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HIGHLIGHT

Glycosylated Biomarker Sensors: Advancements in Prostate Cancer Diagnosis

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Prostate cancer is currently diagnosed using the conventional gold standard methods using prostate-specific antigen (PSA) as a selective biomarker. However, lack of precision in PSA screening has resulted in needless biopsies and delays the treatment of potentially fatal prostate cancer. Thus, identification of glycans as a novel biomarker for the early detection of prostate cancer has attracted with a considerable attention due to its reliable diagnostic platform compared with the current PSA systems. Therefore, biosensing technologies that provide point-of-care diagnostics have demonstrated the ability to detect various analytes, including glycosylated micro- and macro-molecules, thereby enabling versatile detection methodologies. This highlight article discusses recent advances in the biosensor-based detection of prostate cancer glycan biomarkers, as well as innovative strategies for the conjugation of nanomaterials adapted to biosensing platforms. Finally, concluded with prospects and challenges of prostate cancer biosensors and recommendations to overcome the issues associated with prostate cancer diagnosis.

1. Introduction

In the 19th century, prostate cancer (PCa) was described as a "very rare illness" by J. Adams.[1] However, nowadays, PCa is one of the most common illnesses among men, and it is considered a major cause of cancer death in men worldwide.[2,3] PCa currently accounts for up to 29% of all cancer cases and 13% of deaths.[4] In 2018, there were 1.3 million cases of PCa globally, with approximately 359,000 deaths. Notably, the mortality rate of PCa in less developed countries, including all regions of Africa, Asia, and Latin America increases due to the limited resources for screening and detecting PCa, thereby increasing the odds of detection in late stages.[5,6] In the next 15 years, it is projected that PCa will become prodigious cancer diagnosed in men, with an expectation to increase disease incidence and related deaths up to 2.1 million and 6.3 million, respectively.[7]

The high prevalence of PCa can be attributed to several factors. For example, cancer is more likely to occur as age increases. Approximately one-third of men aged > 50 years and 70% of men aged > 70 years suffer from PCa. The number of cases has increased with increasing average human life expectancy.[1] Furthermore, PCa is remarkably a heterogeneous disease. It can be indolent, demonstrating no sign during a patient's lifetime. Most men die off, rather than, showing the disease symptom. In some ways, the disease can be very aggressive, for it can percolate into the seminal vesicles, bladder, and rectum, before further metastasizing to the bone and other organs,[8,9] thereby causing significant morbidity and mortality. Regardless of its manifestation, PCa leads to decrease in quality of life; hence, development of disruptive and reliable diagnostic tools for early-stage diagnosis is crucial for better treatment decisions.

1.1 Conventional diagnosis of prostate cancer

Digital rectal examination (DRE) and prostate-specific antigen (PSA) screening in serum are the major platforms for diagnosing possible cancerous abnormalities of the prostate.[10] DRE is the most widely used and oldest PCa screening method. This invasive technique includes inserting a lubricated finger into the rectum to test the presence of clumps or stiffening. Through DRE, physicians are able to feel if there is a change in prostate size, shape, or texture that could indicate the possibility of cancer or other conditions.[11] Albeit it prevails as a crucial technique in PCa diagnosis with a reported sensitivity of 18–22%, research shows that the contribution of DRE in preventing the percolation of PCa or in lowering the death rate is not significant.[7,12]

For more than two decades, prostate-specific antigen (PSA), a serine protease synthesized by the prostate tissue, has been acclaimed as the gold standard biomarker for PCa detection. Thereafter, clinical management of the disease has been transformed through PSA screening.[10] This biomarker (33–34 KDa) is also known as kallikrein-3, and can be used for assessing patients; its normal concentration should be below 4 ng/mL (cut-off value) in men.[13] Disruption of the prostate gland, such as inflammation or neoplasia, can cause PSA to leak into the bloodstream, thus increasing PSA serum levels and indicating carcinoma. Therefore, further investigation via biopsy is recommended if the PSA level rises higher

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than 4 ng/mL.[14] Nevertheless, several factors including prostatitis, irritation, or benign prostatic hyperplasia (BPH), may influence serum PSA levels.[15] These events highlight the constraints in detecting PCa at an early stage. Therefore, the use of PSA as PCa's biomarker is still exceptionally debatable, with a substantial number of false-positive results associated with the lack of sensitivity and specificity at 20% and 93%, respectively, resulting in extensive overtreatment and unnecessary biopsies.[3,16]

For nearly two decades, the unrestricted use of PSA has led to many men with indolent PCa receiving needless therapies, resulting in life-altering complications such as incontinence or impotence.[7] Furthermore, it is now clear that PSA-level analysis is insufficient to reliably detect PCa, consequently emphasizing the need for a case-specific sensing biomarker, preferably with an early diagnostic procedure.

2. Glycan biomarkers – potential molecular tools in biosensors

Biomarker is a characteristic that may be tested and evaluated scientifically to identify natural or pathological biological processes, as well as pathogenic disorder or disease.[17] For example, as tumors grow, cells or organs release numerous deoxyribonucleic acid (DNA), proteins, and metabolites, which are linked to tumor stages and can be used as biomarkers for cancer screening and clinical diagnosis. Biomarker identification is becoming the preferred approach for detecting diseases, including prostate cancer, due to their unique correlation with genomic modifications in cancer cells and disease processes.[18] In other words, cancer biomarkers are useful for early detection of the disease using tumor cells, blood, or other body fluids (i.e., urine and saliva) of cancer patients.

Despite the well-established role of PSA as a biomarker, its application still faces difficulties in clinically distinguishing indolent and aggressive PCa. Thus, studies have actively searched for alternative PCa biomarkers, including kallikrein-2;[19] prostate cancer antigen 3, also commonly known as DD3;[20] and α-methylacyl-coenzyme A racemase.[21] However, only few of the identified biomarkers have shown therapeutic use. Considering that PSA is a glycoprotein, attempts to improve its diagnostic potential should be focused on establishing cancer-specific PSA forms in amino acids and carbohydrate composition. Proteins with glycosylation are found in about half of all serum proteins. This mechanism is a key feature in tumorigenesis, which suggests that cancer diagnosis targets could include modified glycoproteins with tumor-specific glycan substituents.[3,22] As the disease forms and transforms, glycan modification responds promptly and predictably, making the phenomenon a definitive biomarker for cancer prediction. Sialylation, fucosylation, and truncation of *O*- and *N*-glycan branching are typical cancer-associated changes in glycan structures,[23] as depicted in **Fig. 1**. Accordingly, changes in the glycan profile are also referred to as the "hallmark of cancer". Hence, glycosylated PSA has been proposed as a prognostic biomarker for patients with aggressive PCa. For example, measuring the change in the sialylation of PSA glycans could distinguish patients with PCa from those with BPH.[24] Furthermore, the isoelectric point (pI) of PSA can be altered by increasing or reducing the monosaccharide content in the PSA *N*-glycan chain,[25] hence differentiating between PSA of normal and tumor origins.

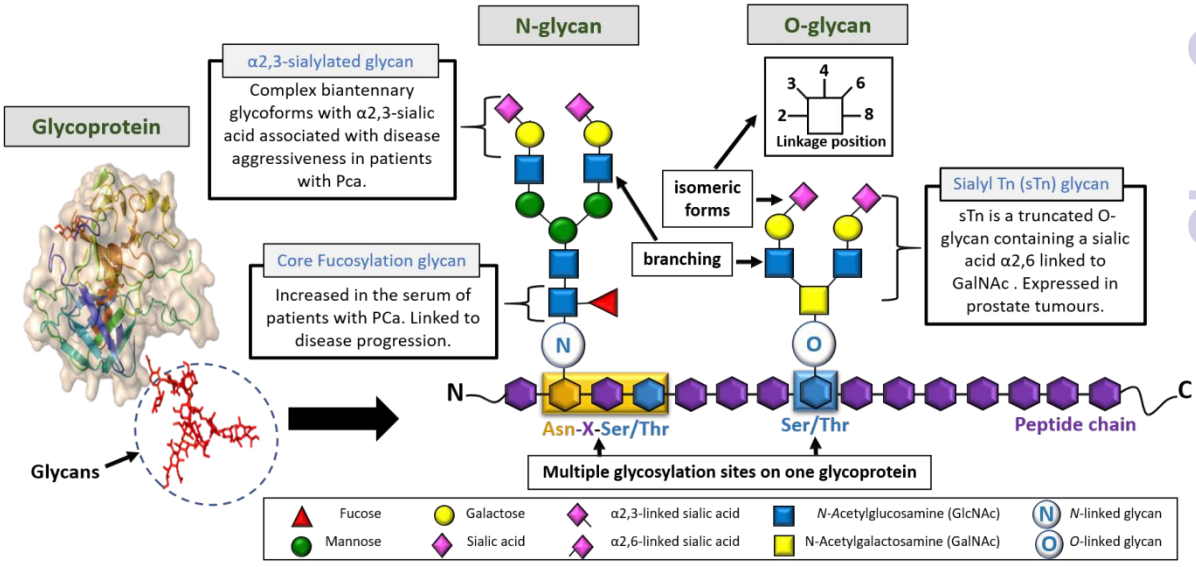

Fig.1 Glycan alterations in protein with the most common carbohydrates moieties.

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Table 1. Overview of lectin-glycan recognition in PCa application.

Lectin	Abbreviation	Origin	Carbohydrate specificity	Glycan linkage	Ref.
Fucose binding lectins					
Aleuria aurantia	AAL	Animal	α-1-6GlcNAc	*N*-linked	[35]
Lens culinaris	LCA	Plant	α-1,6-GlcNAc	*N*-linked	[36]
Lotus tetragonolobus	LTA	Plant	α-1,2Galβ	*N*-linked	[37]
Mannose binding lectins					
Canavalia ensiformis	Con A	Plant	α-D-Man	*N*-linked	[38]
			α-D-Glc	*N*-linked	
Sialic acid binding lectins					
Maackia amurensis	MAA	Plant	α-2-3Galβ1-4GlcNAc	*N*-linked	[16]
Sambucus nigra	SNA	Plant	α-2-6GalNAc	*O*-linked	[39]

Gal: galactose; Glc: glucose; GalNAc: *N*-acetylgalactosamine; GlcNAc: *N*-acetylglucosamine; Man: mannose.

Since PCa is a heterogeneous and multifocal disease, it is probable that multiple glycoprotein biomarkers will be required to direct clinical decisions. At this point, fetuin, a non-PSA biomarker seems to have the ability to avoid drawbacks of the PSA test.[26,27] Fetuin is a member of the cystatin superfamily, and mainly synthesized in the liver and secreted into the bloodstream. A prominent feature of this protein is the functional diversity exerted in human physiology and pathophysiology. Structurally, the protein has binding sites for hydroxyapatite and transforming growth factor-b (TGF-b) superfamily members. Fetuin is modified by phosphorylation and by *N*-linked and *O*-linked glycosylation of several threonines. In this context, fetuin occurs in multidimensional glycoforms comprising *O*-linked glycans, as well as different sialylation of bi-, tri-, and tetraantennary *N*-linked oligosaccharides. Recently, autoantibodies against fetuin were reported to exhibit prognostic value in the detection of metastatic diseases, thus suggesting the role of fetuin in PCa.[27]

N-linked glycosylation and *O*-linked glycosylation are the two most common pathways by which glycans bound to proteins (**Fig. 1**). The presence of a covalent bond via *N*-acetylglucosamine (*N*-GlcNAc) between *N*-linked glycosylation sites and asparagine residues in consequence Asn–X–serine/threonine (the X represents any amino acid excluding proline) makes the affinity strong. For *O*-linked glycosylation, the glycans are attached to the hydroxyl groups of serine or threonine. The major markers for the occurrence of PCa include the appearance of sialylated structures with aberrant fucosylation and excess branching of suspected glycan proteins.[28]

Recent development in the field of glycomics is possible due to advances in sample pretreatment and the use of sophisticated instruments, including nuclear magnetic resonance (NMR), mass spectrometry, chromatography, and electrophoresis.[29,30] While such an approach may effectively recognize the glycan structure, there is a need for professional operators and expensive equipment. Therefore, alternative approaches have arisen for analysing glycan using lectins, the natural glycan-recognizing proteins. The word 'lectin' was coined from the Latin term *legere,* meaning 'to choose',[31] thereby implying that lectins are biorecognition elements that can recognize specific glycan carbohydrate moieties, and therefore, are particularly useful tools for glycoanalysis. Lectins with natural carbohydrate binding functionality have been isolated from a variety of sources, including animals, plants, and bacteria.[32,33] The categorization of lectins is subjected to their monosaccharide units specificity, such as galactose, *N*-acetylglucosamine (GlcNAc), *N*-acetylgalactosamine (GalNAc), glucose, L-fucose, mannose, and sialic acid.[34] The summarized rundown of lectins with carbohydrate specificity, origin, and glycan linkage is specified in **Table 1.**

Identification of glycoprotein using lectin-based strategies, such as lectin affinity chromatography (LAC), lectin histochemistry, lectin blotting, and lectin array that have been applied for cancer biomarker research was excellently summarized by Hashim and group.[34] In this highlight article, special attention is paid to the biosensor-based techniques used in the analysis of PCa-associated glycan biomarkers. This study emphasizes on reviewing the sensing materials, surface binding strategies and sensing performance of the reported assays, followed by suggestions on the issues that need to be addressed to improve their efficacy in detecting biomarkers. Finally, we envision that advances in biosensing technologies will significantly aid future cancer diagnosis.

3. Biosensing strategies for PCa glycan biomarkers detection

Biosensors can be defined as self-contained devices that are able to provide specific analytical information through a combination of biological recognition element and transducer.[40] Historically, the field of biosensors originated in 1962 when Clark and Lyons used glucose-based electrodes for monitoring oxygen in the blood of patients undergoing surgery.[41] Since then, biosensing has developed rapidly drawing on knowledge from various fields, including analytical chemistry, biology, and microelectronics for numerous applications, such as in medicine,[42–44] environmental,[45–47] and food[48–50] analyses. With remarkable progress in nanotechnology and material science along with the advent of glycobiology technologies, glycan-binding ligand assemblage (e.g., natural and synthetic receptors) have been extensively incorporated into various biosensor designs, whereby they represent a feasible diagnostic approach to detect low-level disease biomarkers needed for early diagnosis.[51,52] These techniques are generally based on the concept of immobilizing one interacting partner on a surface in order to detect a binding event. Hence, array could include: (i) lectins; (ii) antibodies; or (iii) aptamers; which are designed as bio-recognition elements in the construction of biosensors, as depicted in **Fig. 2**. The following sections, therefore, addresses the detection of glycan

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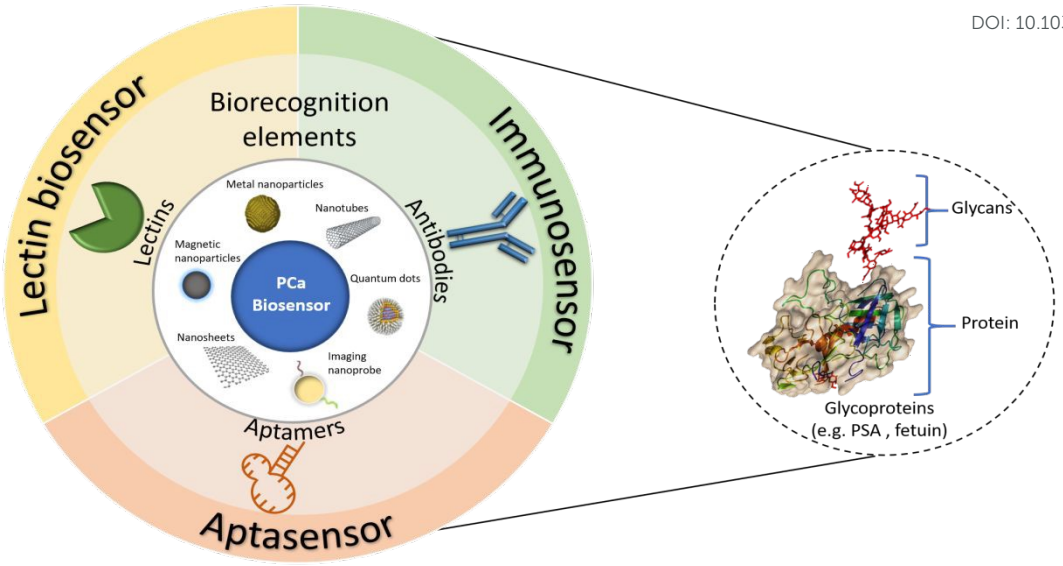

Fig. 2 Overview of different nanomaterials used in combination with biomolecules for glycan biomarkers detection.

biomarker, which is classified as lectin-biosensor, immunosensor, and aptasensor based on the type of biorecognition elements.

In addition, the integration of nanomaterials into biological elements has created new possibilities for the exploitation of advanced biosensors. Several methods for detecting PCa biomarkers have been described in the literature, either label-free or label-based assays. There are numerous techniques for utilizing nanostructured materials in the design of biosensor transducer, such as increasing the surface area of the sensor,[53] providing robust immobilization elements,[54] participating in the detection process by conjugation with the biorecognition element,[55] amplifying the output signal,[56] and using them as tags or labels.[57] On this account, recent advancements in glycan biomarker identification based on biosensor platforms for PCa detection are summarized (**Table 2**.) The scope of this review is highlighted to those remarkable efforts of developing biosensors for detecting glycoconjugates of PSA and fetuin.

3.1 Lectin biosensor – *protein probed sensing strategies*

The development of lectin microarrays was the first step towards the effective utilization of lectins in glycan recognition and diagnostics. The method employs lectin immobilization on a solid substrate to enable high-throughput analysis of complex carbohydrates that are present in serum glycoproteins,[61] with minimal reagent consumption and rapid analysis. Despite the advantages of lectin microarray-based analysis, their inherent low sensitivity detection with a relatively narrow dynamic concentration range, as well as low specificity, limits their detection performance.[62] It should also be noted that the technique is not quantitative, thus requiring fluorescent labelling and involves extensive optimization procedures.[51] Hence, lectin-based biosensing technologies can serve as great alternatives.

Electrochemical biosensing for monitoring lectin-sugar interactions of disease-related glycan markers was first claimed by Dai group in 2006, which involves immobilizing lectin as a carbohydrate recognition element onto the gold surface to develop a voltammetry-based measurement system.[56] In their first scheme, core-shell nanocrystal (CdS) tags have been used to amplify the electrochemical bioaffinity assays of protein. Analytical results demonstrated that the proposed biosensor is very selective towards various glycans analytes using the *Peanut agglutinin* (PNA) lectin-modified gold surface. Additionally, as the tagged sugar CdS-(4-aminophenol-β-D-galactopyranoside) increases, the sensitivity of the biosensor also increases. These findings support the use of lectin biosensors integrated with nanoparticles for high sensitivity and specificity in the glycoprotein identification as an alternative to the existing method of lectin microarray. Indeed, a lower detection limit of 0.1 µM and a wider detection range could be obtained by employing electrochemical readout,[56] which has the appealing advantages of miniaturization and low-cost for interfacing biorecognition events and signal transduction in an elegant way. Although this method offers unique multiplexing abilities in addition to the excellent sensitivity via the use of nanocrystals of different compositions, the need for labelling increases the complexity and duration of the assay procedure.

In 2007, Belle group further proceeded to determine the sensitivity and specificity of developed assay in the binding of fetuin to the immobilized *Sambucus nigra agglutinin* (SNA) lectin on the printed circuit board (PCB) electrodes.[63] The electrodes were first designed using computer-aided design (CAD) software and then fabricated onto a 6 inch × 6 inch presensitized PCB using the photolithography technique. Noteworthy, they have pioneered the electrochemical impedance spectroscopy (EIS)-based measurements to elucidate glycan-lectin interactions in a label-free mode of operation. In their founding work for this field, the SNA lectin was covalently surface immobilized on the layered copper/nickel/gold (Cu/Ni/Au) electrodes. When the glycoconjugates samples were introduced on the functionalized electrodes, impedance measurements indicate the specific binding of SNA lectin towards terminal α2,6-linked sialic acid can be obtained. The developed sensor was capable of detecting fetuin serum at a concentration as low as 150 fM with rapid measurements (< 80 s).[63] Consequently, this implies the novelty of the research conducted in selectively screening of fetuin biomarker by utilizing gold nanoparticles (AuNPs) bio-

conjugated to Thomsen-Friedenreich (TF) antigens, which constitute a good bio-mimetic model of carbohydrate for glycoprotein interactions in biological systems.[64] Although the authors did not specify if the glycoconjugates used in their study were explicitly developed for PCa diagnosis, it is well recognized that the fetuin glycoprotein has been associated with annexin in many cancers, including PCa.[65]

Inspired by the exquisitely low detection levels of previous works,[56,63] a few studies targeting PCa-associated glycan biomarkers utilizing lectin biosensors have been reported. Zhang group implemented lectin-gold nanoparticles-thionine (Lectin-Au-Th) bioconjugates for electrochemical analysis of PCa using glass carbon electrode (GCE).[57] In this study, two lectins were used to design the SNA-based and *Canavalia ensiformis* (Con A)-based biosensors that are specific for sialic acid and mannose on normal and cancer cells, respectively. Thionine (Th) served as electroactive species, while lectins were used for the recognition of cell surface glycans. The surface modification was realized by using gold nanoparticles/multiwalled carbon nanotubes (AuNPs-MWCNTs) composite to provide an effective matrix for the immobilization of lectins, as well as enhancing the sensitivity of the device. The biosensor was constructed in a sandwich-type format, with the complete binding strategy of AuNPs-MWCNTs-lectin, followed by the target glycoprotein interaction, and finally, with the lectin-Au-Th bioconjugates. The differential pulse voltammetry (DPV) method was used to detect the immobilized Th (Con A-Au-Th and SNA-Au-Th) on the electrodes since Th was only present in the sandwich assay when lectins reacted with glycans. The results of the assay revealed that PCa cells (LNCaP) induced a strong response signal with peak currents of 5.1 µA and 6.8 µA on the SNA-based and Con A-based biosensor, respectively. However, they discovered that mannose was expressed at high levels in both normal and cancer cells, while sialic acid was more prevalent in cancer cells than in normal controls. Their analytical findings were in good agreement with the results from fluorescent microscopy studies,[57] establishing a biosensing technique for analyzing glycan expression on living cells.

In several cases, researchers are keen on bioanalytical tools that can ascertain glycosignatures rapidly and, ideally, in a label-free way. In 2013, Bertok group reported the development of an EIS-based biosensor using planar polycrystalline gold electrodes for fetuin glycoprotein detection.[54] They demonstrated that AuNPs attachments on the modified aminoalkanethiol linker layer on surface electrodes resulted in enhanced electron transfer and biocompatibility, and facilitated in effective immobilization of lectin as illustrated in **Fig. 3A (i)**. The biosensor construction consists of three phases of self-assembled monolayers (SAMs). The first SAM is the formation of amine-terminated linker layer on a gold surface, followed by the development of a second mixed SAM layer on AuNPs consisting of 11- mercaptoundecanoic acid (MUA) and 6-mercaptohexanol (MCH), and finally, carboxyl group activation (third SAM) for consequent covalent attachment of SNA I lectin as a biorecognition element for the detection of sialic acid present on a fetuin glycoprotein. Interestingly, they found that the interfacial properties of a hybrid interface via SAMs may have a greater influence on the performance of the final biosensor device than the size of the AuNPs deposited.[54] Although this behavior is unexpected,

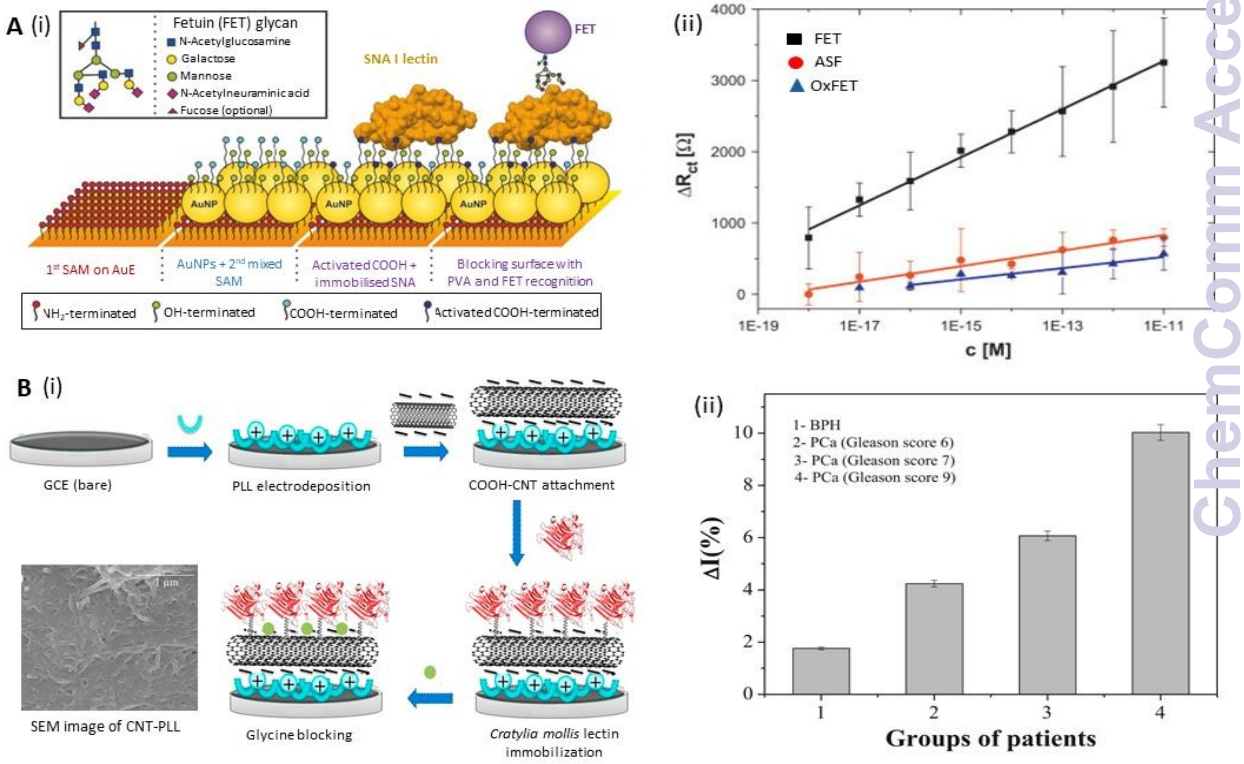

Fig. 3 (A) (i) Surface immobilization of SNA I lectin on the modified AuNPs for fetuin detection. (ii) The developed lectin biosensor selectively responded to the fetuin glycoprotein compared to the controls (i.e., asialofetuin (ASF) and oxidised asialofetuin (OxASF). (Reprinted from ref.[54] with permission from Elsevier). (B) (i) The stepwise modification of the GCE-CNT-PLL for lectin biosensor. (ii) SWV-based electrochemical response of *C. mollis* lectin biosensor for serum samples of PCa patients.(Reprinted from ref.[58] with permission from Elsevier).

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it is consistent with Gooding's observations.[66] Results also demonstrated that the sensor was selective against fetuin, with a detection limit of 0.5 aM.[54] On top of that, the ability of the biosensor to detect quantitative changes in the amount of sialic acid on glycoproteins by calculating an approximate value of lectin molecules per nanoparticle is deemed appealing. This research, however, lacks validation in real sample analysis.

The vital role of PSA glycosylation in PCa specificity detection has been reported and discussed.[22] In 2016, an excellent method for fabricating lectin-based impedimetric biosensors for PSA glycan analysis using polycrystalline gold disk electrodes was reported by Pihíková and coworkers.[67] Two lectins that can detect terminal sialic acids, i.e., SNA and *Maackia amurensis* (MAA) were chosen to investigate lectin binding to glycan parts present on the surface of PSA. Instead of using the nanocomposite technique demonstrated by previous works,[54,68] Pihíková group took advantage of the highly dense SAM layers consisting of MUA:MCH in a ratio of 1:3 and sequentially modified with N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC)/N-hydroxysuccinimide (NHS) for covalent binding of lectin onto the electrodes.[67] It has been reported that the covalent binding technique had influenced the density of biorecognition properties to a high extent, thereby improving the assay efficiency.[69] The SNA-biosensor was shown to detect PSA glycoprotein in human serum, with a recovery index of 95.6% and a lower detection limit of 4 aM at a signal to noise ratio of 3.[67] The effectiveness of this biosensor applied in PSA glycoprofiling of human serum samples combined with the convenience of a one-step preparation method had elevated its potential for clinical use.

Furthermore, it is worth noting that lower detection limit can be expected due to the use of nanomaterials, as exemplified by the results obtained by Bertok and coworkers in 2014.[68] The group demonstrated that the utilization of AuNPs has helped to improve sensor sensitivity, as compared with that of planar Au electrode. In a comparative study, EIS measurements on three-dimensional (3D)-biosensors revealed a detection limit of 1 aM, which was compared to 1 fM for two-dimensional (2D)-biosensors. They attributed this to the fact that 3D architecture of nanostructures provides a larger area for SNA lectin immobilization, as well as improved accessibility for interaction with its fetuin glycoconjugate samples containing sialic acid terminated glycans. The authors postulate that the nanostructured lectin biosensors have a high potential for use in early cancer diagnosis, which is linked to the aberrant glycosylation of protein biomarkers.[68]

The unique properties of carbon nanotube (CNT) has been appreciated in the study of Silva group in 2016, where they investigated the role of fetuin on the metastatic phase of PCa disease diagnosis using a glassy carbon electrode.[58] In this work, poly-L-lysine (PLL) film and carboxylated CNT were deposited on the electrode surface to provide functional groups for *Cratylia mollis* lectin immobilization. The nonspecific sites were then blocked using glycine, as illustrated in **Fig. 3B (i)**. The successful assembly of stable CNT-PLL nanohybrid compound on the GCE electrode was demonstrated via scanning electron microscope (SEM). The micrograph image showed a porous 3D nanostructured film was formed by electrodeposition techniques, consistent with the data reported by Xue et al.[70] It was found that *C. mollis* lectin had a strong interaction with the monosaccharides of fetuin glycoconjugates, as obtained by EIS biosensor.[71] While the approach is label-free and enables rapid detection, the EIS procedure tends to result in frequent noise and misinterpretations.[72] Silva and coworkers, therefore, demonstrated square wave voltammetry (SWV) technique to investigate the interactions between *C. mollis* lectin and fetuin in order to identify glycans moieties from the serum samples of BPH and PCa patients.[58] The reported SWV assay exhibited a linear concentration range of 0.5 to 25 µg/mL with a detection limit of 0.017 µg/mL. The sensor showed a good RSD value of 4.24% for reproducibility against eight different electrodes and exhibited exceptional repeatability with RSD of 1.24% through 20 successive tests of single sensor. Remarkably, their findings revealed that the glycosylation profiles of BPH and PCa patient samples could be detected using *C. mollis*-biosensor and the change in current response (ΔI%) increase proportionally with degree of staging PCa Gleason score of 6, 7, and 9 [**Fig. 3B (ii)**].[58] This led to the suggestion that fetuin can serve as a prognostic marker for tumor metastasis and aggressiveness, which is consistent with the observation made by Mintz et al.[27] The validation of this biosensor using higher number of clinical samples could be implemented in determining its true translational potential for clinical use. Furthermore, additional efforts to reduce the detection limit are required for improved assay performance.

Along the same lines, Filip group has recently developed a lectin-biosensor composed of thionine modified electrochemically reduced graphene oxide (ErGO-Thi) on GCE for the detection of fetuin.[38] Despite the use of noble metal nanoparticles, graphene and its derivatives (reduced graphene oxide, rGO) were found to be promising substrates for the bioreceptor immobilization,[73] mainly due to their high surface area to volume ratio, biocompatibility, and relatively low-price materials. In this work, the oxygen-containing groups on graphene oxide (GO) has been reduce electrochemically to form rGO monolayer and subsequently incubated with thionine (Thi) to form a nanohybrid structure, ErGO-Thi. Thi-containing two aromatic rings and two-primary amine groups can act as surface-immobilization agent to interacts with an aldehyde moiety of glutaraldehyde (GA) in the mixture of Con A lectin and GA. In this context, Con A was attached to the electrode surface via GA linkage to form GCE-ErGO-Thi-ConA-GA and the non-specific interactions was blocked by a carbo-free blocking solution. This Con A-lectin based EIS assay was found to be highly sensitive and selective towards fetuin invertase (INV) with the reported a linear detection range of 10^{-14} to 10^{-18} mol and a sensitivity of 6.1% of charge-transfer resistance (R_{CT}) change per decade of INV concentration.[38] The acclaimed implementation of invertase reported earlier by Mislovicova et al.[74] gives the advantages of the strong biospecific interactions of heavily mannosylated glycoprotein towards Con A. Though this work did not highlight the lowest detection limit that can be obtained, it serves as a proof-of-concept for further work in this direction for developing Con A-biosensor for the detection of PCa through integration of invertase in future.

3.2 Immunosensor – *antibody probed sensing strategies*

Immunosensors rely on the recognition of an antibody immobilized onto a sensing surface for antigen coupling and also in sandwich patterns with other probes. Immunosensor is considered a promising analytic method due to its high specificity and sensitivity. The

combination of immunosensor and lectin technology presents an excellent platform for the detection of glycan biomarkers irrespective of the complex samples. This valuable strategy was pioneered by Chen group in 2007 and known as the antibody-lectin sandwich array (ALSA).[75] One of the major strengths of ALSA is the capacity to measure the patterns of glycosylation on specific glycoproteins – a requirement for the diagnosis of early-stage cancer. This specific feature of ALSA was employed in the first attempt of developing the assay for the quantitative determination of glycosylation pattern of PSA, otherwise known as lectin-based immunoassay (LIA). The assay uses anti-PSA monoclonal antibody (C4E6) as a biorecognition element, whereas glycoprotein-lectin interactions were verified using biotinylated lectin (i.e., SNA, AAL and MAA II). Furthermore, the potential application of LIA in bioanalysis was elucidated upon confirming the presence of fucosylated and sialylated glycans on the PSA detected from the serum of PCa patients.[76]

As precedent technologies to the LIA, sandwich-based immunosensor has also been used in several studies including those of cancer biomarkers.[77,78] In 2015, Damborský and coworkers employed primary and secondary anti-PSA antibodies to detect free PSA (fPSA) using surface plasmon resonance (SPR) immunosensor.[79] Specifically, a sandwich configuration consists of two recognition steps. In the first step, primary antibody (Ab10187 specific for fPSA, epitope 1) immobilized on a sensor chip surface is allowed to bind with the injected fPSA. In the second step, a secondary antibody (Ab10185 specific for both fPSA and PSA/α1-antichymotrypsin (ACT), epitope 5) targeting yet another epitope of the antigen (fPSA) is added to bind with the previously captured fPSA. The two separate recognition steps result in enhanced sensitivity and specificity. The sensitivity of the SPR biosensor was recorded at 3.63 nM^{-1} with the detection limit of 0.27 nM.[79] The study also indicated that the nanoparticle-based immunosensors would be notably useful for large multi-epitope (e.g., multi-glycan) targets.

In 2016, Pihikova and coworkers constructed an EIS-based immunosensor for sensitive detection of PSA glycoprotein using gold electrode.[16] In a typical design, the anti-PSA antibody was immobilized onto the electrode surface and in the present of PSA and lectin, the specific binding generates an inert layer that alters the electrons flux to the electrode surface. Their impedance-based strategy reached an excellent limit of detection (LOD) of 100 ag/mL with a linear detection range of 100 ag/mL to 1mg/mL. In addition, compared to the serum samples from healthy individuals, those from PCa patients had a significant increment of terminal α-2,3-linked sialic acid on PSA identifiable by MAA lectin.[16] This finding highlights the usefulness of the sensor in clinical practice. In the same year, the study was extended by applying three lectins, i.e., SNA, MAA, and *Lotus tetragonolobus agglutinin* (LTA), to detect α-2,6-linked sialic acid, α-2,3-linked sialic acid, and α-L-fucose, respectively.[80] The procedure was employed for qualitative impedimetric assessment of the glycan composition on the PSA surface. In the study, the PSA glycoprotein was captured on an immobilized anti-PSA monoclonal antibody on the gold disk electrodes and followed by the addition of lectin to complete the sandwich form, as shown in **Fig. 4A (i).** The change in charge-transfer resistance (ΔR_{ct}) increased by (35 ± 2)% upon incubating the PSA-immunosensor with SNA lectin compared

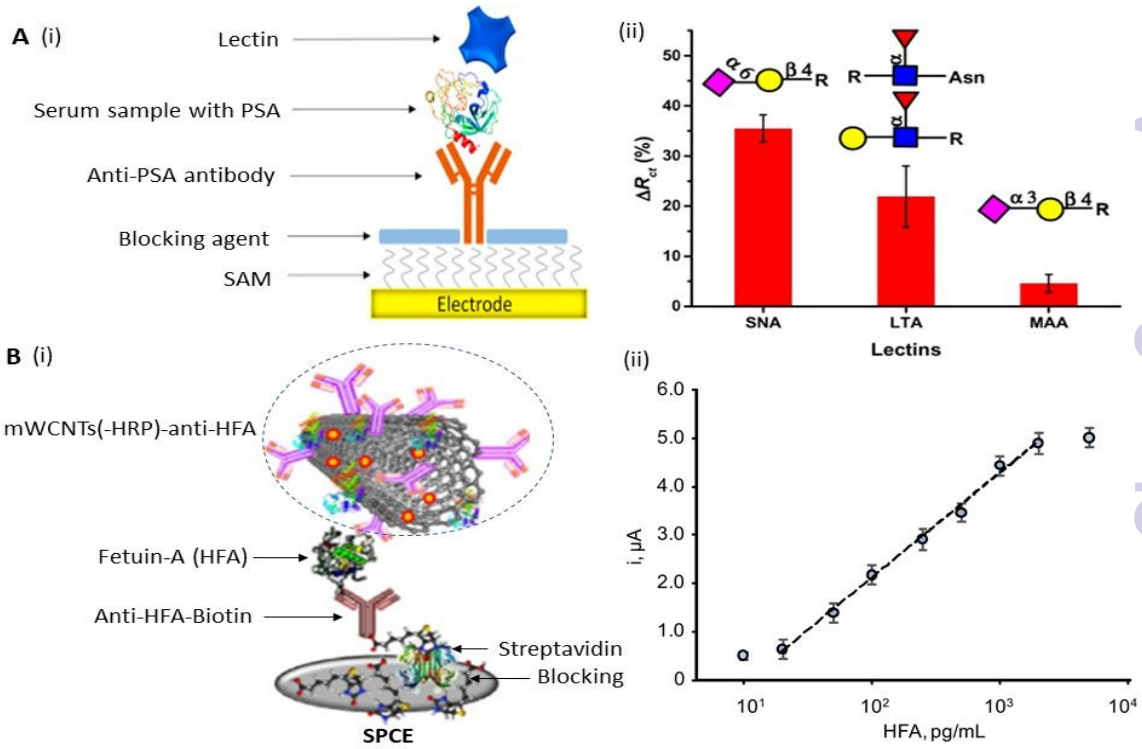

Fig. 4A (i) Construction of the sandwich immunosensor for sensing of PSA glycosylation. (Reprinted from ref.[16] with permission from Elsevier). (ii) Lectins i.e. SNA, LTA and MAA were used in the detection of PSA glycan, with the CFG nomenclature. (Reproduced from ref.[80] with permission from Wiley). (B) (i) Construction of the SPCE/ anti-HFA-Biotin-Strept/HFA/m-MWCNTs-HRP-anti-HFA. (ii) A linear detection range of 20 to 2000 pg/mL for HFA using developed immunosensor. (Reprinted from ref.[55] with permission from Elsevier).

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to that with LTA (22 ± 6)% and MAA (5 ± 2)% [**Fig. 4A (ii)]**. The findings were consistent with that of Reiding et al.[81] who performed a glycoprofiling of PSA in healthy persons using matrix-assisted laser desorption/ionization (MALDI) time-of-flight (TOF) mass spectrometry (MS) to distinguish between α-2,6- and α-2,3-linked of sialic acid. Thus, the authors concluded that the immunosensor can detect the glycoprofiling of PSA's glycan and its concentrations as the composition varies throughout the formation or progression of PCa.[80] In particular, the increase in the core fucosylation and α-2,3-linked sialic acid of PSA was more pronounced in aggressive tumors.[82] This is because Gleason 8 patients (high-risk PCa) contain mainly fucose and α-2,3-linked sialic acid compared with Gleason 7 and 6 patients (intermediate- and low-risk PCa, respectively), with a cutoff value of 30% of PSA.[39] Furthermore, the low reactivity of SNA toward α-2,6-linked sialic acid of PSA in PCa patients with metastatic tumors compared to those with localized tumors suggests that lectin biosensors can quantitatively discriminate between healthy individuals and patients with PCa.[80,82]

Using a different strategy, Kekki and coworkers developed label-based sandwich immunosensors for analyzing PSA fucosylation secreted by tumor prostate cell lines from lymph nodes (LNCaP), metastasis in bones (PC-3), and seminal plasma using *Aleuria aurantia* lectin (AAL).[83] fPSA was captured following the immobilization of biotinylated fPSA-specific recombinant Fab-fragment 5A10 on a streptavidin-coated surface. Thereafter, the sandwich assay formats (Europium (Eu)-labeled AAL and AAL-Eu(III)-chelate-dyed nanoparticle) were completed using two lectin strategies. The outcomes of the two immunoassays on PSA fucosylation were presented in fluorescence signals (cps). Both approaches could strongly detect Fucα1-6/3GlcNAc on PSA derived from LNCaP and PC-3 cells more than 30 and 5 times, respectively, compared to that on seminal plasma PSA. Additionally, the authors reported a statistically significant (p-value = 0.030) increase in the Fuc1-6/3GlcNAc content on PSA in the urine of PCa patients compared to that of BPH. Immunosensors with AAL-Eu(III)-chelate-dyed nanoparticle generated stronger fluorescence signals than immunosensors with Eu-labeled AAL, which corresponded to detection limits of 5.1 µg/L and 49.1 µg/L, respectively. Therefore, nanoparticles were reported to enhance the efficiency of immunosensors.[83] It served as a proof-of-concept for further work in this direction to develop a sensitive biosensor for the detection of PCa by integrating the advances in nanotechnology.

A recent study reported the application of magnetic multiwalled carbon-nanotubes (m-MWCNTs) as nanocarrier tags for fetuin-A detection.[55] Fetuin-A (HFA) is a relevant biomarker of PCa and the results were produced using a screen-printed carbon electrode (SPCE). The sensing strategy involved the use of a biotinylated capture antibody (anti-HFA) immobilized through streptavidin-biotin interaction (anti-HFA-Biotin-Strept) on the modified electrode. The m-MWCNTs were added into a mixed solution containing the detection antibody (anti-HFA) and HRP to form m-MWCNTs-HRP-anti-HFA conjugates. A sandwich assay configuration was implemented with SPCE/anti-HFA-Biotin-Strept/HFA/m-MWCNTs-HRP-anti-HFA as illustrated in **Fig. 4B (i)**. Next, EIS and cyclic voltammetry were used to measure all the detection steps involved in the sandwich immunosensors. The authors emphasized that magnetic nanoparticles play crucial roles in the development of immunosensors by providing a large specific surface area and improved analytical performance attributed to their weak pseudo-peroxidase activity. The sensor exhibited a linear detection range of 20 to 2000 pg/mL [**Fig. 4B (ii)**] and a LOD value of 16 pg/mL with good reproducibility (7.4% relative standard deviation, n=5) and stability for 40 days.[55] Additionally, an impressive detection of HFA glycoprotein in saliva was demonstrated with minimal sample treatments, which could be extended as a non-invasive platform technology to detect other PCa biomarkers.

3.3 Aptasensor – *aptamer probed sensing strategies*

Aptamers are nucleic acid-based recognition probes made up of single-stranded DNA or short ribonucleic acid (RNA) oligonucleotides. Aptamers were first recognized following an in-vitro selection technology known as systematic evolution of ligands by exponential enrichment, or SELEX.[84] They are the synthetic counterparts of bioreceptors with unique properties. For instance, they possess three-dimensional structures capable of recognizing and binding to a diverse set of targets with high specificity and affinity.[85] In comparison to antibodies, aptamers are beneficial based on certain characteristics such as smaller size, high stability, cost-effectiveness, easily generated, and the internal components can be easily manipulated – a process known as structural switching.[86] Nevertheless, the major value of aptamers is attributed to the simplicity of engineering its molecules into sensors and actuator devices.

The nucleic acid-based probe was first reportedly used in diagnostic applications by Davis group in 1996, following the use of fluorescent-tagged DNA ligand to detect human neutrophil elastase using optical platform.[87] In 2016, a DNA aptamer-based biosensor known as aptasensor was established for the diagnosis of PCa.[59] The team demonstrated a microfluidic chemiluminescence sensor to comparatively determine both conventional and glycosylation of PSA biomarker. The process of soft photolithography resulted in the formation of polydimethylsiloxane (PDMS) microchannels with a dimension of 200 µm in width, 20 µm in height, and 1 cm in length and sealed to a glass substrate via UV-ozone treatment. The PSA-specific DNA aptamers demonstrated by Savory et al.[85] were used and labeled with Cyanine3 fluorophore on the 3' end. Single-stranded aptamers were detected lying perpendicular to the surface and with a hydrodynamic radius of 1.4 nm as validated using a computer-controlled microspotter. Thereafter, the amount of fPSA and its glycosylation levels on the immobilized DNA aptamers were detected using secondary antibody and biotinylated lectins in a sandwich format assay. These setups led to the formation of Aptamer-Antibody Assay and Aptamer-Lectin Assay. The fabricated assay using SNA lectin and MAA II lectin was able to identify terminal α-2,6-sialylated and α-2,3-sialylated fPSA as a marker for distinguishing healthy men from those with PCa. The sandwich aptasensor performed well for the dual quantification of biomarkers with a detection limit ranging from 0.5 to 3.0 ng/mL, which was of utmost clinical significance.[59] Notwithstanding the novelty of the platform, it is predicted that the integration of microfluidic with a field-effect transistor (FET) (i.e., an electrode device) would be particularly effective for multi-glycan targets in a label-free way. This disposable assay offers a convenient method for quantifying both PSA and glycan profiling PSA biomarkers with high sensitivity and

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selectivity, paving the way for multiplexable assays for more reliable PCa screening.

In the same year of 2016, a related work by the same authors described an ancillary interface for the detection of PCa biomarkers.[88] The authors stated that further work with aptamer-integrated molecularly imprinted polymer (MIP) had the potential to lower the sensor detection limit to picomolar levels. Molecular imprinting was executed in this approach by the electropolymerization of dopamine following 13 cycles of cyclic voltammetry. This process led to the formation of a layer of thin polydopamine on the aptamer-PSA modified gold electrode. The surface binding mechanisms need to be transduced efficiently to the underlying electrodes, thus necessitating a polymer of an optimal thickness (~10 nm). Also, rigid polymers with good insulator properties are advantageous for capacitive measurements.[89] To remove the PSA protein, the grown polydopamine layer is incubated in a washing solution containing acetic acid and sodium dodecyl sulphate, thus, exposing the hybrid aptamer-MIP template. The EIS system detects a capacitive alteration, which signals the re-bound of the target PSA. This event is attributed to the fact that the binding of the PSA to the cavities of the aptamer–MIP exposes a lower influx of DNA charge to the outer electrolyte, resulting in lower resistance. The authors demonstrated an improved linear detection range of 100 pg/mL to 100 ng/mL with a limit detection down to 1 pg/mL.[88] Accordingly, they hypothesized that a synergistic identification of target PSA by the aptamer and imprinted cavity would result in a three times higher sensitivity compared to the results from their previous work.[59] Moreover, the developed hybrid aptasensor revealed an excellent selectivity when tested with human Kallikrein 2 protein (i.e., 80% homologous to PSA) and enabled a low-fouling sensing mechanism of the bound human serum albumin (HSA) plasma protein.[88] In addition to the prospects of the hybrid strategy in detecting biomarkers of PSA, the method could also be implemented in future research to improve the sensitivity and affinity detection of biomarkers of PSA glycans.

Lectins are natural carbohydrates binders and the most used receptor for glycan identification. Several studies have reported the development of synthetic receptors such as aptamer for the specific recognition of carbohydrates including monosaccharides, poly-, and oligo-saccharides as well as glycoproteins.[90,91] Cancer biomarkers could be detected by using aptamers that are rationally tailored to the glycosylation site of glycoprotein. Although the current generation of aptasensor provides information regarding total PSA, they are not sensitive to alterations in the glycosylation pattern. This limitation inspired Díaz-Fernándeza and coworkers to develop a novel aptamer capable of acting as signalling receptors for the detection of PSA glycosylation using screen-printed gold electrodes.[60] Thereafter, counter-SELEX was introduced by the authors in 2019 to evolve highly selective aptamers against the desired target (**Fig. 5A**). In this technique, the DNA library is tested against a protein isoform (i.e., a compound with similar structural patterns to the DNA library). Unbound sequences are used in the next phase of selection. The use of recombinant PSA (rPSA) without the glycan moiety permitted the isolation of a novel DNA aptamer targeting human PSA (hPSA) with an inherent preference for the glycosylation site. Herein, they developed a sandwich aptasensor for quantifying hPSA using their novel glycan-targeting PSA aptamer (designated PSA-1) and anti-PSA aptamer, with the latter having the capacity to bind to PSA protein regions.[60]

As shown in **Fig. 5B (i),** the anti-PSA aptamer was covalently attached on the modified gold electrode to serve as a capture aptamer. Following the interaction with hPSA, PSA-1 was used as a detection aptamer in chronoamperometric system. The incorporation of PSA-1 into the aptasensor construction led to the detection of hPSA serum at concentrations as low as 0.66 ng/mL,[60] significantly lower than the detection limit reported in the previous work.[59] Hence, the new approach showed promising results based on

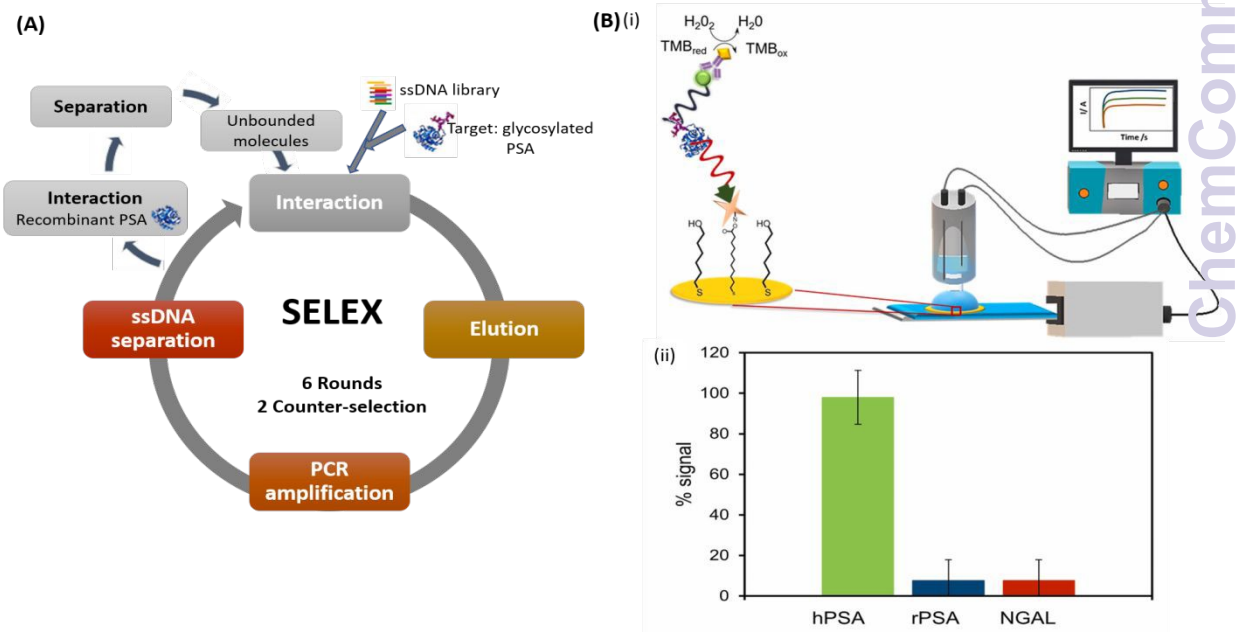


Fig. 5 (A) A schematic illustration of the SELEX procedure applied in directing the selection toward the glycan chain. (B) (i) Sandwich-based aptasensor using PSA-1 for the detection of glycans in human PSA. (ii) Sensor response to human PSA (hPSA), recombinant PSA (rPSA) and the glycoprotein NGAL. (Reprinted from ref.[60] with permission from Elsevier).

the high affinity and selectivity of the novel aptamer, as opposed to lectins, which are limited by their low affinity.[92] Furthermore, the selectivity study revealed that PSA-1 has a strong preference for hPSA over the non-glycosylated target, rPSA, and a single-glycosylated target, lipocalin-2 (NGAL) [**Fig. 5B (ii)**]. This finding is consistent with SPR studies, suggesting that PSA-1 is a glycan-binding aptamer that has a distinct feature that may not be attained by that selected by a non-directed SELEX process (i.e., denoted here as anti-PSA; protein-binding aptamer). Ultimately, the clinical significance of the aptasensor was evaluated using serum samples from PCa patients (n=15) with varying levels of total PSA. An automated ADVIA Centaur® (Siemens) enzyme-linked immunosorbent assay (ELISA) was used to measure the total PSA and validation of the results.[60] Though the authors claimed that a fraction of PSA with diverse glycan structures could be detected by the sensor, its capacity to distinguish between different PSA glycan forms remains unclear. This information is vital since some PSA glycans are associated with more aggressive forms of PCa. Further efforts to validate this strategy using a large cohort of clinical samples are required to fully elucidate the sensor's diagnostic utility for PCa diagnosis.

More recently in 2021, Díaz-Fernándeza and coworkers proved that the previously developed novel aptamer, PSAG-1[93] exhibited high affinity and selectivity towards the PSA glycosylation site, including fucose. The PSAG-1 aptamer was immobilized on the gold electrodes through thiol groups modified AuNPs.[53] A similar immobilization step was employed using a second aptamer, denoted as anti-PSA for the comparison study. Both aptasensors were challenged with varying concentrations of hPSA, rPSA, and hPSA serum. Furthermore, the analytical detection was performed using impedimetric-based EIS in which the charge-transfer resistance (R_{ct}) is modified after the direct binding of the protein to a target-recognizing aptamer. The results were validated using SPR measurements and PSAG-1 sensing interfaces produced significantly higher signals than the anti-PSA in response to the binding of hPSA and hPSA serum. However, the PSAG-1 sensor produced a lower response to rPSA than the anti-PSA sensor. This highlights the excellent sensitivity of using PSAG-1 in recognizing core-fucosylated PSA in serum with a dynamic range of the response covering the clinical range. In specific terms, good reproducibility of 5.4% and an improved detection limit of 0.26 ng/mL were obtained compared to their previous work.[60] Notably, the PSAG-1 aptasensor was successfully used in detecting changes of PSA glycans in clinical serum samples from healthy and patients with different pathologies such as benign prostate and PCa. The results showed good correlation with the commercial ELISA.[53] Although the use of synthetic aptamers for specific recognition of the target glycan biomarkers may require further steps in the counter-SELEX process,[93] this novel feature of a direct label-free approach using glycan-binding aptamers could be extrapolated to other types of biomarkers for PCa diagnosis.

3.4 Analysis of glycosylated biomarkers for clinical use

Table 2 summarizes the clinical studies of different sensing platforms for detecting PCa associated with glycan alterations. Only one study has used lectin nanoelectrodes to distinguish the degree of correlation of PCa stages with the Gleason score (GS).[58] The authors evaluated serum samples obtained from BPH and PCa patients who are classified as GS 6, 7, and 9. The PCa with GS 9 samples exhibited the highest SWV current responses (ΔI) of ~10% compared to that of GS 7 (~6%), GS 6 (~4%), and BPH (~1.8%) with a statistical significance of $p < 0.002$. The identification of patients with a high risk of metastasis tumors (GS≥7) aids their proper management. Further studies to improve the detection limit and trials in large cohort samples are required to confirm these results on the clinical use of fetuin biomarkers. On the other hand, Díaz-Fernándeza and co-workers developed aptamer-based glycan biosensors for better diagnostic performance of PCa, specifically for quantifying patients with serum PSA levels in the grey zone (4–10 ng/mL).[60] They evaluated serum samples from 15 patients and found 60% concordance with those obtained from chemiluminescence ELISA. Further, they accurately classified the discordant results with PSA values below the grey zone, and ELISA showed a PSA value above 29.69 ng/mL. This is attributed to the ability of the developed sandwich assay with the anti-PSA/PSA/PSA-1 aptamer to detect a fraction of PSA with a distinct glycan structure. Implementing this noninvasive technique in clinical practice can significantly improve the effectiveness of diagnosis and treatment of PCa and have an effective impact on costs compared to the use of antibodies and lectins. Further validation in larger independent cohorts and the use of efficient blocking agents for nonspecific interactions on

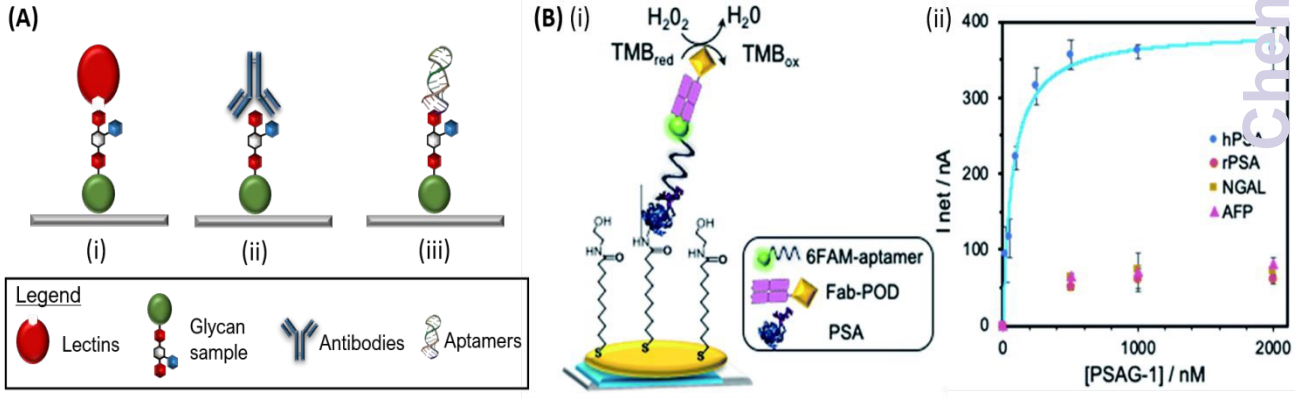


Fig. 6 (A) Glycans as biorecognition elements for the detection of different target analytes such as (i) lectins, (ii) antibodies and (iii) aptamers**.** (B) (i) The construction of glycan biosensor for the detection of target aptamer using screen-printed gold electrodes. (ii) Comparison study of the selectivity PSAG-1 towards hPSA, rPSA, NGAL and AFP. (Adapted from ref.[93] with permission from The Royal Society of Chemistry).

biorecognition interfaces are required to improve the accuracy of glycosylated PSA biomarkers in detecting PCa.

4. Glycan biosensor: an innovative recognition strategy for PCa detection

Glycosylation-based detection predominately relies on well-known biological recognition elements, such as lectins, antibodies, and nucleic acids. Several applications will be hindered if there is no recognition unit available for adequate affinity, scale, or sufficient signal transduction. Interestingly, glycans immobilized on biosensing surfaces could effectively imitate the functional role of cell surface glycans, allowing glycan-binding biomolecules to be identified in a manner comparable to natural recognition by constructing glycan-modified sensor-interface (**Fig. 6A**).

In 2017, Belicky group pioneered a method of glycan-based hanging mercury drop electrode (HMDE) for the detection of sialylated protein biomarker PSA.[37] Electrochemical reaction at mercury electrodes triggers a potential in proteins to catalyse hydrogen evolution, producing a constant current chronopotentiometric stripping (CPS), known as peak H. The authors studied the interactions between the glycans and some vital lectins, such as SNA and MAA. Based on the CPS and voltametric behavior, the developed method was capable to differentiate between the interaction of MAA lectin with PSA, which is specific for PCa, and the interaction of SNA lectin with PSA, which occurs in healthy men.[37] Although this system is favourable for sensing distinct lectins in PSA glycosylation, challenges in such constructs remain, as HMDE is an ill-fitting electrode to be used in biosensor development.

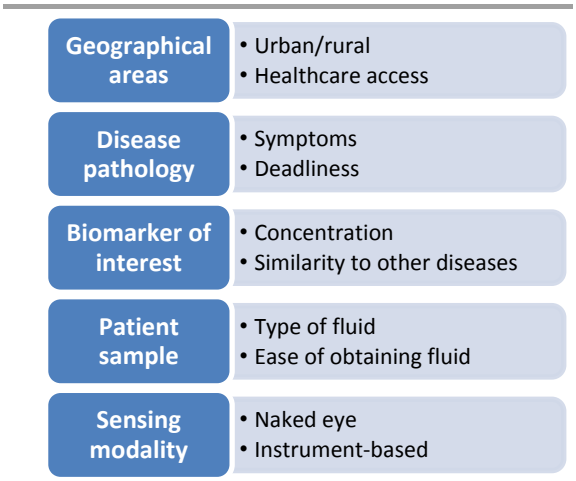
Despite the efficacy of lectin-glycan interaction, the low affinity of natural lectins limits their detection capabilities. Díaz-Fernándeza and coworkers have since focused their efforts on improving the sensor performance through the use of synthetic aptamers, which have been shown to have high affinity and selectivity for the glycosylation site of glycoproteins.[60,92] In recent work, the team implemented an innovative strategy to develop a novel aptamer, PSAG-1, by using a natural *Pholiota squarrosa* lectin (PhoSL) in a counter-SELEX round as a specific binder of α1-6-core-fucose.[93] The strategy was reported to have improved the probability of achieving aptamers specific to the glycan structure in hPSA. The authors proposed that the PSAG-1 not only acts a glycan-binder, but it is able to distinguish hPSA from other core-fucosylated proteins by recognizing the peptide region surrounding the hPSA glycosylation site. This assumption was predicted computationally using the RNA/DNA-COMPOSER tool and molecular dynamics (MD) simulations to identify sugars and nucleotides associated with the formation of glycoprotein-PSAG-1.[93]

The technology was later developed into a glycan biosensor using hPSA, rPSA, lipocalin-2 (NGAL), or α-fetoprotein (AFP) that are covalently bound to screen-printed gold electrodes (SPAuE) for electrochemical detection of PSAG-1 [**Fig. 6B (i)**].[93] The authors assembled a sandwich-based format by introducing 5'-fluorescein-labelled PSAG-1 and antifluorescein (Fab fragment)-peroxidase (POD) conjugate onto the modified electrodes to form a complete binding of SPAuE/hPSA/PSAG-1/Fab-POD, SPAuE/rPSA/PSAG-1/Fab-POD, SPAuE/NGAL/PSAG-1/Fab-POD, and SPAuE/AFP/PSAG-1/Fab-POD. Through the use of the chronoamperometry technique, sensors in the presence of 3,3',5,5'-tetra-methylbenzidine (TMB) and H_2O_2 was reported to have yielded the highest signal for hPSA (359 ± 27 nA); in contrast, it produced a much lower signal against rPSA, NGAL, and AFP. This entails preferential identification of the hPSA glycan by PSAG-1 and discrimination from a non-glycosylated form of rPSA. Likewise, PSAG-1 is a glycan-binder that can distinguish hPSA from other core-fucosylated proteins with a similar glycan structure, such as NGAL, and AFP, by recognizing the peptide region surrounding the glycosylation site [**Fig. 6B (ii)**].[93] This was the first study involving an application of glycan as affinity recognition probes targeting PCa-associated aptamer. This approach, in turn, provides a framework for establishing a patient "fingerprint" through the assay of multiple biomarkers for a specific disease stage.

Overall, these inspiring examples of the glycans as novel probe for numerous functionalities in a flexible way can shed light on interaction between glycan and diverse range of target binding, i.e., lectins, aptamers, and antibodies, making it ideal for glycol-diagnosis in PCa biomarkers.

5. Point-of-care (POC) diagnostics for PCa-associated biomarkers

As previously elucidated in the introductory section of this paper, evidently, PCa is considered as one of the major contributing factors of cancer death among men in low- and middle-income countries. Several scholars[6,7,94] believed that the variance in lifestyle, health care coverage, and limited screening practices may account for the disparities in the rate of both occurrence and deaths in the abovementioned countries. Furthermore, a lack of biopsy materials, dependency on hospital-based sampling rather than population sampling, and a scarcity of histopathologists may all be significant contributing elements to the late detection of the disease. The incidence of cancer rises dramatically with age, in which many men with PCa are symptomless until late in the course of the disease. In accordance with World Health Organization (WHO) guidelines, the Union for International Cancer Control (UICC) emphasizes the need for urgent action to increase early-stage cancer detection and



Scheme 1 Design considerations for point-of-care (POC) diagnostics.

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diagnosis, as well as on the importance of routine examination for PCa in men over the age of 50.

The next principle of screening is quicker and more economical and robust, which can be used regularly with reasonable sensitivity and specificity to allow detection in an asymptomatic phase. A paper-based technology known as the lateral flow assay (LFA) is a powerful technique with high relevance in biosensing, as it meets the requirements for point-of-care (POC) devices due to its simplicity, rapidity, low-cost, and user-friendliness detection-based device, all of which can allow more people with access in resource-limited areas to conveniently receive cost-effective health care. It should be noted that the LFA approach is the most advantageous technique for in situ and disposable sensor, i.e., one-shot analysis. The technical aspects of detection, such as the identity of the biomarker that will be detected, must be considered in the design of POC diagnostics **(Scheme 1)**.

The direct screening of PCa biomarker in urine does reasonably well in fulfilling this requirement, allowing for non-invasive method. Hence, a biomarker assay in urine might be favored over blood/serum sampling. However, there are a few critical issues to consider when analyzing biomarkers in urine. Some glycomic biomarkers are not found in urine and may only be detected in serum, where a highly glycosylated protein that forms large aggregates in urine should be removed prior to glycan analysis. Therefore, exploration of novel diagnostic biomarkers with improved sensitivity, specificity, concentration level, and easier sample collection technique must be developed.

The aforementioned issue can be tackled by using microRNAs (miRNAs), a prominent alternative among PCa biomarkers. Altered miRNA expression profiles have been related to PCa progression, aggressiveness, and metastasis, thereby underlining the prognostic significance of miRNAs.[95] Owing to their small size (18–25 nt long) and their resistance capabilities towards intracellular RNase activity, miRNAs are robust and strong biomarkers that are easily detectable in serum, plasma, urine, and other bodily fluids. Due to these attributes, miRNAs have the prospective in the utilization in cancer prognosis as non-invasive biomarkers. Screening and confirmation studies revealed that miR-107 and miR-574-3p expression profiles were elevated in the urine of men with PCa,[30] which can be used as urinary diagnostic biomarkers.

The final consideration in the development of POC diagnostics is the readout modality **(Scheme 1)**, which determines whether a reader is needed or if the readout is done visually. From this point of view, colorimetric-based LFA is a decent area for advancement because it enables naked-eye readable and rapid diagnostics in field conditions, along with its simplicity in usage. This strip-based assay is similar to the home diagnostic tests for pregnancy in terms of design specifications and response notion. The distinctively colored bands enable the detection of target analyte by the visual observation even through naked eye.

A vast number of nucleic acid-based LFAs (NALFAs) conjugated nanoparticles have been developed towards a wide range of biomarker targets, in which the probe hybridizes to a specific complementary target sequence. NALFAs-based peptide nucleic acid (PNA) probes are exceptional for sensing short nucleic acids, including miRNAs, which are typically difficult to detect using conventional oligonucleotide hybridization probe. The hybridization process generates a colorimetric signal that signifies the presence of the target biomarkers (miRNAs). Notably, the technique of incorporating nanoparticles such as gold, silver, and quantum dots as label plays an important role in improving the visual effect of LFAs with increased detection sensitivity. Thinking beyond best practices, it is also important to measure many biomarkers simultaneously for accurate cancer diagnosis, hence, the use of multiplex LFAs can aid in the detection and diagnostic process.

Although the specificity and false negative/positive signals of the paper-based detection technique remain to be assessed in future research, it is envisioned that LFAs have the potential to empower health care diagnostic with less cost, simpler tools, and more rapid detection of miRNAs without any complex sample treatment and expensive instrument. Thus, the blood test-free approach using urinary diagnostic biomarkers could provide a remarkable breakthrough in clinical analysis and at-home usage, which is particularly well-suited to resource-limited applications and remote settings in developing countries.

6. Conclusions and future perspectives

Since the introduction of glycobiology by Rademacher in 1988, glycosylation has been found to alter protein function and play a key role in the diagnosis of oncological diseases.[96] In particular, glycan alterations are widely recognized as a reliable biomarker and may even indicate disease progression. Biomarkers relying on nanomaterials and biosensing technologies have enabled early cancer diagnosis aimed at improving patient outcomes. Accordingly, numerous prostate cancer screening tests have been developed. The studies highlighted in this review cover prominent related developments published between 2010 and 2021. Although lectin biosensors have received much attention for the detection of glycan biomarkers, a new challenge in affinity biosensor development has emerged regarding the use of aptamers. The capabilities of the aptasensors described in this review highlight their great potential for multiplexed detection of relevant PCa biomarkers, such as PSA and PSA glycosylation, which reduce the risk of false positives and improve the diagnostic accuracy. Additionally, the use of nanomaterials in the biosensor's constructions has been shown to significantly improve the device sensitivity and specificity for the detection of PCa biomarkers. Moreover, aptasensors can be adapted to detect different PCa biomarkers such as miRNA in combination with the lateral flow assay platform for developing next-generation non-invasive POC testing. A considerable shift in research on glycan biomarkers and cutting-edge biosensor technology suggests the upcoming adoption of glycan biosensors as effective devices for monitoring various diseases.

Conflicts of interest

There are no conflicts to declare.

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HIGHLIGHT

Table 2. Glycan biomarkers for detecting PCa.

Method	Nanomaterial	Detection strategy	LOD	Sample	Clinical Utility	Remarks	Ref.
Lectin biosensor							
glassy carbon electrode	AuNPs/MWCNTs	Differential pulse voltammetry	5.0×10^4 cells/mL	Cell	Can quantify the average amount of sialic acid on a single PCa cell surface (LNCaP = 6.4×10^8 molecules)	Sandwich-based assay using lectin (i.e. SNA and Con A)-AuNPs-Th for specific PSA glycan interaction in PCa cell.	[57]
glassy carbon electrode	CNTs	Square wave voltammetry	0.017 µg/mL	Serum	Can screen glycosylation profiles of PCa patients with the Gleason scores of 6, 7, and 9, and BPH samples ($p < 0.01$)	*Cratylia mollis*-biosensor successfully detect monosaccharides of fetuin profile from BPH and PCa samples	[58]
glassy carbon electrodes	reduced graphene oxide	Electrochemical impedance	10^{-14} mol	Serum	Not reported	Using invertase for a strong binding between highly mannosylated fetuin towards Con A-biosensor.	[38]
Immunosensor							
gold electrode	Not reported	Electrochemical impedance	100 ag/mL	Serum	Sensitive discrimination between samples from PCa patients and healthy individuals with PCa/H = 5.5	MAA lectin was employed in glycoprofiling for IgG/HAS depleted serum samples to study PSA glycans in a sandwich format.	[16]
Screen-printed carbon electrode	magnetic-MWCNTs	Electrochemical impedance	20 pg/mL	Saliva	Not reported	Sandwich assay configuration using detection antibody conjugated with nanoparticles for selectively detect fetuin-A.	[55]
Aptasensor							
Disposable microfluidic	Not reported	Chemiluminescence signal	3 ng/mL	Seminal fluid	Not reported	Dual quantification of biomarkers (i.e. PSA and PSA glycosylation) using SNA and MAA II lectin	[59]
Screen-printed gold electrode	Not reported	Electrochemical impedance	0.66 ng/mL	Serum	Can discriminate serum PSA levels in the diagnostic grey zone (4–10 ng/mL) or above	Use counter-SELEX to produce glycan-binding aptamer (PSA-1) for detection of PSA glycans in sandwich form.	[60]
Gold electrode	AuNPs	Electrochemical impedance	0.26 ng/mL	Serum	Distinguishes PSA glycan alterations between patients with benign prostate pathology, diagnosed PCa, and healthy ones using the glycan score	Immobilization of glycan-binding aptamer (PSAG-1) for selective detection of fucose in PSA glycosylation.	[53]

AuNPs: gold nanoparticles; CNTs: carbon nanotubes; Con A: *Canavalia ensiformis*; HAS: human serum albumin; IgG: human immunoglobulin G; LTA: *Lotus tetragonolobus agglutinin*; MAA: *Maackia amurensis*; MWCNTs: multiwalled carbon nanotubes; PCa: prostate cancer; PSA: prostate specific antigen; SELEX: systematic evolution of ligands by exponential enrichment; SNA: *Sambucus nigra agglutinin*; Th: thionine.