is used for tandem mass tag of stable isotope labels.

Source: [37]

4.5.18 isoelectric focusing (IEF) (in proteomics)

Separation technique used to separate or fractionate proteins and peptides in a gel (or similar type of media) according to their isoelectric point.

Note: IEF is typically the first dimension of two dimensional *gel electrophoresis*.

Source: [38]

4.5.19 mass fingerprinting (in proteomics)

peptide mass fingerprinting

mass mapping

Protein analysis where an unknown protein is chemically or enzymatically cleaved into peptide fragments whose masses are determined by mass spectrometry. The peptide masses are compared to peptide masses calculated for known proteins in a database and analyzed statistically to determine the best match.

Source: [31] page 1576

4.5.20 MS/MS data based identification (in proteomics)

Mass spectrometric method used for protein/peptide identification based on *protein sequencing*.

Note: Using peptide ion fragments, peptide sequence is read and in combination with *database searching* a list of hit candidates is obtained.

Source: [39]

4.5.21 multi-dimensional protein identification technology (MudPIT)

Non-gel step-wise multi-dimensional technique for separating and identifying individual components of complex protein and peptide mixtures. It combines fractionation of proteolytic peptides in steps on a strong cation exchanger (SAX) using ion exchange chromatography (IEC); each step uses higher elution force. Then, each fraction is separated on reversed phase liquid chromatography and analysed using mass spectrometry.

Source: [31] page 1570

4.5.22 N-terminal analysis

Chemical method for N-terminal sequencing of proteins and peptides starting at their N-terminus (See *N-terminus* and *Edman degradation*).

Source: [40], [30] page 102

4.5.23 N-terminus

The end of an amino acid chain (protein or polypeptide), terminated by a free primary amine group.

Source: [30] page 71

4.5.24 off-gel isoelectric focusing (OFFGEL™)

Separation technique used to fractionate proteins and peptides into discrete liquid fractions according to their isoelectric point.

Source: [41]

4.5.25 off peptide product ions

Protein/peptide sequencing approach using overlapping complementary information on sequence acquired using proteolysis in bottom-up proteomic analysis by at least two different proteolytic approaches.

Source: [13]

4.5.26 peptide

Amides derived from two or more amino carboxylic acid molecules (the same or different) by formation of a covalent bond from the carbonyl carbon of one to the nitrogen atom of another with formal loss of water (peptide bond).

Note 1: The term is usually applied to structures formed from α-amino acids, but it includes those derived from any amino carboxylic acid.

Note 2: Usually, when the number is higher than ten, the peptide is expressed using the term *polypeptide*. An oligopeptide consists of between 2 and 20 amino acids.

Source: [20] page 1356

4.5.27 peptide *de novo* sequencing

Analytical process that derives an amino acid sequence of a peptide from its tandem mass spectrum without the assistance of a sequence database.

Note: It is in contrast to protein/peptide identification using a database search. An advantage of *de novo* sequencing is its applicability to both, known and novel peptides from organisms with unsequenced genome.

Source: [42]

4.5.28 peptide fragment ion

Product ion which originates in a *peptide precursor ion* fragmentation process. They are generated in pairs in regard to what kind of mechanism dissociates the chemical bonds around the peptide bond.

Source: [43] page 243

4.5.29 peptide fragmentation technique

Mass spectrometric technique, which serves to generate peptide fragment ions through fragmentation of precursor ion via different physico-chemical processes in an ion source to a mass analyser, such as collision induced dissociation, electron transfer dissociation, etc.

Source: [43] pages 251-258

4.5.30 peptide precursor ion

Peptide ion, generated during ionisation of peptides in an ion source or during *tandem mass spectrometry* of an intact protein in *top-down proteomic* analysis, selected for further analysis by means of tandem mass spectrometry.

Note: Once generated a peptide fragment ions may become a precursor ion for tandem mass spectrometry of higher orders (MS^n , n=3).

Source [43] page 243

4.5.31 peptidomics

Study of the set of *peptides* in an organism, its peptidome.

Note: It uses similar approaches and methods as *proteomics*.

Source: [44]

4.5.32 phenotype

Set of observable organismal characteristics.

Note 1: The characteristics can be morphology, development, biochemical or physiological properties, or behaviour.

Note 2: Diversity of the phenotype is given by a combination of the gene expression, social and environmental factors. *Proteome* plays an important role in transformation of *genotype* into phenotype.

Source: [21] page 1477

4.5.33 polypeptide

Peptide containing ten or more amino acid residues.

Source: [24] page 1359

4.5.34 primary protein structure

Ordered amino acid sequence of the protein (polypeptide).

Source: [21] page 1579

4.5.35 protein

Naturally occurring and synthetic *polypeptide* having a molecular weight greater than about 10000.

Source: [20] page 1361

4.5.36 protein complex

Ensemble of two or more associated proteins linked by non-covalent protein-protein interactions with a specific function.

Note: Protein complexes are required for most biological processes. Together they form molecular machineries that perform biological functions.

Example Anaphase promoting complex.

Source: [21] page 1572

4.5.37 protein complex purification

Set of methods serving to purify the intact *protein complex* for further analysis.

Note: The methods used may employ single step affinity purification or tandem affinity purification (two affinity purification steps). These approaches include direct application of an epitope on a bait protein for immunopurification or genetic insertion of affinity tag to bait protein, both used to co-purify the prey components of the protein complex.

Source: [43] page 273

4.5.38 protein database

Collection of *protein sequences* from several sources, including translations from annotated genetic coding regions, as well as original protein and peptide sequences.

Note: A protein database is fundamental for determination of protein structure and function via bioinformatics.

Source: [30] page 109

4.5.39 protein folding

Physico-chemical process by which a polypeptide folds into its characteristic and functional three-dimensional structure from random coil.

Source: [21] page 1574

4.5.40 protein fractionation
protein separation

Methodical approach to simplify a complex mixture of proteomic sample.

Note: Target compounds can be either separated into individual protein/peptides or fractionated into groups with similar physico-chemical properties (polarity, charge, molecular mass, *etc*.). Different analytical separation methods are deployed to achieve it – liquid chromatography, gel electrophoresis, isoelectric focusing, affinity chromatography, etc.

Source: [38]

4.5.41 protein fragmentation

Process which happens during *tandem mass spectrometry* of intact proteins.

Note: This process is used in *top-down proteomics*. It generates specific *peptide fragment ions* from a selected protein precursor ion.

Source: [43] page 242

4.5.42 protein identification

Analytical approaches leading to the identity of a *protein*.

Note: Identity of the protein is given in respect to the proteoform by its primary structure, post-translational modification or tertiary structure.

Source: [45] page 14

4.5.43 protein machine

Supramolecular interacting system such as metabolic circuit, intracellular signal transduction, or cell-to-cell communication.

Note: These systems are operated under process control strategies involving integrated feedback control. The input and output of the circuits or modules are coordinated to assure the normal or adaptive function of the cell or organism.

Source: [46]

4.5.44 protein primary structure

Sequence of the *amino acids* in the *polypeptide* chain.

Note : The primary structure of a protein is crucial information for further proteomics analyses.

Source: [21] page 1579

4.5.45 protein purification

Series of processes conducted to isolate either the whole proteome or a subset from a biological sample.

Note: Protein purification involves many different methods like chromatography, immunopurification, precipitation, etc.

Source: [38]

4.5.46 protein quantitation

Analytical methods and related techniques to measure the *protein* content.

Note 1: Protein quantitation can be further divided into relative quantitation and absolute quantitation. Basic analytical methods used in proteomics to quantitate protein content are immunoanalysis (see *Western blotting*), mass spectrometry or densitometry of differential staining. Especially mass spectrometry offers many techniques based either on stable isotope labelling or label-free approaches.

Note 2: Absolute quantitation of proteins (AQUA) is protein quantitation via measurements on constituent proteolytic peptides using chemically synthesized isotope-labeled peptides as surrogate internal standards for mass spectrometry.

Source: [47, 31 page 1519]

4.5.47 protein sequencer

Equipment designed for the automatic determination of amino acid sequence of peptides using a method of N-terminal analysis.

See *Edman degradation*.

4.5.48 protein sequencing

Determination of protein primary structure.

Source: [38]

4.5.49 protein staining

Technique used to visualise *proteins* separated on electrophoretic slab gels.

Note: Typically, three types of staining are commonly used: – silver staining, highly sensitive and non-quantitative; Coommassie Brilliant Blue staining, quantitative and less sensitive than silver staining; fluorescent dye staining, highly sensitive and quantitative.

Source: [9] page 832.

4.5.50 proteoform

All of the different molecular forms in which the protein product of a single *gene* can be found, including changes due to genetic variations, alternatively spliced RNA transcripts and post-translational modifications.

Source: [48]

4.5.51 proteogenomics

Field of biological research in-between *proteomics* and *genomics* using proteomic information (mostly derived from mass spectrometry) to improve *gene* annotations.

Source: [49]

4.5.52 proteolysis

Protein cleavage to generate *proteolytic peptides* for *bottom-up proteomic* analysis, either enzymatic or chemical.

Note: Both, enzymatic and chemical proteolysis may be sequence specific or unspecific. Proteolysis is specific, where cleavage appears at few specific sequence motives (e.g. proteolysis by trypsin), or unspecific, being connected to wide range of sequence motives (e.g. proteolysis by chymotrypsin).

Source: [21] page 1586, [30] page 103, [50] pages 78-86

4.5.53 proteolytic digestion

Exposure of a sample to proteolytic reactions.

See *proteolysis*

4.5.54 proteolytic enzyme

Group of enzymes that cleave the polypeptide into shorter oligopeptides (peptides) and amino acids.

Source: [21] page 1570

4.5.55 proteolytic peptide

Peptide generated via proteolysis.

Source: [43] page 144

4.5.56 proteome

Set of all proteins and their modifications in an organismal compartment (organelle, cell, tissue or organism).

Note 1: The proteome is an analogue of the *genome*.
Note 2: Proteome may be viewed as an actual proteome, a set given by the actual level of protein expression (depending on conditions of growth), or as an overall proteome, a set of all potentially possible proteins expressed by a given genome.

Source: [21] page 1586

4.5.57 proteomics

Complex and comprehensive study of *proteomes*, particularly *proteins*, the way they are expressed, modified, involved in metabolism and of their interactions.

Source: [21] page 1587

4.5.58 quaternary protein structure

Interconnections and organisation of multiple polypeptide chains including any ligands such as cofactors, pigments, etc.

Source: [21] page 1579

4.5.59 relative quantification (in proteomics)

Type of protein/peptide quantitation, where relative quantities of one protein in different samples is determined, i.e. ratio of the content of a protein/peptide in sample 1 to sample 2.

Source: [47]page 184

4.5.60 secondary protein structure

Regular sub-structures favoured by specific groups of amino acid sequences; examples include α-helix and β-sheet.

Source: [21] page 1579

4.5.61 stable isotope labeling (in proteomics)

Techniques which serve to quantify *protein* content by means of mass spectrometry. The principle lies in introduction of a mass tag to internal standard protein/peptide containing different stable isotopes than protein/peptide in sample. Mass resolved signals originated in internal standard and sample are read individually and evaluated.

Note: In the case of complex sample, use of multiple reaction monitoring allows resolution of multiple overlapping signals.

Source: [51]

4.5.62 structural proteomics

Field in *proteomics* which focuses on higher orders of *protein* structure, mainly tertiary or quaternary.

Source: [52]

4.5.63 targeted proteomics

Approach in *proteomics*, which is focused on selected *proteins* of a sample.

Note: It is reflected in sample separation, mass spectrometer tuning and acquisition methods to achieve the highest sensitivity and throughput for hundreds or thousands of samples in regard to the intended aspect (namely amount).

Source: [53]

4.5.64 tertiary protein structure

Three-dimensional fold/organisation of one polypeptide chain, containing specific arrangements of secondary structures.

Source: [21] page 1579

4.5.65 top-down proteomics

Approach to identify proteins, characterise their amino acid sequences or post-translational modifications either by direct mass spectrometric analysis of an ionised

protein molecule, or by sequential combination of protein/peptide fragmentation in the ion source or mass analyser with ion trapping mass spectrometers.

Source: [27, 54, 31 page 1596].

4.5.66 trypsin (in proteomics)

Serine protease with highly specific primary endoproteolytic activity which serves to generate proteolytic peptides in *bottom-up proteomics* analysis.

Note: It may be modified by reductive methylation or glycosylation to increase its thermal stability.

Source: [21] page 2041

4.5.67 two-dimensional gel electrophoresis (2D PAGE) (in proteomics)

Technique used to separate proteins using *isoelectric focusing* in a gel, then the proteins are further separated in the second dimension using *denaturing PAGE*.

Note: 2D PAGE greatly enhances capacity of the separation and facilitates further analysis such as *difference gel electrophoresis* or mass spectrometric analysis of gel separated proteins.

Source: [55]

4.5.68 Western blot

Immunoanalytical technique for identification of polypeptides separated using gel electrophoresis. The polypeptide is transferred by blotting to a membrane where it undergoes specific interaction with primary antibody that is specific to the protein of interest. The peptides are detected as complexes by a *label* and/or enzymatic reaction, which may be conjugated directly to the primary antibody or via a separate (secondary) antibody.

Source: [21] page 2101

General References:[9, 21, 56]

4.6 Metabolomics

4.6.1 cultivation (in metabolomics)

Sample pretreatment to degrade complex metabolites in the presence of oxygen. It is a method for amplifying the concentration of native metabolites in solution.

4.6.2 design of experiment (in metabolomics)

Metabolomic workflow composed of sampling, metabolism quenching, metabolite extraction, metabolite analyses, data analysis and interpretation.

Source: [57]

4.6.3 endometabolome

Total content of metabolites inside a cell (intracellular metabolites).

4.6.4 excretion

Process of removal of compounds and their metabolites from the body, usually through the kidneys (to urine) or in the feces. Unless excretion is complete, accumulation of foreign substances can adversely affect normal metabolism.

4.6.5 exometabolome

Total content of metabolites outside the cell (extracellular environment).

4.6.6 fermentation (in metabolomics)

Degradation of the sample compounds in the absence of oxygen. It produces metabolites for intracellular metabolic profile characterization.

4.6.7 fingerprinting (in metabolomics)

Qualitative analysis of the *endometabolome*.

Example: Profiling, screening the matrix profile with known standards.

Source: [57].

4.6.8 fluxomics

In vivo measurements of metabolic fluxes and their integration with stoichiometric network models.

Source: [58].

4.6.9 footprinting (in metabolomics)

Qualitative analysis of the *exometabolome*.

Source: [57]

4.6.10 glucuronide

Glycoside of glucuronic acid.

Note: Glucuronides of xenobiotics (such as drugs, toxic substances etc.) are formed to *excrete* them from the organism.

Example: Drug glucuronides and sulfate conjugates are identified in excreted fluids.

4.6.11 metabolic pathway

All metabolic products of the main compound (e.g. phase 1 and phase 2 metabolites) with the routes of metabolism and the excreted metabolites in body fluids, e.g. in urine or blood.

Source: [7]

4.6.12 metabolism

Set of continuous life-sustaining chemical processes within cells that can be divided into two categories: whereas in catabolism some substances are broken down to yield energy for vital processes and provide the precursors for biosynthesis, in anabolism part of this energy and the precursors formed are used to construct new cell components.

Note 1: Metabolism means the continuously progressive physical and chemical processes involved in the maintenance and reproduction of life in which

nutrients are broken down to generate energy and to give simpler molecules (catabolism) which by themselves may be used to form more complex molecules (anabolism). In the case of heterotrophic organisms, the energy evolving from catabolic processes is made available for use by the organism.

Note 2: In medicinal chemistry the term metabolism refers to the biotransformation of xenobiotics and particularly drugs.

Source: Adapted from [7]

4.6.13 metabolism quenching

Physical or chemical methodology used for rapid breaking all biochemical processes in *metabolomic* samples.

Source: [57].

4.6.14 metabolite

Any chemical compound of the biological system that is not genetically encoded and is a substrate, intermediate or product of metabolism; or is consumed from the external environment or comes from co-habiting microorganisms such as gut microflora.

Source: Adapted from [7]

4.6.15 metabolite fingerprinting and footprinting

Unique pattern characterizing the metabolism in a given living phase that does not attempt to identify or quantify all the metabolites.

See *fingerprinting* and *footprinting*

4.6.16 metabolite profiling

Strategy to identify and quantify a particular set of metabolites belonging to a specific metabolic pathway or class of compounds.

Source: [57].

4.6.17 metabolome

Total quantitative collection of organic or inorganic low molecular-weight compounds (from 50 to 1000 Da) - *metabolites* - present in the cell or organism which participate in metabolic reactions required for growth, maintenance and normal functioning.

Note: The metabolome forms a large network of metabolic reactions, where outputs from one enzymatic chemical reaction are inputs to other chemical reactions.

Source: [59]

4.6.18 metabolomics

Complex and comprehensive study of cells by measuring the profiles of all, or a large number of their metabolites, an essential part of systems biology in addition to genomics, transcriptomics, proteomics, interactomics and fluxomics. Qualitative and quantitative analysis (determination) of metabolites.

Source: [60, 61]

4.6.19 metabonomics

Measurement of the dynamic multiparametric response of a living system to pathophysiological stimuli or genetic modification.

Note: This term is sometime misapplied to refer to the whole of "metabolomics".

Source: [62]

4.6.20 primary metabolites

Metabolites directly involved in the normal life processes of each cell.

Examples: alcohols, amino acids, monosaccharides, nucleotides, organic acids.

Source: [7].

4.6.21 secondary metabolites

Metabolites not required for the survival of the corresponding organism but which have other important functions.

Examples: antibiotics, pigments, attractants, flavours, drugs, biomarkers.

Source: Adapted from [7]

General References: [7, 61, 63-65]

4.7 Glycomics

4.7.1 activated nucleotide sugars

Activated forms of *monosaccharides* linked to *nucleotides* to be available in a high-energy state for *glycosyl* transfer.

Source: [66, 67]

4.7.2 aglycon(e)

Non-carbohydrate part of a *glycoconjugate* or a *glycoside*.

Source: [66, 67]

4.7.3 aldose

Monosaccharide with an aldehyde group or potential aldehydic carbonyl group.

Source: [66]

4.7.4 anomeric hydroxy(l) group
glycosidic hydroxy group

Hydroxy(l) group that originates from the aldehyde or keto group of a *monosaccharide* after ring closure. A functional group in one of two stereoisomers of a cyclic saccharide that differs only in its configuration at the hemiacetal or hemiketal carbon, which is also called the anomeric carbon. An anomer is a special type of epimer.

See *hemiacetals* and *hemiketals*
Source: [66]

4.7.5 carbohydrate
See *glycan*

4.7.6 carbohydrate-binding protein
See *glycan-binding protein*

4.7.7 comparative glycoproteomics

Field of *glycoproteomics* studies that the changes in the structures and expression levels of *glycoproteins* under different conditions.

4.7.8 comparative proteoglycomics

Field of *proteoglycomics* that studies the changes in the structures and expression levels of *proteoglycans* under different conditions.

Source: [68]

4.7.9 glycan
carbohydrate
polysaccharide
saccharide

Naturally occurring polyhydroxyaldehydes and polyhydroxyketones or compounds derived from these usually having the formula $[C_n(H_2O)_n]$. Compounds consisting of a large number of *monosaccharides* linked glycosidically.

Note 1: The term glycan is usually used for oligo- and polysaccharide, either free or bound to other chemical entities (*glycoconjugate*).

Note 2: Glycans composed of a single type of monosaccharide residue (homopolysaccharide, synonym homoglycan) are named by replacing the ending '-ose' of the sugar by '-an' (e.g. mannans, fructans, xylans, arabinans).

Source: Adapted from [20] page 1337, [69] and [67].

4.7.10 glycan-binding protein
carbohydrate-binding protein

Protein that recognizes and binds to specific *glycans* and mediates their biological function.

See: lectin

Source: [66, 67]

4.7.11 glycan microarray

Glycans attached to a surface of a *microarra*y.

Note 1: Glycan microarrays are used for characterization of *lectins* and other glycan-binding proteins via specific interactions with labelled glycan-binding proteins.

Note 2: Specific lectin-glycan interactions are used also for characterization of glycans in the opposite set-up (see: lectin microarray, glycan profiling).

Source: [66, 67, 70]

4.7.12 glycan profiling

Determination of the diversity of *glycan* structures expressed on *glycoconjugates* or cells.

See: *lectin microarray*.
Source: [67]

4.7.13 glycoconjugate

Generic term for a compound in which *glycans* are covalently bound to other chemical species such as *glycoprotein* or *glycolipid*.

Note: The glycan part of a glycoconjugate is called glycone, the non-saccharide part is called aglycon(e).

Source: [66, 67]

4.7.14 glycoform

Specific form of a *glycoprotein* containing a distinct *glycan* structure per glycosylation site.

Note: Natural glycoprotein consists of a population of glycoforms resulting from variable glycan structure and/or glycan attachment site occupancy.

Source: [66, 67]

4.7.15 glycogene

Gene encoding an enzyme or other functional protein for *glycan* synthesis.

Note 1: The term should not be confused with *glycogen* (an energy storage glucose polysacharide in animals and fungi).

Note 2: Proteins for glycan synthesis include glycosyltransferases, sugar-nucleotide synthases, sugar-nucleotide transporters and sulfotransferases (see: glycosylation, activated nucleotide sugars).

Source: [67]

4.7.16 glycolipid

Compound containing a *glycan* covalently linked to a lipid *aglycone*.

Note: The term “lipid” comprises several types of compounds; therefore different classes of glycolipides are described. For example, typical glycolipids in higher organisms are glycosphingolipids.

Source: [66]

4.7.17 glycome

Complete range of *glycans* and *glycoconjugates* produced by a species, tissue or cell in specified time, space and environment.

Note 1: Glycome is an analogue of the terms *genome*, *proteome* and transcriptome.

Note 2: The terms as for example “sialome” are sometimes used for the specific subclasses of the glycome.

Source: [66, 67, 71]

4.7.18 glycomics

Complex and comprehensive study of the *glycome*. An analogue of the terms genomics or proteomics.

Note: The terms as for example “fucanomics” or “galactanomics” are sometimes used for the specialized fields of glycomics.

Source: [67, 72]

4.7.19 glycone

Saccharide part of a *glycoconjugate*.

Source: [66]

4.7.20 glycoprotein

Protein with one or more covalently bound *glycans*.

See: lectin

Source: [66]

4.7.21 glycoproteomics

Comprehensive analysis of *glycoproteins*.

Note: Glycoproteomics studies the glycosylated subset of the proteome, including sites of glycosylation and glycan structures.

Source: [66]

4.7.22 glycosaminoglycan (GAG)

Long linear, highly charged polysaccharide composed of disaccharide repeating units, each containing hexose or hexuronic acid and *N*-acetylated or substitution-free hexosamine.

Note: GAG could be free complex polysaccharide or covalently linked to a protein core. See proteoglycan.

Source: [66, 67].

4.7.23 glycosidase

Enzyme that catalyzes the hydrolysis of *glycosidic linkages*.

Note: Endoglycosidase hydrolyzes internal glycosidic linkages, exoglycosidase removes terminal monosaccharide from non-reducing end of a glycoside.

Source: [66, 67].

4.7.24 glycoside

Glycan containing at least one *glycosidic linkage* to another glycan or an *aglycone*.

Source: [66]

4.7.25 glycosidic linkage

Linkage of a monosaccharide to another residue via the anomeric hydroxy(l) group.

Source: [66, 67]

4.7.26 glycosidic hydroxy group
See *anomeric hydroxy(l) group*

4.7.27 glycosyl

Monosaccharide ring without the anomeric oxygen.

Source: Adapted from [67]

4.7.28 glycosylation

Covalent attachment of a *saccharide* to a *protein*, *lipid*, *polynucleotide*, *saccharide* or other organic compound, generally catalyzed by enzymes *glycosyltransferases*.

Source: [66]

4.7.29 glycosyltransferase

Enzyme that catalyzes transfer of a *saccharide* from a nucleotide di(mono)phosphate-activated *sugar* to an acceptor molecule

See: *activated nucleotide sugars, glycosylation*
Source: [66, 67]

4.7.30 hemiacetals

Compounds having the general formula R2C(OH)OR' (R' ≠ H).

Note: In *aldoses*, hemiacetals are formed by reaction of an aldehyde with an alcohol group within the sugar molecule, resulting in ring closure.

See: *anomeric hydroxy(l) group*
Source: Adapted from [66]

4.7.31 hemiketals

Compounds formed by reaction of a ketone with an alcohol group, as in ring closure of a *ketose*.

Note: Hemiketals are a subclass of *hemiacetals*.
Source: Adapted from [66]

4.7.32 ketose

Monosaccharide with a ketone group or a potential ketonic carbonyl group.

Source: Adapted from [66]

4.7.33 lectin

Saccharide binding *protein* of non-immune origin.

Note 1: Some lectins participate in immune system processes but they are not products of primary immune response. Lectins also do not display catalytic activity towards their ligands and binding of carbohydrate is reversible and highly specific.

See: glycoprotein
Source: Adapted from [73]

4.7.34 lectin microarray

Lectins with distinct *saccharide*-binding specificity arranged on a *microarray* platform.

Note 1: Lectin microarray is used for characterization of glycans (see: *glycan profiling*) via specific interactions with labelled *glycans*.

Note 2: The specific lectin-glycan interactions are used also for characterization of lectins in the opposite set-up (see: glycan microarray).

Source: [67]

4.7.35 monosaccharide

Glycan that cannot be hydrolyzed into a simpler glycan.

Example: Aldoses, ketoses and a wide variety of derivatives.

Source: Adapted from [66]

4.7.36 nucleotide sugars

See *activated nucleotide sugars*.

4.7.37 polysaccharide

See *glycan*

4.7.38 proteoglycan

Compound consisting of a protein core to which one or more *glycosaminoglycan* chains are covalently attached.

Source: [66, 67].

4.7.39 proteoglycomics

Systematic study of structure, expression, and function of *proteoglycans*.

Source: [68].

4.7.40 saccharide

See *glycan*

4.7.41 sugar

See *glycan*

General References:[71-74]

4.8 Lipidomics

4.8.1 alkaline degradation (in lipidomics)

alkaline hydrolysis

Treatment with different alkaline solutions (e.g. hydrazine, KOH, NaOH) useful for the cleavage of fatty acids.

Note: The resulting (partially or fully) O-deacylated and (fully) N-deacylated products enable the determination of the fatty acid substitution positions by mass spectrometry.

Source: [75]

4.8.2 core oligosaccharide

Intermediate structural region between the O-polysaccharide and *lipid A* moiety of *lipopolysaccharides* (LPS).

Source: [76, 77]

4.8.3 eicosanoids

Biologically active lipid mediators (C20 fatty acids and their metabolites), including prostaglandins, thromboxanes, leukotrienes and other oxygenated derivatives, which are produced primarily by three classes of enzymes, cyclooxygenases (COX-1 and COX-2), lipoxygenases (LOX) and cytochrome P450 mono-oxygenases.

Source: [78]

4.8.4 fatty acyls (FA)

Diverse group of lipid molecules synthesized by chain elongation of an acetyl-CoA primer with malonyl-CoA (or methylmalonyl-CoA) groups.

Note 1: The Fatty Acyl category is further divided into multiple subclasses, including fatty acids and conjugates, eicosanoids, fatty alcohols and esters, glycans and others.

Note 2: Fatty acids are building blocks of the majority of complex cellular lipids like phospholipids, sphingolipids, triacylglycerols and sterylesters.

Examples: Palmitic acid, oleic acid, 1,8-octanedioic acid, prostaglandin A1, leukotriene B4.

4.8.5 glycerolipids (GL)

Esters, ethers and other *O*-derivatives of glycerol with alkyls or alkenyls, including mono- di- and tri-substituted glycerols.

Examples: 1-dodecanoyl-*sn*-glycerol (monoacylglycerol), 1-dodecanoyl-2-hexadecanoyl-3-octadecanoyl-*sn*-glycerol (triacylglycerol).

4.8.6 glycerophospholipids (GP)

phospholipids

Lipids with glycerol backbone esterified with one or two fatty acids in position C-1 and/or C-2 and with phosphoric acid at position C-3. The phosphate group is usually esterified with an alcohol (choline, ethanolamine, serine, inositol, glycerol) to form the so called lipid head group.

Note: GP are ubiquitous in nature and are key components of the membrane lipid bilayers of cells.

Examples: phosphatidyl choline, phosphatidyl ethanolamine, plasmalogens.

4.8.7 lipid A

Glycolipid component of *lipopolysaccharides* (LPSs) that is integrated into the bacterial cell wall and is responsible for the beneficial (immunostimulatory) or toxic (immunopathological) effects of the molecule.

Note: The archetypical (*Enterobacteriaceae*) structure of lipid A consists of a β-1,6-linked D-glucosamine (GlcN) or 2,3-diamino-2,3-dideoxy-D-glucose

(GlcN3N) disaccharide (or a mixture of them), which is acylated by varying numbers (usually four to seven) of ester- and amide-linked acyl groups, so called primary and secondary fatty acids. Phosphate groups and other glycosyl substituents may be linked at C1 and C4' positions. Lipid A is linked to the core oligosaccharide at the C6' position of the distal sugar unit of lipid A.

Source: [76], [79], [75]

4.8.8 lipid droplets

lipid particles
lipid bodies
adiposomes
oleosomes
oil bodies

Round-shaped intracellular inclusions consisting of hydrophobic cores (mainly triacylglycerols, sterol esters, occasionally free fatty acids or squalene) covered by a phospholipid monolayer containing a specific set of proteins.

Note: Originally considered as lipid storage compartments, lipid droplets are currently recognized as organelles with high metabolic activity. Defective formation and/or functions of lipid droplets are involved in various pathogenic conditions, such as obesity, cardivascular diseases, type 2 diabetes or metabolic syndrome.

Source: [80]

4.8.9 lipid extraction

Isolation of lipids in their native state from samples of different origin. Usually based on the use of organic solvents (methanol, chloroform) to facilitate lipid partitioning between acqueous and organic phases.

4.8.10 lipid profiling

Lipidomics technique conferring unbiased information about total lipids within a particular cell type, tissue or body fluid.

4.8.11 lipid remodelling

Changes in the lipid molecules composition after completion of their biosynthesis.

Note 1: In glycerophospholipids, remodelling includes changes in fatty acid composition (for example by acyl chain exchange catalyzed by transacylases) or introduction of unsaturated double bonds (catalyzed by desaturases), synthesis of new lipid species or degradation of existing ones. In phosphoinositides it can involve phosphorylation/dephosphorylation processes.

Note 2: Lipid remodelling is important for adaptation of biological membranes to changing environmental conditions and physiological demands.

4.8.12 lipidome

Complete set of lipid species and their metabolites present within a particular cell type, tissue of body fluid.

Note: It is estimated that lipidome of a eukaryotic cell may contain over 100000 individual molecular species of various lipids.

Source: [81]

4.8.13 lipidomics

Systematic and comprehensive study aimed at identification, profiling and quantification of all lipid species (*lipidome*) present within a particular cell type, tissue or body fluid.

Note 1: In a broader context it includes system-level analysis of subcellular localization and biological activities of individual lipids.

Note 2: Lipidomics is a lipid-targeted *metabolomics* approach aiming at comprehensive analysis of lipids in biological systems.

Source: [81, 82]

4.8.14 lipids

Small biologically active molecules of variable structure, commonly defined by their solubility in non-polar solvents. Hydrophobic or amphipathic small molecules that may originate entirely or in part by carbanion-based condensations of thioesters (fatty acyls, glycerolipids, glycerophospholipids, sphingolipids, saccharolipids, and polyketides) and/or by carbocation-based condensations of isoprene units (prenol lipids and sterol lipids).

Source: Adapted from [83, 84]

4.8.15 lipooligosaccharide (LOS)

Lipopolysaccharides produced by specific wild-type bacteria that give smooth-colony appearance when cultured on agar plates. LOSs are composed of *lipid A* and a *saccharide* moiety limited to an *oligosaccharide* core only.

Note: The lack of O-polysaccharide chains in LOS structures is an inherent property of bacteria.

Source: [85, 86]

4.8.16 lipopolysaccharide (LPS)

Principal structural component of the outer leaflet of the outer membrane of Gram-negative bacteria and cyanobacteria. It forms a tight permeability barrier that prevents the passage of cell-damaging agents (detergents, bile salts, hydrophobic antibiotics) into the cell.

Note 1: The chemical structure of an LPS molecule is divided into three covalently linked segments: the *O-polysaccharide*, the *core oligosaccharide* (featuring an inner and outer region) and the *lipid A* moiety.

Note 2: The biological activity of LPS, LOS or lipid A molecules as endotoxins in terms of host-mediated responses to LPSs or related compounds is called endotoxic activity (endotoxicity).

Note 3: Beyond this general structure, there are variations in all parts of LPS depending on the bacterial strain from which the LPS originate.

Source: [75, 76, 79]

4.8.17 lipopolysaccharidomics

Study of the structure-activity relationship of bacterial *lipopolysaccharides*.

4.8.18 membrane bilayer

Planar structure spontaneously formed by amphipatic *lipids* in a polar (aqueous) environment.

Note: Formation of a lipid bilayer is driven by interaction of the polar heads of lipids with water molecules while minimizing the exposure of the hydrophobic parts of lipids to water. Lipid bilayers represent the basic structure of biological membranes.

4.8.19 membrane rafts

Dynamic, nanometre-sized, highly ordered, membrane domains enriched in sterols and sphingolipids. They contain specific sets of membrane proteins that are sorted into rafts by their specific physical properties and specific lipid-protein interactions.

Note: In functional terms, membrane rafts represent higly specialized membrane domains involved in various cellular processes such as transmembrane transport, membrane trafficking, cell signalling or virus assembly.

Source: [87]

4.8.20 molecular shape of lipids

A concept describing the behaviour of lipid molecules (glycerophospholipids) in aqueous environments and membranes as a function of the ratio between the crossections of their polar heads and hydrophobic tails.

Note: Cylindrical lipids (head ≈ tail; e.g. phosphatidylcholines) form bilayers; conical lipids (head < tail, e.g. unsaturated phosphatidylethanolamines) tend to form inverted hexagonal phases; inverted conical lipids (head > tail, e.g. lysophospholipids) form micelles.

4.8.21 O-polysaccharide

Part of LPS that consists of a polymeric *saccharide* chain extending outwards from the bacterial cell surface.

Source: [79], [76]

4.8.22 plasma lipoproteins

Assemblies of *lipids* and *proteins* in blood *plasma* transporting lipids between tissues.

Note: They are structurally very similar to intracellular lipid droplets. According to their size and buoyant density they are classified into five types with different functions: chylomicrons, very low density liproteins (VLDL), intermediate lipoproteins (IDL), low density lipoproteins (LDL) and high density lipoproteins (HDL).

Source: [88]

4.8.23 polyketides (PK)

Heterogeneous group of compounds assembled from simple acyl building blocks comprising of polyethers, polyenes, polyphenols, macrolides and enediynes.

Note: Many polyketides are cyclic molecules whose backbones are often further modified by glycosylation, methylation, hydroxylation, oxidation, and/or other processes.

Examples: erythromycin, nystatin, tetracyclines.
Source: [89]

4.8.24 polyunsaturated fatty acids (PUFA)

Fatty acids with more than one double bond.

Note: The double bonds usually have cis (Z) configuration and are methylene-interrupted.
Example: linoleic and arachidonic acids.

4.8.25 prenol lipids (PR)

Lipids containing linear chain of isoprenoid groups.

Note: Prenol lipids are synthesized from the five-carbon precursors, isopentenyl diphosphate and dimethylallyl diphosphate. Both precursors are produced mainly via the mevalonic acid pathway.
Example: vitamin A, vitamin E, vitamin K, ubiquinone, beta-carotene.

4.8.26 proteolipids

Proteins with covalently linked lipids, e.g. fatty acid or glycophosphatidylinositol (GPI).

Note: The term 'lipoprotein' is also used to describe such compounds on occasion, but to avoid confusion this might be better reserved for the non-covalently linked lipid-protein complexes of the type found in plasma (*plasma lipoproteins*).

4.8.27 saccharolipids (SL)

Biomolecules where fatty acids are directly bound to a *saccharide* backbone, thus forming structures similar to glycerolipids or glycerophospholipids, with *saccharide* substituting for the glycerol.

Examples: lipid X (diacylaminosugar), Kdo_2LipidA (acylaminosugar glycan).

4.8.28 Shotgun lipidomics

Methodological approach based on direct analysis of the total lipid extracts from cells or tissues without an online chromatographic separation.

Source: [90]

4.8.29 signalling lipids
lipid mediators

Lipid molecules involved in intracellular signalling and regulation of cell death, immune response or inflammation.

Note 1: They include diacylglycerol, phosphatidic acid, phosphatidylinositol-4,5-bisphosphate (the source of polyphosphorylated inositols), arachidonic acid-derived mediators (eicosanoids, prostaglandins and leukotrienes), sphingolipid-derived mediators (ceramides, sphingosine, sphingosine-1-phosphate), or cholesterol-derived steroid hormones (androgens, estrogens, progestogens, mineralocorticoids, glucocorticoids).
Note 2: Broadly defined term, which refers to any lipid messenger that binds a protein target, such as a receptor, kinase or phosphatase, which in turn mediate the effects of these lipids on specific cellular responses.

Source: [91]

4.8.30 structural lipids

Lipids (mainly glycerophospholipids, sterols and sphingolipids) involved in the formation of biological membranes.

4.8.31 sphingolipids (SP)

A complex family of lipids that contain a sphingoid base backbone and a fatty acyl chain.

Note: Together with glycerophospholipids and sterols, sphingolipids are the major lipid components of cellular membranes.

Examples: Subclasses are represented by ceramides, gangliosides, sphingosines, sphingomyelines.

4.8.32 sterol lipids (ST)

Structural derivatives of tetracyclic alcohols with a hydroxyl group in the position C-3 and an aliphatic side chain in the position C-17.

Note: This category primarily comprises sterols - polycyclic compounds of isoprenoid origin, and their various derivatives including steroids, bile acids and sterol esters. Sterols, along with glycerophospholipids and sphingolipids, are important constituents of cellular membranes. In addition, sterol lipids act as hormones (steroids) or fat emulsifiers (bile acids).

Examples: Subclasses are represented by cholesterol, ergosterol, taurocholic acid, glucuronides.

4.8.33 targeted lipidomics

Analytical technique focusing on quantification of specific lipids of interest.

4.8.34 unsaturation index (UI)

A relative number indicating the proportion of double bonds in fatty acid moieties of phospholipids. UI is indicative of membrane physical properties (e.g. fluidity, curvature, or flexibility).

General References: [74, 81, 92].

5 STUDIES OF THE INTERACTIONS BETWEEN BIOMOLECULES

5.1 capture probe

Specifically designed single-strand nucleic acid with a defined (known) nucleotide sequence immobilized on a surface.

Note 1: Capture probe is utilized as a recognition element to test the nucleotide sequence of target DNA or RNA in the sample solution by using DNA or RNA hybridization.

Note 2: Analogous principle based on immobilized nucleic acid probes heas been applied in studied of nucleic acids interactions with proteins or other targets. See also *nucleic acid aptamers*.

Examples: Nucleic acids: ssDNA (alternatively, RNA or a synthetic nucleic acid analogue such as LNA or PNA). Surfaces: microarray, magnetic or other micro/nanoparticle, a transducer of the biosensor.

Source: [11]

5.2 chromatin immunoprecipitation

Method for studying DNA-protein and/or protein-protein interactions in the context of chromatin, suitable for identification of binding sites of proteins in genome DNA etc. It combines chemical cross-linking, isolation of specific protein conjugates by immunoprecipitation and PCR amplification of co-precipitated DNA.

5.3 cross-linking (in biomolecules)

Formation of covalent bridge between two biopolymer molecules or between two segments of the same biopolymer molecule using reactive substances (e.g., formaldehyde, bifunctional aldehydes such as glutaraldehyde, cisplatin) or photochemical processes.

Note: Cross-linking is used to study protein oligomerization, protein-protein and nucleic acid-protein interactions

See chromatin immunoprecipitation

5.4 fluorescent *in situ* hybridization (FISH)

Cytogenetic technique used to detect and localize the presence or absence of specific DNA sequences on chromosomes. It uses fluorescent probes that bind to only those parts of the chromosome with which they show a high degree of sequence complementarity.

Note: FISH is often used for finding specific features in DNA for use in genetic counseling, medicine, and species identification. FISH can also be used to detect and localize specific RNA targets (mRNA, lncRNA and miRNA) in cells, circulating tumor cells, and tissue samples. In this context, it can help define the spatial-temporal patterns of gene expression within cells and tissues.

Source: [93, 94]

5.5 groove binding (in DNA)

Process in which molecules are bound within minor or major groove of the *DNA double helix*.

Source: [11]

5.6 hybridization (nucleic acids)

Process of forming a double-stranded molecule from two *complementary strands* of DNA, RNA or synthetic nucleic acids analogues (e.g. PNA, LNA).

Example: DNA hybridization as a chemical interaction based on the ability of ssDNA to form a DNA double helix (dsDNA) with a counterpart molecule consisting of a complementary nucleotide sequence.

Source: [11]

5.7 hybridization assay

Assay with specifically designed ssDNA probe with a defined (known) *nucleotide sequence* usually immobilized on a surface (in such a case, the NA probe is called the capture probe, CP).

Note: The probe is used as a recognition element to test for the nucleotide sequence within the target DNA in the sample solution. If target DNA contains a sequence complementary to the probe, a hybrid dsDNA is formed.

Source: [11]

5.8 interactome

Virtual map depicting protein-protein interactions.

Note: The interactome is the most visible outcome of *functional proteomics*.

Source: Adapted from [95]

5.9 interactomics

Complex and comprehensive study of interactions and their consequences between various proteins as well as between protein and other molecules within a cell, such as nucleic acids and metabolites.

Source: [96]

5.10 intercalation (in biomolecules)

Thermodynamically favourable, reversible inclusion of a molecule (or group) between two other molecules (or groups).

Example: An insertion of guest molecules between the stacked base pairs of the DNA double helix (double stranded DNA) structure. It typically occurs with compounds of a planar structure with 3–4 condensed aromatic rings. To accommodate an intercalating molecule, the DNA double helix must lengthen and unwind slightly.

Source: [8, 11]

5.11 intermolecular interactions (in biopolymers)

Interactions between (parts of) two *biopolymer* chains (e.g. base pairing in DNA double helix, interactions between two polypeptide chains or between a DNA and a protein molecule).

5.12 intramolecular interaction (in biopolymers)

Interactions between (neighboring or distant) segments within the same *biopolymer* chain (e.g. base pairing in hairpin or monomolecular tetraplex structures of DNA or RNA, interactions stabilizing secondary or tertiary structures of proteins, etc.).

5.13 non-covalent interactions (in biomolecules)

Hydrogen bonds, electrostatic interactions and van der Waals forces taking part in stabilization of proper biopolymer structure and intermolecular interactions.

5.14 non-specific binding (in DNA-protein interactions)

Binding of a protein to DNA independent of nucleotide sequence or conformation of the DNA, often with a strong contribution of electrostatic interaction between the negatively charged sugar-phosphate backbone of the DNA and basic amino acid residues of the protein.

5.15 nucleic acid-protein interaction

Formation of specific or non-specific complexes between DNA or RNA and proteins, mediated and stabilized by a network of non-covalent interactions.

Note: Nucleic acid-protein interactions are critical for basic cellular functions such as replication, transcription, translation, recombination, DNA repair etc. as well as for fine regulation of them. Proteins may bind more tightly to specific sequences in the nucleic acid or non-specifically, in which case their affinity is independent of sequence.

5.16 protein–protein interaction (PPI)

Association of one protein with one or more other proteins to form either homo- or heteromeric proteins or protein complexes.

Note: Such associations are common in biological systems and are responsible for the regulation of numerous cellular functions in addition to the mediation of disease morphology where aberrant interactions play a significant role.

Source: [8]

5.17 pull-down assay

Approach used for studying protein-protein interactions analogous to immunoprecipitation, using (instead of antibody) one of the interacting proteins (the "bait") immobilized at a surface to capture the other protein (the "prey")

5.18 reporter (signalling) probe

Specifically designed ssDNA (alternatively, RNA or a synthetic nucleic acid analogue such as LNA or PNA) with a defined (known) *nucleotide sequence* to detect *complementary DNA* or RNA sequence in a sample.

Note 1: Reporter probe is usually labeled (radioactively, immunologically, by fluorescent, electroactive or other species to produce a specific signal upon hybridization).

Note 2: Similarly as for *capture probe*, nucleic acid signaling probe (or *nucleic acid aptamers*) can be applied in studies of nucleic acids interactions with proteins and other ligands.

5.19 specific binding (in DNA-protein interactions)

Binding of proteins to specific sites in DNA based on the precise recognition of nucleotide sequence and/or conformation of the DNA.

Note: Sequence-specific DNA binding is typical for e.g., restriction enzymes, transcription factors and other proteins involved in cellular regulation.

5.20 stacking interactions

Attractive interaction between planar aromatic rings, in nucleic acids being one of critical forces stabilizing double- and multistranded structures. Stacking interactions are involved in binding of intercalators to DNA.

5.21 TUNEL test

Method for the determination of *strand breaks* and *DNA fragmentation* in cells based on labeling and quantification of free 3'-OH DNA ends.

Note: The abbreviation is for Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL).

5.22 two hybrid system

A molecular biology method for studying protein-protein or protein-DNA interactions in cellular (yeast or bacterial) milieu.

Note: It involves a transcription factor split into two separate domains (DNA binding and and transcription activating), one of which is fused with one of the tested proteins (the "bait") and the latter with another (the "prey"), and a reporter gene construct. Only upon physical interaction between bait and prey is expression of the reporter gene observed.

General References: [7, 11]

6 INDEX OF TERMS

7 INDEX OF ABBREVIATIONS

8 MEMBERSHIP OF SPONSORING BODIES

9 REFERENCES

1. C. A. Burtis, T. Geary. *Pure Appl. Chem.* **66**, 2578 (1994).
2. R. Dybkær. *Pure Appl. Chem.* **73**, 927 (2001).
3. B. Nagel, H. Dellweg, L. Gierasch. *Pure Appl. Chem.* **64**, 143 (1992).
4. H. Lehmann, X. Fuentes-Arderiu, L. Bertello. *Pure Appl. Chem.* **68**, 957 (1996).
5. J. Duffus. *Pure Appl. Chem.* **65**, 2003 (1993).
6. J. H. Duffus, M. Nordberg, D. M. Templeton. *Pure Appl. Chem.* **79**, 1153 (2007).

7. C. Wermuth, C. Ganellin, P. Lindberg, L. Mitscher. *Pure Appl. Chem.* **70**, 1129 (1998).
8. D. R. Buckle, P. W. Erhardt, C. R. Ganellin, T. Kobayashi, T. J. Perun, J. Proudfoot, J. Senn-Bilfinger. *Pure Appl. Chem.* **85**, 1725 (2013).
9. G. Marko-Varga. *Pure Appl. Chem.* **76**, 829 (2004).
10. D. R. Thevenot, K. Toth, R. A. Durst, G. S. Wilson. *Pure Appl. Chem.* **71**, 2333 (1999).
11. J. Labuda, A. M. O. Brett, G. Evtugyn, M. Fojta, M. Mascini, M. Ozsoz, I. Palchetti, E. Paleček, J. Wang. *Pure Appl. Chem.* **82**, 1161 (2010).
12. P. Muller. *Pure Appl. Chem.* **66**, 1077 (1994).
13. A. Manz, P. S. Dittrich, N. Pamme, D. Iossifidis. *Bioanalytical Chemistry: 2nd Edition*. Imperial College Press, London (2015);
14. A. Jenkins, P. Kratochvíl, R. Stepto, U. Suter. *Pure Appl. Chem.* **68**, 2287 (1996).
15. R. van Grieken, M. de Bruin. *Pure Appl. Chem.* **66**, 2513 (1994).
16. A. Ure, L. Butler, R. Scott, R. Jenkins. *Pure Appl. Chem.* **60**, 1461 (1988).
17. M. Nordberg, J. Duffus, D. M. Templeton. *Pure Appl. Chem.* **76**, 1033 (2004).
18. M. De Bolster. *Pure Appl. Chem.* **69**, 1251 (1997).
19. G. Evans. *A handbook of bioanalysis and drug metabolism*. CRC press, Boca Raton (2004);
20. G. Moss, P. Smith, D. Tavernier. *Pure Appl. Chem.* **67**, 1307 (1995).
21. G. P. Rédei. *Encyclopedia of genetics, genomics, proteomics, and informatics*. Springer Science & Business Media (2008);
22. R. J. Reece. *Analysis of genes and genomes*. John Wiley & Sons Hoboken, NJ (2004);
23. A. Lesk. *Introduction to genomics*. Oxford University Press (2012);
24. M. S. Poptsova, ed. *Genome Analysis: Current Procedures and Applications* Caister Academic Press, Norfolk, UK (2014).
25. J. Pevsner. *Bioinformatics and Functional Genomics, 3rd Edition*. John Wiley and Sons Ltd, Chichester, UK (2015);
26. W. W. Grody, R. M. Nakamura, F. L. Kiechle, C. Strom, eds. *Molecular Diagnostics: Techniques and Applications for the Clinical Laboratory, 1st edition*. Academic Press / Elsevier, London, UK (2009).
27. N. L. Kelleher, H. Y. Lin, G. A. Valaskovic, D. J. Aaserud, E. K. Fridriksson, F. W. McLafferty. *J. Am. Chem. Soc.* **121**, 806 (1999).
28. B. T. Chait. *Science* **314**, 65 (2006).
29. V. L. Boyd, M. Bozzini, G. Zon, R. L. Noble, R. J. Mattaliano. *Anal. Biochem.* **206**, 344 (1992).
30. R. H. Garrett, C. M. Grisham. *Biochemistry*. Brooks/Cole (2010);
31. K. K. Murray, R. K. Boyd, M. N. Eberlin, G. J. Langley, L. Li, Y. Naito. *Pure Appl. Chem.* **85**, 1515 (2013).
32. M. Unlü, M. Morgan, J. Minden. *Electrophoresis* **18**, 2071 (1997).
33. P. Edman. *Acta chem. scand* **4**, 283 (1950).
34. C. K. Frese, A. M. Altelaar, H. van den Toorn, D. Nolting, J. Griep-Raming, A. J. Heck, S. Mohammed. *Anal. Chem.* **84**, 9668 (2012).
35. W. P. Blackstock, M. P. Weir. *Trends Biotechnol.* **17**, 121 (1999).
36. M. Monti, S. Orru, D. Pagnozzi, P. Pucci. *Clin. Chim. Acta* **357**, 140 (2005).
37. J. V. Olsen, B. Macek, O. Lange, A. Makarov, S. Horning, M. Mann. *Nat. Methods* **4**, 709 (2007).
38. E. Buxbaum. *Fundamentals of Protein Structure and Function*. Springer International Publishing (2015);

39. D. F. Hunt, J. R. Yates, J. Shabanowitz, S. Winston, C. R. Hauer. *Proceedings of the National Academy of Sciences* **83**, 6233 (1986).
40. J. Cavadore, G. Nota, G. Prota, A. Previero. *Anal. Biochem.* **60**, 608 (1974).
41. P. E. Michel, F. Reymond, I. L. Arnaud, J. Josserand, H. H. Girault, J. S. Rossier. *Electrophoresis* **24**, 3 (2003).
42. B. Ma, R. Johnson. *Molecular & Cellular Proteomics* **11**, O111. 014902 (2012).
43. R. Westermeier, T. Naven, H.-R. Höpker. *Proteomics in practice: a guide to successful experimental design*. John Wiley & Sons (2008);
44. M. Soloviev. *Peptidomics: methods and protocols*. Humana Press (2010);
45. T. Palzkill. *Proteomics*. New York, Kluwer Academic Publishers (2002);
46. B. Alberts. *Cell* **92**, 291 (1998).
47. J. Lill. *Mass Spectrom. Rev.* **22**, 182 (2003).
48. L. M. Smith, N. L. Kelleher. *Nat. Methods* **10** (2013).
49. A. I. Nesvizhskii. *Nat. Methods* **11**, 1114 (2014).
50. T. E. Creighton. *Proteins: structures and molecular properties*. W. H. Freeman (1993);
51. M. Hamdan, P. G. Righetti. *Mass Spectrom. Rev.* **21**, 287 (2002).
52. M. Norin, M. Sundström. *Trends Biotechnol.* **20**, 79 (2002).
53. V. Marx. *Nat. Methods* **10**, 19 (2013).
54. S. K. Sze, Y. Ge, H. Oh, F. W. McLafferty. *Proceedings of the National Academy of Sciences* **99**, 1774 (2002).
55. U. K. Laemmli. *Nature* **227**, 680 (1970).
56. P. K. Ghosh. *Introduction to Protein Mass Spectrometry*. Academic Press, London, UK (2016);
57. S. G. Villas-Boas, J. Nielsen, J. Smedsgaard, M. A. Hansen, U. Roessner-Tunali. *Metabolome analysis: an introduction*. John Wiley & Sons (2007);
58. M. Cascante, S. Marin. *Essays in biochemistry* **45**, 67 (2008).
59. S. G. Oliver, M. K. Winson, D. B. Kell, F. Baganz. *Trends Biotechnol.* **16**, 373 (1998).
60. O. Fiehn. *Plant Mol. Biol.* **48**, 155 (2002).
61. M. Walhout, M. Vidal, J. Dekker. *Handbook of systems biology: concepts and insights*. Academic Press (2012);
62. J. K. Nicholson, J. C. Lindon, E. Holmes. *Xenobiotica* **29**, 1181 (1999).
63. M. Lämmerhofer, W. Weckwerth, eds. *Metabolomics in practice: successful strategies to generate and analyze metabolic data*. John Wiley & Sons (2013).
64. W. L. Cabrera, J. S. Knapp, eds. *Metabolomics: metabolites, metabonomics, and analytical technologies*. Nova Science Publishers (2011).
65. T. O. Metz. *Metabolic Profiling*. Springer (2011);
66. A. Varki, R. D. Cummings, J. D. Esko, H. H. Freeze, G. W. Hart, M. E. Etzler, eds. *Essentials of glycobiology*. Cold Spring Harbor Laboratory Press, USA (2008).
67. H.-J. Gabius, ed. *The sugar code: fundamentals of glycosciences*. John Wiley & Sons (2011).
68. M. Ly, T. N. Laremore, R. J. Linhardt. *OMICS: J. Integrative Biol.* **14**, 389 (2010).
69. R. A. Dwek. *Chem. Rev.* **96**, 683 (1996).
70. C. D. Rillahan, J. C. Paulson. *Annu. Rev. Biochem* **80** (2011).
71. M. Cohen, A. Varki. *OMICS: J. Integrative Biol.* **14**, 455 (2010).
72. V. H. Pomin. *Biochimica et Biophysica Acta (BBA)-General Subjects* **1820**, 1971 (2012).
73. H. Lis, N. Sharon. *Chem. Rev.* **98**, 637 (1998).
74. J. M. Berg, J. L. Tymoczko, L. Stryer, N. D. Clarke. *Biochemistry, 2002*. W H Freeman, New York, USA;

75. A. Kilár, Á. Dörnyei, B. Kocsis. *Mass Spectrom. Rev.* **32**, 90 (2013).
76. H. Mayer, U. R. Bhat, H. Masoud, J. Radziejewska-Lebrecht, C. Widemann, J. H. Krauss. *Pure Appl. Chem.* **61**, 1271 (1989).
77. A. P. Anisimov, Y. Knirel. *Acta Naturae (англоязычная версия)* **4**, 46 (2012).
78. The American Oil Chemists' Society. *AOCS Lipid Library*. http://lipidlibrary.aocs.org/, accessed 7 June 2016.
79. C. R. Raetz, C. Whitfield. *Annu. Rev. Biochem* **71**, 635 (2002).
80. D. J. Murphy. *Protoplasma* **249**, 541 (2012).
81. M. R. Wenk. *Cell* **143**, 888 (2010).
82. A. Shevchenko, K. Simons. *Nature reviews Molecular cell biology* **11**, 593 (2010).
83. E. Fahy, S. Subramaniam, R. C. Murphy, M. Nishijima, C. R. Raetz, T. Shimizu, F. Spener, G. van Meer, M. J. Wakelam, E. A. Dennis. *J. Lipid Res.* **50 Suppl**, S9 (2009).
84. E. Fahy, S. Subramaniam, H. A. Brown, C. K. Glass, A. H. Merrill, Jr., R. C. Murphy, C. R. Raetz, D. W. Russell, Y. Seyama, W. Shaw, T. Shimizu, F. Spener, G. van Meer, M. S. VanNieuwenhze, S. H. White, J. L. Witztum, E. A. Dennis. *J. Lipid Res.* **46**, 839 (2005).
85. A. Preston, R. E. Mandrell, B. W. Gibson, M. A. Apicella. *Crit. Rev. Microbiol.* **22**, 139 (1996).
86. C. R. Raetz, C. Whitfield. *Annu. Rev. Biochem* **71** (2002).
87. D. Lingwood, K. Simons. *Science* **327**, 46 (2010).
88. N. E. Miller. *J. Clin. Pathol.* **32**, 639 (1979).
89. C. Hertweck. *Angewandte Chemie (International ed. in English)* **48**, 4688 (2009).
90. D. Schwudke, K. Schuhmann, R. Herzog, S. R. Bornstein, A. Shevchenko. *Cold Spring Harbor perspectives in biology* **3**, a004614 (2011).
91. C. N. Serhan, S. Hong, Y. Lu. *The AAPS Journal* **8**, E284 (2006).
92. A. D. Watson. *J. Lipid Res.* **47**, 2101 (2006).
93. A. Pernthaler, J. Pernthaler, R. Amann. *Appl. Environ. Microbiol.* **68**, 3094 (2002).
94. M. Wagner, M. Horn, H. Daims. *Curr Opin Microbiol* **6**, 302 (2003).
95. C. Sanchez, C. Lachaize, F. Janody, B. Bellon, L. Röder, J. Euzenat, F. Rechenmann, B. Jacq. *Nucleic Acids Res.* **27**, 89 (1999).
96. S. Feng, L. Zhou, C. Huang, K. Xie, E. C. Nice. *Expert review of proteomics* **12**, 37 (2015).