



Nanobiosensors: Construction and Diagnosis of Disease

29

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Contents

1	Introduction	640
2	Concept of Nanobiosensors	641
2.1	Ideal Features of Nanobiosensors	642
2.2	Components of Nanobiosensors	642
2.3	Nanobiosensors for Diagnosis of Disease	643
3	Conclusion	655
	References	656

Abstract

Nanobiosensors, which are advancing daily, are of interest to many researchers and enabled developments in this field. Considering the increasing population today, nanobiosensors have become widely used methods for the early diagnosis of many diseases. Compared to traditional analytical methods, nanobiosensors have significant advantages such as high sensitivity and selectivity, shorter analysis time, biocompatibility, and easy miniaturization of used devices for on-site analysis. Like biosensors, nanobiosensors can be classified in various ways, either by biological molecules such as enzymes, antibodies, and

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aptamers or by working principles such as optical and electrochemical. This chapter summarizes nanobiosensors designs, characterizations, applications, and advances for detecting various diseases and biomarkers. Current studies of nanobiosensors were given, along with detailed information on nanoparticles' effect, biological molecules, and detection methods. As a result, it can be concluded that the outputs obtained by using nanobiosensor technology will increase the quality of life.

Keywords

Diagnosis · Disease · Electrochemical · Nanobiosensors · Nanomaterials

1 Introduction

Nanotechnology's history dates back to the 1950s and has contributed to the emergence of nanoscale materials with extraordinary properties. In addition, nowadays, developments in nanotechnology have allowed the obtaining of new materials by processing different materials at the atomic level. As the materials approach the nanoscale, they gain many new superior physical and chemical properties. Nano-sized materials exhibit different properties than normal-sized materials due to their surface and quantum effects. Nanomaterials have unique physical, chemical, and mechanical properties with quantum size effects, unique characteristics of surface atoms, size dependence of electronic structure, and high surface/volume ratio. Nanoparticles have found wide applications in many different fields from biomedical, optics, and electronics, and they arouse great interest because they form a bridge between bulk materials and atomic or molecular structures (Asadian et al. 2019; Saleh et al. 2019; Dutta and Das 2021; Shen et al. 2021; Laraib et al. 2022).

Nowadays, the development of sensitive and selective methods for determining and detecting compounds used to diagnose and treat many diseases that threaten human health is possible by integrating nanomaterials into sensors and creating nanosensors. Moreover, biosensors that contain a biological recognition element, such as an enzyme, antibody, or oligonucleotide, and transform the biochemical response resulting from the interaction of this element with the target molecule into a measurable physical signal also enable the development of sensitive and selective analytical methods (Nosrati et al. 2018; Huang et al. 2021a; Tahir et al. 2022; Younis et al. 2022).

To mention the immobilization of nanoparticles on the sensor surfaces, much more sensitive and selective analyses can be performed by expanding the surface area of the biomolecule-modified sensors and increasing their electrical conductivity. This chapter provides recent advancements, salient features, types, and uses of nanobiosensor to determine and detect several diseases.

2 Concept of Nanobiosensors

Sensors are electronic devices that record a physical, chemical, or biological change and convert these changes into a measurable signal. The sensor contains a recognition element that provides a selective response to a particular or group of analytes, thereby minimizing interference from other sample components. Another significant sensor component is the transducer or detector device that generates the signal. It is a signal processor (Balasubramanian and Burghard 2006; Simões and Xavier 2017; Raj and John 2018).

Living organisms have a high sensitivity feature beyond what is known; for example, it has been reported that dogs' sense of smell is 100,000 times more sensitive than humans, eels are sensitive enough to immediately detect a few drops of foreign matter added to tons of water, and algae are very sensitive to toxic substances in the literature (Yonzon et al. 2005; Sekhar and Wignes 2016). All living organisms immediately perceive the changes in their environment and strive to adapt to them to survive. The basis for the *in vitro* use of biosensors is based on this sensing mechanism.

Combining analysis systems with biological substances that make it possible to detect these stimuli in living things has led to the emergence of biosensors. In this way, while only anion and cation analyzes can be made with classical electrochemical methods, adding biomaterial to the system while preparing the sensor makes it possible to determine many substances (Fatima et al. 1986; Vaz et al. 2022).

While the device that determines and records a physical feature is defined as a sensor, the biosensor is defined as a sensor system that combines a biochemical component with a physicochemical converter. The task of a biosensor is to produce a continuous digital electrical signal proportional to the amount of an analyte. In biosensor systems, changes in physical size are measured by converting them to changes in electrical size. Examples of these changes are current, voltage, and temperature (Chandra 2013; Mahapatra et al. 2020; Nazare et al. 2021).

Generally, nanobiosensors consist of the analyte, bioreceptor, transducer combined with nanomaterials, and finally, detector. The analytes serve as a sample for analysis and/or detection of the respective bioreceptors. Next, a transducer coupled with nanomaterials converts the physiochemical response into an electrical signal. These signals can be measured as different responses such as current, electric potential, conductivity, density, impedance, phase of electromagnetic radiation, mass, temperature, and viscosity. Changes in these responses provide information for quantitative and qualitative determination of the analyte. Nanomaterial used in nanobiosensors act as an interlayer between the transducer and biological agents. The transducer is integrated with nanomaterials to create a nanobiosensor (Sharifi et al. 2020; Sellappan et al. 2022; Thakur and Sankar 2022). The schematic diagram for the basic principle of nanobiosensors is presented in Fig. 29.1.



Fig. 29.1 Schematic representation of the nanobiosensors

2.1 Ideal Features of Nanobiosensors

There are main features that an ideal nanobiosensor should have for precise and sensitive determinations. Linearity must be wide enough to detect high analyte concentrations. It should be sufficiently sensitive depending on the analyte concentration exhibiting high selectivity to obtain reliable results. The time to achieve 95% of the total response should be as short as possible. Properties such as biocompatibility, stability at usual storage circumstances, and stabilizability also contribute to the high specificity of nanobiosensors towards the analyte. Nanobiosensors must be distinct and unrestrained of any physical factors such as agitation, pH, etc. In addition, the nanobiosensor designed as a disposable sensing platform is another important feature that attracts users for on-site analysis (Malik et al. 2013; Chamorro-Garcia and Merkoçi 2016; Mahato et al. 2018b).

2.2 Components of Nanobiosensors

Incorporating nanomaterials into biosensors has demonstrated remarkable benefits over conventional sensors and/or last-generation biosensors. These benefits include a relatively higher sensitivity due to a somewhat larger surface area, shorter response time, faster electron transfer capability, high stability, and a long lifetime. Therefore, it is not correct to completely separate the term nanobiosensor or its components from biosensor systems. As with the biosensor, the nanobiosensor has three essential elements: bioreceptor (sensing probe), transducer coupled with nanomaterials, and signal amplification (Parolo and Merkoçi 2013; Srivastava et al. 2018; Gajdosova et al. 2020).

As the first component, bioreceptors are biological components that can capture or interact with the target analyte and respond according to the interaction. Tissues, bacteriophages, proteins, microorganisms, organelles, human cells, nucleic acids, enzymes, and antibodies are the most commonly used biological components to produce nanobiosensors. As the second component, the transducer acts as an interface. It measures external or physical changes, interacts with bioreceptors, and then converts the changes in energy levels into a measurable electrical signal at the detector. Detector elements collect the signals from the transducer and transmit these signals to a microprocessor for analysis.

Moreover, nanobiosensors are a concept that emerged by combining nanomaterials with transducers. Therefore, nanomaterials exhibit a significant role in the current and future advancements of nanobiosensors. Nanomaterials have unique properties such as being more robust, lighter, cheaper, more durable, and sensitive. Common nanomaterials, used in nanobiosensor design, are metallic nanoparticles, carbon nanotubes, magnetic nanoparticles, quantum dots, polymeric nanomaterials, etc. These properties also make nanomaterials attractive in the fabrication of nanobiosensors (Park et al. 2016; Mishra and Rajakumari 2018; Bozal-Palabiyik et al. 2021; Krzyczmonik et al. 2021; Pérez et al. 2021).

Biosensors can be classified according to the types of bioreceptor such as DNA, enzyme or antibody-based, as well as transducer types such as electrochemical, optical, microwave, and mass-based (Chamorro-Garcia and Merkoçi 2016; Thakur and Sankar 2022). Within the scope of this handbook, electrochemical nanobiosensor designs are classified according to bioreceptor type, and information about their structures and their use in the diagnosis of various diseases is presented in this chapter.

2.3 Nanobiosensors for Diagnosis of Disease

In enzyme-based nanobiosensors, enzymes are adsorbed onto electrode surfaces using various immobilization techniques, either by van der Waals force or by chemical bonding. The enzyme and its associated substrate interact on the surface, where the enzyme acts as a biorecognition element with outstanding catalytic properties. The biochemical reactions between enzyme and substrate rely specifically on the biospecificity of enzymes. Monitoring the changes occurring due to the response makes it possible to produce nanobiosensors that offer compassionate, specific, selective, stable, and reproducible results (Yang 2012; Das et al. 2016; Kurbanoglu et al. 2020).

Since the concept of biosensors was introduced by Clark and Lyons in 1962 to measure glucose levels, glucose sensors still maintain their importance today. Because real-time glucose determination in various body fluids is essential to manage the progression of diabetes, one of the most important diseases of our age (Fracchiolla et al. 2013). In their study, Kausaite-Minkstimiene et al. designed a novel amperometric glucose nanobiosensor using a nanobiocomposite consisting of poly(1,10-phenanthroline-5,6-dione) (PPD), poly(pyrrole-2-carboxylic acid) (PPCA), gold nanoparticles (Au NPs), and glucose oxidase (GOx). In this study, firstly, the graphite rod (GR) electrode is a working electrode subjected to the cleaning procedures. Then, PPD was dropped to the electrode surface, and electrodeposition using cyclic voltammetry (CV) was performed to obtain PPD/GR electrode. As a second step, Au NPs-entrapped PPCA ((Au NPs)PPCA) layers were created using electrodeposition. To prepare an enzyme-based sensor, (Au NPs)PPCA/PPD/GR electrode was modified with the GOx, and in this study, a mixture of EDC and NHS was used for covalent coupling of GOx to the modified surface. Before amperometric measurements, GOx-(Au NPs)PPCA/PPD/GR electrode was washed

and left to dry at room temperature. Choosing electrochemical polymerization and immobilization conditions is one of the most critical steps of enzyme-based sensors to achieve the best analyte response. Because the number of potential scan cycles and the scan rate directly affect the polymeric layer formation and the immobilization of the relevant enzyme on the modified surface. For this study, different ranges were investigated, and the most suitable potential scan cycles were found to be ten and the optimal scan rate to be 100 mV/s. Also, as metallic NPs, Au NPs can improve the analytical signal through their ability to facilitate electron transfer. As a result of the experiments, when 0.15 nM Au NPs concentration was used, the (Au NPs)PPCA layer became more porous and provided a much larger surface area. After optimizing the experimental conditions, the developed enzyme sensor exhibited a wide linear range from 0.20 until 500.0 mM with a relatively low detection limit (LOD) of 0.08 mM for glucose. Furthermore, the designed sensor was highly selectively used for glucose detection in human serum (Kausaitė-Minkstienė et al. 2020).

Nowadays, the great development and commercial success of smartphones, tablets, and smartwatches have brought practical usability in many areas. Moreover, comprehensive digitization also contributes to point-of-care testing (POCT). These trends have the advantage of significantly reducing the extended analysis time compared to traditional analysis methods performed in the laboratory and are an alternative to the complex testing system that delays the ability to the worse conditions of patients (Soni and Jha 2017; Xu et al. 2018). In their work, Jędrzak et al. developed a fast, simple, and mobile smartphone-based nanobiosensor with the potential for glucose measurement in diabetic patients (Fig. 29.2). In this study, $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ and $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ were used to prepare magnetite nanoparticles via the

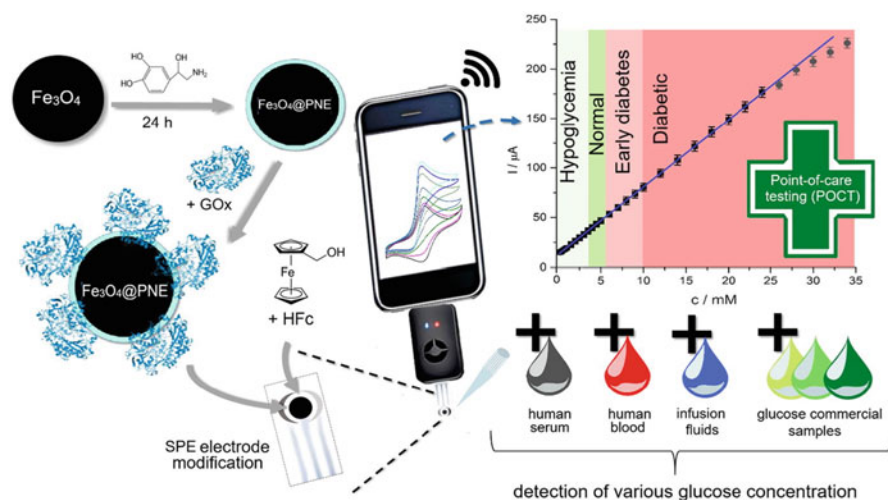


Fig. 29.2 Schematic illustration of the developed sensor. (Reprinted with permission from Jędrzak et al. (2022))

coprecipitation method. In the second step, magnetite nanoparticles were added to the TRIS buffer solution, and after obtaining a homogeneous mixture, norepinephrine solution was added to this solution. Finally, $\text{Fe}_3\text{O}_4@\text{PNE}$ hybrid material was obtained for surface modification. The optimal amount of $\text{Fe}_3\text{O}_4@\text{PNE}$ and glucose oxidase were mixed in the citric buffer to fabricate the enzyme-based nanosensor. As the next step, 1 μL of the $\text{Fe}_3\text{O}_4@\text{PNE}$ -GOx was deposited on the screen-printed electrode (SPE) surface and dried at room temperature. Quinones or the amino groups in the $\text{Fe}_3\text{O}_4@\text{PNE}$ nanosensor supported the creation of hydrogen bonds between GOx molecules and $\text{Fe}_3\text{O}_4@\text{PNE}$. Therefore, it can be said that $\text{Fe}_3\text{O}_4@\text{PNE}$ nanoplatform exhibited a higher capability of GOx immobilization on the surface. After completing the electrochemical and surface characterizations, the designed sensor showed a linear concentration range of 0.2–24 mM and a LOD of 6.1 μM . In addition, the developed sensor exhibited long-term stability for 20 weeks. Finally, the developed sensor could be a valuable platform for POCT by a smartphone and potentiostat. Moreover, the developed sensor as a promising POCT biosensor could detect glucose content in human serum and human blood samples (Jędrzak et al. 2022).

In 2020, Eom et al. proposed an enzyme-based electrochemical nanobiosensor for the rapid and accurate detection of cholesterol in saliva samples of hyperlipidemia patients. The flexible electrode was first prepared using polyimide as a working electrode in this study. Platinum nano-cluster (Pt-NC) was electroplated on the surface by potentiostatic mode (−200 mV, 200 s) to modify the electrode surface. The procedure was carried out separately from 1 to 5 times at 100 s intervals. The optimum amount of cholesterol oxidase, cholesterol esterase, and peroxidase were mixed with fabricating an enzyme-based sensor. Subsequently, 15 μL of the enzyme mixture as an optimal amount was dropped on the modified Pt-NC electrode surface. Finally, Nafion was coated on the enzyme-modified surface and dried at ambient temperature to use in the determination of cholesterol. After morphological, topographical, and electrochemical characterization, the proposed sensor showed a more comprehensive concentration range from 2 μM to 486 μM and a LOD of 2 μM . Here, saliva samples were obtained from three hyperlipidemia patients to investigate of the feasibility of an advanced Pt-NC/enzyme/Nafion sensor using amperometric measurements. The received responses showed that a developed sensor can be utilized to determine cholesterol in saliva immediately without pretreating procedures (Eom et al. 2020).

In a study conducted in 2021, Rahimi-Mohseni et al. developed an enzyme-based sensor to detect phenylketonuria (PKU), a congenital disease that occurs in 1 out of every 10,000 newborns. This sensor is based on zinc oxide (ZnO) nanorods, Au NPs, and enzyme phenylalanine hydroxylase (PHA) available in the extract of mosses leaf-like tissue. In this study, firstly, a graphite screen-printed electrode (GSPE) was covered with ZnO nanorods, then this surface was covered by Au NPs. Finally, the moss extract and potassium ferricyanide were coated onto the modified surface. Differential pulse (DP) voltammograms were recorded in phosphate buffer solution (PBS) as supporting electrolytes to perform the electrochemical measurements. When $\text{ZnO}@Au$ nanohybrid was covered on the surface of GSPE, the current

value was increased, and the ΔE_p value decreased. This phenomenon is due to the electron transfer ability and catalytic activity of ZnO@Au nanohybrid. To investigate surface characterization, field emission scanning electron microscopy (FE-SEM) images of the bare GSPE, ZnO/GSPE, ZnO@Au/GSPE, and ZnO@Au nanohybrid/mosses extract modified GSPE was recorded. Figure 29.3a showed the entire GSPE surface, while Fig. 29.3b showed the presence of ZnO nanorods on the electrode surface. The presence of ZnOs allowed increasing the performance of the developed biosensor due to its small size and high specific surface area. Figure 29.3c showed that Au nanoparticles as shine sphere were dispersed on the ZnO nanorods modified surface.

Moreover, this structure confirmed the successful synthesis of the nanohybrid. Thanks to the changed surface morphology, Fig. 29.3d confirmed the presence of the enzyme layer on the surface of ZnO@Au/GSPE. In addition, the simultaneous presence of Zn, O, and Au on the developed surface was confirmed by energy-dispersive X-ray spectroscopy (EDX) analysis. Under the optimum working conditions, the detection range of 5.0 nM to 100 μ M with a LOD of 3.0 nM was obtained.

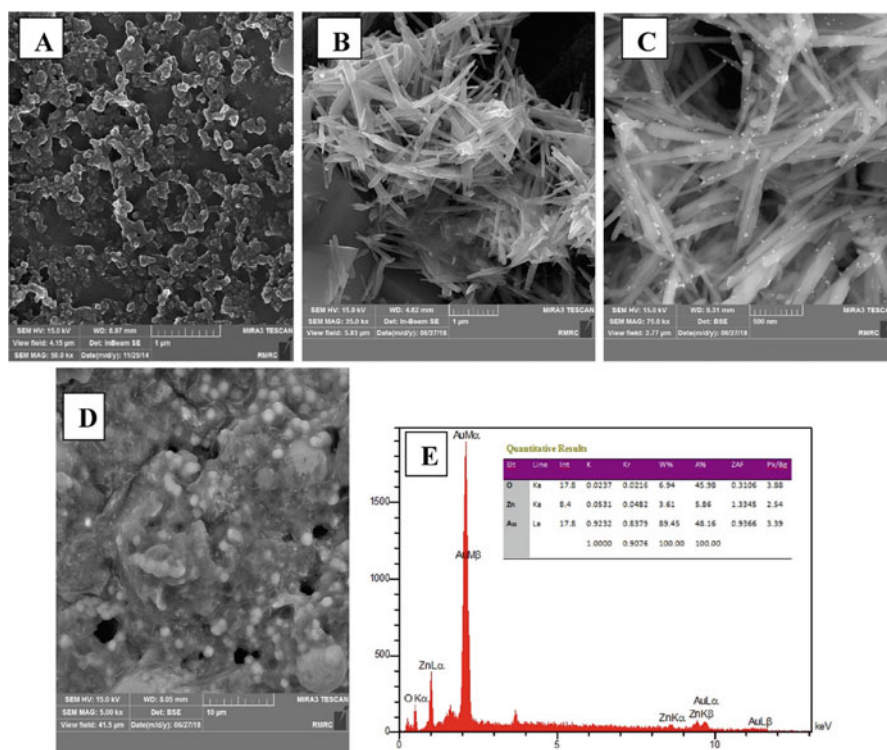


Fig. 29.3 FE-SEM images of bare GSPE (a) ZnO nanorods/GSPE (b), ZnO@Au nanohybrid/GSPE (c), ZnO@Au nanohybrid/mosses leaf-like extract/GSPE (d), and EDX of ZnO@Au nanohybrid (e). (Reprinted with permission from Rahimi-Mohseni et al. (2021))

Furthermore, the proposed nanobiosensor was successfully applied to determine phenylalanine in the human blood serum samples (Rahimi-Mohseni et al. 2021).

Deoxyribonucleic acid (DNA)-based biosensors, which serve as oligonucleotides as another biological molecule, can be used to allow a single-chain nucleic acid molecule to define and bind its complementary strand in samples. The interaction mechanism is based on stable hydrogen bonds between the two nucleic acid strands. As seen in many different applications, DNA-based sensors exhibit several advantages. Because synthesis procedures contain simple steps, these sensors allow fast on-site analysis. Moreover, these sensors are simple, quick, and precise, used for surgical, forensic, environmental, and pharmaceutical applications (Oliveira Brett 2005; Abu-Salah et al. 2010, 2015).

In 2021, Pareek et al. suggested a label-free DNA-based nanobiosensor platform to detect human papillomavirus (HPV). Here, the indium tin oxide (ITO) glass electrode was washed with acetic acid and distilled water. Then, chitosan (CHIT) capped Au NPs (ccAu NPs) were electrodeposited on the surface of ITO by the CV method. Further, probe DNA (PDNA) was coated on the ccAu NPs modified ITO electrode, and subsequently, HPV-16 target DNA (TDNA) at different concentrations was immobilized on the probe-modified surface. When the ITO surface was modified with ccAu NPs, due to the conductive properties of ccAu NPs, electron transfer was facilitated, and a suitable surface was obtained for the binding of PDNAs. Under optimal conditions, the linear concentration range from 1 pM to 1 μ M and a LOD of 1 pM was found to detect HPV-16. Moreover, the long stability and selectivity of the developed sensor have also shown that it can be a suitable platform for detecting HPV-16 from clinical samples (Pareek et al. 2021).

DNA-based sensors have many advantages in determining disease genes as well as drugs, proteins, or biomarkers. In their study, Zhang et al. developed an electrochemical DNA biosensor using carbon dots (CDs) and graphene oxide (GO) to detect PML/RAR α fusion gene. This gene is essential for the early clinical diagnosis of acute promyelocytic leukemia (APL). In this study, the bare glassy carbon electrode (GCE) was rinsed with alumina polishing suspension and distilled water, respectively. Then, the optimal amount of CDs/GO nanocomposites was dropped on the GCE surface and dried at 60 $^{\circ}$ C. Secondly, the prepared electrode was immersed in PBS containing EDC and NHS. Afterward, the capture probe DNA was incubated on the CDs/GO/GCE at room temperature. At the final step, methylene blue (MB) and complementary sequences of DNA solution were immobilized on the surface to construct a measurement sensor. After the prepared sensor was rinsed with TE buffer, DP voltammograms were recorded to detect PML/RAR α fusion gene. Moreover, electrochemical results showed that the excellent conductivity of CDs and the large surface area of GO provided faster electron transfer to enhance the hybridization efficiency of DNA. After experimental conditions such as accumulation time of MB, hybridization time of DNA and hybridization temperature were optimized, the linear detection range from 2.50×10^{-10} M to 2.25×10^{-9} M with a LOD of 83 pM was obtained for use in clinical diagnostic assays (Zhang et al. 2021).

Nowadays, peptides have recently been used as potential antifouling materials due to their unique properties such as easily synthesized processes,

cost-effectiveness, biocompatibility, and tunable or modifiable structures. Peptides have the same chemical structures as proteins. However, they have shorter lengths of peptide bonds than proteins. Peptides are also formed by natural or synthetic short polymers of amino acids linked by these bonds. Artificial peptides can be synthesized via standard solid-phase synthesis protocols by screening from the peptide library to provide a specific sequence. In addition, the peptides exhibit many other advantages, including high stability, standard synthetic protocol, easy modification, and outstanding chemical versatility. Therefore, they are ideal molecules as biorecognition elements in biosensors (Liu et al. 2015; Barbosa et al. 2018; Karimzadeh et al. 2018).

In 2020, Hui et al. proposed a novel biosensor based on specifically designed antifouling peptides and a signal amplification strategy to determine prostate-specific antigen (PSA) in human serum samples. As shown in Fig. 29.4, first, the surface of GCE was cleaned. A mixture of poly(ethylene glycol) (PEG) and poly(3,4-ethylene dioxythiophene) (PEDOT) nanocomposites was electrodeposited on the GCE surface by CV. The second step covered the PEG/PEDOT/GCE with the solution, including streptavidin, NHS, and EDC. To develop a peptide-based sensor, self-designed biotin-labeled peptides (Pep1, biotin-PPPPEKEKEKE, and Pep2, biotin-PPPPEKEKEKEHSSKLQC) were synthesized. The mixed solution of the two kinds of peptides was immobilized onto the PEG/PEDOT/GCE surface. This

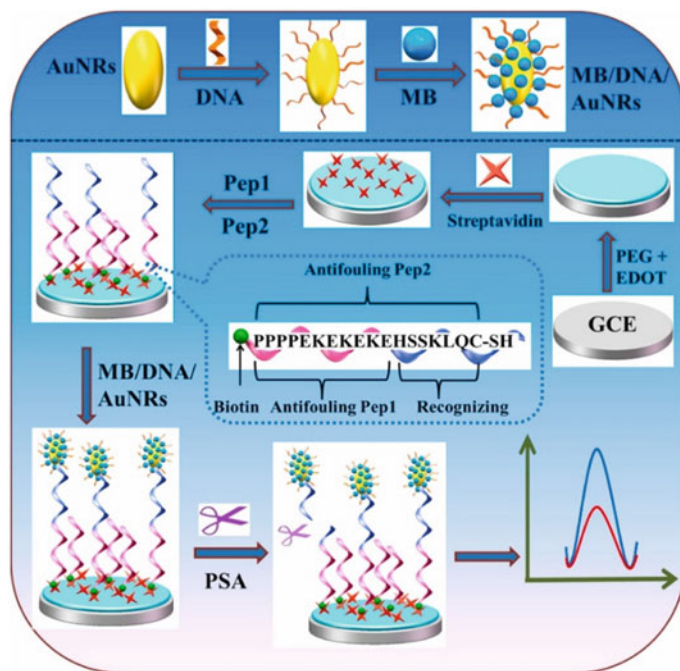


Fig. 29.4 Schematic diagram and working principle of the antifouling electrochemical biosensor. (Reprinted with permission from Hui et al. (2022))

immobilization was based on the strong interaction between biotin and streptavidin. As a signal amplifier, DNA/Au NRs solution was covered onto the Pep/PEG/PEDOT/GCE, and adsorption of MB onto DNA/Au NRs was performed. Before all the electrochemical measurements, the modified surface was immobilized with PSA, and DPV responses were recorded. After the optimum conditions were determined, the developed nanobiosensor showed a wide concentration range from 0.10 pg mL^{-1} to 10.0 ng mL^{-1} with a LOD of 0.035 pg mL^{-1} . Moreover, the developed biosensor was successfully used to determine PSA in human serum samples (Hui et al. 2022).

In their study, Song et al. developed an efficient and simple antifouling biosensor for detecting human immunoglobulin G (IgG) using a Y-shaped peptide constructed with two branches. EKEKEKE and HWRGWVA as peptide sequences were synthesized for antifouling. The bare GCE surface was polished and rinsed before surface modification in this study. To construct modified GCE/PEDOT-citrate surface, electrochemical deposition was performed using a mixed solution consisting of EDOT and sodium citrate. Afterward, GCE/PEDOT-citrate surface was covered with Au NPs by electrodeposition. The PEDOT-citrate/Au NPs modified surface was covered with a Y-shaped peptide solution in the final stage. The developed biosensor was soaked in IgG solutions at room conditions for 90 min; then, measurements were performed. The electrode characterizations showed that unmodified GCE had a higher current value than GCE/PEDOT-citrate. While this indicated the presence of PEDOT-citrate on the GCE surface, it was due to the charge repulsive interaction between negatively charged PEDOT-citrate and negatively $[\text{Fe}(\text{CN})_6]^{3-/4-}$ molecules. After the GCE/PEDOT-citrate surface was coated with Au NPs, the excellent electrical conductivity of Au NPs increased the current signal. Under the optimal experimental conditions, it was observed that as the human IgG concentration increased, the DPV current signals decreased. Moreover, reductions in current signals were observed since the redox molecules inhibit electron transfer after the prepared nanosensor was covered with the Y-shaped peptides and unique IgG molecule. The developed sensor exhibited a linear concentration range from 0.1 to $10,000 \text{ ng mL}^{-1}$. The LOD was estimated to be 0.032 ng mL^{-1} . Moreover, the designed sensor was successfully used to detect IgG in clinical human serum samples (Chen et al. 2021).

Very recently, other examples of peptide-based nanobiosensors were proposed to detect trypsin (Lin et al. 2018), epidermal growth factor receptor (EGFR) (Li et al. 2013), human chorionic gonadotropin (hCG) (Xia et al. 2017), human norovirus (Hwang et al. 2017), and miRNA-21 (Kangkamano et al. 2018). The nanomaterials and composites used in these sensors exhibited several advantages: high electrical conductivity, large surface area and mechanical strength, high selectivity, chemical stabilization, and good electron interaction characteristics. These facts led to the evolution of electrochemical nanobiosensors with good sensitivity, a wide linear concentration range, and low LOD values.

Immunosensors are affinity-ligand-based biosensors in which antibodies are used as bio-components, and the antibody-antigen interaction is monitored with a suitable transducer. The main feature of all immunosensors is their specificity resulting from

molecular recognition of antigens by antibodies to form a stable complex (Mahato et al. 2018a). Electrochemical immunosensors based on the specificity of antigen-antibody interactions by an electrochemical conversion are the most preferred because of their advantages such as cost, simplicity, sensitivity, miniaturization properties, and rapid analytical response (Pei et al. 2013; Karunakaran et al. 2015; Felix and Angnes 2018). Especially label-free immunosensors are preferred in immunoassay systems due to their potential simplicity and affordability. In 2022, Wu et al. developed a label-free sensor based on multilayer nanocomposite modification of ordered mesoporous carbon (CMK-3), Au NPs, ferrocene carboxylic acid (Fc), magnesium (Mg), aluminum (Al), and layered double hydroxide (LDHs) materials for the detection of cancer antigen 125 (CA125), which is a biomarker for the diagnosis of ovarian cancer. In this study, preparation of a modified sensor was designed by layer-by-layer (LBL) self-assembly by electrostatic attraction, as shown in Fig. 29.5. Firstly, the GCE surface was polished and rinsed with ethanol, hydrochloric acid, and deionized water. Then, 1 mg mL⁻¹ of CMK-3 was dropped onto the GCE surface, and Au NPs were coated on the CMK-3 modified electrode. Finally, CMK-3(Au/Fc@MgAl-LDH)_n multilayer nanocomposites were obtained using repeating the modification process with Au NPs and Fc@MgAl-LDH with the optimal number of *n* cycles. To prepare immunosensor, glutaraldehyde (GA) as a crosslinking agent was immobilized on the modified electrode to bind carboxyl (-COOH) of Fc and amino (-NH₂) of antibody. Afterward, 1 mg mL⁻¹ antibody

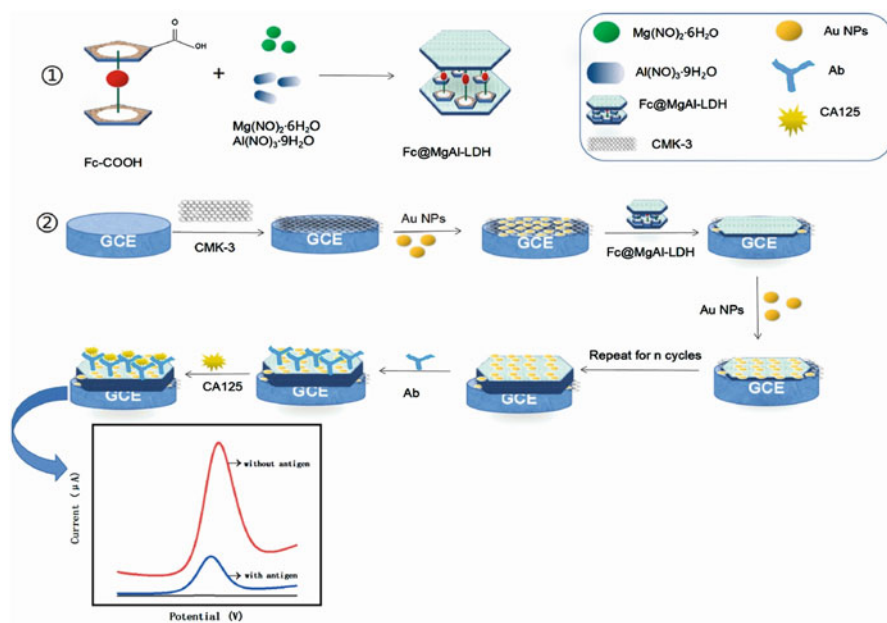


Fig. 29.5 Schematic of the proposed electrochemical immunosensor. (Reprinted with permission from Wu et al. (2022))

(Ab) solution was dropped onto the modified surface, and the unbound antibody was cleaned with the 0.01 M PBS (pH 7.0) solution. Subsequently, bovine serum albumin (BSA) solution was dropped onto the antibody-modified surface to prevent nonspecific binds. CA125 was immobilized on the modified sensor in the last step to perform DPV measurements. Electrochemical impedance spectroscopy (EIS) was used for the electrochemical characterization of the prepared sensors. The resistance was reduced when the GCE surface was modified with CMK-3(Au/Fc@MgAl-LDH)*n* nanocomposites. These results provided that the CMK-3(Au/Fc@MgAl-LDH)*n* were immobilized successfully onto the electrode surface. When the antibody, BSA, and antigen were gradually immobilized on the modified surface, the electrochemical resistance gradually increased due to the rejection of the idioelectric proteins during electronic transfer. These increases demonstrated the successful modification of antibody, BSA, and antigen to the electrode surfaces. In addition, the CV measurements performed also showed the results that nanomaterials and biological samples were surface modified, consistent with the EIS results. Under optimal conditions, the developed immunosensor showed a linear concentration range between 0.01 U mL⁻¹ and 1000 U mL⁻¹ with a LOD of 0.004 U mL⁻¹. Furthermore, the developed immunosensor, which exhibited long-term stability, provided the utility of accurate detection of CA125 in clinical cancer diagnosis (Wu et al. 2022).

In 2021, a novel label-free electrochemical immunosensor to detect carcinoembryonic antigen (CEA) that serves as a tumor marker using reduced graphene oxide (rGO) was proposed by Jozghorbani et al. In this study, first GCE was rinsed with deionized water, and then rGO was dropped onto the GCE surface and dried at 60 °C. Then, the EDC/NHS solution containing 20 mg mL⁻¹ of EDC and 10 mg mL⁻¹ of NHS was coated on the rGO/GCE surface. 20 mg mL⁻¹ antibody was immobilized on the modified sensor, and this electrode was rinsed with PBS to remove non-covalently bound antibodies. BSA was dropped on the antibody-modified surface to block nonspecific sites, and the electrode surface was rinsed with PBS several times. After optimum parameters, pH, incubation time, and anti-CEA concentration were determined, increasing concentrations of CEA were measured using the developed sensor. The developed immunosensor exhibited a linear concentration-response in the range of 0.1–5 ng mL⁻¹ with a LOD of 0.05 ng mL⁻¹ for the detection of CEA. The proposed immunosensor was evaluated to detect CEA in the human blood serum samples. Moreover, the obtained results were compared with the standard enzyme-linked immunosorbent assay (ELISA) method (Jozghorbani et al. 2021).

In the case of the nanomaterials and nanocomposites, Au NPs (Carneiro et al. 2017), trimetallic NPs composed of palladium, platinum, and copper (Zhao et al. 2022), metal oxide NPs such as TiO₂ NPs (Shawky and El-Tohamy 2021), and also polymeric materials including polyethyleneimine and chitosan (Li et al. 2021; Shanbhag et al. 2021) supported suitable surfaces to achieve high performance of detection of diseases biomarkers. In particular, the conductivity and biocompatibility of these materials have provided advantages by creating excellent synergetic effects between materials.

In terms of human health, the need for compassionate and specific analysis methods to diagnose a biomarker or related diseases cannot be ignored. Nowadays, sensitive determination of particular analytes in complex samples can be enhanced by using sandwich or labeled methods. In these methods, the analyte binds to the primary antibodies (captured antibodies) followed by labeled secondary antibodies (detection antibodies) (Pei et al. 2013; Ilkhani et al. 2015). In 2020, Zhang et al. proposed a sandwich-type electrochemical immunosensor to detect the N-terminal B-type natriuretic peptide precursor (NT-proBNP) that serves as a diagnostic biomarker of heart failure. In this study, the GCE surface was polished and washed using alumina powder and ultrapure water. Then, Au NPs were electrodeposited onto the GCE using the CV method. Next, optimal amounts of Ab1, BSA, NT-proBNP, and Au@PdPt RTNs-Ab2 were immobilized on the modified electrode surface, respectively. Before each modification, the prepared electrodes were washed with PBS (pH 7.4). To investigate the developed immunosensors, EIS and CV methods were performed. As shown in Fig. 29.6a, when Au NPs were coated on the bare GCE, the diameter of the semicircle of Au NPs modified electrode decreased significantly compared to bare GCE (curves a and b). This decrease was due to the excellent conductivity of Au NPs. In addition, an increasing trend when the Au NPs modified electrode was modified with Ab1, BSA, NT-proBNP, and the Au@PdPt RTNs-Ab2 (curve c-f) layer by layer, respectively, was observed in the diameter of the semicircle. These increases were because the surface's biological materials significantly inhibited interfacial electron transfer efficiency. In Fig. 29.6b, cyclic voltammograms were presented; after the GCE was coated with Au NPs, the peak current of the redox solution increased significantly more. It was recorded that the peak current of the redox solution decreased when the modified surface was coated with Ab1, BSA, NT-proBNP, and Au@PdPt RTNs-Ab2, respectively. All the results observed in CV and EIS showed that each nanoparticle and biological material was influential in detecting NT-proBNP and successfully modified on the electrode

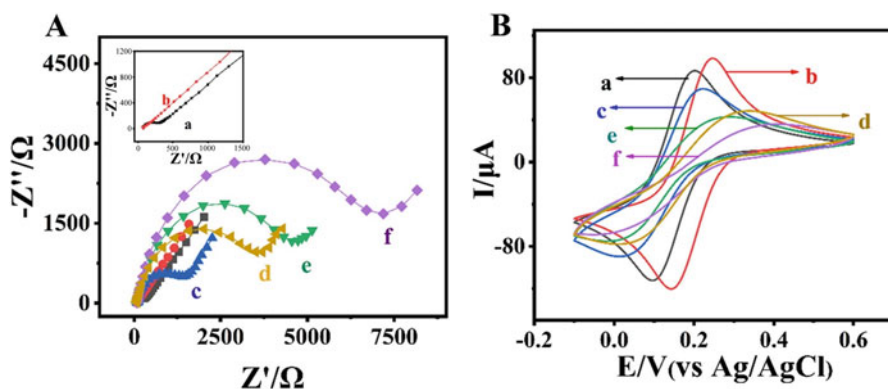


Fig. 29.6 (a) EIS and (b) CV of bare GCE (a), GCE/Au NPs (b), GCE/Au NPs/Ab1 (c), GCE/Au NPs/Ab1/BSA (d), GCE/Au NPs/Ab1/BSA/NT-proBNP (e), and GCE/Au NPs/Ab1/BSA/NT-proBNP/Au@PdPt RTNs-Ab2 (f). (Reprinted with permission from Zhang et al. (2022))

surfaces. Under optimized experimental conditions, the developed immunosensor showed a wide linear range from 0.1 pg mL^{-1} to 100 ng mL^{-1} and a LOD of 0.046 pg mL^{-1} . Moreover, the use of the developed immunosensor was confirmed by the detection of NT-proBNP in human serum samples (Zhang et al. 2022).

In their work, Yang et al. fabricated a sandwich-type electrochemical immunosensor for the effective detection of hepatitis B surface antigen (HBsAg) based on Au@Pd nanodendrites (NDs) functionalized MoO₂ nanosheets (NSs). Firstly, the GCE surface was polished and rinsed, and Ag NPs were coated on the GCE surface by electrodeposition. Subsequently, Ab1 dispersion was immobilized on the Ag NPs/GCE surface. After immobilization of BSA to the surface to block nonspecific binding sites, HBsAg was coated on this surface. Finally, an Ab2-label solution composed of Au@Pd NDs and amino-functionalized MoO₂ NSs was incubated on the modified surface. Au@Pd NDs/NH₂-MoO₂ NSs could catalyze hydrogen peroxide (H₂O₂) reduction effectively. Therefore, HBsAg analyzes were performed by following the current changes in the reduction of H₂O₂ at different concentrations. The current responses showed that NH₂-MoO₂ had a good catalytic capacity for HBsAg detection. Moreover, increased current responses confirmed that Au@Pd NDs/NH₂-MoO₂ NSs had the greater electrocatalytic performance. Under optimal conditions, the developed immunosensor offered a linear concentration range between 10 fg mL^{-1} and 100 ng mL^{-1} with a LOD of 3.3 fg mL^{-1} to detect of HBsAg. Moreover, the accuracy of the developed immunosensor was satisfactory, which confirmed that the immunosensor possessed an excellent application prospect in clinical samples (Yang et al. 2021).

Following the same trend observed for the nanomaterial-based enzyme, peptide, and label-free sensors, many researchers have reported that the modification of nanomaterials to construct labeled sensors has several advantages. As seen in the kinds of literature in these studies, nanoboxes (Cheng et al. 2021), nanorods (Zhang et al. 2020), metal-organic frameworks (Miao et al. 2019), flower-like NPs (Qian et al. 2019), and also three-dimensional composites (Liu et al. 2021) exhibited superior properties such as a large effective surface area and fast electron transport towards diseases and biomarkers detection.

Aptamers are synthetic strands of DNA or RNA produced by SELEX (systematic evolution of ligands by exponential enrichment). Aptamers can specifically attach to target molecules, cells, and proteins. In recent years, aptamers have provided several advantages such as tolerance to environmental conditions and more excellent stability, easier synthesis route, and more accessible storage than antibodies. Many researchers have suggested aptasensors to detect biomarkers due to the high sensitivity and specificity (Charbgoon et al. 2016; Zhou et al. 2016; Kim et al. 2020).

In their study, Negahdary et al. developed an electrochemical aptasensor to detect amyloid beta (A β) based on fern leaves-like Au nanostructure. The detection procedure is based on modifying the thiol-modified RNA aptamer sequence to the electrode surface by Au nanostructures and the binding between the specific aptamer sequence and A β . To detect A β by the aptasensor, after A β binding time was optimized, DP voltammograms were recorded by following redox peaks responses. After optimizing the experimental parameters, the developed aptasensor showed a

linear concentration range of 0.002–1.28 ng mL⁻¹ and a LOD of 0.4 pg mL⁻¹ (88.6 amol L⁻¹). Moreover, the design was successfully used for the determination of A β in human blood serum and artificial cerebrospinal fluid samples (Negahdary and Heli 2019).

In another study, Wang et al. designed a sandwich-type aptasensor based on hollow mesoporous carbon spheres loaded with porous dendritic bimetallic Pd@Pt nanoparticles (Pd@Pt DNPs) to detect cardiac troponin I (cTnI). In this work, a screen-printed gold electrode (SPGE) was coated with a thiol-modified aptamer. After washing with MCH, which was used to block non-specific binding sites, cTnI was incubated on the surface to obtain the aptamer-cTnI complex. Then, Pd@Pt DNPs/NH₂-HMCS/aptamer (with a different sequence) was dropped on the modified surface, and the developed aptasensor was washed for further use in the analysis. Electrochemical characterization results confirmed that the improvement of the dispersibility of Pt@Pd DNPs and the synergistic catalysis between NH₂-HMCS and Pt@Pd DNPs provide the more excellent electrocatalytic ability for the ultrasensitive detection of cTnI. To obtain the best performance of the developed aptasensor, the incubation time and concentration of the Pt@Pd DNPs/NH₂-HMCS/aptamer label were optimized. Under optimum working conditions, the proposed aptasensor exhibited a linear concentration range from 0.1 pg mL⁻¹ to 100.0 ng mL⁻¹ and a LOD of 15.4 fg mL⁻¹ for cTnI detection. Moreover, the aptasensor was successfully used for cTnI detection in human serum samples (Wang et al. 2021).

Aptamer sequences designed specifically for different molecules such as proteins, biomarkers, and pesticides can also be designed for bacteria. In 2020, a novel aptasensor based on quantum dots and metal oxide NPs was developed by Ghalkhani et al., for the sensitive detection of *Staphylococcus aureus* bacterium (*S. aureus*) that can generate several human infections such as pseudomembranous enteritis, respiratory, and some systemic diseases. In this work, firstly, a nanocomposite composed of silver (Ag) NPs chitosan (Cs), graphene quantum dots (Gr QDs), and nitrogen-doped TiO₂ NPs (NTiO₂) was prepared, and then, SPCE surface was modified by this nanocomposite. After the thiol functionalized DNA aptamer was immobilized on the modified electrode, *S. aureus* was immobilized on the aptasensor surface. The experimental conditions were optimized by the pH of the nanocomposite solution and incubation time of bacterium species. According to the obtained DPV responses, the designed aptasensor displayed the linear concentration range of 10–5 $\times 10^8$ CFU mL⁻¹ with a LOD of 3.3 CFU mL⁻¹. Furthermore, the developed aptasensor was also suitable for human serum samples containing *S. aureus* (Ghalkhani et al. 2022).

In general, the potential for some molecules to bind to aptamers from two different binding sites has been reported; therefore, most sandwich-type sensors have focused on conventionally used antibody-based immunoassays or assay methods using aptamer-antibody pairs to increase sensitivity and selectivity (Huang et al. 2021b; Centane and Nyokong 2022). In their study, Chung et al. designed a magnetic force-assisted sandwich-type sensor for the sensitive detection of thrombin. This study is based on biotinylated thrombin Ab, aptamer with functionalized conducting polymer (poly-(2,2':5',5''-terthiophene-3'-p-benzoic acid) (pTBA)), and streptavidin-starch modified magnetic nanoparticle (MNP)

combined with toluidine blue O (TBO). The characterization of probe molecules and modified sensor surface was investigated by CV, EIS, X-ray photoelectron spectroscopy (XPS), and UV–VIS spectroscopy. All results indicated that the prepared probe molecules and modified sensors were suitable materials and platforms to detect thrombin. To obtain the best sensor response, the effect of pH, binding times of thrombin and MNP@Ab-TBO, removal time of unbound bioconjugates, and applied potential were investigated. After the optimum conditions were provided, the linear concentration range was found between 1.0 and 500 nM with a LOD of 0.49 nM. Moreover, the recovery experiment results demonstrated that the proposed sensor could detect thrombin in human serum (Chung et al. 2018).

To sum up, when designing the aptamer-based sensors, studies primarily provided quick analysis, higher sensitivity, selectivity, and cost-effectiveness in recent years to detect diseases and biomarkers (Zhang et al. 2019; Erkmen et al. 2022; Farahani et al. 2022; Sun et al. 2022).

3 Conclusion

Integrating nanotechnology into sensor systems and using it in this field offers the chance to improve the performance of nanobiosensors, which are used as sensitive methods, especially in the early diagnosis of diseases. As the most crucial component of nanobiosensors, different nanomaterials such as metallic nanoparticles, carbon-based nanomaterials, polymeric nanostructures, magnetic nanomaterials, quantum dots, nanowire, or nanomembrane structures provide advanced superiority by being integrated into systems for biosensing. These materials facilitate the immobilization of biological materials added to the surfaces due to their large surface-to-volume ratios while at the same time facilitating electron transfer thanks to their conductors; they provide high sensitivity and allow them to be excellent candidates for the nanobiosensors designs. In addition, the affinity between a bioreceptor molecule such as enzyme, peptide, antibody, and aptamer and target analytes such as protein, biomarker, or gene undoubtedly provides extraordinary sensitivity, high specificity, and selectivity. Compared to traditional methods such as chromatographic and spectroscopic methods, which require a time-consuming, large amount of organic solvents, and the use of toxic chemicals, nanobiosensors are quick, sensitive, and selective analytical tools for the diagnosis of several diseases. The different features of nanobiosensors such as accuracy, reproducibility, dynamic capacity change, and sensitivity to environmental changes such as pressure, pH, and temperature make them excellent analytical tools for diagnosing diseases. However, more awareness is needed regarding the advancement of nanobiosensors in commercial applications. In the future, nanobiosensors can be integrated into smart devices and remotely controlled systems with various techniques. Multipurpose use, such as the simultaneous detection of different biomarkers, can be improved with cost-effectively designed biochips. Furthermore, in these developments, self-propelled sensors such as micromotors or microconsoles can contribute to the applications of nanobiosensors.

References

- Abu-Salah KM, Ansari AA, Alrokayan SA (2010) DNA-based applications in nanobiotechnology. *J Biomed Biotechnol* 2010:715295
- Abu-Salah KM, Zourob MM, Mouffouk F, Alrokayan SA, Alaamery MA, Ansari AA (2015) DNA-based nanobiosensors as an emerging platform for detection of disease. *Sensors (Switzerland)* 15(6):14539–14568
- Asadian E, Ghalkhani M, Shahrokhian S (2019) Electrochemical sensing based on carbon nanoparticles: a review. *Sensors Actuators B Chem* 293(April):183–209
- Balasubramanian K, Burghard M (2006) Biosensors based on carbon nanotubes. *Anal Bioanal Chem* 385(3):452–468
- Barbosa AJM, Oliveira AR, Roque ACA (2018) Protein- and peptide-based biosensors in artificial olfaction. *Trends Biotechnol* 36(12):1244–1258
- Bozal-Palabiyik B, Kurbanoglu S, Erkmen C, Uslu B (2021) Future prospects and concluding remarks for electroanalytical applications of quantum dots. In: *Electroanalytical applications of quantum dot-based biosensors*. Elsevier Inc, pp 427–450
- Carneiro P, Loureiro J, Delerue-Matos C, Morais S, do Carmo Pereira M (2017) Alzheimer's disease: development of a sensitive label-free electrochemical immunosensor for detection of amyloid beta peptide. *Sensors Actuators B Chem* 239:157–165
- Centane S, Nyokong T (2022) Aptamer versus antibody as probes for the impedimetric biosensor for human epidermal growth factor receptor. *J Inorg Biochem* 230:111764
- Chamorro-Garcia A, Merkoçi A (2016) Nanobiosensors in diagnostics. *Nanobiomedicine* 3:1–26
- Chandra P (2013) HER2 protein biomarker based sensor systems for breast cancer diagnosis. *J Mol Biomark Diagn* 5(1):1000e119
- Charbgoos F, Soltani F, Taghdisi SM, Abnous K, Ramezani M (2016) Nanoparticles application in high sensitive aptasensor design. *TrAC – Trends Anal Chem* 85:85–97
- Chen M, Song Z, Han R, Li Y, Luo X (2021) Low fouling electrochemical biosensors based on designed Y-shaped peptides with antifouling and recognizing branches for the detection of IgG in human serum. *Biosens Bioelectron* 178:113016
- Cheng Q, Feng J, Wu T, Guo Y, Sun X, Ren X, Lee JY, Liu L, Wei Q (2021) Hollow performances quenching label of Au NPs@CoSnO₃ nanoboxes-based sandwich photoelectrochemical immunosensor for sensitive CYFRA 21-1 detection. *Talanta* 233:122552
- Chung S, Moon JM, Choi J, Hwang H, Shim YB (2018) Magnetic force assisted electrochemical sensor for the detection of thrombin with aptamer-antibody sandwich formation. *Biosens Bioelectron* 117:480–486
- Das P, Das M, Chinnadayala SR, Singha IM, Goswami P (2016) Recent advances on developing 3rd generation enzyme electrode for biosensor applications. *Biosens Bioelectron* 79:386–397
- Dutta D, Das BM (2021) Scope of green nanotechnology towards amalgamation of green chemistry for cleaner environment: a review on synthesis and applications of green nanoparticles. *Environ Nanotechnol Monit Manag* 15:100418
- Eom KS, Lee YJ, Seo HW, Kang JY, Shim JS, Lee SH (2020) Sensitive and non-invasive cholesterol determination in saliva: via optimization of enzyme loading and platinum nanocluster composition. *Analyst* 145:908–916
- Erkmen C, Tiğ GA, Uslu B (2022) First label-free impedimetric aptasensor based on Au NPs/TiO₂ NPs for the determination of leptin. *Sensors Actuators B Chem* 358:131420
- Farahani FA, Alipour E, Mohammadi R, Amini-Fazl MS, Abnous K (2022) Development of novel aptasensor for ultra-sensitive detection of myoglobin via electrochemical signal amplification of methylene blue using poly (styrene)-block-poly (acrylic acid) amphiphilic copolymer. *Talanta* 237:122950
- Fatima T, Bansal S, Husain S, Khanuja M, Islamia JM, Delhi N (1986) Biosensors. *IEEE Trans Biomed Eng* 33(2):77–268
- Felix FS, Angnes L (2018) Electrochemical immunosensors – a powerful tool for analytical applications. *Biosens Bioelectron* 102:470–478

- Fracchiolla NS, Artuso S, Cortelezzi A (2013) Biosensors in clinical practice: focus on oncohematology. *Sensors (Switzerland)* 13(5):6423–6447
- Gajdosova V, Lorencova L, Kasak P, Tkac J (2020) Electrochemical nanobiosensors for detection of breast cancer biomarkers. *Sensors (Switzerland)* 20(14):1–37
- Ghalkhani M, Sohoul E, Khaloo SS, Vaziri MH (2022) Architecting of an aptasensor for the staphylococcus aureus analysis by modification of the screen-printed carbon electrode with aptamer/Ag–Cs–Gr QDs/NTiO₂. *Chemosphere* 293:133597
- Huang X, Zhu Y, Kianfar E (2021a) Nano biosensors: properties, applications and electrochemical techniques. *J Mater Res Technol* 12:1649–1672
- Huang Z, Chen H, Ye H, Chen Z, Jaffrezic-Renault N, Guo Z (2021b) An ultrasensitive aptamer-antibody sandwich cortisol sensor for the noninvasive monitoring of stress state. *Biosens Bioelectron* 190:113451
- Hui N, Wang J, Wang D, Wang P, Luo X, Lv S (2022) An ultrasensitive biosensor for prostate specific antigen detection in complex serum based on functional signal amplifier and designed peptides with both antifouling and recognizing capabilities. *Biosens Bioelectron* 200:113921
- Hwang HJ, Ryu MY, Park CY, Ahn J, Park HG, Choi C, Do HS, Park TJ, Park JP (2017) High sensitive and selective electrochemical biosensor: label-free detection of human norovirus using affinity peptide as molecular binder. *Biosens Bioelectron* 87:164–170
- Ilkhani H, Sarparast M, Noori A, Bathaie SZ, Mousavi MF (2015) Electrochemical aptamer/antibody based sandwich immunosensor for the detection of EGFR, a cancer biomarker, using gold nanoparticles as a signaling probe. *Biosens Bioelectron* 74:491–497
- Jędrzak A, Kuznowicz M, Rębiś T, Jesionowski T (2022) Portable glucose biosensor based on polynorepinephrine@magnetite nanomaterial integrated with a smartphone analyzer for point-of-care application. *Bioelectrochemistry* 145:108071
- Jozghorbani M, Fathi M, Kazemi SH, Alinejadian N (2021) Determination of carcinoembryonic antigen as a tumor marker using a novel graphene-based label-free electrochemical immunosensor. *Anal Biochem* 613:114017
- Kangkamano T, Numnuam A, Limbut W, Kanatharana P, Vilaivan T, Thavarungkul P (2018) Pyrrolidiny PNA polypyrrole/silver nanofoam electrode as a novel label-free electrochemical miRNA-21 biosensor. *Biosens Bioelectron* 102:217–225
- Karimzadeh A, Hasanzadeh M, Shadjou N, de la Guardia M (2018) Peptide based biosensors. *TrAC – Trends Anal Chem* 107:1–20
- Karunakaran C, Pandiaraj M, Santharaman P (2015) Immunosenors. In: *Biosensors and bioelectronics*. Elsevier Inc, pp 205–245
- Kausaite-Minkstimiene A, Glumbokaite L, Ramanaviciene A, Ramanavicius A (2020) Reagentless amperometric glucose biosensor based on nanobiocomposite consisting of poly(1,10-phenanthroline-5,6-dione), poly(pyrrole-2-carboxylic acid), gold nanoparticles and glucose oxidase. *Microchem J* 154:104665
- Kim SM, Kim J, Noh S, Sohn H, Lee T (2020) Recent development of aptasensor for influenza virus detection. *Biochip J* 14(4):327–339
- Krzyszczonik P, Bozal-Palabiyik B, Skrzypek S, Uslu B (2021) Quantum dots-based sensors using solid electrodes. In: *Electroanalytical applications of quantum dot-based biosensors*. Elsevier Inc, pp 81–120
- Kurbanoglu S, Erkmen C, Uslu B (2020) Frontiers in electrochemical enzyme based biosensors for food and drug analysis. *TrAC – Trends Anal Chem* 124:115809
- Laraib U, Sargazi S, Rahdar A, Khatami M, Pandey S (2022) Nanotechnology-based approaches for effective detection of tumor markers: a comprehensive state-of-the-art review. *Int J Biol Macromol* 195:356–383
- Li R, Huang H, Huang L, Lin Z, Guo L, Qiu B, Chen G (2013) Electrochemical biosensor for epidermal growth factor receptor detection with peptide ligand. *Electrochim Acta* 109:233–237
- Li X, Lin LY, Wang KY, Li J, Feng L, Song L, Liu X, He JH, Sakthivel R, Chung RJ (2021) Streptavidin-functionalized-polyethyleneimine/chitosan/HfO₂-Pr₆O₁₁ nanocomposite using

- label-free electrochemical immunosensor for detecting the hunger hormone ghrelin. *Compos Part B Eng* 224:109231
- Lin Y, Shen R, Liu N, Yi H, Dai H, Lin J (2018) A highly sensitive peptide-based biosensor using NiCo₂O₄ nanosheets and g-C₃N₄ nanocomposite to construct amplified strategy for trypsin detection. *Anal Chim Acta* 1035:175–183
- Liu Q, Wang J, Boyd BJ (2015) Peptide-based biosensors. *Talanta* 136:114–127
- Liu Y, Si S, Dong S, Ji B, Li H, Liu S (2021) Ultrasensitive electrochemical immunosensor for ProGRP detection based on 3D-rGO@Au nanocomposite. *Microchem J* 170:106644
- Mahapatra S, Baranwal A, Purohit B, Roy S, Mahto SK, Chandra P (2020) Advanced biosensing methodologies for ultrasensitive detection of human coronaviruses. In: *Diagnostic strategies for COVID-19 and other coronaviruses*. Springer, pp 19–36
- Mahato K, Kumar S, Srivastava A, Maurya PK, Singh R, Chandra P (2018a) Electrochemical immunosensors: fundamentals and applications in clinical diagnostics. In: *Handbook of immunoassay technologies: approaches, performances, and applications*. Academic Press, pp 359–414
- Mahato K, Maurya PK, Chandra P (2018b) Fundamentals and commercial aspects of nanobiosensors in point-of-care clinical diagnostics. *Biotech* 8(3):1–14
- Malik P, Katyal V, Malik V, Asatkar A, Inwati G, Mukherjee TK (2013) Nanobiosensors: concepts and variations. *ISRN Nanomater* 2013:1–9
- Miao J, Li X, Li Y, Dong X, Zhao G, Fang J, Wei Q, Cao W (2019) Dual-signal sandwich electrochemical immunosensor for amyloid β -protein detection based on Cu–Al₂O₃-g-C₃N₄-Pd and UiO-66@PANI-MB. *Anal Chim Acta* 1089:48–55
- Mishra RK, Rajakumari R (2018) Nanobiosensors for biomedical application: present and future prospects. Present and future prospects. In: *Characterization and biology of nanomaterials for drug delivery*. Elsevier Inc, pp 1–23
- Nazare A, Pal K, Maji S (2021) 14. Electrochemical biosensors. In: *Food, medical, and environmental applications of polysaccharides*. Elsevier Inc, pp 403–441
- Negahdary M, Heli H (2019) An ultrasensitive electrochemical aptasensor for early diagnosis of Alzheimer's disease, using a fern leaves-like gold nanostructure. *Talanta* 198:510–517
- Nosrati R, Dehghani S, Karimi B, Yousefi M, Taghdisi SM, Abnous K, Alibolandi M, Ramezani M (2018) Siderophore-based biosensors and nanosensors; new approach on the development of diagnostic systems. *Biosens Bioelectron* 117:1–14
- Oliveira Brett AM (2005) Chapter 4 DNA-based biosensors. *Compr Anal Chem* 44:179–208
- Pareek S, Jain U, Bharadwaj M, Chauhan N (2021) Sensing and bio-sensing research A label free nanosensing platform for the detection of cervical cancer through analysis of ultratrace DNA hybridization. *Sens Bio-Sensing Res* 33:100444
- Park CS, Lee C, Kwon OS (2016) Conducting polymer based nanobiosensors. *Polymers (Basel)* 8(7):1–18
- Parolo C, Merkoçi A (2013) Paper-based nanobiosensors for diagnostics. *Chem Soc Rev* 42(2):450–457
- Pei X, Zhang B, Tang J, Liu B, Lai W, Tang D (2013) Sandwich-type immunosensors and immunoassays exploiting nanostructure labels: a review. *Anal Chim Acta* 758:1–18
- Pérez DJ, Patiño EB, Orozco J (2021) Electrochemical nanobiosensors as point-of-care testing solution to cytokines measurement limitations. *Electroanalysis* 34(2):184–211
- Qian Y, Feng J, Fan D, Zhang Y, Kuang X, Wang H, Wei Q, Ju H (2019) A sandwich-type photoelectrochemical immunosensor for NT-pro BNP detection based on F-Bi₂WO₆/Ag₂S and GO/PDA for signal amplification. *Biosens Bioelectron* 131:299–306
- Rahimi-Mohseni M, Raouf JB, Aghajanzadeh TA, Ojani R (2021) Phenylketonuria monitoring in human blood serum by mosses extract/ZnO@Au nanoarrays-loaded filter paper as a novel electrochemical biosensor. *Microchem J* 160:105739
- Raj MA, John SA (2018) Graphene-modified electrochemical sensors. In: *Graphene-based electrochemical sensors for biomolecules*. Elsevier Inc, pp 1–41

- Saleh TA, Fadillah G, Saputra OA (2019) Nanoparticles as components of electrochemical sensing platforms for the detection of petroleum pollutants: a review. *TrAC – Trends Anal Chem* 118: 194–206
- Sekhar PK, Wignes F (2016) Trace detection of research department explosive (RDX) using electrochemical gas sensor. *Sensors Actuators B Chem* 227:185–190
- Sellappan L, Manoharan S, Sanmugam A, Anh NT (2022) Role of nanobiosensors and biosensors for plant virus detection. In: *Nanosensors for smart agriculture*. Elsevier Inc, pp 493–506
- Shanbhag MM, Ilager D, Mahapatra S, Shetti NP, Chandra P (2021) Amberlite XAD-4 based electrochemical sensor for diclofenac detection in urine and commercial tablets. *Mater Chem Phys* 273:125044
- Sharifi M, Hasan A, Attar F, Taghizadeh A, Falahati M (2020) Development of point-of-care nanobiosensors for breast cancers diagnosis. *Talanta* 217:121091
- Shawky AM, El-Tohamy M (2021) Signal amplification strategy of label-free ultrasensitive electrochemical immunosensor based ternary Ag/TiO₂/rGO nanocomposites for detecting breast cancer biomarker CA 15-3. *Mater Chem Phys* 272:124983
- Shen Y, Zhang Y, Gao ZF, Ye Y, Wu Q, Chen HY, Xu JJ (2021) Recent advances in nanotechnology for simultaneous detection of multiple pathogenic bacteria. *Nano Today* 38:101121
- Simões FR, Xavier MG (2017) 6 – electrochemical sensors. In: *Nanoscience and its applications*. Elsevier Inc., pp 155–178
- Soni A, Jha SK (2017) Smartphone based non-invasive salivary glucose biosensor. *Anal Chim Acta* 996:54–63
- Srivastava AK, Dev A, Karmakar S (2018) Nanosensors and nanobiosensors in food and agriculture. *Environ Chem Lett* 16(1):161–182
- Sun J, Wang G, Cheng H, Han Y, Li Q, Jiang C (2022) An antifouling electrochemical aptasensor based on hyaluronic acid functionalized polydopamine for thrombin detection in human serum. *Bioelectrochemistry* 145:108073
- Tahir MA, Rafiq A, Dina NE, Amin I, Mansoor S, Zhang L, Mujahid A, Bajwa SZ (2022) Methods for design and fabrication of nanosensors. In: *Nanosensors for smart agriculture*. Elsevier Inc, pp 53–79
- Thakur PS, Sankar M (2022) Nanobiosensors for biomedical, environmental, and food monitoring applications. *Mater Lett* 311:131540
- Vaz R, Frasco MF, Sales MGF (2022) Biosensors: concept and importance in point-of-care disease diagnosis. In: *Biosensor based advanced cancer diagnostics*. Academic Press, pp 59–84
- Wang Z, Zhao H, Chen K, Li H, Lan M (2021) Sandwich-type electrochemical aptasensor based on hollow mesoporous carbon spheres loaded with porous dendritic Pd@Pt nanoparticles as signal amplifier for ultrasensitive detection of cardiac troponin I. *Anal Chim Acta* 1188:339202
- Wu M, Liu S, Qi F, Qiu R, Feng J, Ren X, Rong S, Ma H, Chang D, Pan H (2022) A label-free electrochemical immunosensor for CA125 detection based on CMK-3(Au/Fc@MgAl-LDH)_n multilayer nanocomposites modification. *Talanta* 241:123254
- Xia N, Wang X, Yu J, Wu Y, Cheng S, Xing Y, Liu L (2017) Design of electrochemical biosensors with peptide probes as the receptors of targets and the inducers of gold nanoparticles assembly on electrode surface. *Sensors Actuators B Chem* 239:834–840
- Xu D, Huang X, Guo J, Ma X (2018) Automatic smartphone-based microfluidic biosensor system at the point of care. *Biosens Bioelectron* 110:78–88
- Yang H (2012) Enzyme-based ultrasensitive electrochemical biosensors. *Curr Opin Chem Biol* 16(3–4):422–428
- Yang Q, Wang P, Ma E, Yu H, Zhou K, Tang C, Ren J, Li Y, Liu Q, Dong Y (2021) A sandwich-type electrochemical immunosensor based on Au@Pd nanodendrite functionalized MoO₂ nanosheet for highly sensitive detection of HBsAg. *Bioelectrochemistry* 138:107713
- Yonzon CR, Stuart DA, Zhang X, McFarland AD, Haynes CL, Van Duyne RP (2005) Towards advanced chemical and biological nanosensors – an overview. *Talanta* 67(3):438–448
- Younis S, Zia R, Tahir N, Bukhari SZ, Khan WS, Bajwa SZ (2022) Nanosensors for animal health monitoring. In: *Nanosensors for smart agriculture*. Elsevier Inc, pp 509–529

- Zhang Y, Figueroa-Miranda G, Lyu Z, Zafu C, Willbold D, Offenhäusser A, Mayer D (2019) Monitoring amyloid-B proteins aggregation based on label-free aptasensor. *Sensors Actuators B Chem* 288:535–542
- Zhang Y, Zhang Z, Rong S, Yu H, Gao H, Sha Q, Ding P, Pan H, Chang D (2020) A sandwich-type ECL immunosensor based on signal amplification using a ZnO nanorods-L-cysteine-luminol nanocomposite for ultrasensitive detection of prostate specific antigen. *Anal Chim Acta* 1109: 98–106
- Zhang ZY, Huang LX, Xu ZW, Wang P, Lei Y, Liu AL (2021) Efficient determination of pml/rara fusion gene by the electrochemical dna biosensor based on carbon dots/graphene oxide nanocomposites. *Int J Nanomedicine* 16:3497–3508
- Zhang B, Li F, Han F, Yang H, Jiang C, Tan S, Tu J, Qiao B, Wang X, Wu Q (2022) Bioelectrochemistry A sandwich-type electrochemical immunosensor using trimetallic nanozyme as signal amplification for NT-proBNP sensitive detection. *Bioelectrochemistry* 145:108075
- Zhao H, Cao L, Liu Q, Tang F, Chen L, Wang S, Li Y, Li Y, Li B, Liu H (2022) Label-free electrochemical immunosensor based on PdCuPt/PPY/DCSC as a signal amplification platform for sensitive detection of cardiac troponin I. *Sensors Actuators B Chem* 351:1–7
- Zhou Q, Rahimian A, Son K, Shin DS, Patel T, Revzin A (2016) Development of an aptasensor for electrochemical detection of exosomes. *Methods* 97:88–93