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Review

Application of surface plasmon resonance for the detection of carbohydrates, glycoconjugates, and measurement of the carbohydrate-specific interactions: A comparison with conventional analytical techniques. A critical review

Gulnara Safina*

Department of Chemistry, University of Gothenburg, Kemigården 4, 412 96 Gothenburg, Sweden

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ABSTRACT

Carbohydrates (glycans) and their conjugates with proteins and lipids contribute significantly to many biological processes. That makes these compounds important targets to be detected, monitored and identified. The identification of the carbohydrate content in their conjugates with proteins and lipids (glycoforms) is often a challenging task. Most of the conventional instrumental analytical techniques are time-consuming and require tedious sample pretreatment and utilising various labeling agents. Surface plasmon resonance (SPR) has been intensively developed during last two decades and has received the increasing attention for different applications, from the real-time monitoring of affinity bindings to biosensors. SPR does not require any labels and is capable of direct measurement of biospecific interaction occurring on the sensing surface. This review provides a critical comparison of modern analytical instrumental techniques with SPR in terms of their analytical capabilities to detect carbohydrates, their conjugates with proteins and lipids and to study the carbohydrate-specific bindings. A few selected examples of the SPR approaches developed during 2004–2011 for the biosensing of glycoforms and for glycan–protein affinity studies are comprehensively discussed.

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* Tel.: +46 766 22 90 61.
E-mail address: gulnara.safina@chem.gu.se

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Gulnara Safina is currently assistant professor in the Department of Analytical Chemistry at the University of Gothenburg (Sweden). She received her BS in 2002 and PhD in 2006 from Kazan State University (Russia). Previously she was a postdoc in Department of Pure and Applied Biochemistry and Department of Biochemistry at Lund University (Sweden) for two years. Her research interests include bio-, enzyme- and immunosensors, bioanalytical electrochemistry, surface plasmon resonance and quartz crystal microbalance biosensors, development of analytical methods and tools for multicompound assay.

1. Introduction

The aim of this critical review is to provide a brief but comprehensive comparison of SPR with other modern instrumental analytical techniques in terms of its applicability and usefulness of the detection of different carbohydrates, their conjugates with proteins and lipids, and the study of carbohydrate-specific interactions. It should be noticed that there is no aim to enumerate all works published recently, although they undoubtedly made a significant contribution to this field. The goal is to provide a critical broad overview about instrumental analytical techniques and approaches for screening and quantification of glycoforms, and measurements the carbohydrate-specific interactions with a special emphasis of the benefits of surface plasmon resonance.

Carbohydrates, their conjugates with proteins and lipids and processes associated with them participate in many key physiological functions, and play crucial roles in various biological events ranging from cell-to-cell communication to cell death. For instance, the linking of the carbohydrates to the amino acids of the proteins, the protein glycosylation, appears to play critical roles in various biological functions. Protein glycosylation is involved in a wide range of physiological processes, such as tumor growth, cancer, inflammations, immunological disorders etc. This makes glycoforms and the processes they are associated with important targets for their analysis and monitoring. The brief description of carbohydrates, various types of glycoconjugates and the overview of their functions and role in biological processes is given in Section 2 of this review.

The detection principles of carbohydrates and glycoforms are often based on selective capture of sugar moieties using specific receptors – lectins – followed by the characterisation of sugar–lectin interaction. Using a panel of lectins with known specificities to oligosaccharide epitopes as an array simultaneous probing of several carbohydrate-containing molecules can be carried out. Brief description of lectins, their classification according to carbohydrate specificity and the examples of lectin-based conventional assay formats is provided in Section 3.

The advantages and difficulties of the carbohydrate microarrays as a separate modern tool for the screening and measurement of the carbohydrate-specific bindings are briefly described in Section 4.

Often unraveling of the structural composition of glycoconjugates is a challenging and complicated task. The difficulties arise from the structural diversity of the glycoform molecule after the glycosylation process. The structure of the glycoforms can vary from molecule to molecule and from cell to cell. Complexity of the carbohydrate content in the biological samples often necessitates additional manipulations with the sample prior the detection (e.g., glycan release, separation, purification). Conventional analytical techniques for the carbohydrate assay include different modes of chromatography, electrophoresis and mass spectrometry. These techniques are highly sensitive; however, they are often time-consuming as they comprise the steps of purification, digestion or enrichment of the sample. Moreover, often usage of radioactive or fluorescent labels is required in order to visualise and detect the eluted molecules. The critical overview of the advantages and disadvantages of modern instrumental analytical techniques for the detection and structural characterisation of carbohydrates and carbohydrate-containing molecules is given in Section 5.

Label-free analytical techniques are suitable for both direct detection of the carbohydrate-containing molecules and capability of carrying out the real-time study and monitoring of carbohydrate-specific interactions. Among other label-free analytical techniques surface plasmon resonance has received special attention and has found numerous applications from surface studies to biosensors during last two decades. SPR is used for the real-time monitoring of the biospecific interactions between the free analyte in the solution and the ligand attached to a metal layer by measuring the alteration of the refractive index as a result of increasing the analyte surface concentration in the vicinity of the sensing surface. One of the main advantages of SPR is its ability to provide the information about the binding affinity and kinetics of the biospecific interaction. Special SPR approaches like SPR imaging (SPRi) are characterised as highly-throughput, capable of monitoring simultaneously multiple carbohydrate-specific interactions in a microarray format. The brief description of the working principle of different SPR approaches is given in Section 6.

The selected examples of the application of SPR based techniques and methods for the analysis of both small glycans and their large glycoforms developed between 2004 and 2011 have been critically discussed in Section 7. SPR approaches were compared with other modern analytical techniques in terms of their applicability for the detection of carbohydrates and carbohydrate-containing molecules.

Section 7 also reviews the attempts of coupling of SPR with other analytical techniques like high-performance liquid chromatography, mass spectrometry and electrochemistry. Findings from these studies help us to gain additional insights into the processes occurring on the sensing surface, to improve the selectivity of glycoconjugates analysis and to obtain the structural information about the detected molecules.

2. Carbohydrates, carbohydrate-containing molecules and their role in biological processes

Carbohydrates or glycans (monosaccharides, oligosaccharides and polysaccharides) are one of the most abundant and diverse classes of organic molecules found in nature. They consists of a large class of compounds with the empirical formula $C_m(H_2O)_n$ [1].

Monosaccharides are called simple sugars and can not be broken down into smaller sugars. Monosaccharides consist typically of from three to seven carbon atoms and are described either as

aldoses or ketoses, depending on whether the molecule contains an aldehyde function or a ketone group. Hexoses containing six carbon atoms are the most abundant sugars in nature. Aldoses or ketoses are often given more detailed names to describe both the important functional groups and the total number of carbon atoms, for instance aldohexoses and ketohexoses. Mannose (Man), glucose (Glc) and galactose (Gal) are the typical examples of aldohexoses.

Oligosaccharides (greek *oligo*–"few") consist of from two to ten simple sugar molecules. Disaccharides are common in nature. Maltose (2 units of Glc), sucrose (Glc and fructose) and lactose (Glc and Gal) are the examples of disaccharide molecules. Oligosaccharides are often found as a component of complex biological molecules including glycoproteins or glycolipids.

Polysaccharides are polymers of the simple sugars and their derivatives. They may be either linear or branched polymers and may contain hundreds or even thousands of monosaccharide repeating units. The well-known representatives of polysaccharides are starch, glycogen, cellulose and chitin.

Carbohydrates play vital roles in all forms of life. They are involved in a large number of biological recognition phenomena such as cell–cell interactions [2] and cell death [3], signal transduction [4], inflammatory processes, cancer metastasis [5], bacterial and viral infections [6]. Carbohydrates serve as energy sources (e.g., starch and glycogen); they are the components of coenzymes and the parts of nucleic acids. Glycans and their conjugates with proteins and lipids play key roles in the immune and endocrine systems, fertilisation, brain development, prevention of pathogenesis and blood clotting [7,8].

The major classes of protein- and lipid-linked oligosaccharides include glycoproteins and proteoglycans; glycopeptides; peptidoglycans; glycolipids and lipopolysaccharides [9]. These compounds differ by sugar, protein or lipid contents in the molecule and the role they play in biological processes. In naturally occurring glycoconjugates, the portion of the molecule comprising the glycan can vary greatly in its contribution to the overall size, from being very minor in amount to being the dominant component or even almost the exclusive one. In many cases, the glycans comprise a substantial portion of the mass of glycoform. Here a brief overview of the main classes of glycoconjugates and their biological functions is provided.

Glycoproteins (GPs) are proteins that contain glycan chains covalently attached to polypeptide side chain (Fig. 1A). The carbohydrate may be in the form of a monosaccharide, disaccharide, oligosaccharide, polysaccharide, or their derivatives (e.g., sulfo- or phospho-substituted). The carbohydrate content in glycoprotein may vary from one to 80 percent. The predominant sugars found in glycoproteins are Glc, Gal, Man, fucose (Fuc), N-acetylgalactosamine (GalNAc), N-acetylglucosamine (GlcNAc). The sugar chain can be linked to the protein component in two major ways, through either O-glycosidic or N-glycosidic bonds. The O-glycosidic linkage forms due to the direct attachment of GalNAc, a galactose molecule with an amine group covalently bonded to the second carbon, to the protein part through either serine (Ser) or threonine (Thr). Another type of O-linked glycoproteins consists of a Gal or a glucosyl-galactose disaccharide linked to the hydroxyl of hydroxylysine or arabinose bound to the hydroxyl of hydroxyproline. The other class of glycoproteins contains the N-linked glycans. In this case GlcNAc, a glucose molecule with an amine group covalently bonded to the second carbon, is attached to the N-terminus of an asparagine (Asn) residue. The asparagine must be surrounded by a specific amino acid sequence containing -X-Asn-X-Thr; where the X can be any amino acid, while a large variety of polysaccharide side chains can be linked to the other side of GlcNAc. A typical carbohydrate chain includes a few Man linked to GlcNAc [10,11].

GPs play a crucial role in a number of important cellular processes involving cell-to-cell communication, proliferation, recognition and death. Glycoproteins are also involved in transporting of biologically active substances like vitamins and hormones, play an important role in nervous functions, biosynthesis and tumor metastasis [2–4]. GPs are found in different tissues (e.g., cartilages, nerve and connecting tissues). Such glycoproteins like prothrombin, thrombin, and fibrinogen play a critical role in blood clotting mechanism. Certain glycoproteins have a protective function. For example, high molecular weight polymers like mucins found on internal epithelial surfaces form a layer that protects epithelium form chemical, physical, and microbial disturbances. Some glycoproteins protect the skin from the other excretory products that could be harmful for the skin; also they maintain the structure of the skin (collagen). Many glycoproteins function as hormones, for instance, human chorionic gonadotropin (HCG) presence of which in blood and urine indicates pregnancy. Another example of glycoproteins is erythropoietin which regulates red blood cell production. Enzymes of classes of oxidoreductases, transferases and hydrolases are also glycoproteins. GPs play role in vision, forming the outer membranes of retinal rods. Glycoproteins directly participate in immune response as they are contained on the surface of B and T cells; moreover, immunoglobulins are also glycoproteins [12].

The majority of the proteins targeted by therapeutic compounds are glycoproteins. This includes pharmaceutical products for heart diseases, cancer, neurodegenerative disorder and diabetes [13]. Some GPs are used as a tumor and infection markers in order to monitor the adherence of the bacteria to cells and tissues [14,15].

Removing or altering the sugar chain may affect the structure and the functions of the glycoprotein molecule. It has been reported that protein glycosylation, the linking of the carbohydrates to the amino acids of the proteins, seems appears to play critical roles in biological functions and affect a wide range of physiological processes including tumor growth, cancer, metastasis, inflammations, chronic, immunological and cardiovascular disorders [16–21].

Proteoglycans (PGs) are a subclass of glycoproteins in which the carbohydrate units are polysaccharides that contain amino sugars, glycosaminoglycans (GAGs). Proteoglycans may contain up to about 100 covalently attached GAGs like hyaluronan, chondroitin sulfate, keratin sulfate, dermatan sulfate and heparan sulfate. The GAGs of the proteoglycans are linear polymers of up to about 200 repeating disaccharide units consisting of glucosamine (GlcN) or galactosamine (GalN) alternating with an uronic acid or a neutral sugar. The chain of repeating units is attached through the link protein to the core protein by a linkage region consisting of oligosaccharide of a structure different from the repeating units (Fig. 1B). Proteoglycans may also contain one or more oligosaccharides of structures similar to those found in other glycoproteins. Proteoglycans are very diverse molecules, and various combinations of both different types of proteins and classes of GAG chains are found in vertebrates.

PGs found in the connective tissue including blood vessels, cartilages and skin, on the cell surfaces and intracellular compartments. PGs are the major part of the animal extracellular matrix. Specific interactions between proteoglycans and macromolecules in the extracellular matrix, which take place through both glycosaminoglycan and core protein components, are the key factors in the functions of proteoglycans [22].

PGs are involved in binding cations (Na^+, K^+ and Ca^{2+}) and water, regulating the transfer of molecules through the matrix, affecting the stability and activity of proteins, cell–cell adhesion, cell signaling, migration and proliferation [23]. They play an important role in cell interactions, cell division, viral and tumor invasions, cancer and metastasis, neuroregeneration and differentiation [24]. The inability of certain enzymes to degrade PGs leads to their accumulation within cells and various disorders development [25].

Glycopeptides (GPps) are peptides that contain carbohydrate moieties covalently attached to the side chains of the amino acid residues (oligopeptide) through a glycosyl linkage (*O*-, *N*- or *S*-) (Fig. 1C). Glycopeptides are produced by enzymic or chemical cleavage of glycoproteins, or by chemical or enzymic synthesis. They play a role in fertilisation, the immune system, brain development, the endocrine system and inflammation [26,27]. Many GPps are pharmaceuticals for treatment of infections and inflammations [28].

Peptidoglycan (PpG), or murein, is a polymer containing sugars (GAGs) cross-linked by a peptide bond consisting of 3–5 amino acids (Fig. 1D). PpG is an essential component of the bacterial cell wall, which helps to maintain the cell structure and counteracts the high osmotic pressure of the bacterial cell. This macromolecule is known to occur as a monomolecular layer between the inner and outer membrane in Gram-negative bacteria and as a multimolecular layer, often associated covalently or non-covalently with various additional compounds (teichoic acids, neutral polysaccharides, etc.) in Gram-positive bacteria.

Peptidoglycan regulates the transport of molecules into cells, participates in the regulation of cell division. Bacteria also often release peptidoglycan fragments, which function as signals in cell–cell communication. Bacterial PpG stimulates the production of mediators which cause different biological effects in the host organism like inflammation, fever, hypotension, leukocytosis, shock and multiple organ failure [29].

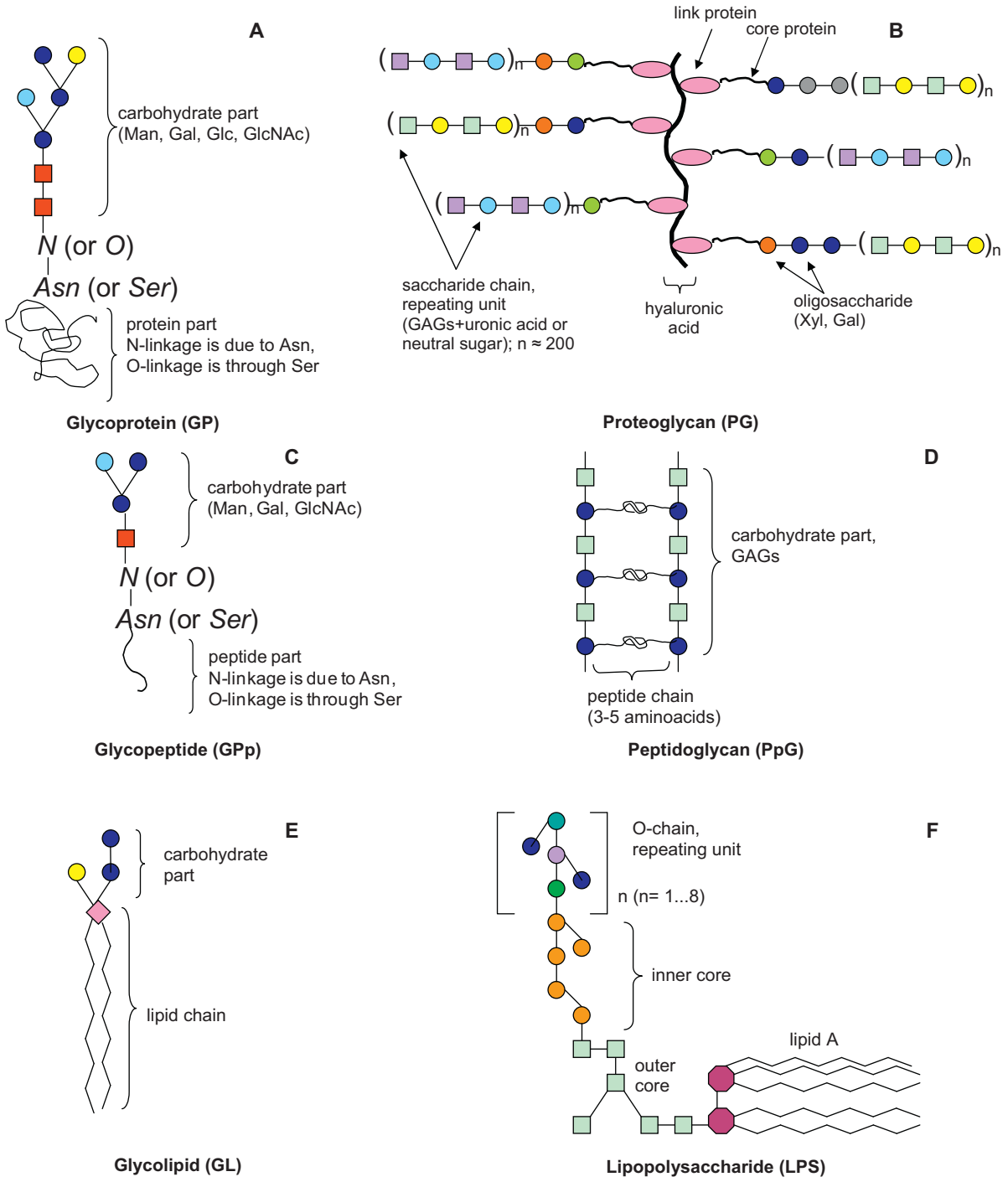

Fig. 1. Schematic structures of different glycoforms.

Lipids attached to a short carbohydrate chain (mono- or oligosaccharides) form *glycolipids (GLs)* (Fig. 1E). Glycolipids include glycosphingolipids, sphingolipids and gangliosides. Glycosphingolipids contain a hydrophobic ceramide anchor N-acylsphingosine and a hydrophilic head group composed of sugars. Glycosphingolipids are usually found at the outer surface of cell membranes. Sphingolipids are consisted in brain and therefore are referred as brain lipids. They can be classified as (1) gray-matter lipids (neuronal lipids) and (2) white-matter lipids (myelin). Neuronal lipids are similar to other tissue and cell membranes, while myelin contains sphingolipids, cholesterol, and phospholipids. Gangliosides are specific for neuronal lipids. Their degradation, i.e. removing the saccharide units, is catalysed enzymatically by transferases. Defects in these enzymes and, therefore, a defect in ganglioside metabolism, lead to glycolipid storage disorder associated with certain neuro-degenerative diseases.

Glycolipids play significant roles in signaling mechanism (brain lipids), blood clotting, cell adhesion, and serve as markers for cell–cell communication and as an energy source [30,31].

Lipopolysaccharides (LPSs), or lipoglycans, are large molecules consisting of a lipid covalently joined to a polysaccharide. They are found in the outer cell membrane of all Gram-negative bacteria. Today the structures of the LPSs of many Gram-negative microorganisms have been characterised, and they all display a common general architecture. The molecules contain three separate groups covalently attached to each other: O-polysaccharide chain, outer and inner cores, and lipid A [32,33] (Fig. 1F). Differences between species and strains exist in all parts of the molecule, but most variation occurs in the O-chain, followed by the core region and at last by the lipid A part.

The repeating O-polysaccharide units are composed of hexoses and consist between one and eight glycosyl residues. Diversity of the structure of the O-polysaccharide units is responsible for multiplicity of serotypes of Gram-negative species. The inner core contains a high proportion of 3-deoxy-D-manno-octulosonic acid and L-glycero-D-manno heptose. The outer core is more likely to consist of hexoses (Glc, Gal) and hexosamines (GalNAc and GlcNAc). The last part, lipid A, is typically composed of the GlcN residues carrying two phosphoryl groups which can be substituted with other groups like phosphates or sugars. To this structure up to four acyl chains are attached, which in turn be substituted by fatty acids, saturated or unsaturated (rarely).

The O-polysaccharide as the outermost part of the LPS molecule is therefore the major antigen targeted by host antibody responses. Due to the high specificity of these responses, the O-chain is often also known as the O-antigen. LPSs cause inflammatory reaction, triggering toxic reactions such as fever, shock and even death [34–36]. Also bacterial LPSs are involved in surface adhesion to host tissue [37] and are essential for the viability of all Gram-negative bacteria.

Protein- and lipid-linked oligosaccharides are a large component of the eukaryotic cell surface where they play a critical role in biological processes of cell–cell and cell–pathogen interactions and adhesion. The selected representatives of different glycoconjugates and the biological functions associated with them are presented in Table 1. Alterations in both the synthesis and catabolism of the oligosaccharides in the content of glycoconjugates may have serious and life threatening consequences, such as tumor metastasis, carbohydrate deficiency syndromes and lysosomal storage diseases [38]. The high biological importance of carbohydrate-containing molecules clearly indicates the necessity of the development of novel and improvement of existing chemical, biochemical and instrumental analytical techniques for their reliable detection, identification and structural characterisation.

3. Lectins as a tool for the detection of glycans and the characterisation of carbohydrate-specific interactions

3.1. General characteristics of lectins and the nature of their binding to carbohydrates

A sensitive detection and mapping of carbohydrate-containing molecules and characterisation of the glycosylation pattern of proteins may be achieved by using carbohydrate-specific lectins. Lectins (lat. *legere*—"to select") are proteins or glycoproteins of non-immune origin found in plants, microorganisms, animals and humans. They recognise and reversibly bind to specific carbohydrate structural epitopes through hydrogen bonds, metal coordination, van der Waals and hydrophobic interactions without altering the glycan molecule [39,40]. For instance, two amino acids residues in the protein bind to both the metal ion and the sugar, while other protein side chains form hydrogen bonds with other hydroxyl groups on the carbohydrate.

Divalent cations, such as Ca^{2+}, Mg^{2+} and Mn^{2+} are involved in carbohydrate recognition either indirectly, by orientation of the amino acid chain, or by acting as a bridge between the protein and the sugar moieties through the interactions with sugar hydroxyl groups.

Despite on the hydrophilic character of carbohydrates caused by the presence of multiple hydroxyl residues, the interactions through hydrogen bonds are not dominating in carbohydrate–lectin binding. Non-polar (hydrophobic interactions) play a major role in stabilising of sugar–lectin complexes [41], for instance, the interaction between aromatic residues and galactose in galactose-specific lectins.

3.2. Conventional lectin-based approaches for carbohydrates discrimination

Large group of methods of separation, isolation and the subsequent identification of mono-, oligo- and polysaccharides, glycoproteins and glycolipids is based on their individual affinity to lectins [42]. In general, in terms of their carbohydrate binding affinity lectins are divided into four major classes: (1) mannose binding lectins, (2) galactose binding lectins, (3) lectins, which recognise sialic acid and (4) fucose discriminating lectins. Table 2 provides general information about the selected lectins, which are classified according to their carbohydrate specificity.

Current research on lectins is focused on two directions. The first direction is based on the characterisation of the lectin molecule structures, their biological roles and carbohydrate-binding specificity. Another research direction is focused on the application of lectins as a powerful tool for isolation, identification, characterisation and study either free forms of glycans or their conjugates with lipids and proteins, both in solution and on solid surfaces [43–45]. This approach finds application, for instance, in purification of glycoconjugates by lectin-based affinity chromatography, which is described in sub-section 5.1. A panel of different lectins with known specificities can be an excellent tool to probe and to map the oligosaccharides epitopes and the carbohydrate moieties on the cell surface [46,47]. The larger number of lectins with different glycan affinities is used, the broader pool of carbohydrate moieties can be mapped. This can be applied either for a better assessment of protein glycosylation or can provide additional insights for studying of the process of bacterial cell adhesion.

Application of lectins as recognition molecules for the discrimination of glycans is well-known and described elsewhere [48]. The choice of the assay format depends on the purpose and the material studied. Semiquantitative method of carbohydrate analysis is based on visual precipitation (agglutination) which occurs after mixing lectin and carbohydrate-containing solutions [49,50]. Although this

Table 1
Selected representatives of the major classes of glycan-containing molecules and their biological function and importance.

Glycoconjugate	MW (kDa)	Carbohydrates composed in its structure	Function, activity, importance
Glycoproteins (GPs)			
Fetuin	48	Neu5Ac, Gal, GalNAc	Important for stimulation of cell proliferation *in vitro*; Antagonist of antiproliferation action of TG1-β; Identified as an apoptosis-inducing protein with anti-cancer activity; Mediates the transport and availability of biosubstances; Calcium ion binding protein
Asialofetuin	48	Gal and GalNAc	Marker for human carcinoma cells
Transferrin	80	GlcNAc-Man, GlcNAc-GlcAc-Man-GlcNAc	Binds, stores and delivers iron; Marker of inflammation reaction
RNAase B	14.7	GlcMan, Man-GlcNAc	Catalyses the hydrolysis of RNA; Protects RNA from viruses
Thyroglobulin	660	Man, GlcNAc, Neu5Ac	Plays an important role in the synthesis and releasing of thyroid hormone; Marker of thyroid cancer
Proteoglycans (PGs)			
Chondroitin sulfate	16.9	-4GlcUAβ1-GalNAcβ1-	Plays role in maintaining the structural integrity of the tissue; Major component of cartilage; Stabilises normal brain synapses as part of perineuronal nets.
Glycopeptides (GPps)			
Oritavancin	1.793	GlcNAc and MurNAc	Antibiotic for the treatment of serious Gram-positive infections
Peptidoglycans (PpGs)			
Murein	$n \times 109$ (n = 3–6)	GlcNAc and MurNAc	Helps maintain the structure of the bacterial cell; Involved in bacterial cell reproduction
Glycolipids (GLs)			
Galactocerebroside	0.840	Gal	A part of myelin
Gangliosides	0.7-1.6	Glc, Gal, Neu5Ac	Serves in cellular recognition and cell-to-cell communication.
Lipopolysaccharides (LPSs)			
Bacterial endotoxins	10–100	Gal and GalNAc	Triggers inflammations, fever and septic shock. Important for viability of the bacterial cell

Abbreviations of the carbohydrates residues (in alphabetical order).
Gal—galactose.
GalNAc—N-acetylgalactosamine.
Glc—glucose.
GlcAc—glucuronic acid.
GlcMan—4-b-D-glucosylmannose.
GlcNAc—N-acetylglucosamine.
Man—mannose.
MurNAc—N-acetylmuramic acid.
Neu5Ac—N-acetylneuraminic acid (sialic acid).
UA—uronic acid.

Table 2
Selected representatives of lectins and their carbohydrate binding specificity.

Lectin	Abbreviation	Source	MW (kDa)	Carbohydrate binding affinity
Mannose binding lectins				
Concanavalin A	Con A	*Canavalia ensiformis*	102	Branched α-mannosidic structures; Mannose type, hybrid type and biantennary complex type N-glycans
Lentil lectin	LCH	*Lens culinaris*	48	Fucosylated core region of bi- and triantennary complex type N-glycans
Snowdrop lectin	GNA	*Galanthus nivalis*	60	α 1-3 and α 1-6 linked high mannose structures
Galactose/N-acetylgalactosamine binding lectins				
Ricinus communis agglutinin	RCA	*Ricinus communis*	120	Galβ1-4GlcNAcβ1-R
Peanut agglutinin	PNA	*Arachis hypogaea*	110	Galβ1-3GalNAcα1-Ser/Thr (T-antigen)
Hairy vetch lectin	VVL	*Vicia villosa*	70	GalNAcα-Ser/Thr (Tn-antigen)
Sialic acid/N-acetylglucosamine binding lectins				
Wheat germ agglutinin	WGA	*Triticum vulgaris*	34	GlcNAcβ1-4GlcNAcβ1-4GlcNAc; Neu5Ac
Maackia amurensis	MAA (MAL)	*Maackia amurensis*	140	Neu5Ac/Glcα2-3Galβ1-4GlcNAcβ1-R
Fucose binding lectins				
Ulex europaeus agglutinin	UEA	*Ulex europaeus*	170	Fucα1-2Gal-R; Fucα1-2Galβ1-4(Fucα1-3/4)Galβ1-4GlcNAc
Aleuria aurantia lectin	AAL	*Aleuria aurantia*	33.4	R2-GlcNAcβ1-4(Fucα1-6)GlcNAc-R1

Abbreviations of the carbohydrates residues (in alphabetical order).
Fuc—fucose.
Gal—galactose.
GalNAc—N-acetylgalactosamine.
Glc—glucose.
GlcNAc—N-acetylglucosamine.
Man—mannose.
Neu5Ac—N-acetylneuraminic acid (sialic acid).
Ser—serine.
Thr—threonine.

method is simple, it requires significant consumption of reagents and can not be applied for the analysis of minute amounts of sample. Enzyme-linked lectinosorbent assay (ELLA), lectinoblotting and lectin–glycan interaction on thin-layer plates are more advanced and sensitive methods for the assay of glycoconjugates and for measuring lectin–carbohydrate interactions [44]. ELLA, a modified version of enzyme-linked immunosorbent assay, where antibodies in the enzyme conjugate are replaced by lectins, does not require large amounts of reagents and sample, and is suitable for multiple samples analysis on a microtiter plate [51,52]. At the same time, the assay procedure consists of several incubation and washing steps, and, therefore, is often time-consuming.

Lectin-based luminescent techniques for the detection of glycans and glycoconjugates are described in [53,54]. A new sensitive detection the very low amount of glycoproteins, the potential biomarkers, using LectinNanoProbeArrays is developed by Nagaraj et al. [55]. This approach is based on creating the lectins array by deposition only a few hundreds of picoliters of lectins utilising a piezoelectric liquid dispenser. The formed lectin spots are only 250 μm in diameter. Then nanoliters of fluorescently labeled probes of asialofetuin and sialyated form of fetuin are dispensed over the lectin spots on the array. The developed approach makes it possible to distinguish the small yet important changes between the closely related glycoforms of fetuin.

Lectin microarrays appeared to be the useful and effective tools for the characterisation of the carbohydrate–protein interactions [56,57]. On the one hand, this approach is extremely sensitive, simple, allows detection of the glycoprotein down to picomolar concentrations and has potential to become a suitable tool for glycoprotein assay. On the other hand, the identification of the carbohydrate moieties in the unknown sample may be complicated. In this case it is necessary first to isolate the glycoprotein of interest from a mixture of other glycoproteins as they may also have specificity to the same lectin and act as interferences. The described approach also requires labeling of the probe before the detection, which makes the assay more complex and time-consuming.

4. Carbohydrate microarrays as an efficient high-throughput tool for measuring the carbohydrate-specific interactions

Carbohydrate microarrays containing the group of mono-, oligo- and polysaccharides immobilised on a solid support are the high-throughput tools for monitoring the carbohydrate–protein interactions and have been intensively developed during last years [58–60]. The most commonly used approach for making the glycan microarrays is covalent attachment of chemically modified carbohydrates to the derivatized glass surfaces. Other approaches including non-covalent immobilisation of the unmodified carbohydrates on an underivatized surface, non-covalent immobilisation of the chemically modified carbohydrates on an underivatized surface, and covalent immobilisation of the unmodified carbohydrate on a derivatized surface are also described in a number of research articles and reviews [59–70].

In carbohydrate microarrays a few nanoliters of solutions containing glycans in concentrations of 1–100 μM are precisely deposited on the glass surface in 5–15 μm-diameter spots. Array is incubated for several hours with fluorophore-labeled sample in order to allow the biospecific binding with the immobilised glycans, and the fluorescence intensity is measured by optical detectors.

Glycan microarrays have been successfully applied for:

(1) High-throughput measurements of carbohydrate–lectin interactions and characterisation of novel carbohydrate-binding proteins [58,59,62];
(2) Rapid estimation of enzymatic activity of carbohydrate-processing enzymes and determination of their substrate specificity;
(3) Profiling of carbohydrate–antibody interactions for the purposes of medical diagnostics;
(4) Detection of bacterial and viral pathogens and study of the adhesion mechanism of the bacterial cells to the carbohydrate surfaces [63,71];
(5) Characterisation or novel pharmaceuticals and therapeutic targets.

Lectin-binding assays performed using carbohydrate microarrays, in contrast to the conventional agglutination tests, are highly sensitive, enable to recognise below 1 nM of lectin [62], and require much less amount of sample. The most significant advantage of the carbohydrate microarrays-based assay is the possibilities to rapidly screen hundreds or even thousands samples at a time, and to study numerous protein–carbohydrate interactions simultaneously on a miniature array surface.

Although carbohydrate microarray appears to be an efficient tool for measuring even relatively weak glycan–protein bindings, this technique has several limitations. First, because of the lack of functional groups capable of covalently bind to a surface, glycans must be derivatized or modified prior the immobilisation. Second, the immobilised glycans should be properly oriented and spaced on the surface in order to allow the bindings with the tested sample [72]. Third, in order to carry out the detection of the sample using the conventional detectors, the bioprobes need to be labeled with fluorescent or radioactive tags, or gold nanoparticles [60,73–76].

5. Instrumental analytical techniques for the detection of carbohydrates and carbohydrate-containing molecules and characterisation of carbohydrate–protein bindings

In general, structural characterisation of glycoconjugates is a challenging and difficult task. It is more complicated than, for instance, characterisation of nucleic acids or proteins [77]. The difficulties are attributed to the structural diversity of the conjugate after the glycosylation process, so called microheterogeneity. The extent of the structural diversity can vary from one glycosylation site to another, from glycoprotein to glycoprotein, and from cell type to cell type. This indicates that at any given glycan attachment site on a given protein synthesised by a particular cell type, a range of variations can be found in the structures of the attached glycan chain. Because of the complexity of the carbohydrate content in the biological probe, the additional manipulation with the sample, such as separation, purification and glycan release prior the identification are often required [78]. Despite on the mentioned difficulties, the intensive development of modern analytical instrumental techniques during the last years made a significant progress in the structural analysis of glycoconjugates and in studying the protein glycosylation [79]. The brief overview and critical discussion of modern techniques for isolation, detection and structural characterisation of glycans and glycoforms are given in this section.

5.1. High-performance liquid chromatography for isolation and detection of glycan-containing molecules

Normal-phase, reversed-phase, ion-exchange, thin-layer, affinity and size-exclusion chromatography coupled with UV, fluorescent, electrochemical, mass spectrometric (MS) and light scattering detectors are successfully used for isolation of glycoconjugates followed by their detection and quantification [78,80–98]. Such hyphenated techniques accomplished with programs containing glycan database and tools to assist the interpretation and

assignment of HPLC-glycan profiles [99] are powerful and are capable of providing both qualitative and quantitative detailed information about the complex structure of the unknown glycan species with little sample consumption. However, these techniques require a few pretreatment steps prior the detection. These steps include tryptic digestion of the bioprobe, releasing/isolation of glycans, purification, derivatization and often labeling [100], which make the overall assay tedious and time-consuming. Therefore, often the complete analysis of the sample requires several days. On the other hand, despite on these difficulties, recent advances in sample preparation and carboohydrate modification made sufficient improvements in isolation, identification and quantification of glycans and their conjugates using chromatographic techniques.

Isolation, fractionation and characterisation of glycans or their conjugates can be successfully performed using lectin affinity chromatography [101]. In this format of chromatography a buffer consisting of a mixture of glycans passes through the column containing the immobilised lectin. Different lectins are able to specifically recognise distinct oligosaccharide epitopes in the structure of the analysed molecule. The glycans elution profile gives information about ligand binding specificity and interaction strength. The separation can be facilitated if the column containing several immobilised lectins with different carbohydrate specificities (multi-lectin column) is utilised. In this case the entire pool of glycoproteins can be recognised and characterised [102,103]. Although lectin affinity chromatography is simple, this technique provides only a limited information about the binding affinity, for instance, it is difficult to deduce the values of association and dissociation constants (K_A and K_D), especially if multiple-ligand column is used.

Frontal affinity chromatography (FAC) is more efficient and sophisticated way for measuring and assessment of the lectin–carbohydrate interactions [104,105]. In FAC the known amount of glycan labeled with fluorescent tag is applied to the column containing the immobilised lectin, and the elution of the carbohydrate from the column is monitored. The eluted volume is a measure of the binding affinity. The inversed mode, where lectins are applied to the column with the attached glycans is also possible. The latter approach requires only a few micrograms of protein for each analysis. FAC is a powerful, accurate, convenient and highly sensitive approach capable of detection of picomoles of oligosaccharides [106–108]. This technique is simple, does not require the use of sophisticated equipment and involvement of skilled personnel. FAC is suitable for the characterisation even weak bindings. If the binding affinity is high, the assay requires a very low amount of analyte. Conversely, if the binding affinity is low, the relatively large amounts of analyte may be needed. The important issue is the stability of lectin immobilised on the column matrix. In this case the protein retains sufficient binding affinity for many column runs. The drawback of FAC is that many different column runs must be made in order to perform the complete analysis of the glycan of interest from a complex sample containing several different saccharides. Thereby, the assay may take weeks to months.

5.2. Capillary electrophoresis for the separation and quantification of glycans and glycan-containing molecules

Electromigrative separation techniques provide high-speed separations, high resolution and nowadays are frequently applied for glycoforms analysis. The separation of glycans is based on differences in their effective electrophoretic mobility in the separation medium under the applied external voltage. Several types of electrophoretic techniques such as capillary zone electrophoresis, micellar electrokinetic chromatography, gel electrophoresis and capillary electrochromatography have been utilised for glycans and glycoconjugates separation [109–119]. Despite on that capillary electrophoresis (CE) provides good resolution, rapidity and allows carrying out measurements with little sample consumption, this technique has a number of difficulties in terms of the glycoconjugates assay. For instance, in contrast to HPLC, which are capable of separation both charged and neutral compounds, only ionic or ionizable species can differentially migrate under an applied electric field and, thus, be separated by CE. At the same time, most of the glycans are not readily ionizable, which hinders the application of CE for their separation. Although, as an alternative, neutral compounds can be separated by micellar electrokinetic chromatography, the application of this technique is limited because of the hydrophilic nature of carbohydrates.

Carbohydrates mainly lack the chromophores, fluorophores and, in general, are electrochemically inactive. Therefore, derivatization or labeling of glycans is required in order to make them distinguishable by the detectors, such as optical or electrochemical, which are usually coupled with the CE set up [75]. In order to be able to unravel the chemical composition of the analysed molecules, electrophoretic separation can be hyphenated with mass spectrometric detection (CE-MS). CE-MS forms a powerful tandem technique, which provides the information about the chemical composition of the carbohydrates following after the separation of the glycans [114,116]. In return, the essential contribution of CE in this tandem is in its capability to resolve isomers, which are otherwise indistinguishable by merely MS. In addition, CE-MS is highly sensitive technique and is suitable for the quantification of nanograms of glycan.

5.3. Mass spectrometry for the analysis of carbohydrates and glycoconjugates

Mass spectrometry is a powerful technique suitable for both the structural characterisation and quantification even a minute amounts of glycans (lower fmol range) [120,121]. MS is a useful technique for analysis of even complex glycan mixtures. Different MS techniques, including, electrospray ionisation mass spectrometry (ESI-MS) [122–126] and tandem MS/MS [124,127,128] sequencing can be used for the thorough analysis of the biological samples and can bring insight into the structures of the individual glycans. Indeed, the special contribution is made by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF), applicability of which for the analysis of carbohydrates and glycoconjugates is illustrated in a number of papers and vast and exhaustive reviews [122–124,129–135].

High sensitivity and efficiency allow MS to be utilised for measuring the changes in proteins glycosylation [136–138], though it is possessed of a number of limitations, and study of protein glycosylation by MS still remains to be a challenging task [139]. First, rapidity and dynamics of the glycosylation process complicate obtaining of reproducible results. Another limitation is a poor resolution of the peaks. For instance, MALDI-TOF provides a good resolution of individual glycoforms only for small proteins (~40 kDa) containing a limited number of glycans. The measurements of larger glycoproteins and those containing multiple glycosylation sites usually yield broad and unresolved peaks, and therefore give information only about the average carbohydrate content. It occurs due to microheterogeneity, the phenomenon when one peptide can be glycosylated by different carbohydrates and each of them has its own mass and structure. In addition, many biological samples contain the mixtures of isomeric oligosaccharides of different structures but the same mass, which hinders of obtaining the well-resolved peaks. Luckily, this problem may be partially solved by separation and isolation of the isomeric glycoforms on the reversed-phase or normal-phase HPLC column prior the MS detection [97,140–142]. Third limitation of utilising MS for the analysis of glycoconjugates is attributed to their lower

ionisation efficiency in contrast, for instance, to proteins. Fortunately, certain problems and artifacts during the assay, such as the ones associated with formation of droplets in ESI-MS, can be significantly reduced with application of the nanospray ESI technique [143].

5.4. Application of flow cytometry for the quantification of glycans

Flow cytometry can be used as an alternative method to study the lectin–glycan interactions [144,145]. The approach described by Yamamoto et al. is based on the immobilisation of the biotinylated glycoproteins on streptavidin microsphere beads followed by their mixing with various amounts of fluorophore-labeled lectins [145]. The biospecific binding is detected and quantified by measuring the fluorescence signal by a photomultiplier tube. Although, this strategy is simple, rapid, reproducible and suitable for the measurement of minute samples, probe labeling, high price of the equipment and requirement of highly-trained personnel limit the application of flow cytometry for the particular purpose of glycan analysis.

5.5. Atomic force microscopy for probing the glycan-containing molecules and study the glycan–protein interactions

Atomic force microscopy (AFM) is a very high-resolution scanning probe microscopy, with resolution on the order of fractions of a nanometer. It is sufficiently powerful to be used for imaging the structures of various surfaces and is capable of providing a three-dimensional surface profile. The ability of AFM to operate well in ambient air or liquid environment makes it possible to be applied for study biological molecules and even living cells.

A few attempts of studying lectin–glycan interactions were made by functionalising of an AFM tip with different types of lectins [146,147]. The obtained results have indicated the possibility to differentiate the oligosaccharide moieties on the surfaces of tumor and reference (healthy) cells. One of the major drawbacks of AFM, however, is its relatively low imaging speed. Usually several minutes are required for a typical scan. This makes it impossible to perform the real-time imaging of the dynamic processes, such as protein glycosylation, on a molecular scale. In addition, the application of AFM may not be the optimal and adequate choice for routine analyses of carbohydrates because of the high price of the apparatus and requirement of high-skilled personnel.

5.6. Nuclear magnetic resonance spectroscopy for the determination of glycans structure

Nuclear magnetic resonance (NMR) spectroscopy is a promising technique for the structural analysis of carbohydrates [148–150], the dynamics of the glycan termini of the glycoproteins [151] and the linkage information in biological samples [152,153]. With using the NMR spectrometers equipped with very sensitive probes, ^{1}H NMR profiling of 2–5 nmol of glycans is possible. The measurements can be perform by a combination of two-dimensional NMR techniques such as correlation spectroscopy (COSY) and total correlation spectroscopy (TOCSY) for ^{1}H, which allows assignment of the ^{1}H signals of individual monosaccharide residues. In order to obtain the complete picture of the glycan structure both ^{1}H and ^{13}C NMR spectra should be obtained.

NMR study of glycopeptide–lectin interaction has been demonstrated on lectin *Vicia villosa* and a-D-GalNAc glycosylated β^3-peptides [154]. The comparison of differences in binding the natural a-glycopeptide, Tn antigen and unnatural β^3-glycopeptide by lectin has been shown. The NMR study has indicated that lectin does not distinguish the glycosylated α- and β^3-peptides. The approach seems to be capable of identifying which part of the glycoform is in contact with lectin and to estimate the interaction affinity.

Despite of the powerful possibilities of NMR, the application of this technique for the routine glycan analysis is limited by the high cost of the equipment and the requirement of the high level expertise in order to interpret the NMR spectra. The other limitations are attributed to the poor dispersion of signal obtained from the similar sized molecules and overlapping of resonances, which hampers the data interpretation. The inter-unit linkages in carbohydrates provide a conformational flexibility, which also results in broadening of resonances.

Because of the structural analysis of glycans and glycoconjugates is a complicated and challenging task, in order to obtain more reliable results and to facilitate their interpretation, it is advisable to combine the isolation of glycans by HPLC or CE with their structural analysis using several different techniques. For instance, a combination of such powerful detecting techniques as NMR and MS for the analysis of the separated glycans has a great potential, as it strengthens the efficacy of the assay and is capable of giving the thorough information about the structures of the analysed carbohydrates.

5.7. Application of quartz crystal microbalance for study of glycan-specific interactions

Quartz crystal microbalance (QCM) is one of the label-free techniques successfully applied to detect macromolecules (proteins, polymers), to study surface adsorption and affinity interactions. This technique is capable of detecting the real-time mass changes of a quartz crystal by measuring the change of its frequency when the biospecific binding occurs on the crystal surface [155]. Study and monitoring the interactions between lectins and different glycoforms when one of the partners is fixed on the surface of a quartz crystal immersed in the solution containing the other partner were described elsewhere [156–160].

Modern QCM instruments, due to their capability of simultaneous measurement both frequency and dissipation changes (QCM-D), allow obtaining additional insights into the structural properties and rigidness of the biocomplex formed on the surface of a quartz crystal [157,159]. The limitation of QCM is that this technique requires relatively large volumes of the sample due to the relatively large surface area and working cell volume. Moreover, it may be difficult to obtain a uniformly functionalised surface when the surface area increases. In order to obtain the information about the affinity and binding kinetics, the sample solution must enter and leave the sensing area with minimum dispersion. This can be achieved when QCM crystal is integrated with microfluidic system and the small sample volume is used [161], although, this can not always be easily implemented.

Surface plasmon resonance described in the next two sections does not suffer from these limitations and drawbacks. It allows a label-free real-time detection of small amounts of sample, it can be easily integrated with microfluidic device and is capable of providing the information about the kinetics and the affinity of the biospecific interaction.

6. Surface plasmon resonance: working principle and approaches

Surface plasmon resonance monitors biospecific interactions, which occur on a surface of a metal layer between the immobilised ligand and the free analyte, by measuring changes in resonance angle due to an increase of the mass concentration of the analyte

in the vicinity of surface. SPR phenomenon is described elsewhere [162]. In this section a brief explanation of the SPR phenomenon and 2 major SPR techniques, flow SPR and SPR imaging, are given.

When a beam of light with certain angle of incidence (θ) propagates from a material with a high refractive index (e.g., glass) into a material with a low refractive index (e.g., water) the light is refracted towards the interface. At higher angles of incidence all the light is reflected inside the medium of higher refractive index, and total internal reflection occurs. If the surface of the glass is coated with a thin film of metal, usually gold (Kretschmann configuration), this reflection is not total. The part of the reflected light is transformed to an electric field called evanescent wave, which passes into the lower refractive index medium (Fig. 2A). The wave penetrates beyond the reflecting surface to about a few hundreds nm, interacts with the surface mobile electrons in the metal film called surface plasmons (Fig. 2A), and decays exponentially with the distance from the metal surface. The interaction with surface plasmons causes their oscillation. This results in energy transfer and the light no longer being reflected from the surface. At a certain incidence angle the momentum of the incoming photons matches the momentum of the surface plasmons, and the electrons "resonate"; this gave a name to the phenomenon of surface plasmon resonance. This certain angle, at which surface plasmon resonance occurs is called resonant angle. SPR is seen as a dip in the intensity of the reflected light at a specific angle of reflection (Fig. 2B). When the chemical composition of the medium near to the gold film changes, the refractive index in the vicinity of the surface also changes; this leads to a shift of the reflected angle (Fig. 2A and B, characteristic shift from angle I to angle II—$\Delta\theta$). The signal is recorded by the optical detector.

The physical principles of SPR are complex and can be described by the mathematics and quantum physics. Luckily, the detailed knowledge of the theoretical background of the SPR phenomenon is not required in order to hold the sufficient operating skills with modern SPR analysers [163].

Since the first biosensor based on SPR was developed almost two decades ago, the application of this technique has increased rapidly, from surface studies to bioanalytical applications [162–164]. SPR-based instruments use an optical method to measure the refractive index near a sensor surface (within ~300 nm). Although there are several SPR-based instruments available, so far Biacore analyser produced by Biacore AB (Sweden) is the most widely used one [165].

In a conventional flow SPR performed by the Biacore family instruments the capturing molecule (ligand) is immobilised on the flow channel of the microfluidic sensor chip. The free analyte is injected into the aqueous solution (sample buffer, running buffer) through this channel, under continuous flow (Fig. 2A). The binding of the analyte to the immobilised ligand causes accumulation of the analyte molecules on the sensor surface. The surface plasmon resonance conditions are influenced by the material accumulated onto the gold film. Thereby, when adsorption or specific interaction between the analyte X and the immobilised ligand L, i.e. association process, takes place on the sensor surface, this results in an increase of refractive index in the vicinity of the sensor surface and in a shift of the resonance angle (Fig. 2C). This change in refractive index measured in real time and plotted as response in resonance units (RU) versus time is called a sensorgram (Fig. 2C). For most biological molecules the shift in the refractive index is proportional to the mass of the adsorbed (bound) analyte. Dissociation of an analyte from the formed biocomplex [X-L] leads to a decrease of the concentration of X in the vicinity of the sensor surface and, as a result, in a decrease of the SPR response (Fig. 2C) [166].

Biacore family instruments were primarily intended for the investigation of protein–protein interactions. Nevertheless, it can be applied for the real-time monitoring of biospecific interactions between any compounds with relatively high molecular weight including interactions of glycoproteins and glycolipids with their receptors.

The SPR imaging (SPRi, "SPR microscopy") takes the SPR analysis a step further. SPRi combines the sensitivity of scanning angle SPR measurements with the capability of providing the spatial imaging [167]. SPRi allows studying simultaneously multiple different interactions on an array of the precisely patterned molecules (Fig. 3). Due to such design of the sensor chip in SPRi the entire biosensing surface is imaged in real time by collecting the light reflected from the gold surface, and is visualised by a high resolution CCD camera. High throughput, sensitivity and obtaining the spatially resolved images of biointeractions open up great future for the SPRi to be applied in clinical chemistry and medicine for screening of biomarkers and therapeutic targets.

7. Application of SPR for the detection of carbohydrate-containing molecules and study the carbohydrate-specific bindings

In this section the selected examples of the applications of SPR techniques like conventional flow SPR and SPR imaging for the measurement of carbohydrate-specific interactions and analysis of glycan-containing molecules will be discussed.

7.1. Study the carbohydrate-specific interactions and the detection of carbohydrate-containing molecules using flow SPR

A study of carbohydrate–lectin bindings and SPR measurement of serum glycoproteins was carried out using a created lectin panel and Biacore 3000 analyser [168]. Six lectins with different carbohydrate specificity were immobilised on the surfaces of the four-channel microfluidic sensor chips. Three channels of each sensor chip contained lectins covalently immobilised on the carboxymethyl dextran-based surface using the well-defined standard amine coupling procedure, and one channel with no immobilisation was used as a reference. A study of three different lectin–GP interactions simultaneously at one sample injection was possible. The lectin panel showed a selective glycan recognition pattern and was capable of distinguishing the glycoproteins with similar glycan structure, such as fetuin and asialofetuin. It should be mentioned that the obtained results are in good agreement with study on a similar theme using quartz crystal microbalance [157]. Due to the optimised regeneration conditions the same sensor chip could be re-used several times without deterioration of the binding affinity of the immobilised lectins; it reduces the total cost of the analysis. The developed approach allows not only the rapid screening of serum GPs but is also capable of detecting them with sufficient sensitivity at the concentration of 0.01 mg mL^{-1}, and of estimating the affinity binding constants and the rate constants of lectin–GP interactions.

Foley et al. demonstrated a high resolution differential SPR method for the detection of carbohydrates in sub-nanomolar concentrations and study of carbohydrate-specific bindings under conditions close to the biological ones [169]. High resolution SPR measured the difference in SPR signals obtained from the sensing and reference areas. The sensing areas of a chip containing carboxymethylated dextran were individually modified by covalent immobilisation of anti-PNA, anti-IgG antibodies and BSA. First, lectin PNA and IgG injected into the flow system were bound by their specific antibodies. After that, the disaccharides (T-antigen and lactosamine) were injected and their binding to PNA was measured. The sensing surface modified with anti-IgG and IgG served as a reference in order to control the possible non-specific binding.

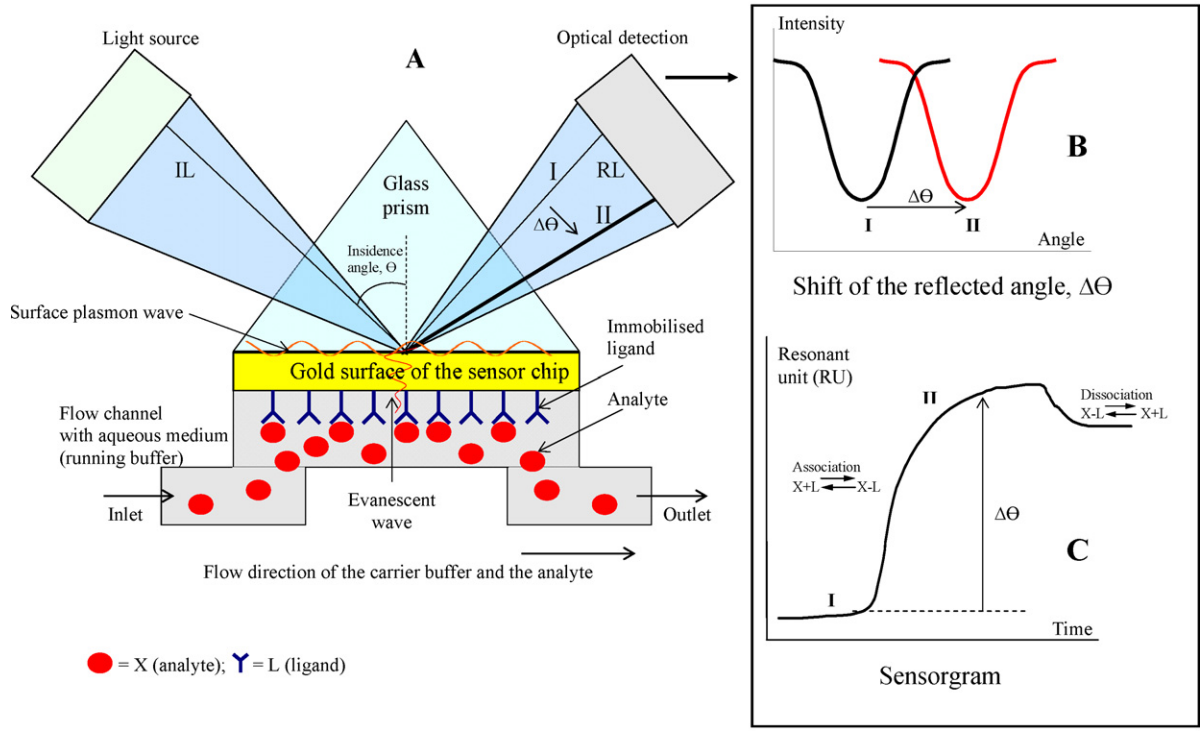

Fig. 2. A schematic illustration of SPR phenomenon and the experimental set up for the flow SPR. (A) Incident light (IL) from the light source is directed on the glass prism coated with thin layer of gold under the angle θ. The part of the reflected light (RL) is transformed to an evanescent wave. The evanescent wave transfers the energy to the surface plasmons. At certain θ the momentum of the IL matches the momentum of the surface plasmons, and the SPR occurs. (B) SPR is seen as a dip in the intensity of the reflected light at a specific angle of reflection. This angle shifts from position I to position II (by $\Delta\theta$), when the chemical composition of aqueous medium near the gold film changes. (A)+(C) One side of the gold film with the immobilised ligand (L) is integrated with microfluidic flow channel; the analyte X is passing through the channel under continuous flow. Binding of the free X to the immobilised L (association) leads to a proportional increase in a reflected angle (shift by $\Delta\theta$). Dissociation of the biocomplex [XL] leads to a decrease in a reflected angle. The changes are plotted as a sensorgram (resonance units (RU) *vs.* time).

In the described approach, the increased sensitivity of the assay can be achieved due to the subtraction of a non-specific adsorption and noise.

A lectin-based sandwich-type immunoassay with SPR detection was developed in order to study the human chrorionic gonadotropin glycosylation patterns [170]. The aberrant glycosylation of HCG may be used for the diagnosis and monitoring both the pregnancy complications and certain types of tumors. Thus, the urine samples taken from the patients with normal pregnancy and from the ones which have different types of tumor were analysed using SPR. Carbohydrate-free antibody fragments covalently immobilised on the surface of the Biacore sensor chip were used to capture HCG. The samples containing HCG with different glycosylation patterns and later individual lectins were injected into the flow system. HCG in the injected urine samples bound to the immobilised antibodies and remained associated. Lectins were screened for their ability to bind the carbohydrate moieties of HCG on the HCG–antibody complex. The tested lectins exhibited an increased affinity to the glycosylated HCG at different pregnancy complications, as it led to an increase of the shift of the resonance

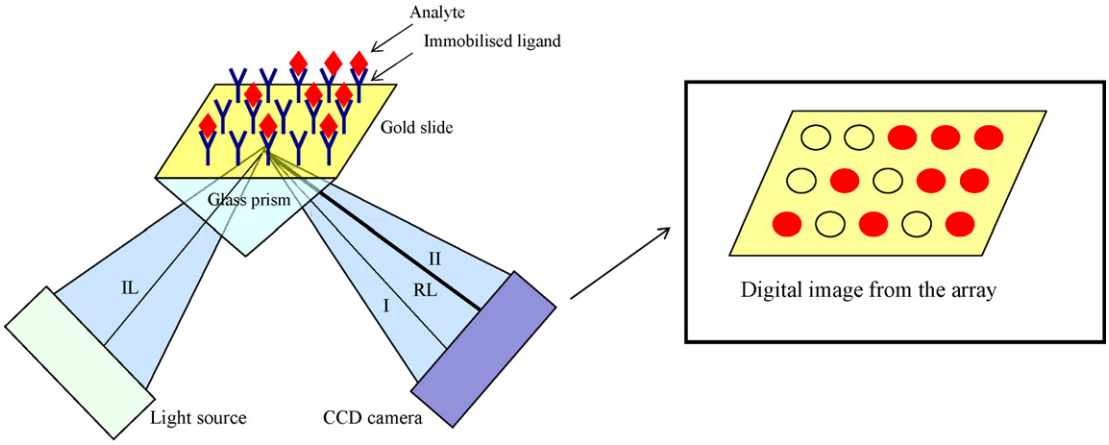

Fig. 3. A schematic illustration of the set-up for the SPR imaging. The molecules of ligand Y are immobilised in an array format on the surface of the gold slide attached to the glass prism. The incident light (IL) from the light source is directed on the prism. The binding of Y to the analyte ◊ causes the shift of the angle of the reflected light (RL) from position I to position II. No characteristic shift is observed at the absence of binding. Digital image from the array is recorded by CCD camera.

frequency. At the same time, the injection of a control sample (taken from non-pregnant woman) did not result in any increase of the resonance frequency shift. The obtained results showed that five out of eight lectins recognised neutral sugars and the other three discriminated sialic acid. Authors claim that as a biological sample (serum) contains a myriad of different glycoproteins and other biomolecules, the simplified approach based only on interaction of HCG with lectin would not have had an appropriate selectivity. The described sandwich-type immunoassay with SPR detection based on the capturing of HCG by the antibody fragment followed by probing with lectin improves the selectivity of HCG detection. Although the overall approach definitely may have potential to be established as a label-free fast screening of biomarkers, more thorough investigations with increased lectin panel need to be carried out in order to obtain statistically representative data.

The approach reversed to the ones described above was proposed in [171–173]. In this approach, the polysaccharides (starch, mannan) [172] or glycopeptides [173] were immobilised on the surface of the sensor chip and lectins, conversely, were injected into the flow system followed by SPR measurement of the specific lectin-glycoform bindings.

The carbohydrate–lectin interaction between a multivalent glycodendrimer and ConA was evaluated using SPR technique [171]. The gold sensing surface was functionalised with mannose derivatives and binding to the injected lectin was detected by measuring the resonance frequency shift. The multivalent glycodendrimers injected next acted as mannose competitors for the ConA binding sites. SPR experiments showed that glycodendrimers efficiently inhibit ConA-mannose interaction and demonstrate higher binding affinity due to the presence of multivalent binding sites. Further developments of such assay format may significantly contribute in understanding of protein–carbohydrate interaction, elucidating biological pathways and cellular mechanisms and, therefore, may be helpful in designing new therapeutic agents for human health.

The described "reversed" approach has both advantages and disadvantages. On the one hand, lectins, while binding to the immobilised carbohydrates, cause larger shifts of the resonance frequency due to their high molecular weight (50–170 kDa) and higher mass concentration accumulated on the sensing surface. Hence, the enhanced sensitivity of the detection can be expected and achieved. This approach may be applied in clinical studies both for the screening and quantification of low concentrations of lectins in physiological samples. On the other hand, carbohydrates lack of functional groups capable of binding directly to the surface of the sensor chip. Hence, additional, often time-consuming, modification of the sensing surface using linkers (spacers) or derivatization of carbohydrates is required. It also may be difficult to achieve a high selectivity of the detection due to the non-specific adsorption of sample components on the functionalised surfaces. Luckily, the problem of interferences and unwanted non-specific binding can be partly solved using a multichannel sensor chip where different carbohydrates are immobilised on the separated channels and one channel containing no immobilised ligand is used as a blank. In addition, it is possible to couple the SPR analyser (Biacore) with mass spectrometer, which described in the next sub-section. This will increase both efficiency and versatility of the technique and will make it possible to resolve the molecular structures of the analytes in complex mixtures.

The affinity interactions between different peptidoglycans with the peptydoglycan recognition proteins (PGRP-Iα and PGRP-S) using surface plasmon resonance were studied in real-time mode [174,175]. PGRPs were immobilised on the surface of the sensor chip and PpGs were used as analytes. The obtained sensorgrams were normalised and K_d were calculated. The obtained values of K_d on the level of μM–nM showed rather high binding affinity. This approach can be used to carry out screening of novel therapeutic agents (e.g., antibiotics) and has potential to be applied for fundamental studies of cellular bioprocesses.

An assessment of the glycolipid–lectin interaction involving ConA-functionalised surfaces was conducted by means of the SPR technique [176]. In this assay format the GL micelles were used as analytes. GL micelles–Con A interactions were found to fit in a bivalent analyte model, in which the micelle would bind to two receptor sites independently. This work showed that SPR technique enables not only to evaluate the biospecific interactions in a real-time mode but also to obtain the detailed information about the mechanism of the interaction such as defining valency, which is difficult with other analytical techniques (e.g., with affinity chromatography).

Many pathogenic bacteria develop infections after adhering to host surfaces due to their specific surface structures. Although several mechanisms of the interaction of bacteria with mammalian cells and virulence factors have been proposed, there is still no good understanding of the occasional transition from usually benign commensal to deadly pathogen. Many of molecules-targets have been characterised as belonging to the family of proteoglycans. For better understanding the mechanism of adhesion of bacteria to the host cell, the interaction between the bacterial glycolipid from *E. faecalis* and biotinylated glycosaminoglycans immobilised on a streptavidin-modified chip was measured by SPR [177]. GLs from the bacterial culture *E. faecalis*, first isolated by preparative layer chromatography, were probed on the functionalysed SPR sensing surfaces to create the GL-GAG binding patterns. This study has significant theoretical and practical potential, since this SPR approach makes the quick label-free screening of bacterial toxins possible. Due to its capability of measuring the binding affinity and the kinetics of the interaction, SPR may be an efficient tool for the development and testing of new antibacterial therapeutic compounds. For instance, in this format a drug can compete with GAGs for the binding sites of the bacterial GLs, so the assessment of the binding affinity and kinetics can help in design of new pharmaceuticals with high antibacterial efficacy. To date, we may hope that intensive research involving SPR will help us to fully understand the mechanism of interaction between the surface bacterial GLs and the carbohydrates of the host tissues, which will be a significant step for the development of new strategies of prevention and treatment of bacterial infections.

Although, in general, in terms of sensitivity SPR is restricted to the study of molecules larger than 5 kDa, recent improvements in SPR instrumentation have enabled detection of small molecules, such as oligosaccharides and even monosaccharides. First attempt on glucose (monosaccharide) detection using Biacore analyser was demonstrated by Hsieh et al. [178]. Authors investigated the affinity of Glc to the glucose/galactose-binding protein (GGBP) and its various mutants covalently attached to the sensor surface. It was reported that the GGBP protein has a high affinity to D-glucose, long term stability, and therefore is suitable to measure D-glucose at the low concentrations in biological fluids [179,180]. The obtained data have shown that despite on the low molecular weight of glucose (180 Da) its relatively small concentration of 0.1 mM can be detected with good reproducibility using SPR. On the one hand, the linear concentration range obtained using this approach was considerably narrower that the physiological range of glucose (1–30 mM). Authors considered that the problem of the narrow concentration range can be solved by modifications of GGBP, for instance by obtaining new mutants, and optimisation of the working conditions. On the other hand, the described approach made it possible to measure the lowest physiological glucose concentrations. This SPR approach can be especially useful for diagnosis of hypoglycemia (low blood glucose), since the widely applied electrochemical assay of glucose using enzyme biosensors may have limited accuracy at low glucose levels [181]. The advantage of the described SPR approach is that, in contrary to the most

other glucose detecting devices [182,183], no enzymes (glucose oxidase or glucose dehydrogenase) are needed. At the same time, glucose oxidase is a commercially available enzyme while GGBP has to be harvested from the grown bacterial culture, isolated and further purified purposefully. Furthermore, it is well-known that enzymatic conversion of glucose by glucose oxidase is a highly specific reaction, while GGBP used in this work has affinity to both glucose and galactose. Therefore, galactose may act as a possible interfering compound, which has to be eliminated or masked. This additional sample pretreatment step may limit applicability of the described approach. Last but not least important moment is that the assay of glucose using commercially available biosensors (test strips) is incomparably cheaper, faster and more convenient than the one performed using the expensive and bulky Biacore analyser.

Until recently high-throughput analysis of glycans using the flow SPR has been challenging, since multiple parallel measurements have not been possible. However, very recent advances in the instrument developments achieved by Bio-Rad allow the construction of the SPR analyser capable of measuring up to 36 ligand–analyte interactions simultaneously in a 6×6 array format, and thereby greatly improve throughput of the flow SPR analysis [184].

7.2. SPR imaging as a tool for the fast screening of glycoconjugates and monitoring of the carbohydrate-specific bindings

Contrary to the flow SPR, SPRi technique provides sensitive and fast imaging of tens and even hundreds of biointeractions simultaneously within one small-sized array.

Lectin recognition was evaluated by SPR imaging using a model carbohydrate microarray containing forty 150 μm diameter spots of biotinylated Man, Gal and GlcNAc derivatives together with negative control printed onto neutravidin-coated gold chips [185]. Affinity binding data could be obtained with as little as 10–20 μg of lectin per experiment. The experiments have shown the ability to assess the carbohydrate selectivity of proteins without prior purification. The obtained results of SPR imaging were in agreement with those obtained from fluorescence based carbohydrate arrays but with the added advantage of label-free analysis.

A study of the interaction between the tumor biomarker mucin and the immobilised anti-mucin antibodies using home-made SPRi device was conducted by Fernandez-Gonzalez et al. [186]. The device allowed the measurement of hundreds of samples at a time with using a microarray of anti-mucin antibodies immobilised on the gold surface by covalent binding or physical adsorption. Successful discrimination between the samples taken from healthy and unhealthy patients was achieved. This opens up possibilities for this approach to be applied for fast and inexpensive screening of biological samples.

Coupling of microfluidic device with SPRi for study the lectin–glycoprotein binding on a model system *Datura stramonium* agglutinin and asialofetuin was described by Grasso et al. [187]. The presence of several parallel microchannels on the same sensing surface allowed in-line referencing in order to control possible non-specific binding. Kinetic and affinity parameters of the biospecific interaction were determined; the obtained results confirmed the suitability of this SPRi device for the real-time kinetic measurements.

An interesting example of the application of SPRi for the differentiation of glycoproteins and proteins was demonstrated by Liu et al. [188]. Glycoproteins ovalbumin (OA) and IgG were used as model components while proteins lysozyme (LYZ) and bovine serum albumin (BSA) were used as controls. The biomolecules were precisely deposited on the thiolised gold dots using a microprinting pin and let covalently bind to the modified surface. Then the immobilised molecules were allowed to react with 200 nM of lectin ConA and the produced resonance image signals were recorded by CCD camera. It was shown that glycoproteins OA and IgG produce higher signals after interaction with ConA than non-glycoproteins LYZ and BSA. Moreover, the signal from OA was stronger than the one obtained after incubation of IgG with ConA. That was explained by the fact that OA contains more mannose residues which can specifically bind to more ConA molecules. The small area of the printed protein spots (100 μm in diameter each, 50 nL) allows to create the larger proteins array and, therefore, to increase the number of potential analytes. Concentration of 10 $\mu g\,mL^{-1}$ of OA and IgG can be detected, recognised and differentiated by reaction with ConA. Minimum measurable sample amount using this approach was 5 pg. Hence, it has potential to become a tool for both high-throughput label-free screening and sensitive analysis of GPs. The described design of the sensor chip allows easy referencing with negative control in order to check the non-specific adsorption. The efficiency of this approach was only demonstrated using ConA, however, expanding the library of lectins allows mapping more glycoproteins. The described approach is highly versatile and can be applied for the determination of not only glycoproteins, for instance screening the presence of tumor biomarkers, but other glycoforms as well.

In summary, from the *Sections 3-5 and 7* it can be deduced that conventional instrumental techniques for the characterisation and quantification of glycans and glycoconjugates like lectin-based and carbohydrate microarrays, HPLC, CE, MS, flow cytometry, due to the isolation, enrichment, derivatization and labeling steps, are often laborious, time-consuming and require trained personnel. Although AFM and NMR are the label-free techniques, their applicability for the fast screening and routine assays of glycoconjugates is restricted because of the slow imaging speed (AFM), high cost of the equipment and the requirement of the high level expertise in order to interpret the obtained results. Hence, the development of novel and application of the existing label-free analytical techniques like quartz crystal microbalance [156–160] and surface plasmon resonance [168,178], which are more user-friendly, have promising future for the analysis of carbohydrate-containing molecules.

Both QCM and SPR are sensitive label-free detection techniques, which have found numerous applications in biosensors development, surface adsorption and bioaffinity studies. Contrary to QCM, uniform coating of the functionalised SPR surface can be achieved due to the relatively small working area of the microfluidic sensor chip (in Biacore family instruments) and precise deposition of the sample on the sensor surface using the robotic microdispensers (in SPRi). In addition, small areas of the microfluidic paths and small sample volumes used in SPR minimise the dispersion of the injected sample. That makes SPR a perfect technique for measuring the reaction rate constants, from which K_a and K_d can be deduced. For most biosensor applications or affinity studies, it is highly desirable to have two detection channels, where one channel is used for the sample analysis and the other channel is used as a reference to control the non-specific adsorption. Microfluidic technology used for the fabrication of the SPR sensor chips enables to create at the same surface two practically identical channels with high uniformity and reproducibility. In case of QCM referencing may be achieved by using 2 separate QCM chips, which is possible with using, for instance, multichannel QCM-D E4 analyser. However, this case has several limitations, because for high uniformity, reproducibility and proper referencing the two QCM crystals must be perfectly identical and the delivery of sample solution to both sensors must be identical. Although QCM and SPR investigations involving glycoproteins have reported that, in fact, SPR has no or small advantage over QCM in terms of sensitivity of the detection [157,168,189,190], SPR has definite benefits in being better for kinetic and affinity studies, in consumption less sample volume for obtaining the response (5 $\mu L\,min^{-1}$ injected for 40 s in SPR

vs. 50 μL min^{-1} injected for 2 h in QCM) [157,168]), and in faster response [157,168,190].

The advantages, disadvantages and analytical performances of selected modern instrumental analytical techniques for the detection and quantification of glycan-containing molecules are summarised in Table 3.

7.3. Hyphenation of SPR with other analytical techniques for unraveling the nature and chemical composition of the glycan-containing molecules

Although SPR allows the sensitive direct label-free detection of the carbohydrates and study of biomolecular interactions, it does not provide any structural data about the interacting species. At the same time, SPR is non-destructive technique, thereby same sample after the SPR measurement can be further characterised by a complementary technique. This provides additional insights into the chemical and structural composition of the analyte. In this sub-section a few examples of coupling SPR with conventional analytical techniques are described.

A tandem technique, which couples the SPR monitoring of the captured molecules with their HPLC profiling, was developed by Gallego et al. for the real-time ligand fishing of oligosaccharides [192]. Mannose or fucose binding lectins were immobilised on the surface of the carboxymethylated dextran-coated SPR sensor chip. A mixture of fluorescently labeled N-glycans was injected across the lectin containing surface at a continuous flow, the biospecific binding was monitored by SPR, and the eluted fraction of the unbound glycans was collected. After regeneration of the SPR sensor chip the effluents of the surface bound glycans were collected, lyophilized and profiled on the normal-phase HPLC with fluorescence detection. Although the approach may look rather complex and time-consuming, it has some important and useful advantages. It can be used both for obtaining the information about the binding affinity and for characterisation the carbohydrate molecules from the unknown sample. This approach also allows selecting a glycan with a particular structure even if it is present as a minor constituent in a given mixture. In addition, this approach may be useful for the detection and characterisation of unknown carbohydrate-binding molecules, present at the surface of intact microorganisms. The detection is highly sensitive, so femtomoles of glycans can be quantified. At last, it is possible to equip the system with an in-line coupled mass spectrometer and to obtain the detailed structural information about the analysed molecules.

Combination of SPR with mass spectrometry offers unique capabilities in terms of not only the quantitative and qualitative analysis of the sample but also of gaining the exhaustive structural information about the tested analyte through the measurement of it molecular weight and fragmentation patterns [193,194]. In tandem SPR-MS approach molecules are initially captured from the solutions by ligands covalently immobilised on the SPR sensor surface followed by their further analysis by MS. Two strategies of integrating the sensor chip with MS instrument are possible. The first strategy is based on the collection of the analyte eluted from the recovered (regenerated) SPR sensor chip and directing it into mass spectrometer [193]. This approach is convenient to use when the SPR sensor chip is integrated to a flow system (e.g., Biacore family instruments). At the same time, this approach is time consuming, the sample losses and contamination may occur between several washing steps, and the quantitative elution may be difficult in the case of high-affinity binding.

Another approach is based on MALDI directly performed on the SPRi chip after capturing the analyte of interest. After the analyte bound to the sensing surface the SPRi-slide is inserted directly in an appropriate MALDI-MS plate holder. Finally MALDI matrix is dropped on each localised spot and mass spectrometry measurements are performed. Bellon with co-workers [195] developed a hyphenated SPR-MALDI technique for the detection of glycoproteins β-lactoglobulin and ovalbumin directly from the biochip modified with covalently attached antibodies. Femtomole amounts of specifically bound analytes determined by SPR were sufficient to obtain good quality mass spectra. This strategy has a number of advantages in contrast to the former approach. First, the array format of the sensor chip allows carrying out the fast screening of multiple interactions, in contrast to the conventional flow SPR, which enables to monitor from one to four interactions at a time (Biacore). Second, the sensor chip can be easily transferred to MALDI support, allowing both direct SPR and MALDI-MS measurement from the same surface, which minimises sample contamination and sample loss. Third, due to the sensor chip design spatially resolved SPR and MS measurements can be performed.

Coupling SPR with electrochemistry (EC-SPR) [196,197] measures changes in the optical properties of an electrode surface or molecules adsorbed on the electrode. EC-SPR provides additional insights into various electrochemical phenomena and processes taking place at the solid–liquid interfaces. Hence, the metallic layer (gold sensor chip), on the surface of which the biospecific interaction occurs, can be at the same time used as a working electrode for the electrochemical investigations. In comparison with the conventional current-based electrochemical techniques, EC-SPR is particularly attractive for studying heterogeneous electrochemical reactions occurring on electrode surfaces in a combination with SPR monitoring of changes in optical properties of the reactions species.

Gondran with co-workers developed EC-SPR for the measurement of lectins specificity [198]. In this work the alternative approach of rapid modification of the SPR sensing surface and immobilisation of the biomolecules was shown. Initially, the gold SPR sensor surface was modified by the in situ electropolymerisation of the pyrrole derivatives of carbohydrates (lactose and 39-sialyllactose) at a constant potential of 0.95 V *vs.* Ag/AgCl during 40–120 s. Then, the specificity of the functionalysed sensing surfaces gold/polyppyrrole-lactosyl (39-sialyllactosyl) towards lectins PNA and MAA was investigated using SPR. Kinetic analysis of the SPR data allowed determination of the equilibrium dissociation constants of the lectin-carbohydrate complexes. The sufficient detection limits of 41 nM for PNA and 83 nM for MAA were achieved.

An interesting version of the miniaturised EC-SPR on-chip biosensor was proposed by Nakamoto et al. [199,200]. The biosensor consisted of two gold films both integrated into the PDMS microchannel. One gold surface was modified with anti-transfferin antibody. Another working surface was functionalised with osmium-poly-vinylpyridine wired horseradish peroxidase (Os-gel-HRP) together with tri-enzyme layer containing creatininase, creatinase and sarcosine oxidase. The device was aimed for the simultaneous detection of both the large (glycoprotein transferrin, MW 75–80 kDa) and the small (creatinine, MW 113) biomolecules. The transferrin concentration was measured from the increase of SPR angle due to an immune complex formation. Creatinine could be detected by measuring, conversely, the decrease of the SPR angle in the Os-gel-HRP surface caused by an oxidation state change from Os^{2+} to Os^{3+} due to the enzymatic reduction of hydrogen peroxide, a final product of the series of enzymatic conversion of creatinine. Biosensor could measure two analytes in human urine within 15 min by one sample injection (50 μL). The detectable concentration ranges were 0.02–10 μg mL^{-1} and 0.010–10 mM for transferrin and creatinine, respectively, which are to be sufficient for clinical tests. The significant advantage of the proposed biosensor is the possibility to simultaneously measure transferrin and creatinine in mild

Table 3
Analytical capabilities of the selected instrumental techniques for the detection and quantification of glycan-containing molecules.

Analytical technique	Analyte	Capturing molecule	Detectable concentration	Time for analysis	Format of analysis	Sample preparation	Labeling required	Advantages	Disadvantages	Ref
NP-HPLC-MS-MS	N-glycans of blood serum GPs	–	1 pg (fM-pM)	Several hours	Indirect	Digestion of blood serum; Solid-phase extraction of N-glycans; Purification; Acid hydrolysis	Yes/No	No derivatization; High sensitivity; Robustness; Reproducibility	Complex sample pretreatment; Lack of suitable software to ease the data analysis	[88]
RP-HPLC-MS	N-glycans	–	70 fM	>50 h	Indirect	Release of N-glycans; Purification	Yes	High sensitivity; High resolution; Small sample consumption	Complex sample pretreatment	[97]
FAC on DC-SIGNR column, fluorescent detection	DC-SIGN, DC-SIGNR, LSECtin	Mono- or biantennary agalactosylated N-glycans	2.5–5 nM	n/a	Indirect	Cell expression	Yes	Simplicity; High sensitivity Information about lectin-glycan affinity can be obtained	Difficulties with obtaining the valency of binding molecules; High dependence of K_d on the applied conditions; Time-consuming	[108]
CE	Sialyloligosaccharides in human milk	–	9 μg mL^{-1}	35 min	Indirect	Extraction	Yes/No	Simple sample preparation; Good resolution; High throughput	Difficult to identify glycans from unknown sample; Ionization of glycan is required	[109]
CE-MS	Glycopeptides	–	1.8 μM	15 min	Indirect	Protease digestion; Sample enrichment	No	High sensitivity; Rapidity; Information about the glycan structure is provided	Decreased efficiency due to signal broadening; High price of the equipment	[113]
MS-MS	RNAse B	–	1 μM	n/a	Direct	Cleavage of the sample; Protease digestion; Sample enrichment	No	Changes in glycosylation can be distinguished	Complex sample pretreatment; Low reproducibility; Difficult to resolve isomers; High price of the equipment	[99]
MALDI–TOF MS	N-glycans	–	20 pmol	n/a	Direct	Releasing of N-glycans; Derivatization with GT reagent	Yes	High sensitivity	Difficult to resolve isomers; High price of the equipment	[115]
Flow cytometry	Glycoproteins	Fluorophore-labeled lectins	0.17 aM	2 h	Indirect	Labeling of glycoproteins; Coupling to streptavidin-microspheres beads	Yes	High selectivity; High sensitivity;	High price of the equipment	[145]

Table 3 (*Continued*)

Analytical technique	Analyte	Capturing molecule	Detectable concentration	Time for analysis	Format of analysis	Sample preparation	Labeling required	Advantages	Disadvantages	Ref
								High reproducibility; High-throughput; Simplicity; Minute amount of sample is required; Rapidity; Possible to measure weak interactions		
AFM	Glycophorin A	*Psathyrella velutina* lectin	nM	n/a	Direct	Centrifugation of the blood cells	No	Detects an interaction at single molecule level; Detects small changes in the carbohydrate moiety of GP	Low imaging speed; Difficult to confirm the valency of the binding; Not suitable for routine analyses; High cost of the equipment	[147]
NMR	a-D-GalNAc glycosylated b3-peptides	*Vicia villosa* lectin	nM	n/a	Direct	Non	No	Detects interaction at single molecule level; Provides structural information about the analyte	Sophisticated equipment is needed; Highly-skilled personnel is required; Not suitable for routine analyses; High cost of the equipment	[154]
EI	Glucose	GGBG	1 μM	30 min	Direct	Non	No	High sensitivity; Simplicity; Rapidity; Low cost	Ligand is not commercially available; Ligand has affinity to other sugar (galactose)	[191]
QCM-D	Serum glycoproteins	Lectins	50 μg mL^{-1}	3 h	Direct	Non	No	Provides the information about rigidness of the biocomplex; Monitors simultaneously four interactions	Difficult to obtain the kinetic data; High price of the equipment	[157]
QCM	Bacteria	Lectins	10^3 cells	30 min	Direct	Washing the sample in PBS; Sample lysis; Centrigugation Resuspension	No	Possible to perform bacteria typing; Simplicity; Rapidity	Difficult to obtain the kinetic data Low throughput	[158]
QCM	ConA	Mannan	0.5 μM	10 min	Direct	Non	No	Rapidity; Simplicity; Robustness; Provides K_d values	Difficult to obtain the precise kinetic data; Non-specific binding may be significant	[160]
SPR	Serum glycoproteins	Lectin	10 μg mL^{-1}	25 min	Direct	Non	No	Rapidity; High reproducibility; Possible to monitor simultaneously several interactions; Provides K_d, K_a values; Provides kinetic values	High price of the equipment	[168]

SPR	Glucose	GGBG	0.1 mM	10 min	Direct	Non	No	High reproducibility; Rapidity	Narrow concentration range; Ligand is not commercially available; Ligand has affinity to other sugar (galactose) Not practical for routine analyses	[178]
SPRi	Glycoproteins	ConA	5 pg	~1 h	Direct	Non	No	High sensitivity; Measures multiple interaction at a time; Easy referencing	Long procedure of preparation of the glycoprotein array	[188]

Abbreviations of the instrumental techniques (in alphabetical order).
AFM—atomic force microscopy.
CE—capillary electrophoresis.
EI—electrochemical impedance.
FAC—frontal affinity chromatography.
MALDI–TOF MS—matrix-assisted laser desorption/ionization time-of-flight mass spectrometry.
MS—mass spectrometry.
NMR—nuclear magnetic resonance.
NP-HPLC—normal phase high-performance liquid chromatography.
QCM—quartz crystal microbalance.
QCM-D—quartz crystal microbalance with dissipation monitoring.
RP-HPLC—reversed-phase high-performance liquid chromatography.
SPR—surface plasmon resonance.
SPRi—surface plasmon resonance imaging.
Abbreviations of the analytes or reagents (in alphabetical order).
2-AB—2-aminobenzamide.
GGBG—glucose/galactose binding protein.
GP—glycoprotein.
GT reagent—carboxymethyl trimethylammonium hydrazide.
DC-SIGN—dendritic cell-specific intracellular adhesion molecule-3-grabbing nonintegrin.
DC-SIGNR—dendritic cell-specific intracellular adhesion molecule-3-grabbing nonintegrin related protein.
LSECtin—liver and lymph node sinusoidal endothelial cell C-type lectin.

Table 4
Detection of carbohydrates and measuring the carbohydrate–protein binding—a comparison of the conventional analytical techniques with flow SPR and SPR imaging.

Analytical technique/ approach	Advantages	Disadvantages
Comparison with flow SPR		
LC with fluorescent detection	Glycans are profiled; Isomers can be resolved; Highly sensitive detection (~fM)	Labeling of glycans; Long retention time prolongs the assay
FAC	Simple equipment; Low cost; Highly sensitive detection (~pM)	Labeling of glycans; Time-consuming; High sample consumption at low affinity; Not recommended for low affinity interactions (K_d > 1 mM); No information about the reaction rate constants
CE-MS	Glycans are profiled; Isomers can be resolved; Structural information about the separated glycans is provided; Highly sensitive detection (~ng)	Only charged molecules are separated, restriction to neutral glycans; Destructive
QCM (QCM-D)	Lower cost of the equipment; Additional information about the structural properties and rigidness of the biocomplex are provided (in QCM-D)	Not easily integrated with microfluidic system; Higher sample and reagents consumption; Harder to achieve uniformity of coating of crystal; Difficulties with referencing; Difficult to obtain reaction rate constants
Comparison with SPR imaging		
ELLA	Slightly lower detection limit (~pM)	Includes several incubation and washing steps; Labeling of glycans; Labeling of lectins with enzyme
Carbohydrates microarrays	Slightly lower detection limit (~pM)	Labeling of proteins
MS	Structural information about the glycans is provided; Highly sensitive detection	Destructive; Ionization of glycans is needed; Extensive sample preparation is required; Fractionation of complex glycan mixtures before analysis; Difficulties with quantification; Difficulties with reproducibility; High level of expertise is required to interpret spectra; Higher cost of the equipment
AFM	Interaction at single molecule level is possible to record	Low imaging speed; Not suitable for high-throughput assay; High level of expertise is required; Higher cost of the equipment
NMR	Structural information about the glycans is provided	Relatively insensitive; Often large amount of sample is required; Not suitable for high-throughput assay; High level of expertise is required to interpret spectra; Higher cost of the equipment

Abbreviations of the instrumental techniques (in alphabetical order).
AFM—atomic force microscopy.
CE-MS—capillary electrophoresis coupled with mass-spectrometric detection.
ELLA—enzyme-linked lectin assay.
FAC—frontal affinity chromatography.
LC—liquid chromatography.
MS—mass spectrometry.
NMR—nuclear magnetic resonance.
QCM—quartz crystal microbalance.

conditions, while the conventional detection of creatinine, using, for instance, Jaffe method [201] is carried out in alkaline media and is unfavorable for the proteins. Non-specific adsorption of urinary proteins can be overcome by 5 times dilution of urine sample before the injection. The rather simple sample preparation like addition of ascorbate oxidase, uricase, and catalase prior the analysis helps to minimise the interferences from ascorbic and uric acids. On the one hand, the original approach, sufficient sensitivity of the detection, miniaturised design, possibility to renew the biosensor surfaces after each experiment undoubtedly make this analytical device a very attractive and promising tool for clinical analysis. On the other hand, the obtained results have shown that the biosensor needs further optimisation of the experimental conditions in order to reduce the signal fluctuations and to improve reproducibility.

The above described attempts of hyphenation of SPR with separation and detection analytical techniques clearly indicate the facilitated and improved capabilities of surface plasmon resonance, as these tandem techniques help to unravel the structural features of the detected molecule and to increase the selectivity of the analysis.

8. Conclusion

The major benefit of SPR in comparison with other modern analytical techniques is its capability of real-time monitoring the carbohydrates–protein interactions by measuring the reaction rate constants and the binding affinity. SPR allows the label-free direct analysis of intact glycoforms omitting the long sample pretreatment step.

The developed simple protocols allow functionalisation the SPR sensing surfaces according to the user-defined task. This makes SPR a versatile and powerful technique for the quantification of different glycoforms. Furthermore, the optimisation of the surface modification together with using the reference sensing surfaces help to minimise the unwanted non-specific binding and mass-transfer effect, which appear to be the major problems in SPR analysis.

Although higher detection sensitivity can be achieved for lager glycoconjugates, modern SPR analysers allow the quantification of even such small molecules as monosaccharides.

Coupling SPR with other analytical techniques like liquid chromatography, mass spectrometry and electrochemistry bring new insights into glycan analysis. These hyphenated techniques are capable of providing the detailed structural and behavioral information about the captured glycan molecules, enhance the selectivity of the carbohydrate analysis from complex mixtures by profiling the individual glycans, and simultaneously monitor the optical and electrochemical processes occurring at the solid–liquid interfaces. The tandem SPR-based analytical techniques and tools offer promising capabilities for measurement, investigation and monitoring the process of protein glycosylation. Thereby, the contribution of the hyphenated SRP-based tools in study of this problem will be very significant for the disease diagnostics, biomarkers screening and optimisation of therapeutic efficacy of novel pharmaceuticals.

The comparison of the flow SPR and the SPR imaging with the selected instrumental analytical techniques in terms of their advantages and disadvantages in gycan and glycoconjugate assay is summarised in Table 4.

References

[1] S.L. Flitsch, R.V. Ulijn, Nature 421 (2003) 219–220.
[2] B.K. Brandley, R.L. Schnaar, J. Leukoc. Biol. 40 (1986) 97–111.
[3] R.W. Johnstone, A.A. Ruefli, K.M. Tainton, M.J. Smyth, Leuk. Lymphoma 38 (2000) 1–11.
[4] K. Olden, R.M. Pratt, C. Jaworski, K.M. Yamada, Proc. Nation. Acad. Sci. 76 (1979) 791–795.
[5] A. Alam, L.I. Singha, V. Singh, Mol. Cell. Biochem. 271 (2005) 177–188.
[6] K.C. Breen, C.M. Coughlan, F.D. Hayes, Mol. Neurobiol. 16 (1998) 163–220.
[7] A. Maton, J. Hopkins, C.W. McLaughlin, S. Johnson, M. Quon Warner, D. LaHart, J.D. Wright, Human Biology and Health, Prentice Hall, Englewood Cliffs, New Jersey, USA, 1993, pp. 52–59.
[8] A. Varki, J.B. Lowe, in: A. Varki, R.D. Cummings, J.D. Esko, et al. (Eds.), Essentials of Glycobiology, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor (NY), 2009.
[9] A. Varki, in: A. Varki, R.D. Cummings, J.D. Esko, et al. (Eds.), Essentials of Glycobiology, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor (NY), 2009.
[10] C.K. Mathews, K.E. van Holde, Biochemistry, The Benjamin/Cummings Publishing Company, Inc., Redwood City, 1990.
[11] W.J. Lennarz, The Biochemistry of Glycoproteins and Proteoglycans, New York, Plenum Press, 1980.
[12] A. Gottschalk, Glycoproteins Their Composition, Structure, and Function, Elsevier Publishing Company, New York, 1972.
[13] M. Rambausek, J. Dulawa, K. Jann, E. Ritz, Eur. J. Clin. Invest. 18 (1988) 237–242.
[14] E.H. Beachey, J. Infect. Dis. 143 (1981) 325–345.
[15] M. Benai, I. Kahane, S. Razin, W. Bredt, Infect. Immun. 21 (1978) 365–372.
[16] V. Sharma, M. Vijayan, A. Surolia, J. Biol. Chem. 271 (1996) 21209–21213.
[17] K. Slaviero, S.J. Clarke, L. Rivory, Lancet Oncol. 4 (2003) 224–232.
[18] G.A. Turner, Clin. Chim. Acta 208 (1992) 149–171.
[19] C.K. Mathews, K.E. van Holde, K.G. Ahern, Biochemistry, 3rd ed., Wesley Publishing Company, San Francisco, USA, 2000.
[20] J. Roth, Chem. Rev. 102 (2002) 285–303.
[21] G. Durand, N. Seta, Clin. Chem. 46 (2000) 795–805.
[22] N. Perrimon, M. Bernfield, Semin. Cell Dev. Biol. 12 (2001) 65–67.
[23] T.N. Wight, M.G. Kinsella, E.E. Qwarnström, Curr. Opin. Cell Biol. 4 (1992) 793–801.
[24] S.-G. Lee, J.M. Brown, C.J. Rogers, J.B. Matson, C. Krishnamurthy, M. Rawat, L.C. Hsieh-Wilson, Chem. Sci. 1 (2010) 322–325.
[25] J. van Horssen, P. Wesseling, L.P. van den Heuvel, R.M. de Waal, M.M. Verbeek, Lancet Neurol. 2 (2003) 482–492.
[26] A. Varki, Glycobiology 3 (1993) 97–130.
[27] C.R. Bertozzi, L.L. Kiessling, Science 291 (2001) 2357–2364.
[28] C.G. Gemmell, J. Infect. Chemother. 10 (2004) 69–75.
[29] J. Royet, R. Dziarski, Nat. Rev. Microbiol. 5 (2007) 264–277.
[30] J. Vogel, G. Bendas, U. Bakowsky, G. Hummel, R.R. Schmidt, U. Kettmann, U. Rothe, Biochim. Biophys. Acta 1372 (1998) 205–215.
[31] P. Barraud, in: D. Kasaki (Ed.), Glycolipids: New Research, Nova Science Publishers, Inc., NY, USA, 2008, pp. 79–98.
[32] C. Erridge, E. Bennett-Guerrero, I.R. Poxton, Microb. Infect. 4 (2002) 837–851.
[33] J.H. Banoub, A. El Aneed, A.M. Cohen, N. Joly, Mass Spec. Rev. 29 (2010) 606–650.
[34] H. Diao, M. Kohanawa, H. Nakajima, Y. Sato, T. Minagawa, A. Nakane, Immunology 105 (2002) 344–349.
[35] J.B. Delehanty, B.J. Johnson, T.E. Hickey, T. Pons, F.S. Ligler, NRL Rev. (2008) 101–107.
[36] A.P. Moran, Int. J. Med. Microbiol. 297 (2007) 307–319.
[37] A. Lundberg, L.A. Wikberg, J. Ilonen, O. Vaarala, M.F. Böttcher, Clin. Vaccine Immunol. 15 (2008) 1878–1883.
[38] A. Vellodi, Br. J. Haematol. 128 (2008) 413–431.
[39] C.L. Nilsson, in: C.L. Nilsson (Ed.), Lectins: Analytical Technologies, Elsevier B.V., USA, 2007, pp. 1–13.
[40] S. Elgavish, B. Shaanan, Trends Biochem. Sci. 22 (1997) 462–467.
[41] K. Drickamer, Nat. Struct. Biol. 2 (1995) 437–439.
[42] E.J.M. Van Damme, W.J. Peumans, A. Puzstai, S. Bardocz, Handbook of Plant Lectins: Properties and Biomedical Applications, Wiley & Sons Ltd., UK, 1998.
[43] H. Lis, N. Sharon, Ann. Rev. Biochem. 55 (1986) 35–67.
[44] A.M. Wu, E. Lisowska, M. Duk, Z. Yang, Glycoconj. J. 26 (2009) 899–913.
[45] A. Monzo, G.K. Bonn, A. Guttman, Tr. Anal. Chem. 26 (2007) 423–432.
[46] H. Annuk, S.O. Hynes, S. Hirmo, M. Mikelsaar, T. Wadström, J. Med. Microbiol. 50 (2001) 1069–1074.
[47] P. Falk, K.A. Roth, J.L. Gordon, Am. J. Physiol. 266 (1994) G987–G1003.
[48] J.P. Chambers, B.P. Arulanandam, L.L. Matta, A. Weis, J.J. Valdes, Curr. Issues Mol. Biol. 10 (2008) 1–12.
[49] K. Hasija, Ind. J. Clin. Biochem. 6 (1991) 89–96.
[50] G.G. Irish, D.D. Maenz, H.L. Classen, Anim. Feed Sci. Technol. 76 (1999) 321–333.
[51] J.P.JJr. McCoy, J. Varani, I.J. Goldstein, Exp. Cell Res. 151 (1984) 96–103.
[52] T. Iskratsch, A. Braun, K. Paschinger, I.B.H. Wilson, Anal. Biochem. 386 (2009) 133–146.
[53] Y. Koshi, E. Nakata, I. Hamachi, ChemBioChem. 6 (2005) 1349–1352.
[54] R. Ballerstadt, J.S. Schultz, Meth. Molec. Biol. 600 (2010) 9–30.
[55] V.J. Nagaraj, S. Eaton, D. Thirstrup, P. Wiktor, Biochem. Biophys. Res. Comm. 375 (2008) 426–430.
[56] M. Lee, S. Park, I. Shin, Bioorg. Med. Chem. Lett. 16 (2006) 5132–5135.
[57] J. Gao, D. Liu, Z. Wang, Anal. Chem. 80 (2008) 8822–8827.
[58] G.T. Carroll, D. Wang, N.J. Turro, J.T. Koberstein, in: K. Dill (Ed.), Microarrays: Preparation Microfluidics Detection Methods and Biological Applications, Springer Science + Business Media, LLC, USA, 2009, pp. 191–210.
[59] M. Fais, R. Karamanska, D.A. Russell, R.A. Field, J. Cereal Sci. 50 (2009) 306–311.
[60] D.F. Smith, R.D. Cummings, in: R. Cummings, J. Pierce (Eds.), Handbook of Glycomics, Elsevier Inc., USA, 2009.
[61] K.-L. Hsu, L.K. Mahal, Curr. Opin. Chem. Biol. 13 (2009) 427–432.
[62] P.-H. Liang, S.-K. Wang, C.-H. Wong, J. Am. Chem. Soc. 129 (2007) 11177–11184.
[63] M.M. Ngundi, C.R. Taitt, S.A. McMurry, D. Kahne, F.S. Ligler, Biosens. Bioelectron. 21 (2006) 1195–1201.
[64] O. Oyelaran, J.C. Gildersleeve, Curr. Opin. Chem. Biol. 13 (2009) 406–413.
[65] S. Park, M.-R. Lee, I. Shin, Chem. Commun. 438 (2008) 9–4399.
[66] E.-H. Song, N.L.B. Pohl, Curr. Opin. Chem. Biol. 13 (2009) 626–632.
[67] M.E. Taylor, K. Drickamer, Glycobiology 19 (2009) 1155–1162.
[68] X. Zhou, J. Zhou, Biosens. Bioelectron. 21 (2006) 1451–1458.
[69] X. Zhou, G.T. Carroll, C. Turchi, D. Wang, in: H.G. Garg, M.K. Cowman, C.A. Hales (Eds.), Carbohydrate Chemistry, Biology and Medical Applications, Elsevier Ltd., USA, 2008.
[70] K. Larsen, M.B. Thygesen, F. Guillaumie, W.G.T. Willats, K.J. Jensen, Carbohydr. Res. 341 (2006) 1209–1234.
[71] O. Oyelaran, J.C. Gildersleeve, Expert Rev. Vaccines 6 (2007) 957–969.
[72] I. Shin, S. Park, M. Lee, Chem. Eur. J. 11 (2005) 2894–2901.
[73] L. Krishnamoorthy, L.K. Mahal, ACS Chem. Biol. 4 (2009) 715–732.
[74] H.S. Mader, O.S. Wolfbeis, Microchim. Acta 162 (2008) 1–34.
[75] L.R. Ruhaak, G. Zauner, C. Huhn, C. Bruggink, A.M. Deelder, M. Wuhrer, Anal. Bioanal. Chem. 397 (2010) 3457–3481.
[76] C. Guo, P. Boullanger, L. Jiang, T. Liu, Biosens. Bioelectron. 22 (2007) 1830–1834.
[77] W. Morelle, J.-C. Michalski, Curr. Anal. Chem. 1 (2005) 29–57.
[78] H. Geyer, R. Geyer, Biochim. Biophys. Acta 1764 (2006) 1853–1869.
[79] K. Mariño, J. Bones, J.J. Kattla, P.M. Rudd, Nat. Chem. Biol. 6 (2010) 713–723.
[80] L. Royle, M.P. Campbell, C.M. Radcliffe, D.M. White, D.J. Harvey, J.L. Abrahams, Y.-G. Kim, G.W. Henry, N.A. Shadick, M.E. Weinblatt, D.M. Lee, P.M. Rudd, R.A. Dwek, Anal. Biochem. 376 (2008) 1–12.
[81] C. Grey, P. Edebrink, M. Krook, S.P. Jacobsson, J. Chrom. B 877 (2009) 1827–1832.
[82] Y. Wang, S. Wu, W.S. Hancock, Glycobiology 16 (2006) 514–523.
[83] Z. He, L.P. Aristoteli, L. Kritharides, B. Garner, Biochem. Biophys. Res. Comm. 343 (2006) 496–503.
[84] J. Irungu, E.P. Go, Y. Zhang, D.S. Dalpathado, H.-X. Liao, B.F. Haynes, H. Des, J. Am. Soc. Mass Spectrom. 19 (2008) 1209–1220.
[85] K. Racaityte, S. Kiessig, F. Kalman, J. Chrom. A 1079 (2005) 354–365.
[86] M. Yodoshi, A. Tani, Y. Ohta, S. Suzuki, J. Chrom. A 1203 (2008) 137–145.

[87] X. Chen, G.C. Flynn, J. Am. Soc. Mass Spectrom. 20 (2009) 1821–1833.
[88] L.A. Hammad, D.Z. Derryberry, Y.R. Jmeian, Y. Mechref, Rapid Commun. Mass Spectrom. 24 (2010) 1565–1574.
[89] A. Harazono, N. Kawasaki, S. Itoh, N. Hashii, Y. Matsuishi-Nakajima, T. Kawanishi, T. Yamaguchi, J. Chrom. B 869 (2008) 20–30.
[90] Q. Luo, T. Rejtar, S.-L. Wu, B.L. Karger, J. Chrom. A 1216 (2009) 1223–1231.
[91] M. Madera, Y. Mechref, I. Klouckova, M.V. Novotny, J. Chrom. B 845 (2007) 121–137.
[92] B.D. Prater, H.M. Connelly, Q. Qin, S.L. Cockrill, Anal. Biochem. 385 (2009) 69–79.
[93] S. Sinha, G. Pipes, E.M. Topp, P.V. Bondarenko, M.J. Treuheit, H.S. Gadgil, J. Am. Soc. Mass Spectrom. 19 (2008) 1643–1654.
[94] J. Wohlgemuth, M. Karas, T. Eichhorn, R. Hendriks, S. Andrecht, Anal. Biochem. 395 (2009) 178–188.
[95] J. Yuan, N. Hashii, N. Kawasaki, S. Itoh, T. Kawanishi, T. Hayakawa, J. Chrom. A 1067 (2005) 145–152.
[96] Y. Kanie, A. Enomoto, S. Goto, O. Kanie, Carbohydr. Res. 343 (2008) 758–768.
[97] X. Chen, G.C. Flynn, Anal. Biochem. 370 (2007) 147–161.
[98] K. Kakehi, S. Honda, Appl. Biochem. Biotech. 43 (1990) 55–71.
[99] M.P. Campbell, L. Royle, C.M. Radcliffe, R.A. Dwek, P.M. Rudd, Bioinformatics 24 (2008) 1214–1216.
[100] G.N. Saddic, S.T. Dhume, K.R. Anumula, Meth. Mol. Biol. 446 (2008) 215–229.
[101] X. Mao, J. Qin, B. Lin, in: C.L. Nilsson (Ed.), Lectins: Analytical Technologies, Elsevier B.V., USA, 2007, pp. 213–238.
[102] Z. Yang, W.S. Hancock, J. Chromatogr. A 1053 (2004) 79–88.
[103] Z. Yang, W.S. Hancock, J. Chromatogr. A 1070 (2005) 57–64.
[104] K. Kasai, in: N. Tanoguchi, et al. (Eds.), Experimental glycoscience. Glycochemistry, Springer, USA, 2008, pp. 117–120.
[105] O. Hindsgaul, R.D. Cummings, in: A. Varki, R. Cummings, J. Esko, et al. (Eds.), Essentials of Glycobiology, Cold Spring Harbor Laboratory Press, Cold Spring Harbor (NY), 1999.
[106] K. Matsumura, K. Higashida, Y. Hata, Junko. Kominami, S. Nakamura-Tsuruta, J. Hirabayashi, Anal. Biochem. 386 (2009) 217–221.
[107] S. Nakamura-Tsuruta, N. Uchiyama, J. Kominami, J. Hirabayashi, in: C.L. Nilsson (Ed.), Lectins: Analytical Technologies, Elsevier B.V., USA, 2007, pp. 239–266.
[108] R. Yabe, H. Tateno, J. Hirabayashi, FEBS J. 277 (2010) 4010–4026.
[109] Y. Bao, L. Zhu, D.S. Newburg, Anal. Biochem. 370 (2007) 206–214.
[110] V. Guryča, Y. Mechref, A.K. Palm, J. Michálek, V. Pacáková, M.V. Novotný, J. Biochem. Biophys. Methods 70 (2007) 3–13.
[111] M. Marchetti-Deschmann, J. Kemptner, C. Reichel, G. Allmaier, J. Proteom. 72 (2009) 628–639.
[112] I. Rustighi, C. Campa, M. Rossi, S. Semeraro, A. Vetere, A. Gamini, Electrophoresis 30 (2009) 2632–2639.
[113] K. Imami, Y. Ishihama, S. Terabe, J. Chrom. A 1194 (2008) 237–242.
[114] M. Nakano, K. Kakehi, N. Taniguchi, A. Kondo, in: N. Volpi (Ed.), Capillary Electrophoresis of Carbohydrates, Springer Science+Business Media, LLC, USA, 2011, pp. 205–235.
[115] K.R. Rebecchi, J.L. Wenke, E.P. Go, H. Desaire, J. Am. Soc. Mass Spectrom. 20 (2009) 1048–1059.
[116] M. Pattky, C. Huhn, Bioanal. Rev. 2 (2010) 115–155.
[117] M.V. Novotny, J. Sudor, Electrophoresis 14 (1993) 373–389;
M.V. Novotny, Meth. Enzymol. 271 (1996) 319–347.
[118] M. Stefansson, D. Westerlund, J. Chrom. 632 (1993) 195–200.
[119] Z. El Rassi, W. Nashabeh, Carbohydrate Analysis: High Performance Liquid Chromatography and Capillary Electrophoresis, pp. 267–360.
[120] T. Patwa, C. Li, D.M. Simeone, D.M. Lubman, Mass. Spectrom. Rev. 29 (2010) 830–844.
[121] B.A. Budnik, R.S. Lee, J.A.J. Steen, Biochim. Biophys. Acta 1764 (2006) 1870–1880.
[122] J.S. Sampson, K.K. Murray, D.C. Muddiman, J. Am. Soc. Mass Spectrom. 20 (2009) 667–673.
[123] P. Gemeiner, D. Mislovičová, J. Tkáč, J. Švitel, V. Pätoprstý, E. Hrabárová, G. Kogan, T. Kožár, Biotechnol. Adv. 27 (2009) 1–15.
[124] M. Wuhrer, A. Van Remoortere, C.I.A. Balog, A.M. Deelder, C.H. Hokke, Anal. Biochem. 406 (2010) 132–140.
[125] P. Dwivedi, B. Bendiak, B.H. Clowers, H.H. Hill Jr., J. Am. Soc. Mass Spectrom. 18 (2007) 1163–1175.
[126] D.S. Levin, P. Vouros, R.A. Miller, E.G. Nazarov, J. Am. Soc. Mass Spectrom. 18 (2007) 502–511.
[127] S.M. Peterman, J.J. Mulholland, J. Am. Soc. Mass Spectrom. 17 (2006) 168–179.
[128] J.M. Prien, D.J. Ashline, A.J. Lapadula, H. Zhang, V.N. Reinhold, J. Am. Soc. Mass Spectrom. 20 (2009) 539–556.
[129] D.J. Harvey, L. Royle, C.M. Radcliffe, P.M. Rudd, R.A. Dwek, Anal. Biochem. 376 (2008) 44–60.
[130] J. Hsu, S.J. Chang, A.H. Franz, J. Am. Soc. Mass Spectrom. 17 (2006) 194–204.
[131] P. Kapkova, Rapid Commun. Mass Spectrom. 23 (2009) 2775–2784.
[132] M.L.M. Robijn, J. Planken, D. Kornelis, C.H. Hokke, A.M. Deelder, Trans. R. Soc. Trop. Med. Hygiene 102 (2008) 79–83.
[133] S.I. Snovida, J.M. Rak-Banville, H. Perreault, J. Am. Soc. Mass Spectrom. 19 (2008) 1138–1146.
[134] B. Xie, C.E. Costello, in: H.G. Garg, M.K. Cowman, C.A. Hales (Eds.), Carbohydrate Chemistry, Biology and Medical Applications, Elsevier Ltd., USA, 2008, pp. 29–57.
[135] D.J. Harvey, Mass Spec. Rev. 30 (2010) 1–100.
[136] S.M. Haslam, S.J. North, A. Dell, Curr. Opin. Struct. Biol. 16 (2006) 584–591.
[137] G.-C. Gil, Y.-G. Kim, B.-G. Kim, Anal. Biochem. 379 (2008) 45–59.
[138] W. Morelle, J.-C. Michalski, Nat. Protocols 2 (2007) 1585–1602.
[139] S.J. North, P.G. Hitchen, S.M. Haslam, A. Dell, Curr. Opin. Struct. Biol. 19 (2009) 498–506.
[140] M. Wuhrer, C.A. Koeleman, A.M. Deelder, Methods Mol. Biol. 534 (2009) 79–91.
[141] T.S. Mattu, L. Royle, J. Langridge, M.R. Wormald, P.E. Van den Steen, J. Van Damme, G. Opdenakker, D.J. Harvey, R.A. Dwek, P.M. Rudd, Biochemistry 39 (2000) 15695–15704.
[142] Q. Luo, T. Rejtar, S.-L. Wu, B.L. Karger, J. Chromatogr. A 1216 (2009) 1223–1231.
[143] T.R. Covey, D. Pinto, Nanospray electrospray ionization development: LC/MS, CE/MS application, in: B.N. Pramanik, A.K. Ganguly, M.L. Gross (Eds.), Applied Electrospray Mass Spectrometry, Marcel Dekker, New York, NY, 2002.
[144] F. Jeffers, C. Fuell, L.E. Tailford, D.A. MacKenzie, R.J. Bongaerts, N. Juge, Carbohydr. Res. 345 (2010) 1486–1491.
[145] K. Yamamoto, S. Ito, F. Yasukawa, Y. Konami, N. Matsumoto, Anal. Biochem. 336 (2005) 28–38.
[146] M. Lekka, P. Laidler, M. Łabe, A.J. Kulik, J. Lekki, W. Zaja, Z. Stachura, Chem. Biol. 13 (2006) 505–512.
[147] C. Yan, A. Yersin, R. Afrin, H. Sekiguchi, A. Ikai, Biophys. Chem. 144 (2009) 72–77.
[148] A.J. Petrescu, T.D. Butters, G. Reinkensmeier, S. Petrescu, F.M. Platt, R.A. Dwek, M.R. Wormald, EMBO J. 16 (1997) 4302–4310.
[149] M. Lundborg, G. Widmalm, Anal. Chem. 83 (2011) 1514–1517.
[150] B. Mulloy, G.W. Hart, P. Stanley, Structural analysis of glycans, in: A. Varki, R. Cummings, J. Esko, et al. (Eds.), Essentials of Glycobiology, Cold Spring Harbor Laboratory Press, Cold Spring Harbor (NY), 2009.
[151] A.W. Barb, J.H. Prestegard, Nat. Chem. Biol. 7 (2011) 147–153.
[152] Z. Liu, S. Masuko, K. Solakyildirim, D. Pu, R.J. Linhardt, F. Zhang, Biochemistry 49 (2010) 9839–9847.
[153] R.E. Lehotzky, C.L. Partch, S. Mukherjee, H.L. Cash, W.E. Goldman, K.H. Gardner, L.V. Hooper, Proc. Natl. Acad. Sci. 107 (2010) 7722–7727.
[154] M. Kaszowska, A.S. Norgren, P.I. Arvidson, C. Sandström, Carbohydr. Res. 344 (2009) 2577–2580.
[155] W.H. King, Anal. Chem. 36 (1964) 1735–1739.
[156] M.M. Pedroso, A.M. Watanabe, M.C. Roque-Barreira, P.R. Bueno, R.C. Faria, Microchem. J. 89 (2008) 153–158.
[157] M.E. Yakovleva, G.R. Safina, B. Danielsson, Anal. Chim. Acta 668 (2010) 80–85.
[158] G. Safina, M. van Lier, B. Danielsson, Talanta 77 (2008) 468–472.
[159] M. Yakovleva, A.P. Moran, G. Safina, T. Wadström, B. Danielsson, Anal. Chim. Acta 694 (2011) 1–5.
[160] Z. Pei, H. Anderson, T. Aastrup, O. Ramström, Biosens. Bioelectron. 21 (2005) 60–66.
[161] N. Doy, G. McHale, M.I. Newton, C. Hardacre, R. Ge, J.M. MacInnes, D. Kuvshinov, R.W. Allen, Biomicrofluidics 4 (2010) 1–7.
[162] F. Markey, in: K. Nagata, H. Handa (Eds.), Real-time Analysis of Biomolecular Interactions: Applications of BIACORE, Springer-Verlag Tokyo, Japan, 2000, pp. 13–22.
[163] P.A. van der Merwe, in: S. Harding, P.Z. Chowdhry (Eds.), Surface Plasmon Resonance in Protein-ligand Interactions: Hydrodynamics and Calorimetry, Practical Approach Series, Oxford University, UK, 2001, pp. 137–170.
[164] U. Jönsson, L. Fägerstam, B. Ivarsson, B. Johnsson, R. Karlsson, K. Lundh, S. Löfås, B. Persson, H. Roos, I. Rönnberg, S. Sjölander, E. Stenberg, R. Ståhlberg, C. Urbaniczky, H. Östlin, M. Malmqvist, BioTechniques 11 (1991) 620–627.
[165] U. Jönsson, M. Malmqvist, Adv. Biosens. 2 (1992) 291–336.
[166] S. Hashimoto, in: K. Nagata, H. Handa (Eds.), Real-time Analysis of Biomolecular Interactions: Applications of BIACORE, Springer-Verlag Tokyo, Japan, 2000, pp. 23–32.
[167] S. Scarano, M. Mascini, A.P.F. Turner, M. Minunni, Biosens. Bioelectron. 25 (2010) 957–966.
[168] G. Safina, I.B. Duran, M. Alasel, B. Danielsson, Talanta 84 (2011) 1284–1290.
[169] K. Foley, E. Forzani, L. Joshi, N. Tao, Analyst 133 (2008) 744–746.
[170] L.S. Kelly, S. Birken, D. Puett, Molec. Cell. Endocrinol. 260–262 (2007) 33–39.
[171] K.H. Schlick, M.J. Cloninger, Tetrahedron 66 (2010) 5305–5310.
[172] W. Vornholt, M. Hartmann, M. Keusgen, Biosens. Bioelectron. 22 (2007) 2983–2988.
[173] C.E.P. Maljaars, A. Carvalho de Souza, K.M. Halkes, P.J. Upton, S.M. Reeman, S. André, H.-J. Gabius, M.B. McDonnell, J.P. Kamerling, Biosens. Bioelectron. 24 (2008) 60–65.
[174] S. Kumar, A. Roychowdhury, B. Ember, Q. Wang, R. Guan, R.A. Mariuzza, G.-J. Boons, J. Biol. Chem. 280 (2005) 37005–37012.
[175] M.J. Spencelayh, Y. Cheng, R.J. Bushby, T.D.H. Bugg, J. Li, P.J.F. Henderson, J. O'Reilly, S.D. Evans, Angew. Chem. Int. Ed. 45 (2006) 2111–2116.
[176] B.N. Murthy, N.H. Voelcker, N. Jayaraman, Glycobiology 16 (2006) 822–832.
[177] I.G. Sava, F. Zhang, I. Toma, C. Theilacker, B. Li, T.F. Baumert, O. Holst, R.J. Linhardt, J. Huebner, J. Biol. Chem. 284 (2009) 18194–18201.
[178] H.V. Hsieh, Z.A. Pfeiffer, T.J. Amiss, D.B. Sherman, J.B. Pitner, Biosens. Bioelectron. 19 (2004) 653–660.
[179] J.A. Tamada, N.J.V. Bohannon, R.O. Potts, Nat. Med. 1 (1995) 1198–1201.
[180] R. Badugu, J.R. Lakowicz, C.D. Geddes, J. Fluoresc. 13 (2003) 371–374.
[181] J. Wang, Chem. Rev. 108 (2008) 814–825.
[182] G. Fortier, E. Brassard, D. Belanger, Biosens. Bioelectron. 5 (1990) 473–490.
[183] S. Salman, S. Soundararajan, G. Safina, I. Satoh, B. Danielsson, Talanta 77 (2008) 468–472.
[184] ProteOn™ XPR36, Protein Interaction Array, Bio-Rad Laboratories, Inc., Bulletin 5390 Rev C (www.biorad.com).

[185] R. Karamanska, J. Clarke, O. Blixt, J.I. MacRae, J.Q. Zhang, P.R. Crocker, N. Laurent, A. Wright, S.L. Flitsch, D.A. Russell, R.A. Field, Glycoconj J. 25 (2008) 69–74.
[186] A. Fernandez-Gonzalez, J. Rychlowska1, R. Badia, R. Salzer, Microchim. Acta 158 (2007) 219–225.
[187] G. Grasso, R. D'Agata, L. Zanoli, G. Spoto, Microchem. J. 93 (2009) 82–86.
[188] W. Liu, Y. Chen, M. Yan, Analyst 133 (2008) 1268–1273.
[189] C. Kößlinger, E. Uttenthaler, S. Drost, F. Aberl, H. Wolf, G. Brink, A. Stanglmaier, E. Sackmann, Sens. Actuators B 24–25 (1995) 107–112.
[190] L. Laricchia-Robbio, R.P. Revoltella, Biosens. Bioelectron. 19 (2004) 1753–1758.
[191] J. Wang, K.S. Carmon, L.A. Luck, I.I. Suni, Electrochem. Solid-State Lett. 8 (2005) H61–H64.
[192] R. Gutiérrez Gallego, S.R. Haseley, V.F.L. van Miegem, J.F.G. Vliegenthart, J.P. Kamerling, Glycobiology 14 (2004) 373–386.
[193] J. Buijs, G.C. Franklin, Brief. Funct. Genom. Proteom. 4 (2005) 39–47.
[194] D. Nedelkov, Meth. Mol. Biol. 627 (2010) 261–268.
[195] S. Bellon, W. Buchmann, F. Gonnet, N. Jarroux, M. Anger-Leroy, F. Guillonneau, R. Daniel, Anal. Chem. 81 (2009) 7695–7702.
[196] R. Kurita, Y. Yokota, A. Ueda, O. Niwa, Anal. Chem. 79 (2007) 9572–9576.
[197] S. Wang, X. Huang, X. Shan, K.J. Foley, N. Tao, Anal. Chem. 82 (2010) 935–941.
[198] C. Gondran, M.-P. Dubois, S. Fort, S. Cosnier, S. Szunerits, Analyst 133 (2008) 206–212.
[199] K. Nakamoto, R. Kurita, O. Niwa, Biosens. Bioelectron. 26 (2010) 1536–1542.
[200] K. Nakamoto, R. Kurita, N. Sekioka, O. Niwa, Chem. Lett. 37 (2008) 698–699.
[201] A.S. Kanagasabapathy, S. Kumari, Guidelines on Standard Operating Procedures for Clinical Chemistry, World Health Organization (2000) pp.113.