

Published in final edited form as:

*Mikrochim Acta*. 2017 July 14; 184(9): 3049–3067. doi:10.1007/s00604-017-2410-1.

## Nanomaterial-based biosensors for detection of prostate specific antigen

Dominika Damborska<sup>1</sup>, Tomas Bertok<sup>1</sup>, Erika Dosekova<sup>1</sup>, Alena Holazova<sup>1</sup>, Lenka Lorencova<sup>1</sup>, Peter Kasak<sup>2</sup>, and Jan Tkac<sup>1</sup>

<sup>1</sup>Department of Glycobiotechnology, Institute of Chemistry, Slovak Academy of Sciences, Dubravská cesta 9, SK-845 38 Bratislava, Slovakia

<sup>2</sup>Center for Advanced Materials, Qatar University, P.O. Box 2713, Doha, Qatar

### Abstract

Screening serum for the presence of prostate specific antigen (PSA) belongs to the most common approach for the detection of prostate cancer. This review (with 156 refs.) addresses recent developments in PSA detection based on the use of various kinds of nanomaterials. It starts with an introduction into the field, the significance of testing for PSA, and on current limitations. A first main section treats electrochemical biosensors for PSA, with subsections on methods based on the use of gold electrodes, graphene or graphene-oxide, carbon nanotubes, hybrid nanoparticles, and other types of nanoparticles. It also covers electrochemical methods based on the enzyme-like activity of PSA, on DNA-, aptamer- and biofuel cell-based methods, and on the detection of PSA via its glycan part. The next main section covers optical biosensors, with subsections on methods making use of surface plasmon resonance (SPR), localized SPR and plasmonic ELISA-like schemes. This is followed by subsections on methods based on the use of fiber optics, fluorescence, chemiluminescence, Raman scattering and SERS, electrochemiluminescence and cantilever-based methods. The most sensitive biosensors are the electrochemical ones, with lowest limits of detection (down to attomolar concentrations), followed by mass cantilever sensing and electrochemiluminescent strategies. Optical biosensors show lower performance, but are still more sensitive compared to standard ELISA. The most commonly applied nanomaterials are metal and carbon-based ones and their hybrid composites used for different amplification strategies. The most attractive sensing schemes are summarized in a Table. The review ends with a section on conclusions and perspectives.

### Keywords

Biochip; Biomarker; Cantilever; PSA; Electroanalysis; Electrochemiluminescence; Nanomaterial; Nanotechnology; Optical Assays; Prostate Cancer

---

Corresponding author: Jan Tkac, Jan.Tkac@savba.sk.

**Compliance with ethical standards** The author(s) declare that they have no competing interests.

## Prostate specific antigen (PSA) as a prostate cancer biomarker

Prostate cancer (PCa) is of a major health concern, with approximately 1.1 million cases, 307,000 related deaths in 2012 worldwide and with statistics that 1 in 7 men will be diagnosed with PCa during his lifetime [1]. PCa ranks 4th place in all types of cancer in the world and is the most frequent type of cancer for men in 91 countries worldwide [2]. During the last 30 years, many US counties experienced significant decrease in mortality rates for men with PCa [3]. Survival rate in PCa are generally very good, about 98% of men diagnosed with PCa will live at least five more years after their initial diagnosis and 65–90% will live for at least 10 more years [4]. An important fact in PCa diagnosis is the age of the patient. It is estimated that 68% of all diagnosed cases are men aged 65 years or more [2], but a recent study suggests that prostatic carcinogenesis is initiated much earlier [5].

One of the key aspects to significantly reduce mortality risk is early and accurate detection of PCa. Nowadays, prostate-specific antigen (PSA) is the most effective serum marker available for the clinical detection of PCa. Together with digital rectal examination, PSA blood test is a base for PCa detection, but definitive diagnosis is given after histopathological verification of adenocarcinoma and transrectal ultrasound examination [6]. However, digital rectal examination is linked with the patient discomfort, a risk of slight bleeding and more importantly, with poor sensitivity (27.1%) and specificity of 49.0% [7]. Regarding serum PSA screening, the estimated sensitivity of a PSA cut-off of 4.0 ng mL<sup>-1</sup> was 21% for detecting any PCa and 51% for identifying high-grade cancers [8].

PSA as a biomarker has revolutionized PCa diagnostics [9] and was first identified in human seminal plasma as  $\gamma$ -seminoprotein in 1969 [10]. PSA (also known as human kallikrein 3 = hK3) is about 28–32 kDa single chain glycoprotein belonging to the family of human glandular kallikrein proteins defined as serine proteases [11]. One of the other member of this family is human glandular kallikrein 2 (hK2) sharing many similarities with PSA (78% and 80% identity at amino acid and DNA levels [12], respectively). Thus, hK2 has been recognized as potential diagnostic marker and also as a negative control in specificity control experiments [13]. Circulating PSA includes few molecular forms of PSA in human body; free PSA (fPSA), complex PSA (cPSA) form with plasma proteins, especially serine protease inhibitor  $\alpha$ 1-antichymotrypsin (ACT) and inactive PSA. Total PSA (tPSA) is referred to a total amount of PSA (fPSA + cPSA).

PSA is not cancer-specific and therefore it may be elevated in both malignant and benign conditions such as benign prostate hyperplasia (BPH), prostatitis and others. Physiologically, serum PSA is present in the range 0–4 ng mL<sup>-1</sup> and with the development of PCa its serum level increases [9]. It is well known that PSA is considered as not being sufficiently specific biomarker for PCa detection in the diagnostic grey zone (4–10 ng mL<sup>-1</sup>), complicating to provide a clear difference between benign and malignant cases. Moreover, it was found a direct correlation between age of men and their serum PSA level [14]. The age-specific upper concentration limits for total PSA in men's serum are 2.5 ng mL<sup>-1</sup> (40–49 years), 3.5 ng mL<sup>-1</sup> (50–59 years), 4.5 ng mL<sup>-1</sup> (60–69 years) and finally 5.5 ng mL<sup>-1</sup> for men up to 79 years (an increase by about 1 ng mL<sup>-1</sup> every 10 years). For the sensitivity improvement,

enhancement of specificity in PSA diagnostic grey zone and for prediction of tumor aggressiveness and PCa morbidity, fPSA/tPSA ratio is commonly used [15, 16].

Protocols and diagnostic methods currently used in clinics are transrectal ultrasound and digital rectal exam with biopsy and histological evaluation of a tumor tissue, and ELISA-based blood test for PSA level screening, offering less sensitive approach compared to almost all types of nanostructure-based biosensors described further in the text. Another issue of PCa detection is the fact, that approximately 68% of all diagnosed cases are without any symptoms, including low back pain, hematuria and/or difficulty with urination [5]. Therefore, development of more reliable, low cost and fast analytical methods for accurate PSA detection is needed. ELISA blood tests for PSA level screening, offer a limit of detection (LOD) in picomolar range (pM level, i.e.  $\sim 10 \text{ pg mL}^{-1}$ ). Pitfalls of ELISA method are quite time-consuming analysis, cross-reactivity of antibodies, high limit of detection, tedious washing steps and laboratory variability resulted in data variation among clinics [17]. A major challenges in development of novel assay methods for determination of PSA are short detection time, reliability and achievement of low detection limits with high sensitivity of detection. Therefore, the new biosensor-based detection as potential point-of-care devices are gaining attention to address such challenges [18, 19].

## Electrochemical biosensors for PSA detection

Among others, electrochemical detection of clinically relevant biomolecules belongs to the most popular ones, because it is generally cheap, easy to perform and offer good sensitivity, a wide linear range and low LOD [20]. Moreover, a broad variety of biorecognition elements and nanomaterials enhancing the biosensors performance may be utilized (e.g. antibodies and their fragments, receptors, lectins, enzymes, peptides, nucleic acids, aptamers, etc. in combination with metal, carbon or other types of nanostructures) [21]. Electrochemical lab-on-a-chip-based biosensors offer miniaturized and user friendly platform for biomarker analysis [22]. On the other hand, electrochemical biosensors have a few limitations such as reproducibility of their preparation, difficulties in regenerating the sensing surface and usually an indirect sensing system [23].

Detection of PSA level in serum (comparable to hospital results) was performed using fully automated, amperometric MiSens prototype in 2016 [24] (Fig. 1). A marketable lab-on-a-chip system (so-called „Fraunhofer ivD-platformB) for detection of nucleic acids and protein markers (PSA and C-reactive protein - CRP, as well) using electrochemical (a redox cycling approach) or an optical (total internal reflectance fluorescence) read-out was however already demonstrated in 2012 [25].

Initial efforts in construction of electrochemical PSA biosensors offered LOD, which are typical for ELISA using either antibodies or aptamers. Antibodies were immobilized on a nanoporous gold surface coated by self-assembled monolayer (SAM) [26], a nanocomposite containing gold nanoparticles (AuNPs) and functionalized multi-walled carbon nanotubes (MWCNTs) [27, 28], AuNPs for a real-time electrochemical profiling on a lab-on-chip device [29], reduced graphene oxide (rGO) modified by silk peptide [30], AuNPs deposited on silica nanospheres together with a redox dye [31] and on an ordered and hierarchically

organized interfacial supramolecular architecture [32]. Aptamers were immobilized on a nanocomposite consisting of AuNPs and mesoporous carbon [33].

### Electrochemistry on modified gold electrodes

ELISA is without any doubts the most common immunoassay method widely used in clinical laboratories, however, with higher detection limits compared to other analytical methods. This is the main reason why in many studies the focus was on improving this feature by combining some of the ELISAs advantages with other assay platforms.

In particular, for detection of PSA a magnetic bead-based electrochemical impedance spectroscopy was applied [34]. The sensitivity was enhanced by working in microwells using electric field (a working and a counter electrode in a microwell array) focusing on bead surfaces with LOD down to  $10 \text{ fg mL}^{-1}$  [34]. Cysteamine modified Au electrode with (ferrocenes-modified) PAMAM dendrimers and anti-PSA attached showed LOD of  $1 \text{ pg mL}^{-1}$  with a wide linear range up to  $100 \text{ ng mL}^{-1}$  of PSA in a human serum [35]. Gold surfaces offer the possibility for an easy, one-step formation of self-assembled monolayers consisting of thiolated molecules, controlling the surface density and/or orientation of biorecognition elements, as well as the overall architecture at nanoscale. Using this patterning protocol, non-specific interactions (i.e. adsorption) can be significantly suppressed.

### Carbon nanomaterial-based electrochemical detection

Use of different nanomaterials is often a way to enhance (not only) the electrochemical biosensor performance. An extremely popular nanomaterial since the 2010 Nobel Prize for physics in the area of sensing technology is graphene [36]. Graphene is often used to increase the conductivity and stability of prepared immunosensors, as in case of disposable (screen-printed electrodes-based) sensors for ultralow detection of PSA down to  $2 \text{ pg mL}^{-1}$  with a wide linear range of 6 orders of magnitude [37]. A common way to introduce graphene to an electrode surface is to prepare (nano)composites using polymer matrices, electrochemical mediators or other types of nanomaterials. Chitosan was used as a dispersant in order to achieve a well-oriented immobilization of anti-PSA antibody in a graphene/methylene blue/chitosan nanocomposite for a label-free detection of PSA down to  $13 \text{ pg mL}^{-1}$  with a linear range up to  $5 \text{ ng mL}^{-1}$  [38]. In both previously mentioned references, the recovery of the PSA from a real clinical human serum samples was in the range of 99–107% [37] and 100–102% [38], respectively. These kinds of biosensors can be further applied for analysis of a wide range of analytes, depending on the binding antibody used for the biosensor preparation – this is a general feature for many types of affinity biosensors.

In addition to graphene, other frequently used platforms for signal amplification by different bioconjugation techniques are carbon nanotubes. The enhanced precipitation of a HRP's co-substrate (4-chloro-1-naphthol) was observed using cyclic voltammetry (CV) and square wave voltammetry (SWV) [39, 40]. In the latter work, MWCNTs/ionic liquid/chitosan/AuNPs-polyamidoamine (PAMAM) dendrimer interface was prepared as a support for a

sandwich protocol using anti-PSA-HRP as a label. Using this interface, the detection limit was 20-fold lower compared with their previous work, i.e.  $1 \text{ pg mL}^{-1}$  [39, 40].

### Hybrid nanoparticle-based electrochemical detection

In previous examples, a sandwich configuration was used for PSA detection, what is currently the most common way to enhance the signal coming from a biospecific interaction. For this purpose, several nanohybrids were used in the past years, such as NPs loaded on graphene (g), on rGO or on MWCNTs i.e. rGO/graphitic carbon nitride (rGO-C<sub>3</sub>N<sub>4</sub>) (LOD =  $16.6 \text{ fg mL}^{-1}$ ) [41], nanogold-functionalized g-C<sub>3</sub>N<sub>4</sub> (LOD =  $5.2 \text{ pg mL}^{-1}$ ) [42] or Pd-Ag/MWCNT as a label for a secondary antibody (LOD =  $0.03 \text{ pg mL}^{-1}$ ) [43]. A composite MWCNT)/AuNP with higher number of HRP molecules co-immobilized on a nanohybrid together with anti-PSA antibody led (compared to a single-HRP strategy) to low LOD for PSA down to  $0.4 \text{ pg mL}^{-1}$  [44].

Besides the use of carbon nanotubes, halloysite nanotubes with polypyrrole (electrically conducting polymer) shell and PdNPs were used for amperometric PSA detection down to  $0.3 \text{ pg mL}^{-1}$  [45].

In order to further enhance the fixation capacity of anti-PSA antibody, mesoporous silica nanoparticles (MSNs) were also used. In the first case, in combination with AgNPs to enhance the electron transfer rate for a label-free immunosensor (LOD =  $15 \text{ pg mL}^{-1}$ ) for PSA detection in human serum [46], and in the second case as a controlled release system [47]. Functionalized silica NPs were used for thionine (Th, an electrochemical mediator) encapsulation by capping the pores with carboxylic acid-modified AuNPs. In acidic pH, an acid-labile linker on MSN-Th-AuNP was hydrolyzed and Th was released. By a specific biorecognition of PSA using a secondary anti-PSA immobilized on MSN-Th-AuNP, the PSA concentration (PSA on an electrode interface) was measured by differential pulse voltammetry (DPV) [47].

Three dimensional and a label free PSA immunosensor with LOD of  $0.59 \text{ ng mL}^{-1}$  (a linear range up to  $10 \text{ ng mL}^{-1}$ ) was constructed using graphene-gold composites [48]. A similar paper suggested an enhanced conductivity of (rGO)/Ag composite compared to bare rGO [49].

If the analysis should be provided by a point-of-care diagnostic device, the detection platform should be disposable or reusable and analysis performed in a cost-effective way. A reusable device (a temperature-induced regeneration of a polymer interface) was introduced in 2016 for quantitation of various biomarkers. In case of PSA a LOD of  $0.9 \text{ pg mL}^{-1}$  was achieved, when horseradish peroxidase (HRP) and antibody-labelled NPs were used for the signal amplification [50]. Surface regeneration is a required attribute of the sensor in case when the price of the sensor is high with surface plasmon resonance (SPR) based immunosensors being good examples.

For a reliable PCa diagnostics, more than a single marker should be evaluated. In a work of Pan et al., a simultaneous detection of VEGF (vascular endothelial growth factor) and PSA in human serum was performed using dual-modality biosensor using GO/ssDNA and poly-

L-lactide nanoparticles on a gold electrode surface [51]. The biosensor showed no difference in signal compared to a standard ELISA analysis of three patient sera with an early stage PCa [51].

A multiplexed analysis using an 8-sensor detection chamber in a microfluidic system was used by immobilization of antibodies on disposable screen-printed carbon electrodes modified by rGO [52]. Moreover, GO nanosheets decorated with Fe<sub>3</sub>O<sub>4</sub> nanoparticles and anti-PSA antibodies for selective PSA enrichment were also applied [52]. This protocol offers several advantages and solutions to the most problematic aspects of biomarker detection, e.g. possible array format of analysis for assay of more biomarkers (PSA and PSMA – prostate specific membrane antigen, respectively). Moreover, the selective magnetic enrichment of a low abundant protein led to an increase in sensitivity of the procedure (ultralow LOD down to 15 fg mL<sup>-1</sup> and 4.8 fg mL<sup>-1</sup> for PSA and PSMA, respectively) in a serum sample. This is an important feature allowing to dilute abundant interfering compounds present in serum [52]. However, the concept of the use of magnetic NP-based enrichment for selective PSA binding was published in 2012 by the group of Gooding, with gold-coated magnetic NPs functioning as „dispersible electrodes,, [53] (Fig. 2).

### Novel types of nanoparticles applied for electrochemical detection

Several new, innovative ways for further sensitivity enhancement were described for electrochemical biosensors for PSA analysis. In 2016, cubic Cu<sub>2</sub>O nanoframes with hollow edges (with good electrocatalytic activity towards ferrocene (Fc) derivatives and hydrogen peroxide) were used for the first time [54]. Signal enhancement by Pt-Cu hierarchical trigonal bipyramid nanoframes as a label and Fe<sub>3</sub>O<sub>4</sub> NPs/rGO/polydopamine composite for anti-PSA capture was described one year later [55]. LODs were very similar in these cases, 0.05 pg mL<sup>-1</sup> [54] and 0.03 pg mL<sup>-1</sup> [55], respectively. Novel conductimetric immunosensing of PSA using metal organic framework offered higher LOD of 0.06 ng mL<sup>-1</sup> compared to previous nanoframes methods, but still sufficient for clinical analysis with a dynamic linear range up to 100 ng mL<sup>-1</sup> [56]. Dual-responsive immunosensor for cancer biomarkers determination including PSA utilize colorimetric and electrochemical approach via double enzymatic signal amplifications by HRP- and antibody-labelled NPs yielded reproducible results with LOD of 0.7 pg mL<sup>-1</sup> or 0.8 pg mL<sup>-1</sup> for PSA, respectively [57]. For further signal enhancement during analysis of PSA, so-called triple signal amplification strategy using different nanostructures was applied [58, 59]. Such approach offered lower LOD compared to the biosensor with enzyme-linked aptamer attached to AuNP/PAMAM with LOD of 10 fg mL<sup>-1</sup> (using DPV) or of 5 pg mL<sup>-1</sup> (using EIS) [60]. A LOD of 146 fg mL<sup>-1</sup> and a wide linear range from 0.5 pg to 100 ng mL<sup>-1</sup> was achieved using bovine serum albumin (BSA) stabilized Cu nanoclusters as redox species and PtNPs for immobilization of labeled anti-PSA [61]. Signal amplification strategies (in general) lead to lower LOD when compared to the standard ELISA and fluorescent microarray techniques [62], but labeling can lead to a change in receptors affinity/avidity to a ligand molecule.



### Electrochemical detection of PSA via its enzymatic activity

The biosensor employing a specific cleavage of surface immobilized short peptide (CEHSSKLQLAK-NH<sub>2</sub>) by PSA offers LOD of 0.11 fg mL<sup>-1</sup> [63]. High initial current signal decreased when Au-poly(methylene blue) and Au-rGO nanocomposite with HRP conjugated to the peptide were released after enzymatic action of PSA [63].

### DNA- and aptamer-based electrochemical detection

Finally, a rolling circle amplification (RCA) reaction with poly(thymine)-templated CuNPs for a signal amplification during PSA analysis should be mentioned. Construction of specific primer-AuNP-aptamer/PSA/anti-PSA sandwich triggered the RCA reaction, where an enormous number of tandem poly-thymine repeats were generated and served as templates for CuNPs formation. DPV signal was generated by dissolving the CuNPs and by measuring the released Cu ions. In this study, LOD of 0.02 ± 0.001 fg mL<sup>-1</sup> was achieved for detection of PSA [64].

In addition to antibodies and peptides also mentioned above (since PSA is an enzyme specifically cleaving HSSKLQL amino acid sequence – a feature also important for PSA analysis) [65, 66], aptamers are very commonly used to specifically bind PSA from a sample (usually with different nanomaterials such as graphene, carbon nanotubes or AuNPs) [67–69].

Estrella et al. published several papers dealing with aptasensors-based PSA detection and different problems specific for affinity biosensors, such as: i) non-specific interactions (their elimination using zwitterionic, sulfo-betaine moieties with efficient antifouling properties) [70], ii) increasing the aptamer affinity towards PSA analyte by preparation of molecularly imprinted polymer-aptamer hybrid receptors with low cross-reactivity of the surface with a homologous hK2 [13] and ultralow LOD for early PCa diagnostics or detection of other low abundant markers present in complex matrices [71].

β-Cyclodextrins (β-CD) were also utilized for the surface modification and subsequent PSA analysis due to their ability to create gates for the electron transfer of electrochemical probes [72] or because of the host-guest interaction between ferrocene and β-CD, offering a higher concentration and electro-stability of ferrocene derivatives on a surface [73].

### Biofuel cell-based electrochemical detection

For the PSA tumor marker detection, a visible light-driven biofuel cell was also utilized, consisting of a nanostructured bioanode for visible light-driven glucose oxidation and a biocathode, where bilirubin oxidase catalyzed O<sub>2</sub> reduction [74]. With an increase of PSA, the amount of bilirubin oxidase molecules increased (BOD is a part of a detection probe with detection antibody), thus leading to a higher current output and the device offered LOD of 0.1 pg mL<sup>-1</sup> [74].

### Glycoprofiling of PSA as a potential biomarker

Slightly different approach for PSA analysis compared to above mentioned ones that should lead to a more reliable differentiation of healthy people and PCa patients, was used by our

group. In a series of papers, we demonstrated preparation of PSA immunosensor using self-assembled monolayers on gold. A nanoscale-controlled interfacial layer and a sandwich configuration using different lectins were applied for glycoprofiling of PSA attached to anti-PSA antibodies immobilized on the electrode [75, 76]. A LOD of  $100 \text{ ag mL}^{-1}$  with a linear concentration range spanning 10 orders of magnitude was achieved using impedimetric assays. *Maackia amurensis* II agglutinin was revealed to be the most promising lectin among the tested ones for the reliable glycoprofiling of PSA from PCa patients (e.g. increase in the  $\alpha$ -2,3-terminal N-acetylneuraminic acid for PCa patients compared to healthy individuals) [77].

Besides use of lectins, there is another way for the (less selective, compared to lectins) glycoprotein analysis – using boronic acid derivatives recognizing diols in glycan structures. 4-mercaptophenylboronic acid-capped AuNPs were used for the voltammetric determination of complex-type N-glycan containing PSA and mannose-rich avidin [78].

Even though impedimetric biosensors belong to most sensitive ones, offering extremely low LOD, it is of great importance to lower the non-specific interactions. In real serum samples, abundant proteins such as albumin are present in concentrations of about 106 times higher compared to the level of oncomarkers. The high selectivity is thus the main priority for affinity biosensors. To make the electrochemical methods competitive to ELISA, for a routine testing, a method as simple as possible should be developed, to provide an easy, fast, reliable, robust and cheap analysis.

## Electrochemiluminescence-based PSA detection

A wide range of PSA sensing protocols has been published based on electrochemiluminescent (ECL) determination, which combines the advantages of chemiluminescence and electrochemistry. ECL is a promising tool in immunoassays and clinical diagnosis due to its high sensitivity, simplicity, good stability, low background and visual imaging capability [79]. ECL is a process of transforming an electrochemical energy into a luminescent energy with emission of light from a luminophore excited-state formed by an electron transfer reaction. There are two best known ECL system: luminol +  $\text{H}_2\text{O}_2$  and  $\text{Ru}(\text{bpy})_3^{2+}$  + tripropylamine (TPA) [80]. In the process of ECL, the electrochemical oxidation of luminophore (luminol or  $\text{Ru}(\text{bpy})_3^{2+}$ ) is necessary for ECL emission. The starting point for oxidation is application of a potential in the presence of a co-reactant ( $\text{H}_2\text{O}_2$  or TPA). Oxidized product reacts with the co-reactant resulting in formation of highly reducing radical. Excited-states of luminophores are subsequently generated in the following reactions resulting in light emission (Scheme 1) [81–83]. An alternative way for generation of chemiluminescence signal is demonstrated using the catalytic effect of ferrocenecarboxylate on the cathodic ECL signal of peroxodisulfate [84].

ECL intensity can be enhanced via immobilization of luminol on the modified electrode surface, especially with various nanoparticles (NPs). A sensitive multi-functionalized BSA encapsulated platinum and silver alloy core-shell nanoparticles ( $\text{Pt/Ag@BSA}$ ) were applied for construction of ECL immunosensor for PSA quantitation [85]. A composite  $\text{Pt/Ag@BSA}$  was used as multifunctional carrier, onto which numerous luminol molecules and PSA



antibodies were immobilized. Indium tin oxide (ITO) covered by AuNPs acted as extraordinary base for this biosensor, due to its ability to promote the electron transfer and to load large amount of Pt/Ag@BSA. Immunosensor had relatively low LOD of  $0.3 \text{ pg mL}^{-1}$  and a linear range from  $0.001$  to  $200 \text{ ng mL}^{-1}$ . Spiking experiments with human serum samples revealed recovery in the range 98–102% [85].

Another example of using luminol in combination with nanomaterials for PSA detection was fabrication of an immunosensor based on dendrimer-encapsulated AuNPs labels [86]. As in the previous case, ITO working electrode was modified with NPs ( $\text{Fe}_3\text{O}_4@\text{SiO}_2$ ), which were covered by a primary antibody and served as ECL labels. The biosensor exhibits a linear response range from  $0.001$  to  $100 \text{ ng mL}^{-1}$  with the same LOD as in previous case, i.e.  $0.3 \text{ pg mL}^{-1}$  [86].

In 2013 Sardesai et al. constructed a microfluidic ECL device for extraordinarily sensitive quantitation of two cancer biomarker proteins PSA and interleukin-6 (IL-6) [87]. Both proteins were detected in synthetic cancer patient serum samples within 1.1 h and the results correlated well with single-protein ELISA with LOD of  $9 \text{ zM}$  ( $100 \text{ fg mL}^{-1}$ ) and  $1 \text{ zM}$  ( $10 \text{ fg mL}^{-1}$ ) for PSA and IL-6, respectively. The potential applied during the assay procedure was  $0.95 \text{ V}$  (vs. SCE), what can be problematic for electrochemical detection in real human sera due to presence of many redox active interfering compounds. The device utilized single-walled carbon nanotube forests decorated with an antibody at the bottom of the wells. After the antigen capture, a RuBiPy-silica-secondary antibody label was applied on the array, followed by addition of reductant TPA to produce ECL [87]. The same team described another type of ECL protein immunoarray using RuBiPy-doped silica nanoparticles and reached LOD down to a few  $\text{fg mL}^{-1}$  range [88].

In addition to “traditional” ECL luminophores, there is an increased attention to apply nanomaterials as ECL labels, including semiconductor nanocrystals such as CdSe, CuS,  $\text{MoS}_2$ -CdS,  $\text{CeO}_2$ , CdS, CdTe quantum dots (QDs), graphene dots, titanium dioxide ( $\text{TiO}_2$ ) and its derivate, etc. The ECL biosensor using flower-like 3D  $\text{TiO}_2$  (fl- $\text{TiO}_2$ ) as signal labels for secondary antibody was demonstrated [89]. Capture antibodies were immobilized on Au/Pd-modified ITO electrode and  $\text{S}_2\text{O}_8^{2-}$  served as a co-reactant of ECL reaction. The immunosensor exhibits LOD of  $0.32 \text{ pg mL}^{-1}$  [89]. There are also many other papers describing use of nanoparticles as ECL luminophores for PSA immunosensing with LOD down to a  $\text{pg mL}^{-1}$  range [90–93].

Very low LOD was reached by the immunosensor based on CdS NPs sensitized  $\text{TiO}_2$  nanotubes (an emitter), a CdTe NPs functionalized multi-wall carbon nanotube (CdTe-MWNT) composite (a quencher) and  $\text{K}_2\text{S}_2\text{O}_8$  as a co-reactant (Fig. 3) [94].  $\text{TiO}_2$  nanotube sensitized with CdS NPs has 14.1 times stronger cathodic ECL intensity than that of pure  $\text{TiO}_2$  nanotube electrode. The biosensor exhibits extremely low LOD of  $1 \text{ fg mL}^{-1}$  with a linear range exceeding 5 orders of magnitude (from  $1 \text{ fg mL}^{-1}$  to  $10 \text{ pg mL}^{-1}$ ). The potential in medicinal application was demonstrated by analysis of PSA in human serum samples with satisfactory results (average recovery of 98%; average RSD of 0.37% ( $n = 3$ )) [94].

The lanthanide-based luminescent probe (EuPO<sub>4</sub> nanowire) has been exploited in the work of Ma et al. due to its special properties, such as a long luminescent lifetime, a narrow emission band, low toxicity and high quantum yield [95]. The authors constructed an ultrasensitive label-free ECL immunosensor for PSA analysis with LOD of 177 fg mL<sup>-1</sup>. In the study chitosan was applied for covalent attachment of captured antibodies (via -NH<sub>2</sub> groups) and for better dispersion of EuPO<sub>4</sub> nanowires on glassy carbon electrode. Recovery index was in range from 96% to 101% and all RSD were less than 6.0%, making the biosensor suitable for PSA detection in serum samples [95].

Aptamers are vital alternatives to antibodies. A signal-switchable ECL aptasensor based on CdS flower-like 3D assemblies was demonstrated [96]. Ferrocene modified graphene sheets were applied for highly efficient quenching of ECL. Captured DNA was immobilized on the modified electrode and served as an anchor for PSA aptamer. After that, conjugate sheets of ferrocene and graphene were applied. Thanks to strong  $\pi - \pi$  stacking interaction between graphene and nucleotides, graphene sheets were adsorbed on the single-stranded DNA. After PSA addition, aptamer tended to form a complex with PSA molecules resulting in graphene-ferrocene dissociation with recovery of an ECL signal. The aptasensor offered low LOD (0.8 pg mL<sup>-1</sup>) and was tested in human serum [96].

A DNA-based supersandwich ECL immunosensor based on carbon nanotube and AuNP modified electrode offered LOD of 4.2 fg mL<sup>-1</sup> [97]. While capture antibody is immobilized on the modified electrode, detection antibody is attached to dendrimer together with an auxiliary DNA probe 1 with Ru complex and supersandwich is formed by addition of an auxiliary DNA probe 2 with histidine. Histidine residue after hybridization of both DNA probes come together to provide ECL signal and in order to enhance the signal incubation with DNA probe 1 and DNA probe 2 can be repeated [97].

## Optical biosensors for PSA detection

Optical biosensing for PSA detection in PCa biomarker research represents a widespread principle due to numerous benefits offered, such as direct and rapid quantitation, high specificity, easy miniaturization and in some cases also real-time monitoring of biospecific interactions [98]. However, some limitations of this sensing principle, i.e. sensitivity, photobleaching of fluorescent probes and high cost of analysis due to certain instrumentation requirements should be overcome [99]. Herein, the main focus is placed on the most widely used optical biosensor platforms published so far, which are surface plasmon resonance (SPR)-based biosensors including SPR imaging and localized SPR (LSPR). In addition to these techniques, other optical biosensor systems are reported below, such as fluorescence, chemiluminescence and surface-enhanced Raman scattering-based biosensors.

### Surface plasmon resonance (SPR) biosensors

SPR-based biosensing of biomolecules has been a topic drawing a substantial scientific interest in the past decade especially due to a label-free, direct and real-time detection with possible kinetic studies. SPR biosensor for analysis of proteolytically-active PSA offering LOD of 100 pg mL<sup>-1</sup> and a short response time was reported using magnetic nanobeads conjugated with a peptide [100]. The same principle of detection was utilized in the cell-on-

chip analytical system for real-time detection of secreted molecules (PSA and  $\beta$ -2-microglobulin) from human prostatic carcinoma cell lines (LNCaP) [101]. Determination of secreted biomolecules was reached using a configuration with alginate beads for trapping of living cells on the top of antibody spots (Fig. 4). SPR method of detection was applied for determination of kinetic parameters of PSA-specific antibodies towards PSA, but also for detection of PSA with LOD of 0.27 nM ( $8.6 \text{ ng mL}^{-1}$ ) [102].

The drawback of SPR biosensing is quite high detection limit, thus a broad variety of nanoparticles were employed for signal amplification [103, 104]. The utilization of both matrix elimination buffer and AuNPs for SPR analysis of PSA was described [105]. By performing a sandwich assay using antibody-modified nanoparticles LOD of  $2.3 \text{ ng mL}^{-1}$  (AuNPs, 20 nm) and  $0.29 \text{ ng mL}^{-1}$  (AuNPs, 40 nm) for tPSA spiked in 75% human serum were achieved [105]. Besides, the authors compared SPR biosensor platform with quartz crystal microbalance (QCM) device indicating that both methods provide comparable performance for PSA analysis [105]. Results from direct and sandwich assays performed in this study using the SPR and the QCM instruments produced linear lines with  $R^2$  values of 0.98 and 0.99, respectively, suggesting that both these methods are useful for PSA determination in diluted human sera.

Furthermore, a novel strategy for improvement of SPR biosensing sensitivity was based on the construction of a new PSA-imprinted SPR sensor chip [106]. The imprinted SPR chip was designed in the presence of methacrylic acid as a functional monomer and ethylene glycol dimethacrylate as a cross-linker via UV polymerization using a microcontact imprinting technique. The microcontact-PSA imprinted SPR chips showed ultrasensitive PSA determination with LOD of  $91 \text{ pg mL}^{-1}$  ( $18 \times 10^{-14} \text{ M}$ ) [106]. A similar imprinting-based approach was applied for the selective binding of a PSA, when PSA was imprinted in the polymer, but the imprinting polymer also contained boronate functional groups for an interaction of the matrix with a glycan part of PSA [107]. Because the boronate-glycan interaction can be disrupted by low pH, a simple pH lowering was applied to remove the PSA template from the surface. Such an approach exhibited a 30-fold higher affinity for its target compared to that of other (glyco)proteins [107].

In addition to the SPR technique, localized surface plasmon resonance (LSPR) is an emerging label-free assay platform due to highly-sensitive and robust determination of biomolecules [108]. Paralleled LSPR lab-on-a-chip device with real time monitoring of 32 sensing sites distributed across 8 independent microfluidic channels offered fast detection of PSA down to  $500 \text{ pg mL}^{-1}$  in a complex matrix (50% human serum) [109].

A response from a single AuNP as the shift value of LSPR maximum wavelength using dark-field microspectroscopy system was applied for determination of PSA-ACT ( $\alpha$ 1-antichymotrypsin, PSA serum inhibitor) complex with a linear dynamic range from  $10^{-4}$  to  $10 \text{ ng mL}^{-1}$ , with LOD of  $0.1 \text{ pg mL}^{-1}$  determined with an LSPR  $\lambda_{\text{max}}$  shift of  $\sim 3.0 \text{ nm}$  [110].

### Plasmonic ELISA-like detection

Several plasmonic-based ELISA sensing platform have been reported for detection of various biomarkers [111]. The method was pioneered by Stevens et al. [112, 113] with initial achievements reviewed [114].

In the case of PSA determination, the development of triangular silver nanoprisms (AgNPRs) etching-based plasmonic biosensor was reported by Liang et al. [115]. Triangular AgNPRs are etched by hydrogen peroxide produced by glucose oxidase (an enzyme label) catalyzed oxidation of glucose with change of the nanoparticle shape [116]. This subsequently causes a concomitant blue shift of the SPR spectra (Fig. 5) depending on the change of the particle architecture [116]. The triangular AgNPRs-based plasmonic ELISA approach detected PSA at concentration as low as  $4.1 \text{ fg mL}^{-1}$  in a wide linear range from  $10 \text{ fg mL}^{-1}$  to  $100 \text{ pg mL}^{-1}$  in patient serum samples from 16 donors [115].

Very sensitive detection of PSA was performed by plasmonic ELISA-like approach based on enlargement of 5 nm AuNPs by  $\text{H}_2\text{O}_2$  produced in the presence of PSA [117]. PSA is captured on the surface by a primary antibody, then reacts with a detection antibody conjugated with glucose oxidase on the surface of magnetic beads. In presence of glucose,  $\text{H}_2\text{O}_2$  is formed, inducing growth AuNPs in the presence of  $\text{HAuCl}_4$  and the solution turns red from colorless. The biosensor offers LOD of  $93 \text{ aM}$  ( $\sim 3.0 \text{ fg mL}^{-1}$ ) and reliable results compared to HRP-based ELISA in 10,000-fold diluted human sera [117]. An advantage of using labeled biorecognition elements here is the fact one can use highly diluted sera, what leads to a minute sample consumption.

### Fiber optic detection

Further improvement in optical biosensor design represents a fiber-optic (FO) technology. The implementation of FO to LSPR biosensing offers many advantages, such as high-precision mechanism for angular or wavelength interrogation of metal films and no need for bulky optics [118].

An emerging lab-on-fiber concept was proposed to establish a new generation of miniaturized configuration at the top of the fiber for sensing applications. An ultrasensitive, robust, uniform gold nanodisk arrays FO nanoprobe for PSA analysis was prepared [119]. The authors reported miniaturized biosensor functionalized with monoclonal antibodies demonstrating very low LOD of  $100 \text{ fg mL}^{-1}$  (3 fM) for fPSA [119].

A fiber optic LSPR sensor combined with a microfluidic channel was introduced that allows for a continuous supply of a fluid for a bio-reaction for real-time analysis of PSA [120]. Furthermore, the same group previously reported in-situ optimization method for antibody immobilization and for construction of LSPR biosensor [121]. Among others, a real-time label-free detection of PSA based on a FO LSPR sensor platform with the application of spherical AuNPs on a flattened end-face of the optical fiber was capable to detect PSA down to  $1 \text{ pg mL}^{-1}$ , however, not in real serum samples but spiked in a bovine serum albumin solution ( $1 \text{ mg mL}^{-1}$ ) [122].

## Fluorescence detection

Fluorescent biosensors are frequently applied for analysis of biomolecules associated with various types of cancer. There have been many recent discoveries and novel approaches in the field of PSA fluorescent biosensing by combining highly sensitive fluorescent detection with the high selectivity provided by analyte-binding proteins.

A multi-channel system combining fluidics and micropatterned plasmonic materials with both fluorescent and SPR analysis on a single chip was described [123]. The dual detection system was based on combination of a small and motorized fluorescence microscope mounted on a portable 4-channel SPR instrument for detection of PSA at clinical concentrations from 10 pM to 50 nM (0.3–1600 ng mL<sup>-1</sup>) in a single assay lasting 12 min [123]. This kind of tests make PSA screening for a routine prevention much more accessible, since the system can be fully automated and thus doesn't need a skilled operator. In the study, however, the authors did not test the system for analysis of a real human sera and a negative control was only human immunoglobulin, a highly abundant glycoprotein in blood (7.5–22 mg mL<sup>-1</sup>) [123].

Another strategy for constructing fluorescent biosensors was application of core-shell composite fluorescent silica nanoparticles for 3-fold enhancement of photoluminescence signal [124]. A highly sensitive, specific and reliable strategy based on metal-enhanced fluorescence and magnetic separation was applied for the detection of PSA in both buffer and serum [124]. Using immunomagnetic nanospheres and immunofluorescent nanoparticles capture and determination of the analyte was performed simultaneously. The authors showed a good linear relationship between the fluorescence intensity and the concentration of PSA (0.1–100 ng mL<sup>-1</sup>) with LOD of 27 pg mL<sup>-1</sup> [124]. However, fluorescent methods working at >500 nm are potentially prone to interferences from background fluorescence and inner filter effects, what has to be taken into account for real samples analysis. Use of magnetic nanoparticles can eliminate these issues by a selective capture and transfer of only desired analyte molecule on a testing surface.

Surface plasmon-enhanced fluorescence analysis using Au nanohole array can detect PSA down to 140 f. (i.e. 0.004 ng mL<sup>-1</sup>) [125]. The authors demonstrated a simultaneous co-excitation of localized surface plasmons and propagating surface plasmons on an Au nanohole array under Kretschmann configuration for PSA detection with the latter one offering approximately seven-fold lower LOD compared to the former one on the same substrate [125].

A variety of nanometer-sized inorganic crystals with unique optical properties i.e. QDs have been applied to enhance detection sensitivity of fluorescence-based biosensors [126]. The authors described novel homogeneous assays that enabled direct analysis of target analytes by monitoring the variations of the peak emission intensity of ZnSe QDs-labelled biomolecular sensors upon binding to specific target analytes. As the proof-of-the-concept, they performed direct homogeneous immunoassays for PSA using ZnSe QD-anti-PSA, where the amount of target PSA was estimated to be 2.6 pmol, or 74 ng [126]. Furthermore, application of QDs was described in the work of Pei and co-workers [127] based on GO QD@silver core-shell nanocrystals enabling fluorescent signal enhancement. The

ultrasensitive PSA immunosensor showed a good linear relationship between the fluorescence intensity and the concentration of PSA in the range from 1 pg mL<sup>-1</sup> to 20 ng mL<sup>-1</sup> with a LOD of 0.3 pg mL<sup>-1</sup> [127].

Besides use of other nanoparticles like nanorods [128], core-shell nanoparticles [129], the QDs can be utilized for the enhancement of LSPR fluorescence assay due to its photostability. In the work by Song et al. novel nanostructured biosensing chips for PSA detection via nanoimprinting onto a glass substrate were constructed [130]. By measuring the plasmon enhanced fluorescence through a conventional dark field microscope they achieved LOD for analysis of PSA as low as 100 pg mL<sup>-1</sup> [130].

A fluorogenic peptide substrate labelled with 4-amino-7-trifluoromethylcoumarin was applied to overcome limitation of autofluorescence of intra-seminal molecules [131]. The authors prepared a novel QDs-conjugated immunosensor for accurate identification of PSA in a seminal fluid (Fig. 6) [131].

A fluorescent detection principle based on an activatable nanoprobe with on signal on activation was described [132]. The quantitation was based on immobilization of capture antibodies on the surface to capture PSA. After attachment of PSA, the subsequent step was incubation of the surface with a complex of detection antibodies and fluorescent dye on AuNPs with fluorescence quenched after binding. After addition of cysteamine, a fluorescent dye was released and generated fluorescence. PSA was detected down to 32 fg mL<sup>-1</sup> [132]. The authors showed a significant increase in assay sensitivity using their probe compared to commercially available ELISA for real serum samples analysis, so the method is of a great interest for real-world applications.

A nanoporous silicon based microarray format of analysis was applied for analysis of PSA and kL2 [133]. A sandwich configuration with standard fluorescent dyes applied offered LOD of 100 fg mL<sup>-1</sup> for both analytes and the biosensor platform of detection was validated in spiked serum samples [133]. Besides antibodies, aptamers were applied for fluorescent detection of PSA. The combination of aptamers and 2D-layered nanomaterial (MoS<sub>2</sub> nanosheets) was presented for the first time in 2015 [134]. This fluorescent biosensor showed a “turn-on” fluorescent response to PSA with a high sensitivity and selectivity, with LOD of 0.2 ng mL<sup>-1</sup> [134].

Very attractive strategy for PSA determination was designed as a portable smartphone in a fluoropolymer microfluidic device [135]. The research group presented a microcapillary film phone for PSA quantitation which was composed of a smartphone integrated with a magnifying lens and a miniaturized immunoassay platform. The microcapillary film phone working principle was based on illuminating the test strip sample with a UV light and capturing the signal with a smartphone camera attached with a magnifying lens. This approach offered LOD of 0.08 ng mL<sup>-1</sup> in whole blood samples with the use of a fluorescent substrate [135].



## Chemiluminescent detection

It is known that chemiluminescence detection is more sensitive compared to fluorescent one because chemiluminescence detection does not require a light source and therefore has a low background noise [136].

Benefits of magnetic microparticles (MMPs) in chemiluminescent enzyme immunoassay were examined [137] due to many advantages, such as reduced incubation time, high surface area for immobilization, high capture efficiency and simple separation [138]. MMP-based assay (Fig. 7) showed the LOD of 0.1 ng mL<sup>-1</sup> for PSA with reduced cost, assay time and amounts of reagents compared to the commercial microplate test for PSA. Furthermore, the selectivity of this method was proved with highly homologous protein to PSA, hK2 [137].

Instead of antibodies, the application of aptamers was used to capture and detect PSA [139]. A guanine-rich chemiluminescent aptasensor prepared on a nanocomposite consisting of MWCNTs and MNPs was introduced to rapidly capture PSA within 30 min without time-consuming procedures (washing and multiple incubation steps). The described aptasensor with a good precision and reproducibility showed a wide linear dynamic range (1.9–125 ng mL<sup>-1</sup>) with LOD of 1 ng mL<sup>-1</sup> [139].

## Other optical detection platforms

Besides the already described optical-based detection platforms, several further strategies of optical-based biosensing have been reported, as well. One very attractive strategy for optical biosensing is surface-enhanced Raman scattering (SERS). SERS has its own inherent advantages over fluorescence including wide excitation wavelength ranges, lower susceptibility to photo-bleaching and capacity for a multiplexed assay [140]. This technique was also employed for PSA detection [141]. The authors introduced a novel wash-free magnetic immunoassay technique within a microdroplet sensor. Various concentrations of PSA ranging from 0.05 to 200 ng mL<sup>-1</sup> were added to the microdroplet sensor, and SERS signals were measured at frequency of 174 droplets min<sup>-1</sup>. The SERS-based microdroplet biosensor can detect as low as 0.1 ng mL<sup>-1</sup> of PSA with a tiny volume of a sample needed for analysis [141]. An interesting approach for highly sensitive detection of PSA was based on attachment of a derivative of a malachite green dye selectively at ends of gold nanorods [142]. Then two types of gold nanorod particles were prepared by covalent immobilization of antibodies at the end of nanorods to have: 1. capture antibodies against PSA and 2. detector antibodies against PSA. Then, both types of modified gold nanorods were mixed together to detect PSA, forming a chain of nanoparticles with nanoparticle separation of ~5.6 nm in presence of PSA. The length of the nanorods chain depended on PSA concentration, resulting into a change of SERS signal with LOD of 0.01 ng mL<sup>-1</sup> for PSA [142].

An improved white-light reflectance spectroscopy can provide LOD for tPSA of 0.2 ng mL<sup>-1</sup> and for fPSA of 0.15 ng mL<sup>-1</sup> [143]. The authors firstly optimized many aspects of the assay, such as material composition and thickness of the transducer and the shape and volume of a microfluidic cell. Moreover, they developed a software for real-time monitoring

of reflectance spectra. This label-free immunosensing system with an appropriate fluidic cell was able to determine both tPSA and fPSA in human serum samples [143].

An improved detection limit of PSA (1 pg) was achieved by application of the optofluidic chip consisting of arrayed nanopore-based sensors fabricated from an anodic aluminum oxide thin film [144]. Such type of the biosensor capable of ultrasensitive determination of dual PCa biomarker (PSA and a neuroendocrine marker) was published for the first time showing 50–100-fold enhancement of sensitivity compared to traditional ELISA [144].

## Piezoelectric and other alternative methods for PSA detection

An alternative to all mentioned biosensing platforms is a piezoelectric assay. Piezoelectric sensors are capable of detecting antigens in the picogram range [145]. Another benefit of this detection method is the potential to detect antigens in the gas phase as well as in the liquid phase. A piezoelectric membrane-based approach with the application of magnetic beads to quantify seven PCa biomarkers including PSA was described [146]. The study dealt with the discrimination between 100 PCa (Gleason score 6 and 7 – a grading system used to evaluate the prostate biopsy samples) and 120 BPH patients. For these seven proteins in serum samples, the highest AUC (area under the curve) discrimination was achieved with a spondin-2 or free/total PSA operation where the area under the curve was 0.84 with a p value below  $10^{-6}$  [146].

An excellent detection limit ( $1 \times 10^{-16}$  g mL<sup>-1</sup>) in a serum for both PSA and CEA (a carcinoembryonic antigen) biomolecules was reached using a hybrid mechanical and optoplasmonic nanosensor [147]. Silicon cantilevers possess excellent mechanical attributes for translating biomolecular recognition on their surface into a nanoscale bending (a static mode) and variations of the resonance frequency (a dynamic mode) of the device [148]. Using a dynamic mode (Fig. 8), an extremely low LOD, which is at least seven orders of magnitude lower than required for a routine clinical practice was achieved [147].

Moreover, a planar split-ring resonator as a transducer has been used in the label-free biosensing of PSA in the microwave region [149]. The split-ring resonators are increasingly popular because their application as an artificial magnetic element or electromagnetic metamaterials to produce even terahertz responses [150]. In the work, a change in the resonant frequency was measured using micro-cantilevers offering a minimum detectable level of 100 pg mL<sup>-1</sup> PSA [149]. Their finding showed, that an antigen-antibody system can be rapidly detected by the split-ring resonator-based biosensor in a local high-impedance microstrip line system [149].

An interesting approach based on the combination of DNA aptamer-beacon and microfluidic Love-wave biosensor for real-time PSA determination was presented [151]. The Love-wave sensors are a special type of surface acoustic wave biosensors having inherent benefits, such as low cost, high sensitivity, low power requirement and real-time monitoring [152]. The microfluidic device showed good performance for PSA detection between 10 ng mL<sup>-1</sup> to 1 µg mL<sup>-1</sup>, with LOD of 10 ng mL<sup>-1</sup> [151].

A completely different approach for PSA biosensing was described by Sun et al. by exploiting magnetic-based biosensors [153]. They used a micro-fluxgate based device with a rectangular magnetic core for the determination of PSA labelled with Dynabeads. The micro-fluxgate sensors are type of magnetic sensors having benefits such as light weight, quick response and compatibility with interface circuits and microfluidic chips [154]. By applying a DC magnetic fields, PSA was detected with LOD as low as  $0.1 \text{ ng mL}^{-1}$  [153]. The same group [155] applied a contactless detection method for PSA detection with a giant magnetoresistance sensor. With DC magnetic field, PSA can be detected with LOD down to  $0.1 \text{ ng mL}^{-1}$  [153]. To detect even lower levels of PSA, Jiang et al. used the loop-mediated isothermal amplification method to measure the levels of reporter DNA molecules [156]. The LOD of the method is 1 f. ( $32.5 \text{ fg mL}^{-1}$ ) [156], which is approximately 100-fold lower than LOD of the two other assays [153, 155]. The dynamic linear range also increased, from 5 [153, 155] to 7 orders of magnitude [156].

## Conclusions and perspectives

A requirement to have reliable, fast, accurate and cheap detection of cancer biomarkers create a great opportunity for development of novel, advanced biosensing platforms, which can be applicable for diagnostics purposes. In summary, advances and challenges in biosensor construction have pushed forward the analysis of cancer biomarkers including PSA. Herein we reported most recent (i.e. last 5 years) efforts and biosensing approaches exploiting various nanomaterials (metallic and carbon nanoparticles, QDs, hybrid nanocomposites) and strategies for sensitivity enhancement. There is no doubt that the progress in the field of biosensing technology will contribute to the effort of construction of point-of-care and bedside medical diagnostics devices. Many properties of the presented nanomaterials are of great importance in nanobiotechnology because of their unique properties, used depending on the detection platform – such as high conductivity for electrochemical biosensors, large surface area, and unique shape allowing multivalent binding or optical properties for plasmon resonance-based detection. Even though it can be hard to say which nanomaterial is the most commonly employed, current trend in biosensor development is the use of hybrid nanomaterials and nanocomposites.

Ultrasensitive analysis of PSA by the biosensors is possible using all assay techniques discussed in this review paper with the most sensitive principle of detection being an electrochemical one with 4 different electrochemical configurations offering detection limit down to  $\text{ag mL}^{-1}$  limit ( $20\text{--}740 \text{ ag mL}^{-1}$ ), while only one other detection scheme based on mass cantilever based sensing offered LOD down to  $100 \text{ ag mL}^{-1}$  (Table 1). SPR technique was successfully utilized for characterization and/or optimization studies, but cannot offer very sensitive quantitation of PSA, with only a moderate increase of sensitivity achieved using nanoparticles. Highly parallel analysis similar to fluorescent arrays/biochips is possible only using the SPR imaging method. Microcantilever arrays can provide some level of multiplexed analysis, however, for the detection of various cancer diseases including PCa most likely an array for detection up to 8 different cancer biomarkers is adequate. Furthermore, the biosensor devices should be available in the form of a compact device to laboratories worldwide to become an alternative to widely used ELISA method and their reliability has to be tested by analysis of PSA directly in human serum samples. The vast

majority of biosensors are still used only in the laboratory and for the research purposes and for the mass production of point-of-care devices there is a need to have really robust devices with assay reliability, storage stability and throughput of sample assays comparable to ELISA with optimized/automated sample/reagent delivery.

## Acknowledgements

The financial support received from the Slovak Scientific Grant Agency VEGA 2/0162/14 and the Slovak Research and Development Agency APVV-14-0753 is acknowledged. The research leading to these results received funding from the European Research Council under the European Union's Seventh Framework Program (FP/2007-2013)/ERC grant agreement number 311532. This publication was made possible by NPRP grant number 6-381-1-078 from the Qatar National Research Fund (a member of the Qatar Foundation). The statements made herein are solely the responsibility of the authors. This publication is the result of the project implementation: Centre for materials, layers and systems for applications and chemical processes under extreme conditions – Stage I, ITMS No.: 26240120007, supported by the Research & Development Operational Program funded by the ERDF.

## References

1. Ferlay J, Steliarova-Foucher E, Lortet-Tieulent J, Rosso S, Coebergh JWW, Comber H, Forman D, Bray F. Cancer incidence and mortality patterns in Europe: Estimates for 40 countries in 2012. *Eur J Cancer*. 2013; 49(6):1374–1403. [PubMed: 23485231]
2. Bray, F., Kiemeny, LA. Epidemiology of Prostate Cancer in Europe: Patterns, Trends and Determinants. *Management of Prostate Cancer*. Springer; 2017. p. 1-27.
3. Mokdad AH, Dwyer-Lindgren L, Fitzmaurice C, Stubbs RW, Bertozzi-Villa A, Morozoff C, Charara R, Allen C, Naghavi M, Murray CJ. Trends and patterns of disparities in cancer mortality among US counties, 1980-2014. *JAMA*. 2017; 317(4):388–406. [PubMed: 28118455]
4. Prostate cancer. 2015. <http://www.nhs.uk/Conditions/Cancer-of-the-prostate/Pages/Introduction.aspx>
5. Miller DC, Hafez KS, Stewart A, Montie JE, Wei JT. Prostate carcinoma presentation, diagnosis, and staging: an update from the National Cancer Data Base. *Cancer*. 2003; 98(6):1169–1178. [PubMed: 12973840]
6. Mottet N, Bellmunt J, Bolla M, Briers E, Cumberbatch MG, De Santis M, Fossati N, Gross T, Henry AM, Joniau S, Lam TB, et al. EAU-ESTRO-SIOG Guidelines on Prostate Cancer. Part 1: Screening, Diagnosis, and Local Treatment with Curative Intent. *Eur Urol*. 2017; 71(4):618–629. [PubMed: 27568654]
7. Parpart S, Rudis A, Schreck A, Dewan N, Warren P. Sensitivity and specificity in prostate cancer screening methods and strategies. *J Young Invest*. 2007 <http://www.jyi.org/issue/sensitivity-and-specificity-in-prostate-cancer-screening-methods-and-strategies/>.
8. Wolf AM, Wender RC, Etzioni RB, Thompson IM, D'Amico AV, Volk RJ, Brooks DD, Dash C, Guessous I, Andrews K, DeSantis C, et al. American Cancer Society guideline for the early detection of prostate cancer: update 2010. *CA Cancer J Clin*. 2010; 60(2):70–98. [PubMed: 20200110]
9. Stamey TA, Yang N, Hay AR, McNeal JE, Freiha FS, Redwine E. Prostate-specific antigen as a serum marker for adenocarcinoma of the prostate. *N Engl J Med*. 1987; 317(15):909–916. [PubMed: 2442609]
10. Hara M, Inoue T, Koyanagi Y, Fukuyama T, Iki H. Immunochemical characteristics of human specific component “ $\gamma$ -Sm”. *Nippon Hoigaku Zasshi*. 1969; 23:333.
11. Oesterling JE. Prostate specific antigen: a critical assessment of the most useful tumor marker for adenocarcinoma of the prostate. *J Urol*. 1991; 145(5):907–923. [PubMed: 1707989]
12. Yousef GM, Diamandis EP. The new human tissue kallikrein gene family: structure, function, and association to disease. *Endocr Rev*. 2001; 22(2):184–204. [PubMed: 11294823]
13. Jolly P, Tamboli V, Harniman RL, Estrela P, Allender CJ, Bowen JL. Aptamer–MIP hybrid receptor for highly sensitive electrochemical detection of prostate specific antigen. *Biosens Bioelectron*. 2016; 75:188–195. [PubMed: 26318788]

14. Oesterling JE, Jacobsen SJ, Chute CG, Guess HA, Girman CJ, Panser LA, Lieber MM. Serum prostate-specific antigen in a community-based population of healthy men. Establishment of age-specific reference ranges. *JAMA*. 1993; 270(7):860–864. [PubMed: 7688054]
15. Ito K, Yamamoto T, Ohi M, Kurokawa K, Suzuki K, Yamanaka H. Free/total PSA ratio is a powerful predictor of future prostate cancer morbidity in men with initial PSA levels of 4.1 to 10.0 ng/mL. *Urology*. 2003; 61(4):760–764. [PubMed: 12670561]
16. Catalona WJ, Partin AW, Slawin KM, Brawer MK, Flanigan RC, Patel A, Richie JP, deKernion JB, Walsh PC, Scardino PT, Lange PH, et al. Use of the percentage of free prostate-specific antigen to enhance differentiation of prostate cancer from benign prostatic disease: a prospective multicenter clinical trial. *JAMA*. 1998; 279(19):1542–1547. [PubMed: 9605898]
17. Velonas VM, Woo HH, dos Remedios CG, Assinder SJ. Current Status of Biomarkers for Prostate Cancer. *Int J Mol Sci*. 2013; 14(6):11034–11060. [PubMed: 23708103]
18. Sharma S, Zapatero-Rodríguez J, O'Kennedy R. Prostate cancer diagnostics: Clinical challenges and the ongoing need for disruptive and effective diagnostic tools. *Biotechnol Adv*. 2017; 35(2): 135–149. [PubMed: 27939303]
19. Shan J, Ma Z. A review on amperometric immunoassays for tumor markers based on the use of hybrid materials consisting of conducting polymers and noble metal nanomaterials. *Microchim Acta*. 2017; 184:969–979.
20. Paleček E, Tkáč J, Bartošík M, Ts B, Ostatná V, Paleček J. Electrochemistry of Nonconjugated Proteins and Glycoproteins. Toward Sensors for Biomedicine and Glycomics. *Chem Rev*. 2015; 115(5):2045–2108. [PubMed: 25659975]
21. Labib M, Sargent EH, Kelley SO. Electrochemical Methods for the Analysis of Clinically Relevant Biomolecules. *Chem Rev*. 2016; 116(16):9001–9090. [PubMed: 27428515]
22. Rackus DG, Shamsi MH, Wheeler AR. Electrochemistry, biosensors and microfluidics: a convergence of fields. *Chem Soc Rev*. 2015; 44(15):5320–5340. [PubMed: 25962356]
23. Grieshaber D, MacKenzie R, Vörös J, Reimhult E. Electrochemical Biosensors - Sensor Principles and Architectures. *Sensors (Basel, Switzerland)*. 2008; 8(3):1400–1458.
24. Uludag Y, Narter F, Sağlam E, Köktürk G, Gök MY, Akgün M, Barut S, Budak S. An integrated lab-on-a-chip-based electrochemical biosensor for rapid and sensitive detection of cancer biomarkers. *Anal Bioanal Chem*. 2016; 408(27):7775–7783. [PubMed: 27562751]
25. Schumacher S, Nestler J, Otto T, Wegener M, Ehrentreich-Forster E, Michel D, Wunderlich K, Palzer S, Sohn K, Weber A, Burgard M, et al. Highly-integrated lab-on-chip system for point-of-care multiparameter analysis. *Lab Chip*. 2012; 12(3):464–473. [PubMed: 22038328]
26. Pandey B, Demchenko AV, Stine KJ. Nanoporous gold as a solid support for protein immobilization and development of an electrochemical immunoassay for prostate specific antigen and carcinoembryonic antigen. *Microchim Acta*. 2012; 179(1):71–81.
27. Tian J, Huang J, Zhao Y, Zhao S. Electrochemical immunosensor for prostate-specific antigen using a glassy carbon electrode modified with a nanocomposite containing gold nanoparticles supported with starch-functionalized multi-walled carbon nanotubes. *Microchim Acta*. 2012; 178(1):81–88.
28. Yang J, Wen W, Zhang X, Wang S. Electrochemical immunosensor for the prostate specific antigen detection based on carbon nanotube and gold nanoparticle amplification strategy. *Microchim Acta*. 2015; 182(9):1855–1861.
29. Uludag Y, Köktürk G. Determination of prostate-specific antigen in serum samples using gold nanoparticle based amplification and lab-on-a-chip based amperometric detection. *Microchim Acta*. 2015; 182(9):1685–1691.
30. Wang Y, Qu Y, Liu G, Hou X, Huang Y, Wu W, Wu K, Li C. Electrochemical immunoassay for the prostate specific antigen using a reduced graphene oxide functionalized with a high molecular-weight silk peptide. *Microchim Acta*. 2015; 182(11):2061–2067.
31. Zhao J, Guo Z, Feng D, Guo J, Wang J, Zhang Y. Simultaneous electrochemical immunosensing of alpha-fetoprotein and prostate specific antigen using a glassy carbon electrode modified with gold nanoparticle-coated silica nanospheres and decorated with Azure A or ferrocenecarboxylic acid. *Microchim Acta*. 2015; 182(15):2435–2442.

32. Gutiérrez-Zúñiga GG, Hernández-López JL. Sensitivity improvement of a sandwich-type ELISA immunosensor for the detection of different prostate-specific antigen isoforms in human serum using electrochemical impedance spectroscopy and an ordered and hierarchically organized interfacial supramolecular architecture. *Anal Chim Acta*. 2016; 902:97–106. [PubMed: 26703258]
33. Liu B, Lu L, Hua E, Jiang S, Xie G. Detection of the human prostate-specific antigen using an aptasensor with gold nanoparticles encapsulated by graphitized mesoporous carbon. *Microchim Acta*. 2012; 178(1):163–170.
34. Shin KS, Ji JH, Hwang KS, Jun SC, Kang JY. Sensitivity Enhancement of Bead-based Electrochemical Impedance Spectroscopy (BEIS) biosensor by electric field-focusing in microwells. *Biosens Bioelectron*. 2016; 85:16–24. [PubMed: 27152445]
35. Çevik E, Bahar Ö, Şenel M, Abasiyanik MF. Construction of novel electrochemical immunosensor for detection of prostate specific antigen using ferrocene-PAMAM dendrimers. *Biosens Bioelectron*. 2016; 86:1074–1079. [PubMed: 27641479]
36. Novoselov KS, Geim AK, Morozov SV, Jiang D, Zhang Y, Dubonos SV, Grigorieva IV, Firsov AA. Electric field effect in atomically thin carbon films. *Science*. 2004; 306(5696):666–669. [PubMed: 15499015]
37. Yan M, Zang D, Ge S, Ge L, Yu J. A disposable electrochemical immunosensor based on carbon screen-printed electrodes for the detection of prostate specific antigen. *Biosens Bioelectron*. 2012; 38(1):355–361. [PubMed: 22770827]
38. Mao K, Wu D, Li Y, Ma H, Ni Z, Yu H, Luo C, Wei Q, Du B. Label-free electrochemical immunosensor based on graphene/methylene blue nanocomposite. *Anal Biochem*. 2012; 422(1): 22–27. [PubMed: 22266207]
39. Salimi A, Kavosi B, Fathi F, Hallaj R. Highly sensitive immunosensing of prostate-specific antigen based on ionic liquid–carbon nanotubes modified electrode: Application as cancer biomarker for prostate biopsies. *Biosens Bioelectron*. 2013; 42:439–446. [PubMed: 23235113]
40. Kavosi B, Salimi A, Hallaj R, Amani K. A highly sensitive prostate-specific antigen immunosensor based on gold nanoparticles/PAMAM dendrimer loaded on MWCNTS/chitosan/ionic liquid nanocomposite. *Biosens Bioelectron*. 2014; 52:20–28. [PubMed: 24016535]
41. Feng J, Li Y, Li M, Li F, Han J, Dong Y, Chen Z, Wang P, Liu H, Wei Q. A novel sandwich-type electrochemical immunosensor for PSA detection based on PtCu bimetallic hybrid (2D/2D) rGO/g-C<sub>3</sub>N<sub>4</sub>. *Biosens Bioelectron*. 2017; 91:441–448. [PubMed: 28064129]
42. Ding L-L, Ge J-P, Zhou W-Q, Gao J-P, Zhang Z-Y, Xiong Y. Nanogold-functionalized g-C<sub>3</sub>N<sub>4</sub> nanohybrids for sensitive impedimetric immunoassay of prostate-specific antigen using enzymatic biocatalytic precipitation. *Biosens Bioelectron*. 2016; 85:212–219. [PubMed: 27179136]
43. Liu L, Li Y, Tian L, Wei Q, Cao W. Ultrasensitive sandwich-type prostate specific antigen immunosensor based on Ag overgrowth in Pd nano-octahedrons heterodimers decorated on amino functionalized multiwalled carbon nanotubes. *Sensors Actuators B Chem*. 2016; 237:733–739.
44. Akter R, Rahman MA, Rhee CK. Amplified Electrochemical Detection of a Cancer Biomarker by Enhanced Precipitation Using Horseradish Peroxidase Attached on Carbon Nanotubes. *Anal Chem*. 2012; 84(15):6407–6415. [PubMed: 22793977]
45. Li Y, Khan MS, Tian L, Liu L, Hu L, Fan D, Cao W, Wei Q. An ultrasensitive electrochemical immunosensor for the detection of prostate-specific antigen based on conductivity nanocomposite with halloysite nanotubes. *Anal Bioanal Chem*. 2017:1–7. [PubMed: 27837266]
46. Wang H, Zhang Y, Yu H, Wu D, Ma H, Li H, Du B, Wei Q. Label-free electrochemical immunosensor for prostate-specific antigen based on silver hybridized mesoporous silica nanoparticles. *Anal Biochem*. 2013; 434(1):123–127. [PubMed: 23201390]
47. Fan D, Li N, Ma H, Li Y, Hu L, Du B, Wei Q. Electrochemical immunosensor for detection of prostate specific antigen based on an acid cleavable linker into MSN-based controlled release system. *Biosens Bioelectron*. 2016; 85:580–586. [PubMed: 27236723]
48. Jang HD, Kim SK, Chang H, Choi J-W. 3D label-free prostate specific antigen (PSA) immunosensor based on graphene–gold composites. *Biosens Bioelectron*. 2015; 63:546–551. [PubMed: 25150936]



49. Han L, Liu C-M, Dong S-L, Du C-X, Zhang X-Y, Li L-H, Wei Y. Enhanced conductivity of rGO/Ag NPs composites for electrochemical immunoassay of prostate-specific antigen. *Biosens Bioelectron.* 2017; 87:466–472. [PubMed: 27591721]
50. Hong W, Lee S, Jae Kim E, Lee M, Cho Y. A reusable electrochemical immunosensor fabricated using a temperature-responsive polymer for cancer biomarker proteins. *Biosens Bioelectron.* 2016; 78:181–186. [PubMed: 26606310]
51. Pan L-H, Kuo S-H, Lin T-Y, Lin C-W, Fang P-Y, Yang H-W. An electrochemical biosensor to simultaneously detect VEGF and PSA for early prostate cancer diagnosis based on graphene oxide/ssDNA/PLLA nanoparticles. *Biosens Bioelectron.* 2017; 89(Part 1):598–605. [PubMed: 26868935]
52. Sharafeldin M, Bishop GW, Bhakta S, El-Sawy A, Suib SL, Rusling JF. Fe<sub>3</sub>O<sub>4</sub> nanoparticles on graphene oxide sheets for isolation and ultrasensitive amperometric detection of cancer biomarker proteins. *Biosens Bioelectron.* 2017; 91:359–366. [PubMed: 28056439]
53. Chuah K, Lai LMH, Goon IY, Parker SG, Amal R, Justin Gooding J. Ultrasensitive electrochemical detection of prostate-specific antigen (PSA) using gold-coated magnetic nanoparticles as 'dispersible electrodes'. *Chem Commun.* 2012; 48(29):3503–3505.
54. Ma H, Li Y, Wang Y, Hu L, Zhang Y, Fan D, Yan T, Wei Q. Cubic Cu<sub>2</sub>O nanoframes with a unique edge-truncated structure and a good electrocatalytic activity for immunosensor application. *Biosens Bioelectron.* 2016; 78:167–173. [PubMed: 26606308]
55. Jiao L, Mu Z, Miao L, Du W, Wei Q, Li H. Enhanced amperometric immunoassay for the prostate specific antigen using Pt-Cu hierarchical trigonal bipyramid nanoframes as a label. *Microchim Acta.* 2017; 184(2):423–429.
56. Bhardwaj SK, Sharma AL, Bhardwaj N, Kukkar M, Gill AAS, Kim K-H, Deep A. TCNQ-doped Cu-metal organic framework as a novel conductometric immunosensing platform for the quantification of prostate cancer antigen. *Sens Actuat B: Chem.* 2017; 240:10–17.
57. Hong W, Lee S, Cho Y. Dual-responsive immunosensor that combines colorimetric recognition and electrochemical response for ultrasensitive detection of cancer biomarkers. *Biosens Bioelectron.* 2016; 86:920–926. [PubMed: 27497199]
58. Li F, Li Y, Feng J, Dong Y, Wang P, Chen L, Chen Z, Liu H, Wei Q. Ultrasensitive amperometric immunosensor for PSA detection based on Cu<sub>2</sub>O@CeO<sub>2</sub>-Au nanocomposites as integrated triple signal amplification strategy. *Biosens Bioelectron.* 2017; 87:630–637. [PubMed: 27619526]
59. Duangkaew P, Wutikhun T, Laocharoensuk R. Triple signal amplification strategy based on size and shape transformation of ultrasmall sub-10 nm gold nanoparticles tag towards sensitivity improvement of electrochemical immunosensors. *Sensors Actuators B Chem.* 2017; 239:430–437.
60. Kavosi B, Salimi A, Hallaj R, Moradi F. Ultrasensitive electrochemical immunosensor for PSA biomarker detection in prostate cancer cells using gold nanoparticles/PAMAM dendrimer loaded with enzyme linked aptamer as integrated triple signal amplification strategy. *Biosens Bioelectron.* 2015; 74:915–923. [PubMed: 26257183]
61. Zhao L, Ma Z. New immunoprobes based on bovine serum albumin-stabilized copper nanoclusters with triple signal amplification for ultrasensitive electrochemical immunosensing for tumor marker. *Sensors Actuators B Chem.* 2017; 241:849–854.
62. Klukova L, Bertok T, Petrikova M, Sediva A, Mislovicova D, Katrlík J, Vikartovska A, Filip J, Kasak P, Andicsová-Eckstein A. Glycoprofiling as a novel tool in serological assays of systemic sclerosis: A comparative study with three bioanalytical methods. *Anal Chim Acta.* 2015; 853:555–562. [PubMed: 25467503]
63. Tang Z, Wang L, Ma Z. Triple sensitivity amplification for ultrasensitive electrochemical detection of prostate specific antigen. *Biosens Bioelectron.* 2017; 92:577–582. [PubMed: 27829569]
64. Zhu Y, Wang H, Wang L, Zhu J, Jiang W. Cascade Signal Amplification Based on Copper Nanoparticle-Reported Rolling Circle Amplification for Ultrasensitive Electrochemical Detection of the Prostate Cancer Biomarker. *ACS Appl Mater Interfaces.* 2016; 8(4):2573–2581. [PubMed: 26765624]
65. Strzemińska I, Sainte Rose Fanchine S, Anquetin G, Reisberg S, Noël V, Pham MC, Piro B. Grafting of a peptide probe for Prostate-Specific Antigen detection using diazonium

- electroreduction and click chemistry. *Biosens Bioelectron.* 2016; 81:131–137. [PubMed: 26938492]
66. Parnsubsakul A, Safitri RE, Rijiravanich P, Surareungchai W. Electrochemical assay of proteolytically active prostate specific antigen based on anodic stripping voltammetry of silver enhanced gold nanoparticle labels. *J Electroanal Chem.* 2017; 785:125–130.
  67. Heydari-Bafrooei E, Shamszadeh NS. Electrochemical bioassay development for ultrasensitive aptasensing of prostate specific antigen. *Biosens Bioelectron.* 2017; 91:284–292. [PubMed: 28033557]
  68. Tahmasebi F, Noorbakhsh A. Sensitive Electrochemical Prostate Specific Antigen Aptasensor: Effect of Carboxylic Acid Functionalized Carbon Nanotube and Glutaraldehyde Linker. *Electroanalysis.* 2016; 28(5):1134–1145.
  69. Rahi A, Sattarahmady N, Heli H. Label-free electrochemical aptasensing of the human prostate-specific antigen using gold nanospears. *Talanta.* 2016; 156–157:218–224.
  70. Jolly P, Formisano N, Tkáč J, Kasák P, Frost CG, Estrela P. Label-free impedimetric aptasensor with antifouling surface chemistry: A prostate specific antigen case study. *Sensors Actuators B Chem.* 2015; 209:306–312.
  71. Tzouvadaki I, Jolly P, Lu X, Ingebrandt S, de Micheli G, Estrela P, Carrara S. Label-Free Ultrasensitive Memristive Aptasensor. *Nano Lett.* 2016; 16(7):4472–4476. [PubMed: 27341189]
  72. Deng H, Li J, Zhang Y, Pan H, Xu G. A new strategy for label-free electrochemical immunoassay based on “gate-effect” of  $\beta$ -cyclodextrin modified electrode. *Anal Chim Acta.* 2016; 926:48–54. [PubMed: 27216392]
  73. Xie S, Zhang J, Yuan Y, Chai Y, Yuan R. An electrochemical peptide cleavage-based biosensor for prostate specific antigen detection via host-guest interaction between ferrocene and [small beta]-cyclodextrin. *Chem Commun.* 2015; 51(16):3387–3390.
  74. Gao C, Zhang L, Wang Y, Yu J, Song X. Visible-light driven biofuel cell based on hierarchically branched titanium dioxide nanorods photoanode for tumor marker detection. *Biosens Bioelectron.* 2016; 83:327–333. [PubMed: 27135937]
  75. Pihíková D, Belický Š, Kasák P, Bertok T, Tkac J. Sensitive detection and glycoprofiling of a prostate specific antigen using impedimetric assays. *Analyst.* 2016; 141(3):1044–1051. [PubMed: 26647853]
  76. Pihikova D, Pakanova Z, Nemcovic M, Barath P, Belicky S, Bertok T, Kasak P, Mucha J, Tkac J. Sweet characterization of prostate specific antigen using electrochemical lectin-based immunosensor assay and MALDI TOF/TOF analysis: Focus on sialic acid. *Proteomics.* 2016; 16(24):3085–3095. [PubMed: 26920336]
  77. Pihikova D, Kasak P, Kubanikova P, Sokol R, Tkac J. Aberrant sialylation of a prostate-specific antigen: Electrochemical label-free glycoprofiling in prostate cancer serum samples. *Anal Chim Acta.* 2016; 934:72–79. [PubMed: 27506346]
  78. Xia N, Deng D, Zhang L, Yuan B, Jing M, Du J, Liu L. Sandwich-type electrochemical biosensor for glycoproteins detection based on dual-amplification of boronic acid-gold nanoparticles and dopamine-gold nanoparticles. *Biosens Bioelectron.* 2013; 43:155–159. [PubMed: 23298627]
  79. Cao Y, Yuan R, Chai Y, Mao L, Niu H, Liu H, Zhuo Y. Ultrasensitive luminol electrochemiluminescence for protein detection based on in situ generated hydrogen peroxide as co-reactant with glucose oxidase anchored AuNPs@MWCNTs labeling. *Biosens Bioelectron.* 2012; 31(1):305–309. [PubMed: 22088259]
  80. Xu Y, Liu J, Gao C, Wang E. Applications of carbon quantum dots in electrochemiluminescence: A mini review. *Electrochem Commun.* 2014; 48:151–154.
  81. Jiao T, Leca-Bouvier BD, Boullanger P, Blum LJ, Girard-Egrot AP. Electrochemiluminescent detection of hydrogen peroxide using amphiphilic luminol derivatives in solution. *Colloids Surf A Physicochem Eng Asp.* 2008; 321(1–3):143–146.
  82. Liu D-Y, Xin Y-Y, He X-W, Yin X-B. A sensitive, non-damaging electrochemiluminescent aptasensor via a low potential approach at DNA-modified gold electrodes. *Analyst.* 2011; 136(3): 479–485. [PubMed: 20938512]
  83. Parveen, S., Aslam, MS., Hu, L., Xu, G. *Electrogenerated Chemiluminescence: Protocols and Applications.* Springer; 2013.

84. Zhang F, Mao L, Zhu M. Ultrasensitive immunoassay for free prostate-specific antigen based on ferrocenecarboxylate enhanced cathodic electrochemiluminescence of peroxydisulfate. *Microchim Acta*. 2014; 181(11):1285–1291.
85. Xu X. Sensitive Electrochemiluminescence Immunosensor for Determination of Tumor Biomarker PSA Based on Multifunctionalized Pt/Ag@BSA Core–Shell Nanoparticles. *Bull Kor Chem Soc*. 2016; 37(4):452–457.
86. Ge S, Yu J, Jiao X, Chen D. Ultrasensitive Electrochemiluminescence Immunoassay for Protein Specific Detection Based on Dendrimer-Encapsulated Gold Nanoparticles Labels. *J Inorg Organomet Polym Mater*. 2013; 23(5):1113–1121.
87. Sardesai NP, Kadimisetty K, Faria R, Rusling JF. A microfluidic electrochemiluminescent device for detecting cancer biomarker proteins. *Anal Bioanal Chem*. 2013; 405(11):3831–3838. [PubMed: 23307128]
88. Kadimisetty K, Malla S, Sardesai NP, Joshi AA, Faria RC, Lee NH, Rusling JF. Automated multiplexed ECL Immunoarrays for cancer biomarker proteins. *Anal Chem*. 2015; 87(8):4472–4478. [PubMed: 25821929]
89. Deng W, Chu C, Ge S, Yu J, Yan M, Song X. Electrochemiluminescence PSA assay using an ITO electrode modified with gold and palladium, and flower-like titanium dioxide microparticles as ECL labels. *Microchim Acta*. 2015; 182(5):1009–1016.
90. Li W, Dai W, Ge L, Ge S, Yan M, Yu J. Electropolymerized Poly(3,4-ethylenedioxythiophene)/Graphene Composite Film and its Application in Quantum Dots Electrochemiluminescence Immunoassay. *J Inorg Organomet Polym Mater*. 2013; 23(3):719–725.
91. Ma H, Li X, Yan T, Li Y, Zhang Y, Wu D, Wei Q, Du B. Electrochemiluminescent immunosensing of prostate-specific antigen based on silver nanoparticles-doped Pb (II) metal-organic framework. *Biosens Bioelectron*. 2016; 79:379–385. [PubMed: 26735872]
92. Wu D, Liu Y, Wang Y, Hu L, Ma H, Wang G, Wei Q. Label-free Electrochemiluminescent Immunosensor for Detection of Prostate Specific Antigen based on Aminated Graphene Quantum Dots and Carboxyl Graphene Quantum Dots. *Sci Rep*. 2016; 6:20511. [PubMed: 26842737]
93. Zhao Y, Wang Q, Li J, Ma H, Zhang Y, Wu D, Du B, Wei Q. A CeO<sub>2</sub>-matrical enhancing ECL sensing platform based on the Bi<sub>2</sub>S<sub>3</sub>-labeled inverted quenching mechanism for PSA detection. *J Mater Chem B*. 2016; 4(17):2963–2971.
94. Tian CY, Zhao WW, Wang J, Xu JJ, Chen HY. Amplified quenching of electrochemiluminescence from CdS sensitized TiO<sub>2</sub> nanotubes by CdTe-carbon nanotube composite for detection of prostate protein antigen in serum. *Analyst*. 2012; 137(13):3070–3075. [PubMed: 22624149]
95. Ma H, Zhou J, Li Y, Han T, Zhang Y, Hu L, Du B, Wei Q. A label-free electrochemiluminescence immunosensor based on EuPO<sub>4</sub> nanowire for the ultrasensitive detection of Prostate specific antigen. *Biosens Bioelectron*. 2016; 80:352–358. [PubMed: 26855165]
96. Yang J-J, Cao J-T, Wang H, Liu Y-M, Ren S-W. Ferrocene-graphene sheets for high-efficiency quenching of electrochemiluminescence from Au nanoparticles functionalized cadmium sulfide flower-like three dimensional assemblies and sensitive detection of prostate specific antigen. *Talanta*. 2017; 167:325–332. [PubMed: 28340728]
97. He Y, Chai Y, Yuan R, Wang H, Bai L, Liao N. A supersandwich electrochemiluminescence immunosensor based on mimic-intramolecular interaction for sensitive detection of proteins. *Analyst*. 2014; 139(20):5209–5214. [PubMed: 25122008]
98. Damborský P, Švitel J, Katrlík J. Optical biosensors. *Essays Biochem*. 2016; 60(1):91–100. [PubMed: 27365039]
99. Vigneshvar S, Sudhakumari CC, Senthikumar B, Prakash H. Recent Advances in Biosensor Technology for Potential Applications – An Overview. *Front Bioeng Biotechnol*. 2016; 4:11. [PubMed: 26909346]
100. Esseghaier C, Suaifan GARY, Ng A, Zourob M. One-Step Assay for Optical Prostate Specific Antigen Detection Using Magnetically Engineered Responsive Thin Film. *J Biomed Nanotechnol*. 2014; 10(6):1123–1129. [PubMed: 24749406]
101. Berthuy, OI., Blum, LJ., Marquette, CA. In: Baldini, F.Homola, J., Lieberman, RA., editors. *Optical Sensors 2015; Proceedings of SPIE*; 2015. pp 950615–950615-950618

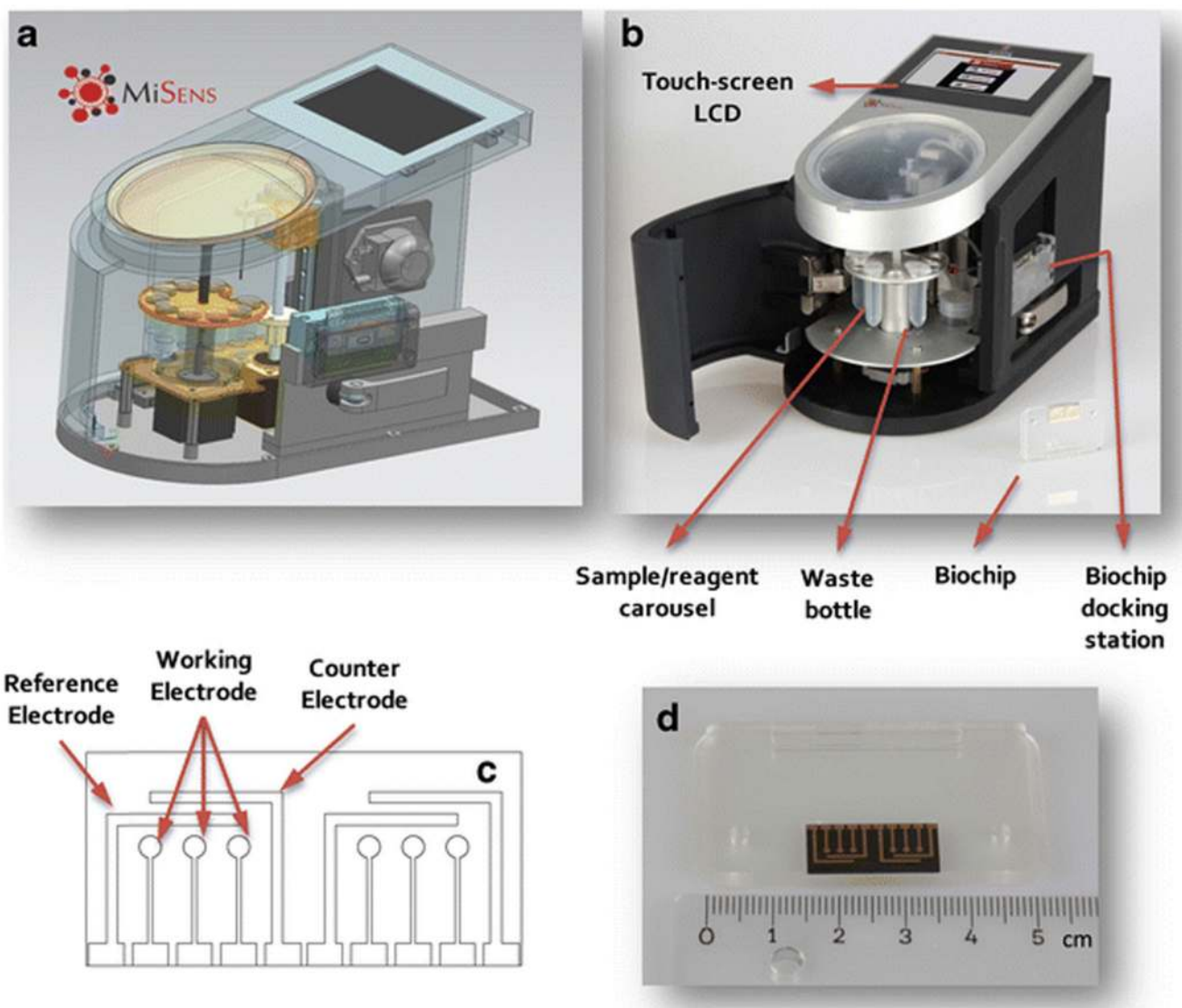
102. Damborský P, Madaboosi N, Chu V, Conde JP, Katrlík J. Surface plasmon resonance application in prostate cancer biomarker research. *Chem Pap.* 2015; 69(1):143–149.
103. Soelberg SD, Stevens RC, Limaye AP, Furlong CE. Surface Plasmon Resonance Detection Using Antibody-Linked Magnetic Nanoparticles for Analyte Capture, Purification, Concentration, and Signal Amplification. *Anal Chem.* 2009; 81(6):2357–2363. [PubMed: 19215065]
104. Lyon LA, Musick MD, Natan MJ. Colloidal Au-Enhanced Surface Plasmon Resonance Immunosensing. *Anal Chem.* 1998; 70(24):5177–5183. [PubMed: 9868916]
105. Uludag Y, Tothill IE. Cancer Biomarker Detection in Serum Samples Using Surface Plasmon Resonance and Quartz Crystal Microbalance Sensors with Nanoparticle Signal Amplification. *Anal Chem.* 2012; 84(14):5898–5904. [PubMed: 22681722]
106. Ertürk G, Özen H, Tümer MA, Mattiasson B, Denizli A. Microcontact imprinting based surface plasmon resonance (SPR) biosensor for real-time and ultrasensitive detection of prostate specific antigen (PSA) from clinical samples. *Sens Actuat B: Chem.* 2016; 224:823–832.
107. Stephenson-Brown A, Acton AL, Preece JA, Fossey JS, Mendes PM. Selective glycoprotein detection through covalent templating and allosteric click-imprinting. *Chem Sci.* 2015; 6(9): 5114–5119.
108. Hong Y, Huh Y-M, Yoon DS, Yang J. Nanobiosensors Based on Localized Surface Plasmon Resonance for Biomarker Detection. *J Nanomater.* 2012; 2012:13.
109. Aćimović SS, Ortega MA, Sanz V, Berthelot J, Garcia-Cordero JL, Renger J, Maerkl SJ, Kreuzer MP, Quidant R. LSPR Chip for Parallel, Rapid, and Sensitive Detection of Cancer Markers in Serum. *Nano Lett.* 2014; 14(5):2636–2641. [PubMed: 24730454]
110. Hwang WS, Truong PL, Sim SJ. Size-dependent plasmonic responses of single gold nanoparticles for analysis of biorecognition. *Anal Biochem.* 2012; 421(1):213–218. [PubMed: 22146558]
111. Xuan Z, Li M, Rong P, Wang W, Li Y, Liu D. Plasmonic ELISA based on the controlled growth of silver nanoparticles. *Nano.* 2016; 8(39):17271–17277.
112. De La Rica R, Stevens MM. Plasmonic ELISA for the ultrasensitive detection of disease biomarkers with the naked eye. *Nat Nanotechnol.* 2012; 7(12):821–824. [PubMed: 23103935]
113. De La Rica R, Stevens MM. Plasmonic ELISA for the detection of analytes at ultralow concentrations with the naked eye. *Nat Protoc.* 2013; 8(9):1759–1764. [PubMed: 23975259]
114. Howes PD, Rana S, Stevens MM. Plasmonic nanomaterials for biodiagnostics. *Chem Soc Rev.* 2014; 43(11):3835–3853. [PubMed: 24323079]
115. Liang J, Yao C, Li X, Wu Z, Huang C, Fu Q, Lan C, Cao D, Tang Y. Silver nanoprism etching-based plasmonic ELISA for the high sensitive detection of prostate-specific antigen. *Biosens Bioelectron.* 2015; 69:128–134. [PubMed: 25721976]
116. Xia Y, Ye J, Tan K, Wang J, Yang G. Colorimetric Visualization of Glucose at the Submicromole Level in Serum by a Homogenous Silver Nanoprism–Glucose Oxidase System. *Anal Chem.* 2013; 85(13):6241–6247. [PubMed: 23706061]
117. Liu D, Yang J, Wang H-F, Wang Z, Huang X, Wang Z, Niu G, Hight Walker AR, Chen X. Glucose Oxidase-Catalyzed Growth of Gold Nanoparticles Enables Quantitative Detection of Attomolar Cancer Biomarkers. *Anal Chem.* 2014; 86(12):5800–5806. [PubMed: 24896231]
118. Mullett WM, Lai EP, Yeung JM. Surface plasmon resonance-based immunoassays. *Methods.* 2000; 22(1):77–91. [PubMed: 11020321]
119. Sanders M, Lin Y, Wei J, Bono T, Lindquist RG. An enhanced LSPR fiber-optic nanoprobe for ultrasensitive detection of protein biomarkers. *Biosens Bioelectron.* 2014; 61:95–101. [PubMed: 24858997]
120. Uh M, Kim JS, Park JH, Jeong DH, Lee HY, Lee SM, Lee SK. Fabrication of localized surface plasmon resonance sensor based on optical fiber and micro fluidic channel. *J Nanosci Nanotechnol.* 2017; 17(2):1083–1091.
121. Uh M, Kang S-K, Park J-H, Jeong DH, Lee H-Y, Lee S-M, Lee SK. Analysis and Optimization of Antibody Immobilization for Immunoassay Using Fiber-Optic Localized Surface Plasmon Resonance Biosensors. *Nanosci Nanotechnol Lett.* 2016; 8(1):8–12.
122. Jeong HH, Erdene N, Park JH, Jeong DH, Lee HY, Lee SK. Real-time label-free immunoassay of interferon-gamma and prostate-specific antigen using a Fiber-Optic Localized Surface Plasmon Resonance sensor. *Biosens Bioelectron.* 2013; 39(1):346–351. [PubMed: 22951530]

123. Breault-Turcot J, Poirier-Richard HP, Couture M, Pelechacz D, Masson JF. Single chip SPR and fluorescent ELISA assay of prostate specific antigen. *Lab Chip*. 2015; 15(23):4433–4440. [PubMed: 26467689]
124. Xu DD, Deng YL, Li CY, Lin Y, Tang HW. Metal-enhanced fluorescent dye-doped silica nanoparticles and magnetic separation: A sensitive platform for one-step fluorescence detection of prostate specific antigen. *Biosens Bioelectron*. 2017; 87:881–887. [PubMed: 27662582]
125. Zhang Q, Wu L, Wong TI, Zhang J, Liu X, Zhou X, Bai P, Liedberg B, Wang Y. Surface plasmon-enhanced fluorescence on Au nanohole array for prostate-specific antigen detection. *Int J Nanomedicine*. 2017; 12:2307–2314. [PubMed: 28392689]
126. Wang J, Mountziaris TJ. Homogeneous immunoassays based on fluorescence emission intensity variations of zinc selenide quantum dot sensors. *Biosens Bioelectron*. 2013; 41:143–149. [PubMed: 22960008]
127. Pei H, Zhu S, Yang M, Kong R, Zheng Y, Qu F. Graphene oxide quantum dots@silver core-shell nanocrystals as turn-on fluorescent nanoprobe for ultrasensitive detection of prostate specific antigen. *Biosens Bioelectron*. 2015; 74:909–914. [PubMed: 26257182]
128. Ming T, Zhao L, Yang Z, Chen H, Sun L, Wang J, Yan C. Strong polarization dependence of plasmon-enhanced fluorescence on single gold nanorods. *Nano Lett*. 2009; 9(11):3896–3903. [PubMed: 19754068]
129. Ma H, Li B, Zhang L, Han D, Zhu G. Targeted synthesis of core-shell porous aromatic frameworks for selective detection of nitro aromatic explosives via fluorescence two-dimensional response. *J Mater Chem A*. 2015; 3(38):19346–19352.
130. Song HY, Wong TI, Sadovoy A, Wu L, Bai P, Deng J, Guo S, Wang Y, Knoll W, Zhou X. Imprinted gold 2D nanoarray for highly sensitive and convenient PSA detection via plasmon excited quantum dots. *Lab Chip*. 2015; 15(1):253–263. [PubMed: 25360665]
131. Frascione N, Gooch J, Abbate V, Daniel B. Fluorogenic displacement biosensors for PSA detection using antibody-functionalised quantum dot nanoparticles. *RSC Adv*. 2015; 5(9):6595–6598.
132. Liu D, Huang X, Wang Z, Jin A, Sun X, Zhu L, Wang F, Ma Y, Niu G, HightWalker AR, Chen X. Gold Nanoparticle Based Activatable Probe for Sensing Ultra-Low Levels of Prostate Specific Antigen. *ACS Nano*. 2013; 7(6):5568–5576. [PubMed: 23683064]
133. Lee S, Hosokawa K, Kim S, Jeong OC, Lilja H, Laurell T, Maeda M. Porous silicon-microarray for simultaneous fluorometric immunoassay of the biomarkers prostate-specific antigen and human glandular kallikrein 2. *Microchim Acta*. 2016; 183(12):3321–3327.
134. Kong R-M, Ding L, Wang Z, You J, Qu F. A novel aptamer-functionalized MoS<sub>2</sub> nanosheet fluorescent biosensor for sensitive detection of prostate specific antigen. *Anal Bioanal Chem*. 2015; 407(2):369–377. [PubMed: 25366976]
135. Barbosa AI, Gehlot P, Sidapra K, Edwards AD, Reis NM. Portable smartphone quantitation of prostate specific antigen (PSA) in a fluoropolymer microfluidic device. *Biosens Bioelectron*. 2015; 70:5–14. [PubMed: 25775968]
136. Tsunoda M, Imai K. Analytical applications of peroxyoxalate chemiluminescence. *Anal Chim Acta*. 2005; 541(1–2):13–23.
137. Liu R, Wang C, Jiang Q, Zhang W, Yue Z, Liu G. Magnetic particle-based, ultrasensitive chemiluminescence enzyme immunoassay for free prostate-specific antigen. *Anal Chim Acta*. 2013; 801:91–96. [PubMed: 24139579]
138. Xie X, Ohnishi N, Takahashi Y, Kondo A. Application of magnetic nanoparticles in full-automated chemiluminescent enzyme immunoassay. *J Magn Magn Mater*. 2009; 321(10):1686–1688.
139. Cha T, Cho S, Kim YT, Lee JH. Rapid aptasensor capable of simply diagnosing prostate cancer. *Biosens Bioelectron*. 2014; 62:31–37. [PubMed: 24973540]
140. Porter MD, Lipert RJ, Siperko LM, Wang G, Narayanan R. SERS as a bioassay platform: fundamentals, design, and applications. *Chem Soc Rev*. 2008; 37(5):1001–1011. [PubMed: 18443685]

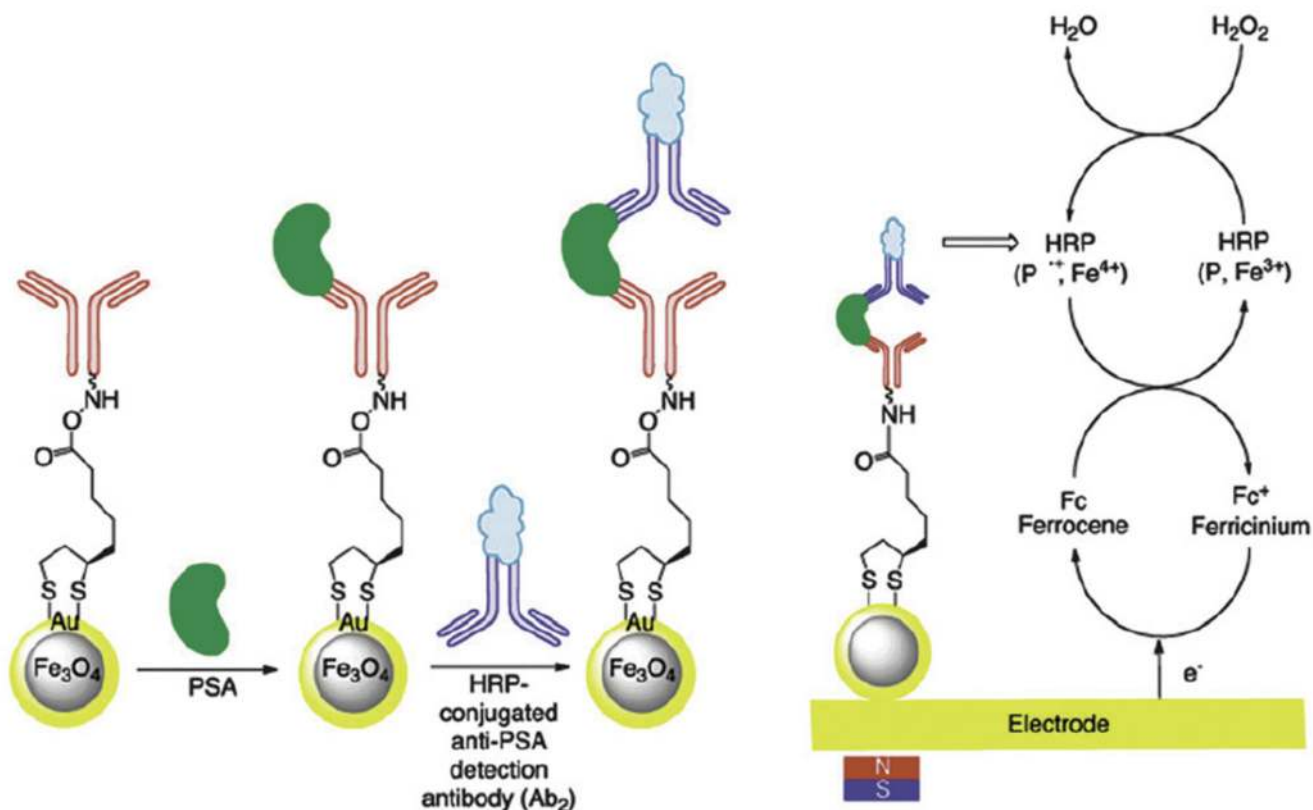


141. Gao R, Cheng Z, deMello AJ, Choo J. Wash-free magnetic immunoassay of the PSA cancer marker using SERS and droplet microfluidics. *Lab Chip*. 2016; 16(6):1022–1029. [PubMed: 26879372]
142. Yang, A-q, Wang, D., Wang, X., Han, Y., X-b, K., Wang, H-j, Zhou, X., Ren, L. Rational design of Au nanorods assemblies for highly sensitive and selective SERS detection of prostate specific antigen. *RSC Adv*. 2015; 5(48):38354–38360.
143. Koukouvinos G, Petrou PS, Misiakos K, Drygiannakis D, Raptis I, Goustouridis D, Kakabakos SE. A label-free flow-through immunosensor for determination of total- and free-PSA in human serum samples based on white-light reflectance spectroscopy. *Sens Actuat B: Chem*. 2015; 209:1041–1048.
144. Alzghoul S, Hailat M, Zivanovic S, Que L, Shah GV. Measurement of serum prostate cancer markers using a nanopore thin film based optofluidic chip. *Biosens Bioelectron*. 2016; 77:491–498. [PubMed: 26457734]
145. Kumar A. Biosensors based on piezoelectric crystal detectors: theory and application. *JOM-e*. 2000; 52(10):1–6.
146. Jakerst JV, Chen Z, Xu L, Nolley R, Chang E, Mitchell B, Brooks JD, Gambhir SS. A Magnetic Bead-Based Sensor for the Quantification of Multiple Prostate Cancer Biomarkers. *PLoS One*. 2015; 10(9):e0139484. [PubMed: 26421725]
147. Kosaka PM, Pini V, Ruz JJ, da Silva RA, González MU, Ramos D, Calleja M, Tamayo J. Detection of cancer biomarkers in serum using a hybrid mechanical and optoplasmonic nanosensor. *Nat Nano*. 2014; 9(12):1047–1053.
148. Arlett JL, Myers EB, Roukes ML. Comparative advantages of mechanical biosensors. *Nat Nano*. 2011; 6(4):203–215.
149. Lee H-J, Lee J-H, Moon H-S, Jang I-S, Choi J-S, Yook J-G, Jung H-I. A planar split-ring resonator-based microwave biosensor for label-free detection of biomolecules. *Sens Actuat B: Chem*. 2012; 169:26–31.
150. Moser HO, Casse BDF, Wilhelmi O, Saw BT. Terahertz Response of a Microfabricated Rod Split-Ring-Resonator Electromagnetic Metamaterial. *Phys Rev Lett*. 2005; 94(6):063901. [PubMed: 15783730]
151. Zhang F, Li S, Cao K, Wang P, Su Y, Zhu X, Wan Y. A Microfluidic Love-Wave Biosensing Device for PSA Detection Based on an Aptamer Beacon Probe. *Sensors*. 2015; 15(6)
152. Lange K, Rapp BE, Rapp M. Surface acoustic wave biosensors: a review. *Anal Bioanal Chem*. 2008; 391(5):1509–1519. [PubMed: 18265962]
153. Sun X, Lei C, Guo L, Zhou Y. Sandwich immunoassay for the prostate specific antigen using a micro-fluxgate and magnetic bead labels. *Microchim Acta*. 2016; 183(8):2385–2393.
154. Lei J, Wang T, Lei C, Zhou Y. Detection of Dynabeads using a micro-electro-mechanical-systems fluxgate sensor. *Appl Phys Lett*. 2013; 102(2):022413.
155. Sun X, Zhi S, Lei C, Zhou Y. Investigation of contactless detection using a giant magnetoresistance sensor for detecting prostate specific antigen. *Biomed Microdevices*. 2016; 18(4):60. [PubMed: 27379844]
156. Jiang X, Cheng S, Chen W, Wang L, Shi F, Zhu C. Comparison of oligonucleotide-labeled antibody probe assays for prostate-specific antigen detection. *Anal Biochem*. 2012; 424(1):1–7. [PubMed: 22343190]



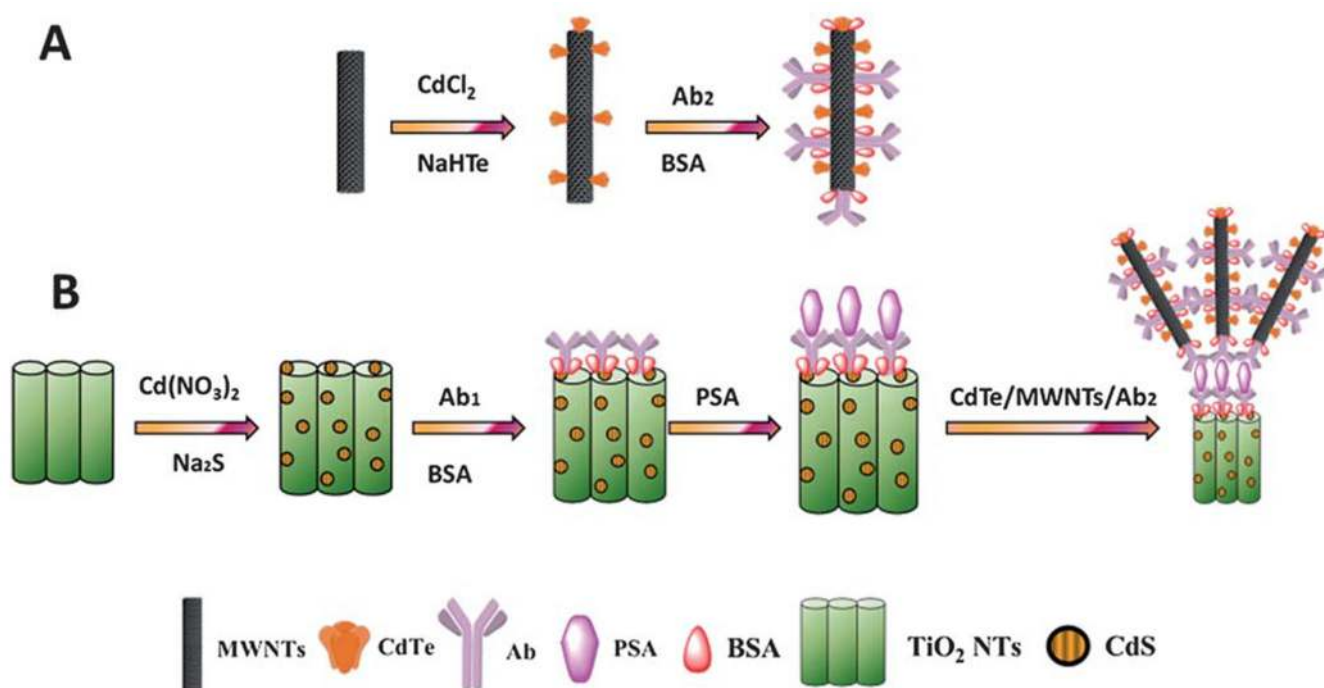


**Fig. 1.** (a, b) Fully integrated and automated MiSens biosensor device controlled wirelessly using a tablet with the developed MiCont™ software (TUBİTAKBİLGEM, Kocaeli, Turkey); (c) the biosensor chip includes two electrode arrays that were fabricated on a 10 × 20 mm silicon dioxide wafer with each array consisting of three working electrodes ( $d = 1$  mm) with shared Au counter and quasi-reference electrodes; (d) the electrode array formed on silicon wafers were attached to a poly(methyl methacrylate) holder by means of a double-sided tape for easy handling. Images taken from [24] with permission of Springer



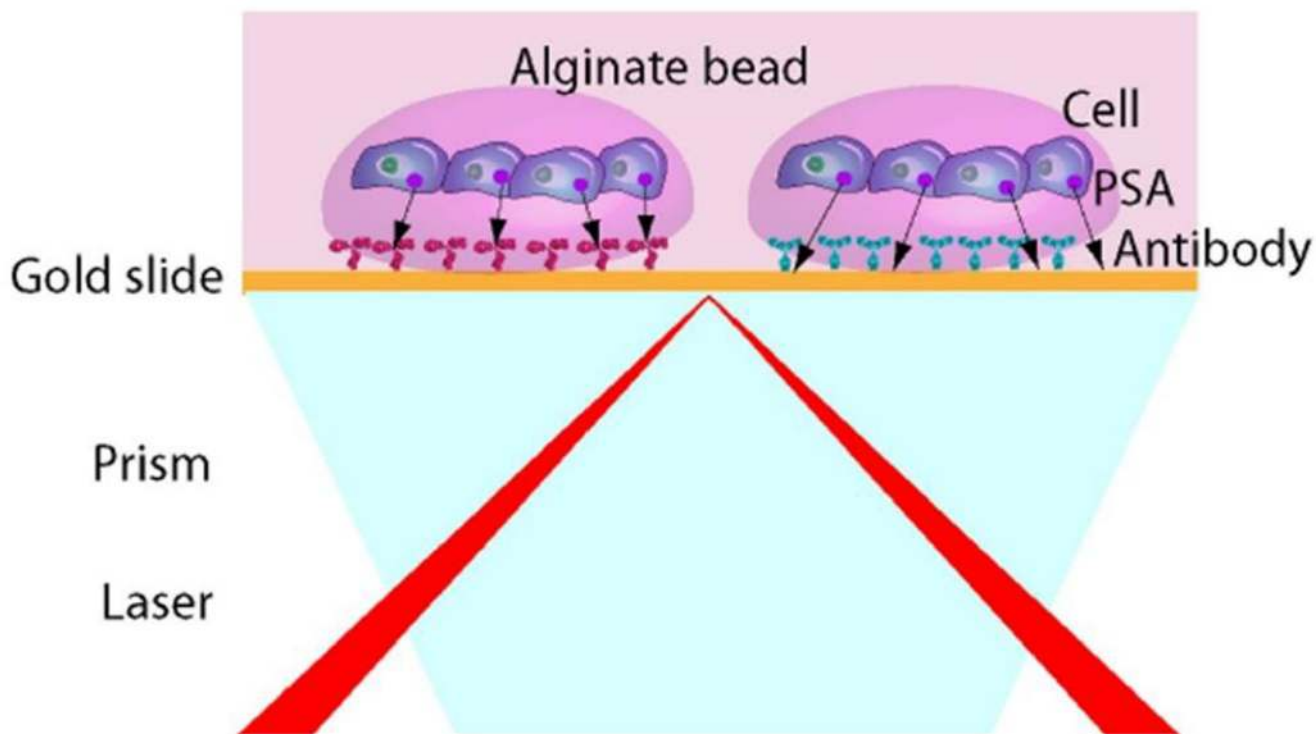
**Fig. 2.**

Outline of the steps required for the capture and detection of PSA using Ab1-Au@MNPs via the sandwich ELISA method. To convert the Au@MNPs into biosensors for PSA, the gold surface of Au@MNPs was covalently modified with anti-PSA antibody. The modified Au@MNPs were dispersed in a PSA sample solution to capture the PSA present in the sample. Then, an enzyme-conjugated anti-PSA antibody was added and a magnetic field was applied to bring the analyte to the transducing electrode for electrochemical sensing. Reproduced from [53] with permission of The Royal Society of Chemistry

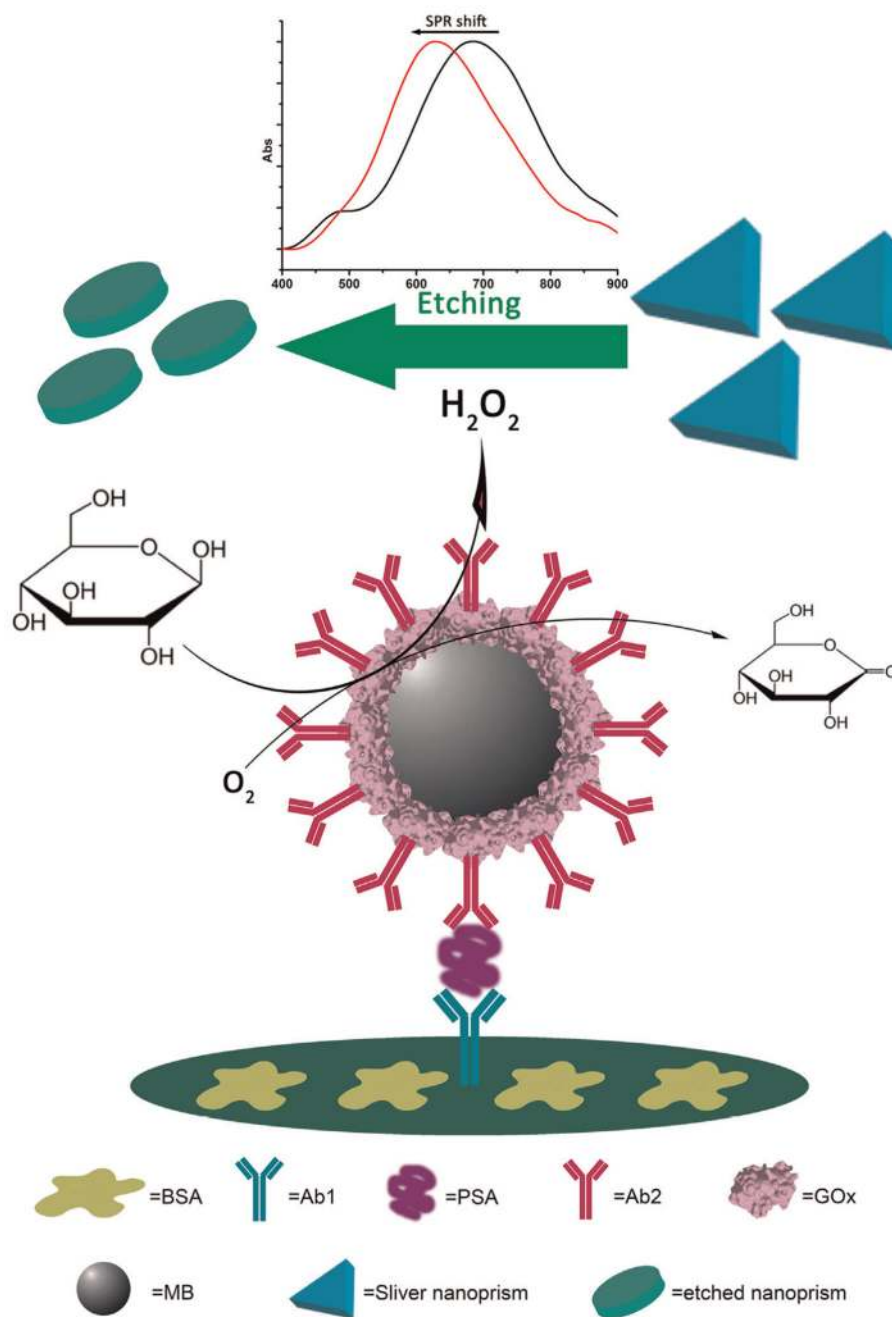
**Fig. 3.**

(A) The preparation of CdTe–MWNTs composites, followed by covalent conjugation of antibody ( $\text{Ab}_2$ ) to the composite with the surface blocked by bovine serum albumin (BSA).

(B) Formation of CdS–TiO<sub>2</sub> nanotubes-based electrode, followed by covalent immobilization of anti-PSA antibody ( $\text{Ab}_1$ ) and blocking by BSA. The final step was incubation with PSA and formation of a sandwich configuration using CdTe–MWNTs composites as ECL quenchers. Reproduced from [94] with permission of The Royal Society of Chemistry



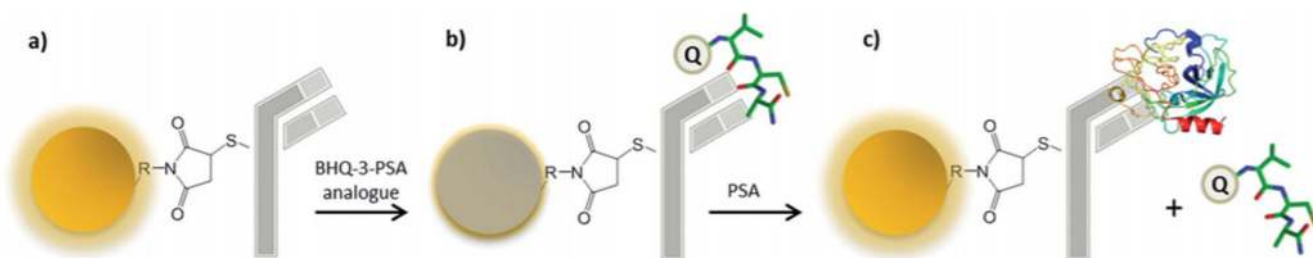
**Fig. 4.** Configuration of a cell-on-chip SPR analytical system. PSA secreted by human prostatic carcinoma cell lines (LNCaP) can be detected by the biosensor in response to specific stimuli, the dihydrotestosterone, transported by a microfluidic network, thanks to the specific antibodies immobilized at localized positions. The image was reproduced from [101]



**Fig. 5.** Scheme of the quantitative immunoassay based on glucose oxidase-catalyzed etching of triangular silver nanoprisms (AgNPRs) into smaller spherical silver nanoparticles. PSA is first immobilized by the capture antibody (Ab<sub>1</sub>), and then PSA is recognized by the detection antibody (Ab<sub>2</sub>) conjugated with glucose oxidase (GOx) on the surfaces of the magnetic beads (MBs). The immobilized GOx catalyzes the oxidation of glucose to generate hydrogen peroxide, which induces the etching of triangular AgNPRs into smaller spherical

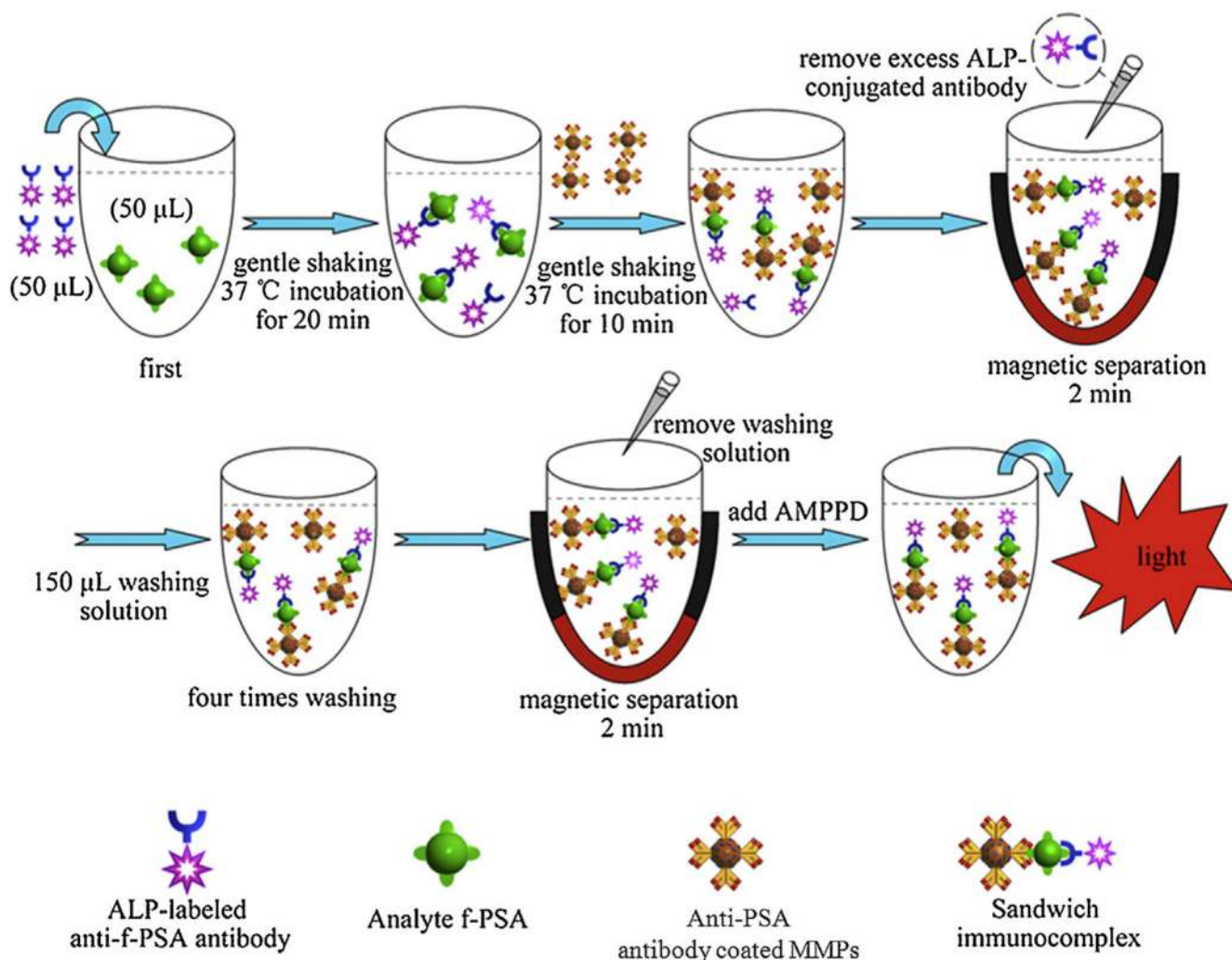
silver nanoparticles. With the etching, the solution turns blue to purple. Reprinted from [115] with permission from Elsevier





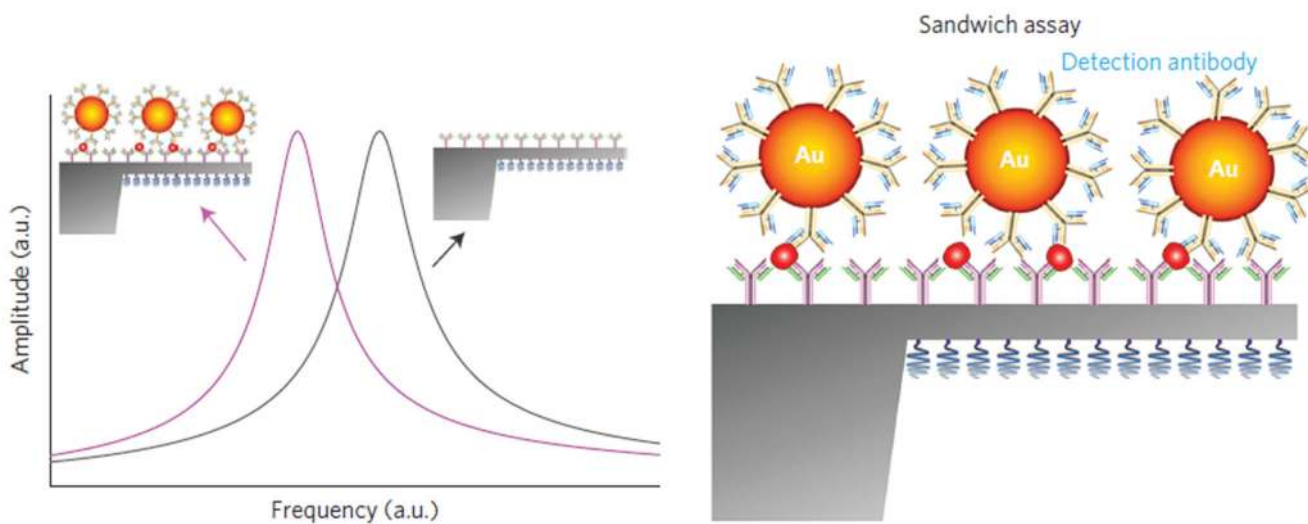
**Fig. 6.**

PSA nanosensor assembly: (a) antibody–quantum dots complexes are prepared using amine-thiol crosslinking; (b) conjugates are incubated with a quencher-labelled epitope peptide quenching fluorescence; (c) binding of higher affinity PSA displaces the peptide from the antibody and restores fluorescence. Reproduced from [131] with permission of The Royal Society of Chemistry (RSC) on behalf of the Centre National de la Recherche Scientifique (CNRS) and the RSC

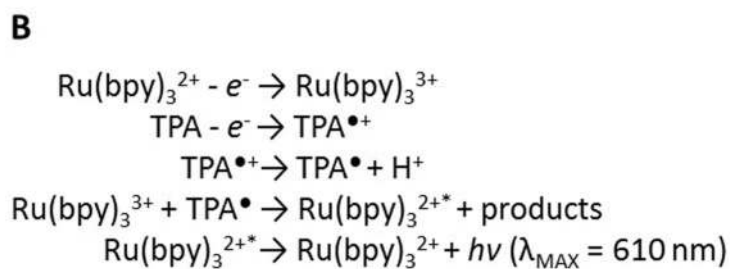
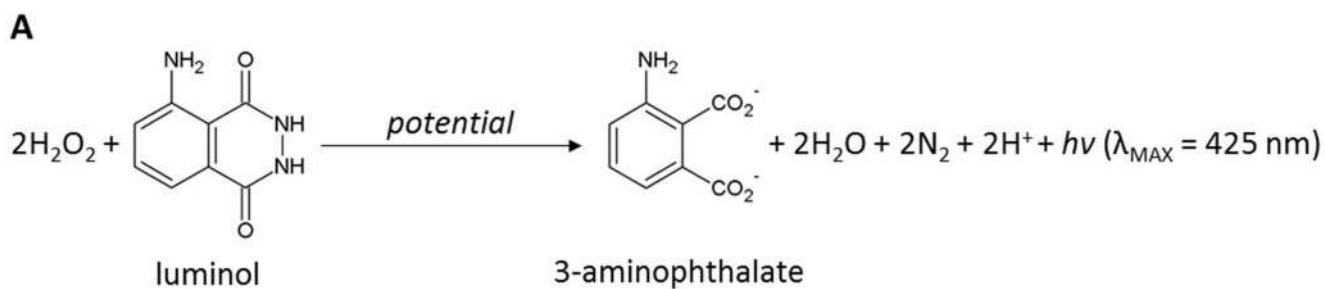


**Fig. 7.**

Schematic demonstration of MMP-based chemiluminescent enzyme immunoassay for PSA detection. First, alkaline phosphatase (ALP) was conjugated to anti-fPSA antibody and then was mixed with a fPSA solution and the resulting mixture was incubated with gentle shaking. Next, anti-PSA antibody coated magnetic microparticles (MMPs) were added to the above solution and the resulting mixture was incubated. At this point, the sandwich immunocomplex MMP-target-ALP was formed. The formed sandwich immunocomplex was magnetically separated and the excess of ALP-conjugated antibodies were removed. Finally, the solution containing the chemiluminescent substrate was added to the sandwich immunocomplex. The resulting mixture was incubated in the dark at room temperature, and the light emissions were measured. Reprinted from [137] with permission from Elsevier



**Fig. 8.** Schematic representation of the effect of the AuNPs mass loading on the resonance frequency of the cantilever. The resulting shift of the resonance frequency is proportional to the added mass. Reprinted by permission from Macmillan Publishers Ltd.: Nature Nanotechnology [147], copyright (2014)

**Scheme 1.**

(A) Luminol ECL reaction with  $\text{H}_2\text{O}_2$ . Reprinted from [81], Copyright (2008), with permission from Elsevier. (B)  $\text{Ru}(\text{bpy})_3^{2+}$  ECL reaction with tripropylamine. Taken from [83]

Table 1

Summary of the best selected biosensor devices for detection of PSA with a list of detections platforms, signal enhancement protocols and key performance characteristics

Biosensing platform	Method	Strategy for signal enhancement	Linear range	LOD	Ref.	
Electrochemical	CV, SWV	Fe <sub>3</sub> O <sub>4</sub> NPs loaded onto GO sheets	61 fg mL <sup>-1</sup> to 3.9 pg mL <sup>-1</sup>	15 fg mL <sup>-1</sup>	[52]	
	SWV	Platinum NPs, BSA-stabilized copper nanoclusters	0.5 pg mL <sup>-1</sup> to 100 ng mL <sup>-1</sup>	146 fg mL <sup>-1</sup>	[61]	
	SWV	Au-rGO nanocomposite, Au-poly(methylene blue)	1.0 fg mL <sup>-1</sup> to 100 ng mL <sup>-1</sup>	0.11 fg mL <sup>-1</sup>	[63]	
	DPV	Poly(thymine)-templated CuNPs, rolling circle amplification	0.05 to 500 fg mL <sup>-1</sup>	0.020 ± 0.001 fg mL <sup>-1</sup>	[64]	
	EIS	Si-nanowires, DNA aptamers	33 aM to 330 fM	23 aM (0.74 fg mL <sup>-1</sup> )	[71]	
	EIS	Au electrode based on mixed SAM, glycoprofiling	4 aM – 40 nM	4 aM (0.13 fg mL <sup>-1</sup> )	[75]	
	LSPR	Fiber-optic nanoprobe	50 fg mL <sup>-1</sup> to 5 ng mL <sup>-1</sup>	100 fg mL <sup>-1</sup>	[119]	
	Optical	Plasmonic ELISA	Triangular silver nanoprisms	10 fg mL <sup>-1</sup> to 100 pg mL <sup>-1</sup>	4.1 fg mL <sup>-1</sup>	[115]
		Fluorescence ELISA	GO quantum dots@silver core-shell nanocrystals	1 pg mL <sup>-1</sup> to 20 ng mL <sup>-1</sup>	0.3 pg mL <sup>-1</sup>	[127]
		SERS	Au nanorods	0.05 to 100 ng mL <sup>-1</sup>	0.01 ng mL <sup>-1</sup>	[142]
Colorimetric immunoassay		AuNPs in presence of GOx	10 to 105 fg mL <sup>-1</sup>	93 aM (3.0 fg mL <sup>-1</sup> )	[117]	
ECL	Chemiluminescence	Magnetic microparticles	0.1 to 30 ng mL <sup>-1</sup>	0.1 ng mL <sup>-1</sup>	[137]	
	ECL immunoassay	CdS NPs sensitized TiO <sub>2</sub> nanotube array, functionalized CdTe-MWCNTs composite	1 fg mL <sup>-1</sup> to 10 pg mL <sup>-1</sup>	1.0 fg mL <sup>-1</sup>	[94]	
Cantil.	ECL immunoassay	MWCNTs coated with polydopamine AuNPs	0.01 pg mL <sup>-1</sup> to 40 ng mL <sup>-1</sup>	4.2 fg mL <sup>-1</sup>	[97]	
	Hybrid mechanical and optoplasmonic sensor	AuNPs	-	100 ag mL <sup>-1</sup>	[147]	
ELISA	Optical	No	~0.01 - 10 ng mL <sup>-1</sup>	~10 pg mL <sup>-1</sup>		

**Abbreviations used:** CV – cyclic voltammetry, SWV – square wave voltammetry, DPV – differential pulse voltammetry, EIS – electrochemical impedance spectroscopy, LSPR – localized surface plasmon resonance, ELISA – enzyme linked immunosorbent assay, SERS – surface enhanced Raman spectroscopy, ECL – electrochemiluminescence, NPs – nanoparticles, GO – graphene oxide, BSA – bovine serum albumin, SAM – self-assembled monolayer, GOx – glucose oxidase, MWCNTs – multiwalled carbon nanotubes.