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# Glycan and lectin biosensors

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A short description about the importance of glycan biorecognition in physiological (blood cell type) and pathological processes (infections by human and avian influenza viruses) is provided in this review. Glycans are described as much better information storage media, compared to proteins or DNA, due to the extensive variability of glycan structures. Techniques able to detect an exact glycan structure are briefly discussed with the main focus on the application of lectins (glycan-recognising proteins) in the specific analysis of glycans still attached to proteins or cells/viruses. Optical, electrochemical, piezoelectric and micromechanical biosensors with immobilised lectins or glycans able to detect a wide range of analytes including whole cells/viruses are also discussed.

## Introduction

For centuries, people believed that sugars were only important to us as a source of energy. With progress in many scientific disciplines, new functions of saccharides in the human body started to emerge. It was revealed that they can serve as energy storage molecules or building blocks for nucleic acids, and be a part of enzymes and their cofactors and other important molecules. In recent years, considerable attention has been paid to saccharides covalently attached to proteins or lipids, called glycans, which can have a linear or branched structure, consisting of monosaccharide units connected together by a glycosidic bond (Figure 1) [1].

It is estimated that approximately 70–80 % of all human proteins are glycosylated, making glycosylation one of the most common post-translational modifications along with phosphorylation or acetylation [2]. Glycans participate in various physiological and pathological processes, such as intermolecular and cell–cell recognition events, they affect the cell cycle, cell differentiation and apoptosis, host–pathogen interactions and inflammation [2–5]. To illustrate the importance of glycans in the immune system, letu focus on red blood cells. There are four different blood types, depending on the type of antigen (glycan) present on the surface of the red blood cell. As can be seen from Figure 2, only a subtle change in the glycan composition (i.e. a single carbohydrate) is behind the classification into four blood types, which has huge consequences as the improper choice of blood type in an emergency can cause death [6].

As aberrant glycosylation can be the result of a disease, monitoring changes in the glycan structure of proteins is becoming a popular diagnostic tool [2]. Only recently, research focus has moved from the study of proteins and deoxyribonucleic acid (DNA) to the study of glycans, creating a new field of science – glycomics. Analogously to proteomics, which studies a proteome, glycomics studies a glycome, i.e. the sum of all glycans and glycoconjugates expressed in an organism, tissue or cell in a particular time, space and environment [1]. Glycomics studies glycan structures, their function and their mutual interactions. When compared with peptides, proteins or DNA, glycan chains have much higher structural variability with a theoretical number of glycan hexamers eight orders of magnitude larger in comparison with peptide hexamers and 11 orders of magnitude larger than six nucleotide sequences [2].

Monosaccharides in the glycan can be attached to proteins either via nitrogen of the amino acid asparagine (N-linked glycans) or via oxygen of serine and threonine (O-linked glycans) (Figure 1). Glycans can be linear or branched with $\alpha$- and/or $\beta$-linkages between neighbouring carbohydrates and thus are considered to be a better information-coding tool than the afore mentioned proteins or DNA [2]. Even though the structural variability of glycans is huge, with an estimated number of unique glycan structures

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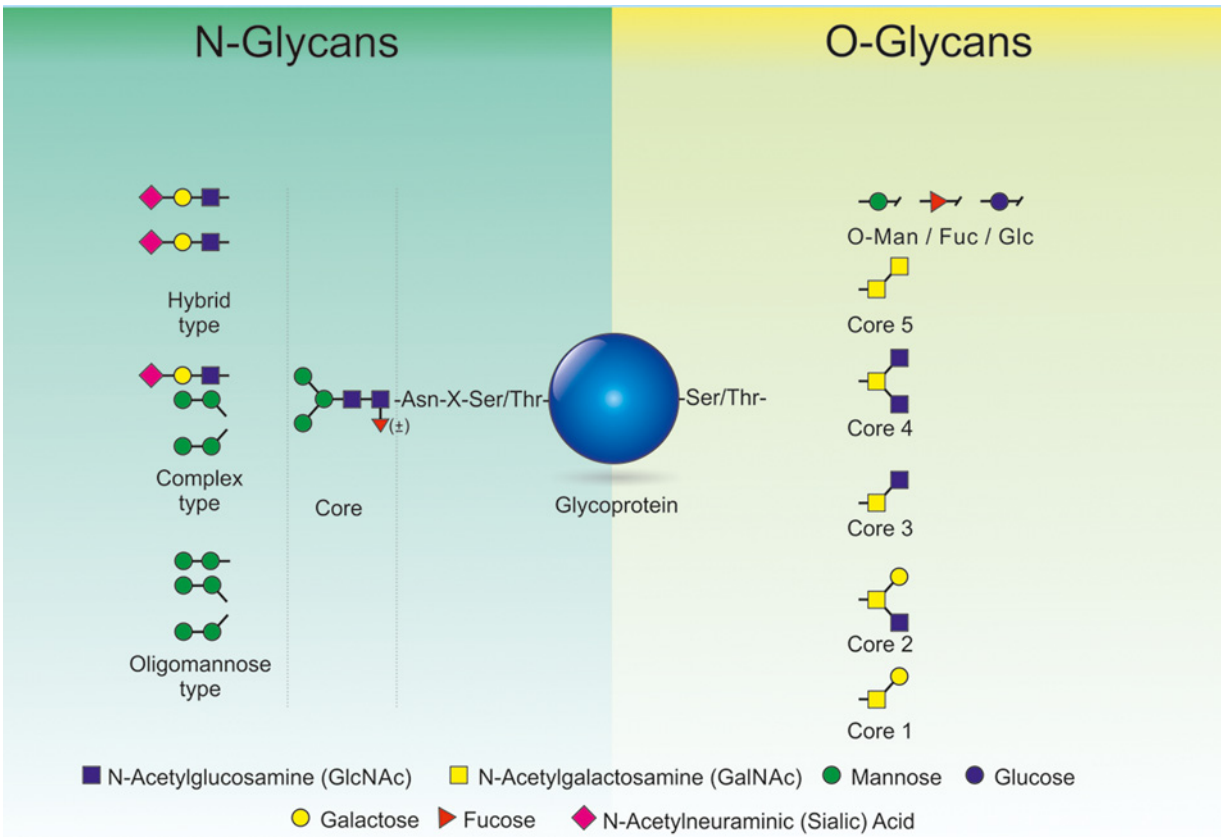


**Figure 1. Graphical representation of naturally occurring glycans, which can be attached to proteins via –OH of Ser or Thr (O-glycans) or via –$NH_2$ of a peptide sequence –Asn-X-Ser/Thr (X might be any amino acid except proline)**

Variability of sugar building blocks, multiple branching and attachment points is shown, as well. Asn, asparagine; Ser, serine; Thr, threonine.

of 5000 [2], carbohydrates as a building block of glycans have similar physico-chemical properties and this is why it is complicated to elucidate the exact glycan structure using instrumental techniques (nuclear magnetic resonance (NMR), mass spectrometry, chromatography or electrophoresis). Recent progress in the field of glycomics was only possible due to advances in sample pretreatment and the use of sophisticated instrumentation [7]. Even though such an approach can identify an exact glycan structure, there is the requirement for skilled operators and costly instrumentation. This is why alternative ways for the analysis of glycans emerged – using natural glycan-recognising proteins, i.e. lectins.

Lectins (from the Latin word 'legere' meaning 'to choose') are a large family of proteins of non-immune origin isolated from natural sources able to bind both free glycans and glycans attached to glycoconjugates, such as intact glycoproteins or even intact cells or viruses [2,8]. Lectins have complex specificities that can recognise not only different monosaccharides within the glycan chain, such as mannose, *N*-acetylglucosamine, sialic acid (SA) or galactose, but also different linkages between saccharide monomers or glycan branching. A good example is a glycan terminated in SA linked to galactose either via an $\alpha$2-6 or $\alpha$2-3 glycosidic bond (Figure 3). *Sambucus nigra* agglutinin (SNA) can recognise terminal SA linked to galactose via an $\alpha$2-6 linkage, whereas *Maackia amurensis* agglutinin (MAA) specifically recognises terminal SA linked to galactose via an $\alpha$2-3-linkage. There is also another very interesting consequence in the selective recognition of either an $\alpha$2-6- or $\alpha$2-3-linked SA to galactose. The influenza virus which attacks humans specifically recognises $\alpha$2-6-linked SA, whereas avian influenza binds to $\alpha$2-3-linked SA present on the surface of the bird's tissue. The difference is only in the position at which SA is bound to galactose (Figure 3) and this means avian flu cannot infect humans and human flu cannot infect birds.

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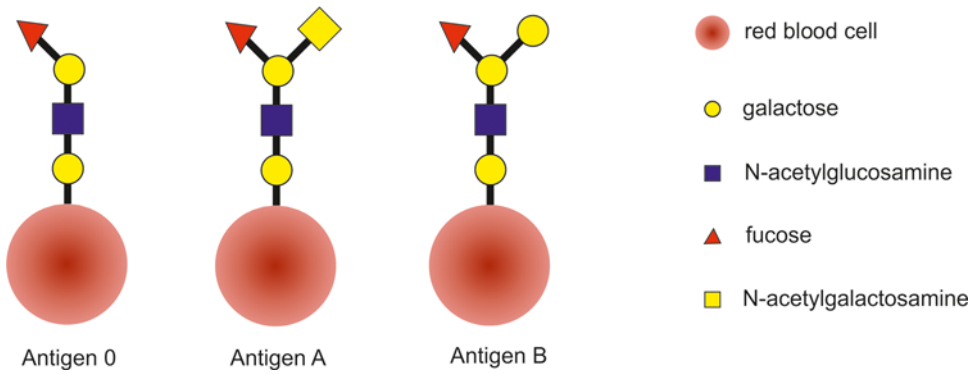


**Figure 2. Different glycan structures present on the surface of blood cells determine the blood type**
Blood type AB erythrocytes have both antigen A and antigen B present on their surface.

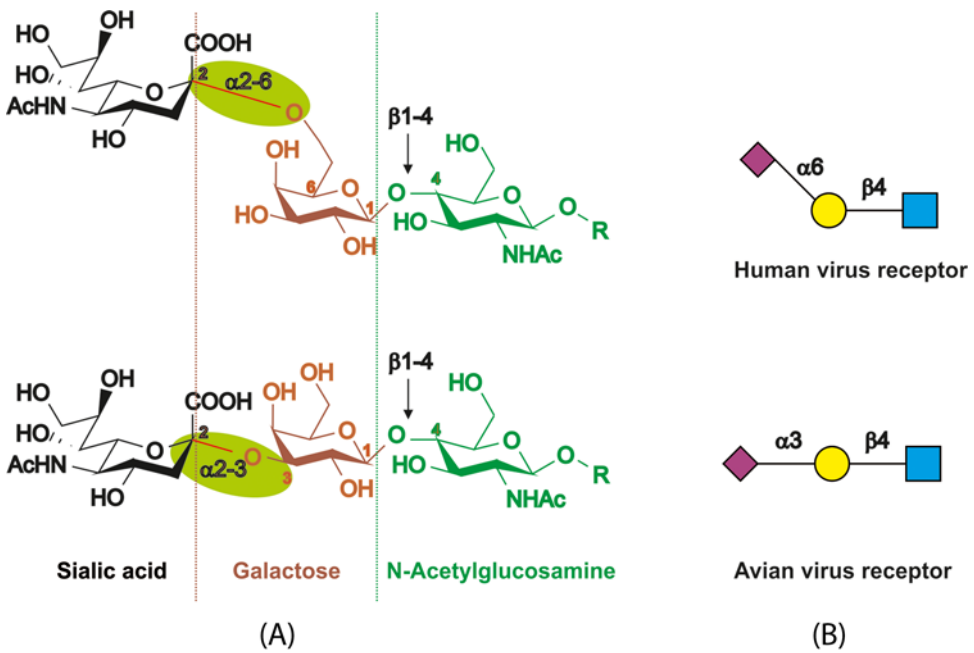


**Figure 3. (A) Difference between SA determinant recognised by human influenza (α2-6-linked SA to galactose) and by avian influenza (α2-3-linked SA to galactose). (B) Labels of saccharides and shortened structure of glycans used are according to the Consortium for Functional Glycomics (CFG).**

Since lectins can be applied to glycan analysis on intact glycoproteins or even cell/virus surfaces without the need to remove them from the protein backbone (a requirement when using instrumental techniques), different lectin-based formats for glycan analysis can be applied. For decades, enzyme-linked immunosorbent assay (ELISA)-like analysis with lectins applied instead of antibodies has been successfully applied to the analysis of a wide range of samples [9]. In recent years, microarray-based approaches with either immobilised lectins or glycans for highly parallel analysis have become increasingly popular with applications in diagnostics [10]. Even though both methods offer a multiplexed format of analysis with huge success, a low sensitivity of assays, a quite narrow dynamic concentration range and a need to apply labels are the main drawbacks of fluorescent lectin/glycan microarrays that need to be addressed (Table 1). With remarkable progress in material science and nanotechnology, lectins or glycans became popularly integrated into various biosensor designs in order to address these issues [2,11].

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**Table 1. Key characteristics of various types of biosensors compared with fluorescent microarrays and ELISA-like method**

| Method | Sensitivity | Multiplexing | Dynamic range | Reproducibility | Label-free? |
|---|---|---|---|---|---|
| ELISA-like | + + | + + | + | + + + | ☒ |
| F. microarray | + | + + + | + + | + + | ☒ |
| Electrochemical | + + + | +[#] | + + + | +[#] | ☑ or ☒ |
| Optical* | + + | + + | + + | + + | ☑ |
| QCM | + | + | + + | + + | ☑ |
| Microcantilever | + | + + | + + | + + | ☑ |

ELISA, enzyme-linked immunosorbent assay; F. microarray, fluorescent microarray; QCM, quartz crystal microbalance. [#]multiplexing is possible, resulting in an increased reproducibility of biosensor preparation; surface plasmon resonance, other plasmonic and ellipsometry-based biosensors, including imaging mode.

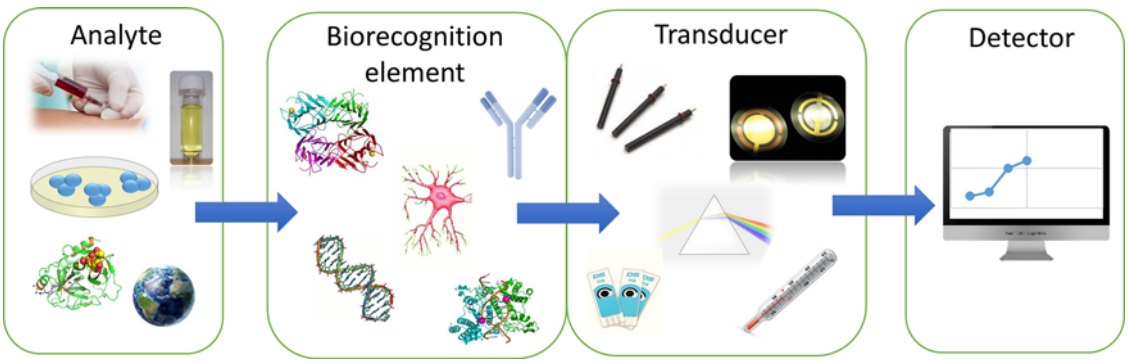


**Figure 4. A scheme of the biosensor with an analyte, a biorecognition element, a transducer and a detector**
Reproduced from De Gruyter [12].

# Lectin/glycan biosensors

Over the last few decades biosensing has become a rapidly developing sector built upon knowledge from various fields including chemistry, biochemistry, biomedicine, biotechnology, nanotechnology and material sciences. Biosensors with high sensitivity and specificity of detection, when integrated with advanced nanomaterials, represent a viable diagnostic approach for the detection of a low level of disease biomarkers needed for early-stage diagnostics. The basic feature of a biosensor is the interaction of an analyte with a biorecognition element (i.e. lectin or glycan), which is in direct contact with a physico-chemical transducer (electrochemical, optical, piezoelectric or microcantilever-based) [12]. The main role of the transducer is to change a biorecognition event into a measurable signal, proportional to the analyte concentration (Figure 4).

## Electrochemical lectin/glycan biosensors

Electrochemical biosensing provides a low-cost, fast and sensitive analytical platform with applications in biotechnology, medicine and pharmacy. There are several electrochemical techniques available, which can be applied in biosensing [13,14], but one of the most intensively applied electrochemical techniques is electrochemical impedance spectroscopy (EIS), which can detect analytes down to a single-molecule level in a label-free way [2]. EIS is a technique able to detect changes in the resistance of the layer on the electrode surface towards a redox probe in the solution as a result of interaction with an analyte, i.e. the higher the amount of proteins/DNA present on the biosensor surface, the

higher the resistance of the layer is, which can be used for analyte quantification. Other electrochemical techniques based on field-effect transistor (FET) sensing or using redox/enzymatic labels can also be applied.

**Electrochemical glycan biosensors** have been applied for the analysis of glycan-binding proteins and viruses. FET-based glycan biosensors have been used for the detection of influenza haemagglutinins (HAs). A biosensor with underivatised glycans immobilised on the amino-oxy-modified silicon surface of the FET device could detect as few as 60 H5N1 or 6000 H1N1 proteins in a 20 $\mu$l sample, corresponding to one H5N1 virus or 12 H1N1 viruses with a dynamic range spanning nine orders of magnitude (attomolar to nanomolar concentration range) [15]. FET-based glycan biosensors prepared on a silicon nanowire, with underivatised glycans covalently coupled via an amino-oxy-modified surface, were also applied in the detection of lectins down to 1 fM with a dynamic range of five orders of magnitude (femtomolar to nanomolar level). Moreover, real-time monitoring of lectin binding was possible [16]. EIS biosensor devices, based on electrodes modified by gold nanoparticles with thiolated glycans attached to nanoparticles via the sulphur–gold interaction backfilled with various thiols to stabilise a self-assembled monolayer (SAM) of thiols on gold, were applied for the detection of lectins down to 7 nM with a linear response within two orders of concentration magnitude [17]. A mannosylated polyaniline film was applied for the impedimetric detection of a lectin down to 0.12 nM with a linear range up to 15 nM [18]. Recently, our group developed an EIS-based glycan biosensor able to detect HAs down to a single-molecule (attomolar) level with a controlled density of sialylated glycans on the surface using the covalent immobilisation of amine-modified glycan on a mixed SAM containing functional –COOH groups. The working concentration range of the biosensor was quite large both for lectins and influenza HAs (attomolar to nanomolar) [19]. Later, this approach was also applied for the impedimetric detection of inactivated whole H3N2 influenza viruses down to 13 viruses/$\mu$l with a large dynamic working range and improved surface chemistry resisting non-specific interactions with covalent immobilisation of amine-modified glycans (unpublished work). The glycan biosensor constructed by antraquinone-modified glycans attached to a graphene surface via $\pi$–$\pi$ stacking interactions was also applied in the analysis of intact bacterial and cancerous cells. Cyclic voltammetry was in this case applied to monitor changes in the redox behaviour of the quinone moiety as a redox probe upon interaction with an analyte. The hepatoma (liver cancer) cell line HepG2 could be detected down to a concentration of 5000 cells/ml with a narrow dynamic range [20].

**Electrochemical lectin biosensors** have been applied for the analysis of viral glycoproteins, P-glycoprotein (responsible for multidrug resistance of cancerous cells), carcinoembryonic antigen (CEA, colorectal cancer), $\alpha$-fetoprotein (different types of cancer) and intact cancerous cells (gastric, liver, colon and lung cancer, and leukaemia); in addition, viruses could also be detected [2]. An interesting strategy of how to detect a biomarker of colorectal cancer (CEA) together with a drug (where both a biomarker and a drug are glycoproteins) applied to cure such a disease was recently proposed. Two different lectins were covalently attached to an aldehyde-modified glass surface and both analytes were detected on the same biosensor surface by detecting quantum dots electrochemically, as the two different quantum dots could be detected at a distinct non-overlapping potential. Both glycoproteins were detected down to a concentration one order of magnitude lower than a required diagnostic cut-off value for both proteins (i.e. low ng/ml for a biomarker and low $\mu$g/ml for a drug) [21]. A typical CEA concentration level in healthy individuals is lower than 3 ng/ml and in patients with cancer it is above 20 ng/ml.

Another cancer biomarker, prostate-specific antigen (PSA), could be detected down to a single-molecule (attomolar) level by an electrochemical biosensor with an immobilised antibody against PSA and a final step being the interaction with lectin (Figure 5C). Such an approach allows specific fishing out PSA from complex human serum with the subsequent ultrasensitive *in situ* glycan analysis by lectins, which is not possible using an assay configuration with immobilised lectins (Figure 5A). It is believed that the analysis of glycans present on PSA, together with the PSA concentration in blood, can be applied for a more robust diagnostics of prostate cancer (PC) and/or for the identification of various disease stages [22]. A sandwich configuration with the lectin concanavalin A (Con A) immobilised on a nanostructured tree-like gold surface followed by incubation with norovirus particles, with a final incubation including a primary antibody and an enzyme-labelled secondary antibody, could detect viruses with cyclic voltammetry down to 60 copies/ml [23]. A hybrid nanomaterial composed of carbon nanotubes and gold nanoparticles modified with Con A lectin was applied for the electrochemical detection of lung cancer cells down to a concentration of 12 cells/ml with a dynamic range spanning six orders of magnitude and with the ability to detect a number of glycans present on a single cell [24]. Cancer cells could be detected down to 50 cells/ml using a quite sophisticated electrochemical amplification strategy employing three different nanomaterials (quantum dots, gold nanoparticles and carbon nanotubes) and two different bioaffinity molecules (DNA and lectin) [25]. Besides the use of electrochemical lectin biosensors in the analysis

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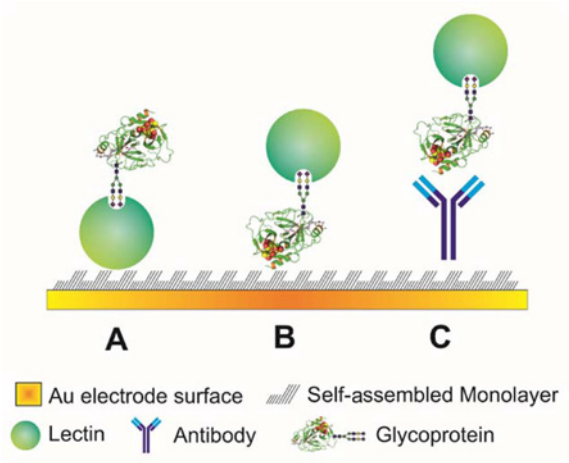


**Figure 5. Configuration of lectin-based analysis with applied direct (A), reverse (B) or sandwich (C) immobilisation protocol**

of standard glycoproteins or cell lines, such devices were utilised by our group for the analysis of glycoproteins in serum plasma from patients with rheumatoid arthritis or systemic sclerosis with the ability to distinguish healthy individuals from those suffering from the disease. The lectin biosensors were built up on a gold surface modified with a mixed SAM resisting non-specific protein binding based on zwitterionic thiols as diluting molecules. Such biosensors offer limits of detection at the femtomolar concentration level with a dynamic range covering a few orders of concentration magnitude [26–28].

## Microcantilever-based lectin/glycan biosensors

This label-free micromechanical detection platform detects the interaction with an analyte by the bending of a cantilever (similar to the cantilever applied in atomic force microscopy), which is detected by a laser beam. In this case, only one side of the cantilever is modified by biorecognition molecules and the other side is passivated to resist non-specific binding.

**Microcantilever glycan biosensors** were utilised for the detection of an antiviral protein, cyanovirin-N, down to 91 pM using a gold electrode modified by glycans via thiol–gold surface chemistry with a dynamic range covering orders of magnitude. Three different types of glycans (galactoside, trimannoside and nonamannoside) were applied in the study, with the best performance obtained with the device patterned by nonamannoside [29]. The same group extended the same biosensor concept for the detection of three *Escherichia coli* strains with as few as eight cells detected on a cantilever surface with a wide dynamic range covering five orders of magnitude [30].

## Quartz crystal microbalance (QCM)-based lectin/glycan biosensors

Quartz crystal microbalance (QCM) is a label-free biosensor platform based on the detection of mass changes by measuring the changes in oscillation frequency of a piezoelectric crystal. This technique has proved to be an efficient tool to study molecular interactions with increasing interest in the qualitative and quantitative evaluation of lectin–glycan interactions [31]. The cell suspension of cancer cell lines was captured onto a QCM chip surface via interaction of the cell-surface glycans with immobilised lectin Con A. The interactions between the set of various lectins and captured cells were monitored in real time by injecting lectin solutions on to the sensor surface [32]. Another strategy based on growing cancer cells directly on the QCM biosensor surface without fixation was used for the determination of cell-surface glycosylation by detecting the interactions with a range of lectins, together with studying the thermodynamic and kinetic parameters of the interactions between lectins and the cell-surface glycans [33]. This approach provides an insight into the cell-surface glycosylation and complex molecular recognition on the intact cell surface, which may have an impact on disease diagnosis and drug discovery. A QCM biosensor with immobilised lectins was developed

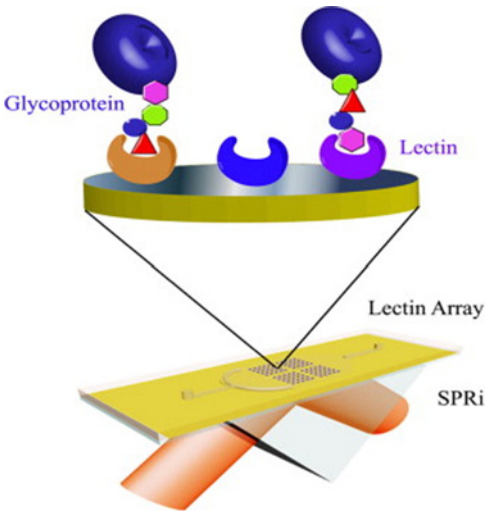


**Figure 6. Schematic representation of lectin array on SPRi**
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for the detection of bacteria characterising seven *Campylobacter jejuni* strains [34] and this method can be applied to strain discrimination of other bacterial species, particularly pathogens.

## Optical lectin/glycan biosensors

Optical-based methods are well suited for kinetic/affinity analysis of the interactions and detection of biomolecules in many areas including immunochemistry, biomedicine and diagnostics. Among them, surface plasmon resonance (SPR), intensively developed over the last two decades, is one of the most appropriate methods for fast and reliable label-free real-time biosensing with an immobilised ligand [35,36]. Details on the general principles of SPR and other optical biosensors are in Chapter 10 "Optical biosensors" of this book. They have two major applications for the analysis of lectin–glycan interactions: (i) detailed characterisation of the interaction (e.g. specificity, affinity, kinetics); and (ii) screening and quantitative analysis of lectin/glycan/glycoprotein interactions for the diagnosis and identification of ligands [35]. Optical biosensors have been used for the analysis and glycoprofiling of a broad spectrum of analytes such as lectins, glycoproteins, biomarkers, stem cells and viruses.

An **SPR lectin biosensor** based on SPR imaging (SPRi) (Figure 6) was used for the rapid profiling of stem cells. SPRi combines the sensitivity of SPR with spatial imaging allowing the simultaneous study of multiple interactions. Forty lectins with different glyco-binding motifs were immobilised on a microarray-based SPR lectin chip and three different cell lines were glycoprofiled. Pluripotent mouse stem cells were clearly distinguished from non-pluripotent stem cells and only eight lectins out of 40 were sufficient to define the pluripotency of mouse stem cells [37].

An **SPR glycan biosensor** containing two types of acetyl-lactosamine glycans simulating the glycans on the cell surface was used for the analysis of interactions with five plant lectins and the lectins were detected over the concentration range 7 nM Quartz crystalmicrobalance 1.4 $\mu$M [38]. An SPR glycan chip modified by single- and double-layered graphene sheets was used for the detection of a lectin down to 20 nM [39]. Another SPR glycan biosensor was developed for rapid HA quantification and vaccine release in pandemic scenarios. This antibody-independent SPR assay utilised synthetic glycans with SA linked to galactose via different linkages. The SA Quartz crystalmicrobalance glycan SPR assay demonstrated a broad dynamic range (0.33 Quartz crystalmicrobalance 30 $\mu$g/ml) for the quantification of HA content in influenza vaccines from different manufacturers for both seasonal and pandemic influenza strains, which were in good agreement with a reference assay [40].

The SPR chip operating in a **sandwich configuration** with anti-PNA (peanut agglutinin) immobilised on the surface, later loaded with PNA lectin, was applied for glycan analysis down to the nanomolar range [41]. Sandwich surface plasmon field-enhanced fluorescence spectrometry biosensing was applied for ultrasensitive PSA analysis and for the detection of glycan GalNAc$\beta$1–4GlcNAc-linked to PSA in serum, to discriminate between PC and benign prostate hyperplasia (BPH). The detection limit for total PSA in serum was 0.04 pg/ml with a wide dynamic range using a biosensor with immobilised anti-PSA IgG. The glycan part of PSA was detected down to 20.0 pg/ml on the biosensor,

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using an immobilised antibody to catch PSA, with a final incubation with lectin which allowed specific distinction between PC and BPH within the PSA grey zone (4–10 ng/ml) [42].

The plasmon light-scattering properties of gold nanoparticles printed into an array format were used in the construction of a **plasmonic glycan biosensor** for the characterisation of the glycosylation of human and porcine fibrinogen via the interaction with lectins. The detection limits for the lectins Con A and wheatgerm agglutinin (WGA) were 10 ng/ml (0.23 nM) and 100 ng/ml (0.93 nM), respectively. The array technology has the potential to perform multi-lectin screening of a large number of proteins providing information on protein glycosylation and their microheterogeneity [43]. A plasmonic waveguide resonance glycan biosensor was used for screening carbohydrate–lectin interactions. Planar optical waveguides were derivatised with mannose or lactose moieties and the association with selected lectins was assessed with a limit of detection of 0.5 nM [44].

Label-free **ellipsometric biosensors** are based on measuring changes in polarisation of the reflected light. An imaging ellipsometry technique was used for the quantitative and qualitative multiplexed analysis of the complex kinetic interactions between lectins and lectin combinations printed in microarray format and a set of glycoproteins [45]. The ellipsometry-based glycan microarray assay platform enabled acquiring binding profiles of influenza A virus strains against 24 glycan receptors of diverse structures immobilised on a solid surface [46].

# Challenges ahead

The main problem of lectin/glycan biosensors is their limited ability to work in a multiplexed format. Highly parallel analysis is only possible using SPRi and, to a lesser extent, microcantilever-based assays. Further work is needed to focus on electrochemical techniques working in an array format, which should be feasible since there are commercial electrodes deposited within ELISA plates (i.e. 96 electrodes) (http://www.dropsens.com/en/screen_printed_electrodes_pag.html). It is anticipated that lectin/glycan biosensors would be more suitable for diagnostic purposes (with the requirement for highly sensitive analyses rather than for highly parallel analysis with portable devices) and not for preliminary assays of a large set of samples, for which the microarray format of determination is ideal. There is also the additional challenge ahead of lectin/glycan biosensors and their application in the analysis of real complex samples, but such an initiative has already started.

Although the controlled orientation during immobilisation of glycans can be quite easily achieved using glycan derivatives (i.e. one terminal $-NH^2$ group for covalent amine coupling), controlled immobilisation of lectin for the construction of lectin microarrays and lectin biosensors is quite challenging due to the larger complexity of a protein molecule compared with a glycan molecule. Mahal's laboratory, for example, employed recombinant lectins with a binding tag for the controlled orientation of lectins on the surface. Such an approach resulted in a much lower limit of detection (LOD) of 12 ng/ml compared with random lectin immobilisation with an LOD of 10 $\mu$g/ml using fluorescent lectin microarrays [47]. The application of recombinant lectins can also solve the problem of the presence of a lectin isoform in commercial products. A notorious example is *Maackia amurensis* haemagglutinin (MAH) and *Maackia amurensis* leukoagglutinin, which can be present in an isolate from *Maackia amurensis* (Amur maackia tree). These two lectins have different glycan specificity and in many cases commercial suppliers do not distinguish between these two isoforms, which can complicate assay reproducibility from laboratory to laboratory [48]. An additional solution might be to focus on artificial glycan-binding molecules such as DNA and peptide aptamers with proper glycan binders identified from huge combinatorial libraries [14].

# Conclusions

Glycan and lectin biosensors have distinct advantages compared with the state-of-the-art glycoprofiling method based on a microarray format of analysis. For example, optical biosensors could be successfully applied for the real-time analysis of a binding event with the possibility of obtaining kinetic/affinity constants of such interactions. Another area of application of optical biosensors is for ultrasensitive analyte analysis (surface plasmon field-enhanced fluorescence spectrometry) or for a highly parallel assay format (SPRi). An electrochemical assay protocol and, in particular, the EIS method of analysis are suitable for label-free glycoprofiling in an ultrasensitive fashion, in which the analyte can be detected down to a single molecule level. An electrochemical assay protocol was successfully applied in the analysis of intact cancerous cells with a possibility of estimating the number of glycan entities present on a single cell. The most important message is that biosensor analysis of the glycan composition of various glycoproteins, which are

biomarkers of numerous diseases (including cancer), together with serological levels of such biomarkers can enhance the reliability of disease diagnosis with a prospect of also detecting different disease stages.

# Summary

- Glycans are information-rich molecules applied for initial cell–cell (or cell–virus) interactions and also in numerous intracellular processes.
- Glycans are involved in physiological (i.e. blood types) and pathological (viral infections, numerous diseases) processes.
- Lectins as natural glycan-recognising proteins are suitable for *in situ* (on intact proteins, cells or viruses) glycan analysis with specificity to detect subtle changes in the glycan structure, such as differences in the linkage between two carbohydrates.
- Plasmonic glycan and lectin biosensors are mainly used for the kinetic/affinity analysis of the interaction of glycans with its binding partners, but recent studies suggest ultrasensitive glycan analysis by plasmonic biosensors is also feasible.
- Electrochemical glycan and lectin biosensors, due to inherently high sensitivity, can be successfully applied in ultrasensitive glycoprofiling with detection limits down to a single-molecule level.
- The most important message is that analysis of the glycan composition of various glycoproteins, which are biomarkers of numerous diseases (including cancer), together with serological levels of such biomarkers can enhance the reliability of disease diagnosis with a prospect to also detect different disease stages.

## Abbreviations

BPH, benign prostate hyperplasia; CEA, carcinoembryonic antigen; Con A, concanavalin A; EIS, electrochemical impedance spectroscopy; FET, field-effect transistor; HA, haemagglutinin; LOD, lower limit of detection; PC, prostate cancer; PNA, peanut agglutinin; PSA, prostate-specific antigen; QCM, quartz crystalmicrobalance; SA, sialic acid; SAM, self-assembled monolayer; SPR, surface plasmon resonance; SPRi, SPR imaging.

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## Competing interests

The Authors declare that there are no competing interests associated with the manuscript.

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