

Chapter 7

Micro/Nano Material-Based Biosensors

Xianxin Qiu, Jie Zhou, and Ping Wang

Abstract Novel nanomaterial for use in bioassay applications represents a rapid advancing field. Various kinds of nanomaterial have been investigated to determine their properties and possible applications in biosensor. The nanomaterial's ultrafine grain, high-concentration grain boundary and the adjacent condition of interfacial atoms determine their specific performance which was obviously different from amorphous, normal polycrystal and monocrystal. The common properties of micro/nano material contain volume effect, also known as small size effect, surface effect, quantum size effect, macroscopic quantum tunnel effect, and dielectric confinement effect. Besides, different nanomaterials also have their unique characteristics. Because of these extraordinary effects and properties, nanomaterial shows incredible macroscopic physical properties, which lay a broad prospect for its application. Biosensor is a kind of special sensor which consists of biomolecule recognition element and all kinds of physical or chemical transduces. They are often applied to analyze and detect living and chemical matter with high specificity and accuracy and low cost. Biosensors including electrochemical biosensors, optical biosensor, piezoelectric sensor, and FET-biosensor have got very great developments with the use of micro/nano material. The structures, properties, and applications in biosensors of several main groups, including metal nanomaterial, carbon nanomaterial, semiconductor material, magnetic nanomaterial, etc., are studied in this chapter.

Keywords Nanomaterial • Biosensor • Application

X. Qiu • J. Zhou • P. Wang (✉)
Biosensor National Special Laboratory, Department of Biomedical Engineering,
Zhejiang University, Hangzhou, China
e-mail: cnpwang@zju.edu.cn

7.1 Introduction

The history of biosensors started in 1967 with the development of enzyme electrodes by scientists Updike and Hicks [1]. Since then, research communities from various fields such as very large-scale integration (VLSI), physics, chemistry, and material science have come together to develop more sophisticated, reliable, and mature biosensing devices. Applications for these devices are in the fields of medicine, agriculture, biotechnology, as well as the military and bioterrorism detection and prevention [2].

Biosensors are known as immunosensors, optrodes, chemical canaries, resonant mirrors, glucometers, biochips, and biocomputers. Two commonly cited definitions by S.P.J. Higson [3] and D.M. Frazer [4], respectively, are “a biosensor is a chemical sensing device in which a biologically derived recognition entity is coupled to a transducer, to allow the quantitative development of some complex biochemical parameter,” and “a biosensor is an analytical device incorporating a deliberate and intimate combination of a specific biological element (that creates a recognition event) and a physical element (that transduces the recognition event).”

Nanotechnology involves the study, manipulation, creation, and use of materials, devices, and systems typically with dimensions smaller than 100 nm. Nanotechnology is playing an increasingly important role in the development of biosensors [5, 6]. Sensitivity and other attributes of biosensors can be improved by using nanomaterials because of effects such as the quantum size effect, mini size effect, surface effect, and macro-quantum tunnel effect.

Because of their submicron size, nanosensors, nanoprobes, and other nanosystems are revolutionizing the fields of chemical and biological analysis, to enable rapid analysis of multiple substances *in vivo* and *in vitro*. As shown in Fig. 7.1, nanoparticles can be modularly assembled from different materials composition with different physical and chemical properties and functionalized with a myriad of ligands for biological targeting [7]. In this chapter, we mainly discuss the structures and properties of nanomaterial and its application in biosensor design.

Nanoparticles can be modularly assembled from different materials composition with different physical and chemical properties and functionalized with a myriad of ligands for biological targeting [7].

7.2 Structures and Properties of Nanomaterial

Novel nanomaterial for use in bioassay applications represents a rapid advancing field. Various kinds of nanomaterial have been investigated to determine their properties and possible applications in biosensor. According to different bulk material, nanomaterial can be classified into several main groups, including metal nanomaterial, carbon nanomaterial, semiconductor material, magnetic nanomaterial, etc. The structures and properties of these nanomaterials are discussed in this section.

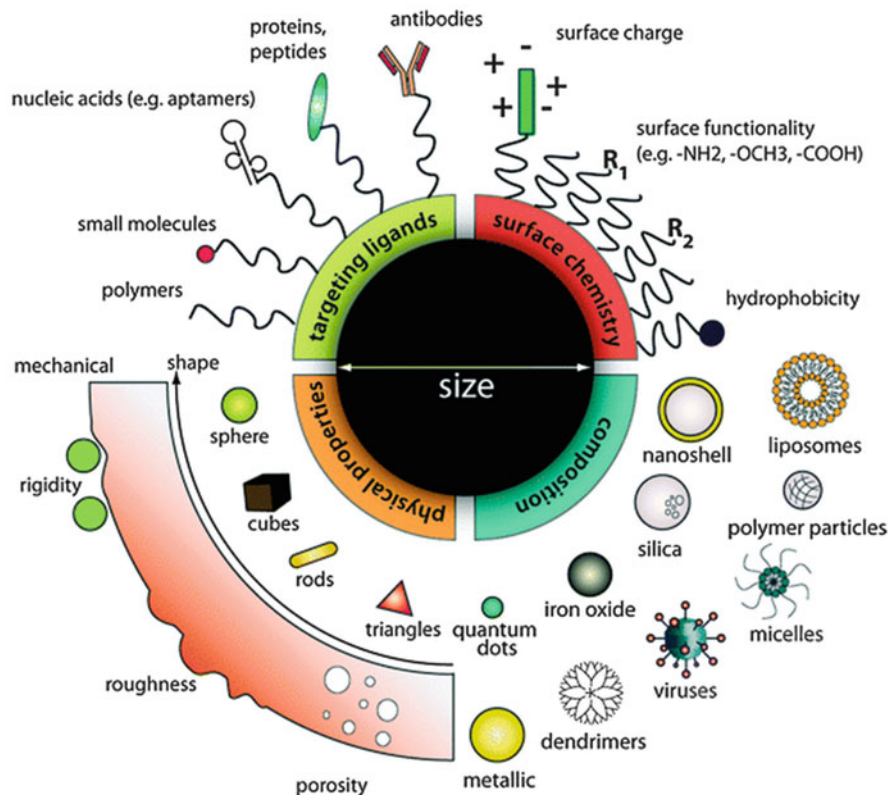


Fig. 7.1 Designing nanoparticles for bioapplications (Reproduced with permission from [7]. Copyright 2010 Royal Society of Chemistry)

7.2.1 Basic Properties of Nanomaterial

The nanomaterial's ultrafine grain, high-concentration grain boundary and the adjacent condition of interfacial atoms determine their specific performance which was obviously different from amorphous, normal polycrystal and monocrystal. We firstly introduce common properties of nanomaterial:

1. Volume effect, also known as small size effect. When the size of nanoparticles is close to or even smaller than the wavelength of conduction electrons and the coherent wavelength of superconducting state, the periodic boundary condition will be destroyed. And the melting point, magnetic properties, optical absorption, heat resistance, chemical activity, and catalytic properties would change significantly.
2. Surface effect. Surface effect refers to the variation of properties induced by the rapid increased ratio of surface atomic number and the total atom number along with the decreasing particle size.

3. Quantum-size effect. When the size of particle decreased to a certain value, the electronic energy levels near Fermi level changed from quasi-continuous energy level to discrete energy level, which makes nanomaterial owning a series of particular properties, such as specific catalysis, strong oxidizability, and reducibility.
4. Macroscopic quantum tunnel effect. It refers to the ability of microscopic particles through the barrier. Magnetic nanoparticles have tunnel effect. So they can pass through the barrier of macroscopic system.
5. Dielectric confinement effect. The dielectric confined effects of nanoparticles have important impacts on the light absorption, photochemical and optical nonlinearity properties of nanoparticles.

Besides, nanomaterial also has other characteristics. Because of these extraordinary effects and properties, nanomaterial shows incredible macroscopic physical properties, which lay a broad prospect for its application.

7.2.2 Metal Nanoparticles

Among all the metal nanomaterials, the precious metal nanomaterial is most widely used. Next we'll primarily introduce the properties of precious metal nanomaterial.

Precious metal nanomaterial refers to material containing precious metal (gold, silver, platinum, palladium, and other expensive elements) and in the size of 0.1–100 nm developed by nanotechnology [8]. Precious metal material itself has unique physical and chemical properties. When the size of the precious metal material is in a state of mesoscopic, its macroscopic properties will change greatly. When the size of precious metal material decreased to nanoscale scope, it not only has all the properties of bulk precious metal but also presents the novel properties of nanomaterial [9]. Thus precious metal nanomaterial has broad application prospect in the field of chemical catalysis, biological medicine, energy and environment, and electronic technology and attracts more and more attention of researchers [10, 11].

7.2.2.1 The Properties of Precious Metal Nanomaterial

Optical Properties

Precious metal nanoparticles have strong light absorption when the size decreased to a certain degree. The smaller the size is, the darker the color is. Besides, compared with bulk material, the light absorption peak of precious metal nanomaterial showed obvious spectral migration properties. It would shift to short-wave direction or long-wave direction that is blue shift or red shift [12]. The quantum confinement effect of precious metal nanoparticles would cause significant quantum energy-level splitting of nanoparticles and the

broadening of bandgap between the conduction band and valence band, inducing the blue shift of spectrum; the interface or surface effect of precious metal nanoparticles leads to increased surface tension of nanoparticles, which further prompts the narrowing of bandgap and causes the red shift of spectrum [13, 14]. The spectral migration characteristics of metal nanomaterial provide solid theoretical support for designing novel nano-optical sensor and colorimetric probe [15–17].

Surface Plasmon Resonance Properties

Under the light exposure, free electrons on metal surface are undergoing electromagnetic disturbance, resulting in the oscillation of partial charges and the formation of plasma [18]. When the frequency of applied electromagnetic wave equals the oscillation frequency of the plasma inside the metal, the surface plasmon resonance (SPR) occurs [19–21].

Noble metal nanoparticles are different from precious bulk metal, for the surface oscillation of electron cannot form electromagnetic wave which oscillated along the surface, but forms electromagnetic wave which oscillated confined on the surface of nanoparticles that is localized surface plasmon resonance (LSPR) [18]. The surface functionalization and modification could be achieved by Au-S bond or electrostatic adsorption, which is the key to its analytical applications. Figures 7.2 and 7.3 show the characteristics of precious metal nanomaterial, respectively, including easy modification and color-gradient characteristics.

Thermal Properties

The melting point of large-sized solid matter is fixed. After it being ultra-micronized, the melting point was significantly decreased, especially when the

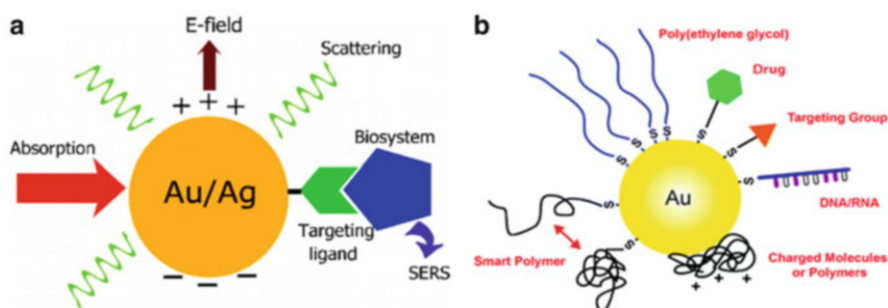


Fig. 7.2 (a) The optical properties of noble metal nanomaterial (Reproduced with permission from [10]. Copyright 2008, American Chemical Society); (b) NMNMs can be conjugated with a wide variety of functional moieties, both through the gold-thiolate bond and by passive adsorption (Reproduced with permission from [22]. Copyright 2010 Royal Society of Chemistry)

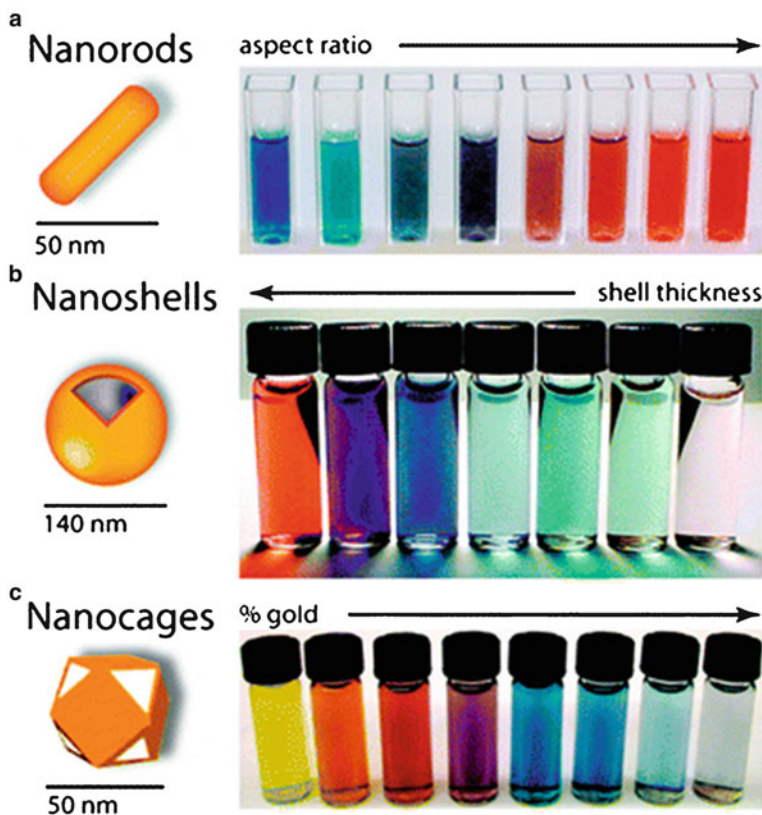


Fig. 7.3 Biomedical applications of gold nanoparticles. (a) Gold nanorods, (b) nanoshells, and (c) gold nanocages. The intense color arises from the collective excitation of their conduction electrons, or surface plasmon resonance modes, because of photon absorption at wavelengths which varies with (a) aspect ratio, (b) shell thickness, and/or (c) galvanic displacement by gold (Reproduced with permission from [23]. Copyright 2011 Royal Society of Chemistry)

particle size is less than 10 nm magnitude. The conductive material made from superfine silver powder can be sintered under low temperature, which could not only save raw material but also produce high-quality membrane with uniform thickness and large coverage [24].

Catalytic Properties

The catalytic activity and selectivity of precious metal nanomaterial can be controlled by regulating its morphology, element composition, and surface modification. The crystal face has important influence on the catalytic activity and selectivity of nanoparticles [24].

7.2.3 Carbon Nanomaterial

Carbon nanomaterial refers to carbon material which at least one dimension is smaller than 100 nm. Novel carbon nanomaterial in different structure and morphology has been successively synthesized, such as zero-dimensional fullerenes, one-dimensional carbon nanotubes, two-dimensional graphene, etc.

7.2.3.1 Fullerene

Fullerene is a novel type of all-carbon molecule. And it is the third allotrope of carbon and famous for its unique cage structure, in which one of most prominent representatives is C_{60} . C_{60} is spherical in shape. It could be expressed as that each carbon atom forms two single bonds and one double bond with adjacent three carbon atoms [25]. Because of the structural characteristics of C_{60} molecule, it presents many special physical and chemical properties such as anti-pressure capacity, the strong intramolecular force, weak molecular pressure, and low surface energy, which make it act as good solid lubricant in the tribology. All π electrons of C_{60} tightly bind together and produce the negative inductive effect. So it has strong electrophilic ability and can be used as electron acceptor. Some kinds of metal atom could be embedded in C_{60} molecule to form the intercalation compound. In 1991, Hebard first reported the superconducting temperature of potassium-doped C_{60} : $T_c = 18$ K, exceeding the highest organic superconductivity temperature ($T_c = 12.8$ K), which aroused great interest of physicists and materials scientists [26]. Lately the T_c of Rb_3C_{60} was found to be 28 k, the T_c for Rb_2CsC_{60} is 31 k, and the T_c for $RbCs_2C_{60}$ is 33 k.

Fullerene molecules such as C_{60} and C_{70} present a unique performance due to its unique structure. So far C_{60} , C_{70} , and their derivatives have been found to have a series of excellent performance in optical, electrical, and magnetic fields. Especially, the nanotubes prepared by C_{60} have broad application prospects.

7.2.3.2 Carbon Nanotubes

Carbon nanotube is a one-dimensional quantum material with special structure (the radial size of CNTs is at nanoscale, the axial size of CNTs is at microscale, and both ends of nanotubes are sealed). According to the number of layers of carbon nanotubes, carbon nanotubes can be generally divided into single-walled carbon nanotubes (SWCNTs), double-walled carbon nanotubes (DWCNTs), and multi-walled carbon nanotubes (MWCNTs).

Carbon nanotubes have good electrical conductivity, mechanical properties, and biocompatibility. Researches have showed that carbon nanotubes not only have high strength and toughness but also have excellent electrical and magnetic properties, making it a recognized super one-dimensional reinforced material.

Mechanical Property

CNTs have good mechanical properties. The tensile strength of CNTs is up to 50–200 GPa, 100 times higher than that of steel. The tensile strength of SWCNTs with ideal structure is about 800 GPa. The composite material, which is made by fabricating carbon nanotubes on other engineering materials as matrix, would have excellent strength, elasticity, antifatigue, and isotropic properties, which may greatly improve the performance of composite materials. The hardness of CNTs is comparative to that of diamond, but it has good flexibility and can be stretched. In addition, the melting point of CNTs is the highest among known materials.

Conductive Property

CNTs have good electrical performance due to the lamellar structure similar with graphite. Theories predicted that the conductive properties of CNTs depend on its diameter and helical angle of CNTs. When the diameter of CNTs is greater than 6 nm, the conductivity of CNTs decreased.

Heat-Transfer Properties

CNTs have good heat transfer properties. CNTs have big L/D ratio; thus the heat transfer performance along the length direction is high, and the heat transfer performance along the vertical direction is low. In addition, CNTs have relatively high thermal conductivity. So the thermal conductivity of the composite material could be greatly improved as long as trace CNTs are doped in the composite material.

7.2.3.3 Graphene

For many years it has been used to describe the properties of carbon material, such as fullerenes, carbon nanotubes, and graphite. Almost all allotropes of carbon except diamond can be obtained from graphene, as shown in Fig. 7.4 [27]. Graphene is normally in a thickness of an atomic layer, but can also be collectively called single-layer graphene, double-layer graphene, and graphene with less than ten layers. Ten atomic layers are the critical thickness between the 2D and 3D materials.

Basic Electronic Properties

The most worth exploring physical property of graphene is its electronic properties [27–29]. Firstly, the mostly discussed aspect is the electronic spectroscopy of

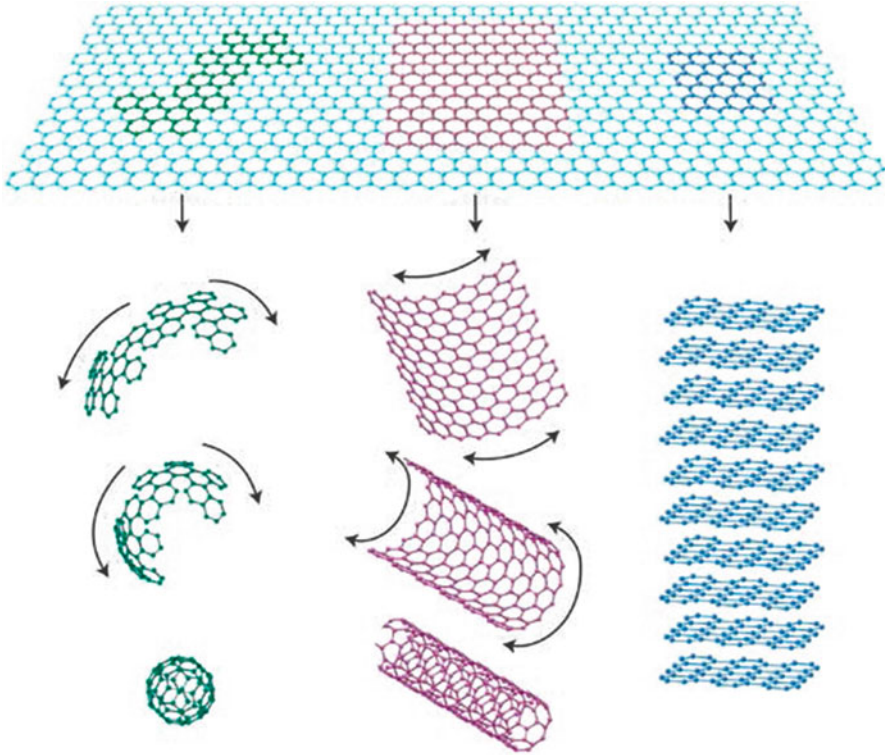


Fig. 7.4 Graphene is a 2D building material. It can be changed into 0D buckyballs, rolled into 1D nanotubes or stacked into 3D graphite (Reproduced with permission from [27]. Copyright 2007 Nature Publishing Group)

graphene (dispersion relations). Secondly, the propagation of electronic wave in graphene is confined in a one-atom thick layer, making them detectable by a variety of probes. At the same time, it makes them sensitive to other materials close to graphene, such as dielectric, superconductor, ferromagnetic, etc. Thirdly, electrons of graphene can transmit in submicron range without scattering; even graphene is put on rough substrate. Fourthly, the quantum effect of graphene is very strong even at room temperature.

Electron Transport Properties

Owing to highly symmetric crystal structure of graphene, at room temperature, the mobility of graphene almost has nothing to do with temperature and is mainly influenced by impurities and defects [30].

Nonelectronic Properties

In 2008, C. Lee reported the first measurement on the mechanical and thermal properties of graphene. The breaking strength of graphene is ~ 40 N/m and reaches the theoretic limit [31]. He also reported that the thermal conductivity of graphene was recorded at room temperature (~ 5000 Wm⁻¹ K⁻¹) [32] and Young's modulus (~ 1.0 TPa) [31]. Graphene can be elastically stretched up to 20 %, which is larger than most crystals [31, 33]. Similarly, graphene has both high flexibility and high vulnerability [34]. In addition, graphene is composed of single-atom thick layers, which make it impermeable to gas, including helium [35].

7.2.4 *Semiconductor Nanomaterial*

The size of particles is greater than that of atom clusters and less than usual particles, generally 0.1–100 nm. It includes two parts with similar volume: one is particles in a diameter of several or dozens of nanometer and the other is the interface between particles.

It is more sensitive to confined space. Semiconductor nanomaterial doesn't exist in nature and it is mainly artificially made by energy-band engineering. The unique properties of semiconductor nanomaterial make it play an important role in the development of various functional devices. Except the common properties of nanoparticles, semiconductor nanoparticles also have special optical effects and photoelectrochemical properties.

Among many outstanding optical properties, the ultrafast optical nonlinear response and photoluminescence (room temperature) features have attracted most attentions. The blue shift of absorption spectrum and fluorescence spectrum forms a series of discrete energy levels in the band.

7.2.5 *Magnetic Nanomaterial*

The properties of nanomaterial are different from that of conventional magnetic material. The reason is that the characteristic physical length associated with magnetism is exactly at the nanometer level. Magnetic nanomaterial has many special properties, such as superparamagnetism, quantum size effect, macroscopic quantum tunneling effect, small size effect, etc. Among them, its magnetic properties are the most outstanding property distinguishing it from any other nanomaterial.

When nanoparticles' size reaches a critical value, it will enter the super paramagnetic state and coercive force is nearly zero. When there is no additional magnetic field, the particles (domain) are in disordered arrangement showing paramagnetism. When magnetic field is applied, the particles are ordered in terms of magnetic field showing the ferromagnetism (but weaker than block).

There are mainly five kinds of magnetic material, metal alloy (Fe, Co, Ni), ferric oxide (Fe_2O_3 , Fe_3O_4), iron nitride (FeN), ferrite (CoFe_2O_4 , $\text{BaFe}_{12}\text{O}_{19}$), and chrome oxide (CrO_2). Among them, Fe_3O_4 is mostly used magnetic nanoparticles. It can be easily prepared by coprecipitation or oxidative coprecipitation in aqueous solution. And its size, shape, and composition can be controlled by adjusting the reaction conditions.

Ferroferric oxide crystal can be expressed as $\text{Fe(III)[Fe(II)Fe(III)]O}_4$. The size of ferromagnetic oxide nanoparticle normally distributed at around 5–100 nm. Owing to the small size effect and surface effect, when the diameter of ferroferric oxide nanoparticle is smaller than 16 nm, it presented superparamagnetism [36]. The unique performance of ferromagnetic oxide nanoparticles affords great potential in biomedical research and separation process study [37]. The nanoparticles are easily to be synthesized and functionalized, which provides great commonality for research. Figure 7.5 shows the properties and modification of ferroferric oxide nanoparticles [38].

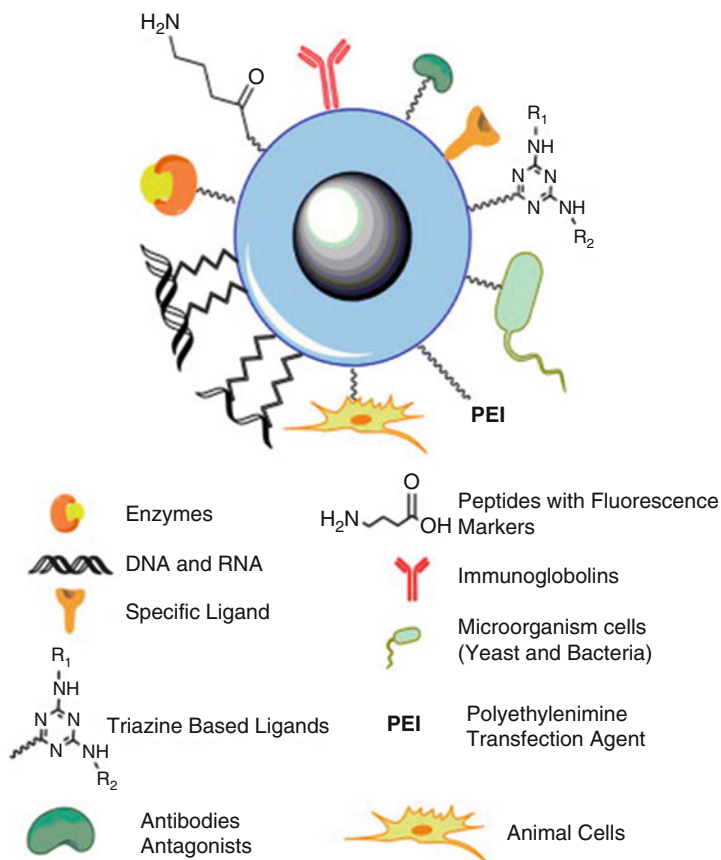


Fig. 7.5 Magnetic nanoparticles decorated with multifunctional receptors and polysaccharides (Reproduced with permission from [38]. Copyright 2008 Elsevier B.V.)

7.3 Recent Developments in the Use of Micro/Nano Material in Biosensors

7.3.1 Electrochemical Biosensors

Nanomaterials mainly have the following functions when it is applied in the field of electrochemical biosensor: (1) accelerate electron transfer and increase the reaction reversibility of redox on electrode surface, (2) catalyze reaction, (3) act as the carrier of biological molecules, (4) label biological molecules, (5) control chemical reaction, and (6) act as reactants.

7.3.1.1 Accelerating Electron Transfer

Metal nanoparticles (such as gold, silver, etc.) and carbon nanotubes can be used as a conductor which connects protein (enzyme) and electrodes and promotes the electron transfer between electrodes and the active center of protein, making it applicable to prepare biological sensors with special performance. Willner et al. [39] linked 1.4 nm gold nanoparticles with flavin adenine dinucleotide (FAD), the cofactor of glucose oxidase (GOx) together, and then recombine FAD-AuNPs composite and cofactor-deleted GOx into new GOx. The recombinant enzyme was assembled onto the gold electrode through double thiol molecules. When detecting glucose with this electrode, the electrons were transferred between enzyme and electrodes through AuNPs making electron transfer rate seven times higher than that between a natural enzyme and the natural oxygen. Because the electron transfer rate of AuNPs is quicker than that of oxygen, the sensor is not affected by the concentration of dissolved oxygen and the interference of reducing substances – ascorbic acid when detecting glucose. After that, they fixed one end of carbon nanotubes on electrodes, while connecting the other end to the FAD, and to form a new GOx like AuNPs [40]. Carbon nanotubes can also transfer electrons between recombinant enzyme and electrodes like a wire. And the electron transfer rate is six times faster than that between a natural enzyme and its oxygen substrate. What's more, the electron transfer rate is related to the length of carbon nanotubes. For example, the electron transfer rate of carbon nanotubes with the length of 25 nm is 1.5 times than that with a length of 50 nm. Xu et al. introduced 17 nm AuNPs into assembled composite membrane for fabricating a glucose biosensor [41].

In addition, the direct electrochemical reaction of many biological molecules such as myoglobin (Mb), hemoglobin (Hb), cytochrome C (Cyt C), and horseradish peroxidase (HRP) on the electrode can also be conducted through the conduction of the metal, metal oxide nanoparticles, or carbon nanotubes. Ju et al. fixed a hybrid composite membrane of zirconium dioxide and Mb on the surface of graphite electrode [42]. The existence of composite membrane accelerated the electron transfer between the electrode and Mb, making the sensor catalyze the reduction of hydrogen peroxide in the absence of any medium. Li et al. [43] assembled gold

nanoparticles on gold electrode using thiol-DNA and then immobilized Cyt_c. Gold nanoparticles transfer electrons between Cyt_c and electrode, by which we can observe the direct electrochemistry of Cyt_c. And in a certain range, with the increased layer of gold nanoparticle, the electrochemical current of Cyt_c increased.

In addition to being electronic wire, metal nanoparticles [44] and carbon nanotubes can also help improve oxidation-reduction reversibility of active substances in electrochemical sensitive membranes.

7.3.1.2 Acting as Catalyst

Metal nanomaterial could catalyze the oxidation or reduction of various electroactive substances (e.g., nicotinamide adenine dinucleotide (NADH) and H₂O₂) and reduce its overpotential in electrochemical reactions. Raj et al. [45] found that NADH was oxidized at -0.3 V on electrodes modified with sol-gel film doped with gold nanoparticles and reached the maximum value at 0 V. Compton et al. [46] found that the silver nanoparticles can catalyze the reduction of H₂O₂, based on which the electrodes have a detection limit of 2.2 $\mu\text{mol/L}$ for H₂O₂. Platinum nanoparticles can also catalyze the oxidation of H₂O₂. Halaoui et al. [47] prepared a highly sensitive H₂O₂ sensor by immobilizing platinum nanoparticles on indium tin oxide (ITO) electrode surface modified with poly diene propyl dimethylamine (PDDA) by self-assembly method. Since NADH is coenzyme for more than 300 kinds of dehydrogenase. While H₂O₂ is the reaction product of oxidase and the substrate of peroxidase, through the high selective determination of NADH and H₂O₂, we can effectively detect the concentration of many kinds of enzymes or the substrate. Palladium and iridium nanoparticles have similar functions with platinum, so they are frequently used as material to immobilize oxidase on electrodes.

Because of the small diameter, high surface energy, and the insufficient atom ligand, carbon nanomaterial such as carbon nanotubes are easily to transfer electron with other substances, making it suitable for broad applications in electrocatalysis. Gorski et al. [48] dissolved carbon nanotubes in chitosan, forming carbon nanotubes-chitosan composites. The compound had good film-forming property and showed the good catalytic capability on the oxidation of NADH. The NADH oxidation potential on the composite membrane was reduced by 300 mV. After fixing glucose dehydrogenase on the electrode surface, the sensor can quantitatively detect glucose of 5.0–300 $\mu\text{mol/L}$. Chen et al. [49] fixed carbon nanotubes-chitosan composites on glassy carbon electrode surface with one step of electrodeposition method, which can catalyze the reaction of O₂ and H₂O₂ at the same time. The prepared biosensor can be used to detect the substrate at different potentials, which effectively eliminates the interference of electroactive material such as ascorbic acid. Using electrical catalysis of carbon nanotubes modified electrodes on H₂O₂ or NADH, we can indirectly detect substrates and inhibitors of many enzymes, such as choline, ethanol, organophosphorus pesticide, and so on.

7.3.1.3 The Immobilization of Biomolecules

With such features as large specific surface area, high surface free energy, good biocompatibility, and rich in functional groups on surface, nanomaterial makes it possible to fix a large number of biological molecules on the electrode surface and keeps its good biological conformation and activity, which plays a vital role in the construction of biosensor.

Gold nanoparticles were widely applied in immobilizing protein. AuNPs have been used to immobilize a lot of protein, such as Hb, HRP, tyrosinase, and glucose oxidase on the electrode surface. Studies have shown that protein (enzyme) can attach on the surface of AuNPs well through the interaction between groups like NH_2 and SH and gold atom. What's more, AuNPs have good biocompatibility. So, fixed biological molecules can maintain their biological activity. Chen et al. [50] deposited chitosan-AuNPs-GOx composite on electrode surface using electrodeposition method and prepared an amperometric sensor with fast response for the determination of glucose. Recently, they used AuNPs which was synthesized in situ on the surface of polydimethylsiloxane (PDMS) to develop polymer-nanomaterial composite film-modified electrodes with good biocompatibility and strong protein adsorption ability [51]. Taking advantage of different adsorption sites of choline oxidase and acetyl cholinesterase on composite membrane, the selective detection of acetyl cholinesterase in homogenate neural tissue can be realized. Except for immobilizing protein, gold nanoparticles have also played a huge role in immobilizing antigen, antibody, DNA, and cells. Zhu et al. [52] synthesized a three-dimensional nanostructured gold film which provided a biocompatible microenvironment for antibodies and then developed a novel unlabeled immunosensor. Ju et al. [53] built a bionic interface using AuNP-chitosan gel and cultured K562 leukemia cells on the interface. The number of cells, the proliferation, and apoptosis of cells can be reflected by the change of impedance value.

Oxide nanomaterial is mainly used for protein immobilization in the field of electrochemical biosensors. Because of the good hydrophilicity and biological compatibility of nano-oxides' surface, nano-oxides are beneficial for maintaining the biological configuration of proteins and can realize the direct electron transfer between active center of protein and the electrode surface. Hu et al. [54] immobilized heme proteins on the graphite electrode through layer-by-layer assembly method by using the interaction between SiO_2 nanoparticles and Hb, which realized direct electrochemistry of HB. In addition, they have discussed the immobile driving force of the protein on oxide nanoparticles based on the model of heme protein [55]. In 2004, for the first time, by introducing SiO_2 nanoparticles and GOx onto grid surface of FET, Chen et al. [56] prepared enzyme-based FET which presented increased sensitivity and prolonged service life for glucose determination compared to enzyme-based FET without SiO_2 nanoparticles. Besides the electrostatic interaction, there is chelation between transitional metal nanoparticles and proteins. Therefore, they are more suitable for immobilizing protein. Nanomaterials such as TiO_2 , Fe_3O_4 , ZrO_2 , and MnO_2 also achieved good results for immobilizing protein on electrochemical biosensors.

7.3.1.4 Biomolecule Labeling

Among metal nanomaterials, gold and silver nanoparticles can be used as biomarkers in electrochemical analysis by labeling biomolecules with molecular recognition ability, such as antigen, antibody, DNA, etc., which play a role in electrochemical immunoassay and DNA testing. Limoges et al. [57] labeled antibody with gold nanoparticles and assembled gold nanoparticles on electrode surface through the immunoreaction between antibody and antigen. Then the concentration of gold ions was determined by anodic stripping voltammetry, thus indirectly measuring the concentration of antibody. The method is of high sensitivity and can be used to measure pmol/L level of immunoglobulin. On the basis of that, the developed AgNP- and CuNP-enhanced stripping method was also applied to the antigen and antibody detection.

With multiple energy state, semiconductor nanomaterials such as CdS, CdTe, PbS, and ZnS can glow when they are excited at multiple wavelengths. The nanoparticle biomarkers used in electrochemical analysis are mainly metal nanoparticle and semiconductor nanoparticles, namely, quantum dots. In the field of electrochemical biosensors, quantum dots are often applied to label biological molecules. Biological molecules labeled by quantum dots were assembled to the sensor interface through specific interaction between antigen and antibody or biotin and avidin. After that, the concentration of targets can be quantitatively detected through stripping voltammetric response of the constituent elements of a quantum dot. Wang et al. [58] labeled CdS quantum dots onto polysaccharides. Due to the specific interaction between the polysaccharide and lectins fixed on surface of gold electrode, CdS quantum dots labeled polysaccharide (fixed concentration) and unlabeled polysaccharide (concentration need to be detected) competitively combined with lectins. So concentration of polysaccharide can be indirectly measured by measuring the stripping voltammetric response of Cd caught by lectins. In addition, they developed an electrochemical method which can detect a variety of kinds of DNA at the same time [59]. Recently, Ju et al. [60] assembled a novel sugar monolayer film that established a method for in situ detection of sugar on cell surface by using CdTe quantum dots as the biomarker.

7.3.1.5 Acting as Reactant

It is known that the chemical properties of nanoparticles are different from that of its body material. Due to high surface energy, the chemical activity of nanomaterial is usually higher than its ontology. As is known to all, MnO_2 can catalyze the decomposition of H_2O_2 , but MnO_2 nanoparticles can directly react with H_2O_2 , generating Mn^{2+} and O_2 , while consuming H^+ . Obviously, the consumption of H^+ will cause the change of pH, which can be monitored by H^+ -sensitive FET. Based on this principle, Chen et al. [61, 62] prepared glucose biosensor and lactic acid biosensor with high sensitivity and good selectivity. In addition, MnO_2

nanoparticles can also react with ascorbic acid. Taking advantage of this property, Chen et al. [63] deposited a layer of MnO_2 -chitosan composite film on the surface of prepared glucose sensor, which can effectively eliminate the influence of ascorbic acid and improve the precision of the sensor in the determination of glucose. More recently they developed H_2O_2 sensor with double catalytic ability by using the property of changing valence of MnO_2 nanoparticles, which were successfully applied for detection of choline at low potential [64].

7.3.2 Optical Biosensor

7.3.2.1 The Application of Metal Nanoparticles in Optical Biosensor

Photochemical sensing research of precious metal nanoparticles has attracted much attention in recent years. Applications of precious metal nanomaterial in the field of optical sensing are mainly concentrated in the following aspects:

1. The optical colorimetric analysis
2. The surface plasmon resonance detection
3. Local surface plasmon resonance (LSPR) and surface-enhanced Raman scattering (SERS)
4. The fluorescence detection

Chromaticity sensor has attracted many attentions due to its simplicity, high sensitivity, low cost, easiness to read by naked eyes, no need for complex instruments, etc. Metal nanoparticles, due to its high extinction coefficient in visible light area, have become one kind of colorimetric probe. The dispersion of AuNP solution presents a red color, while the aggregation of AuNPs presents a purple or blue color. So far, AuNPs modified by different functional molecules with high recognition ability have been widely used.

For the first time, Mirkin et al. [65–67] used thiol-DNA-modified AuNP probe, since DNA can combine with a lot of target molecules, causing the cross-link of AuNPs. So they can detect different kinds of target materials such as oligonucleotides, proteins, small molecules, enzyme activity, metal ion, etc. AuNPs modified with thiolated aptamer acted as colorimetric probe. Based on the color changes in the process of gathering and depolymerization, sensitive colorimetric detection of adenosine can be realized.

Xia group established a universal colorimetric sensor platform by using unlabeled ssDNA, unmodified AuNPs, and cationic conjugated polymers, which can be used in the detection of a variety of target materials including DNA, proteins, small molecules, and inorganic ions [68].

In 2010, Parab et al. reported an optical sensor to detect target DNA by using gold nanorods as molecular probe. This sensor is based on that the hybridization of target DNA with two different DNA probes leads to the aggregation of gold nanorods. This sensor has been successfully applied to detect pathogen gene of

Chlamydia trachomatis in human urine samples with a detection range of 0.25–20 nM.

Surface plasmon resonance sensor is a new type of biological sensing technology which appeared in the 1980s. By utilizing SPR principle, SPR sensor transferred the interaction of biological molecules into optical signal. SPR sensor owns the advantage of real-time, online detection and highly sensitive detection.

Localized surface plasmon resonance refers to a physical optic phenomenon when the incident light radiates on the surface of nanoparticles, in which occasion the frequency of incident light resonates with the oscillation frequency of metal free electron aggregation and presents strong absorption spectra in specific wavelength range.

In addition to the inheritance of SPR sensor's merits, LSPR sensor also has the following features:

1. Small test instrument and simple system setup
2. High sensitivity and strong anti-interference ability
3. Rapid and multichannel detection

The biomacromolecular detection based on the technology of LSPR is an important branch of LSPR sensing research. Gold nanorods are the most commonly used LSPR sensing substrate which is easy to be prepared. Gold nanorods own strong local surface plasmon resonance absorption peak and can be assembled into a one-dimensional (1D), two-dimensional (2D), and three-dimensional (3D) structure under suitable conditions. The resonance absorption peak of gold nanorods is relatively sensitive to shape, size, and space between nanorods and the surrounding medium environment. Using this feature, LSPR sensors based on gold nanorods has begun to be widely used in biological macromolecules detection, immunoassay, etc. [69].

In 1996, Mirkin et al. [65] firstly reported gold nanoparticle-based DNA detection by LSPR method. He added a single-stranded DNA sequence which was complementary to different DNA fragments modified on the surface of AuNPs to the AuNPs solution, leading to the red shift of the absorption peak of LSPR. At the same time, the color of AuNP solution turned from red to blue. Although this method is sensitive, the surface of AuNPs needs complex modification process and the operation is complicated. In 2000, by using gold nanoparticles with negative charge on its surface, Li et al. [70] wrapped single-stranded DNA on the surface of AuNPs through electrostatic interactions. When target DNA fragments appeared in the system, the hybridization of target DNA with DNA probe on the surface of AuNPs would occur, which would induce the aggregation and color change of gold nanoparticles. Immune analysis based on the technology of LSPR is an important branch of LSPR sensing research. In 2002, Thanh et al. [71] develop a method which can detect anti-protein antibody at 1 $\mu\text{g}/\text{mL}$ based on the aggregation of AuNPs.

Huang et al. [72] prepared a photochemical biosensor based on unlabeled and high-throughput LSPR for the first time. The author prepared five LSPR sensors with different throughput by using five kinds of gold nanorods with different length

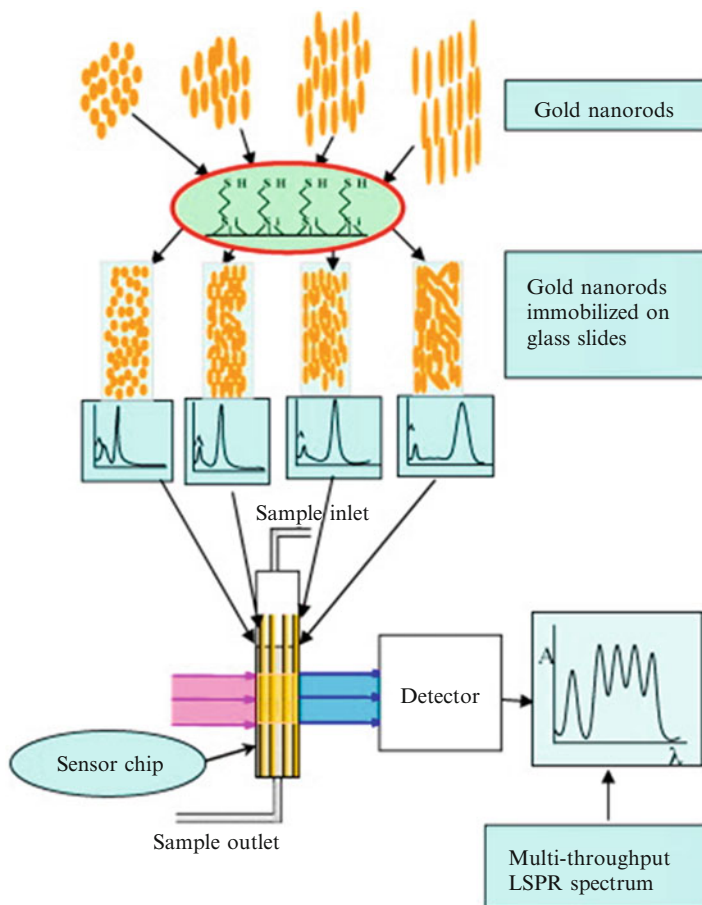


Fig. 7.6 Multi-throughput localized surface plasmon resonance (MLSPR) (Reproduced with permission from [72]. Copyright 2008 Elsevier B.V.)

to diameter ratio. Different receptor-ligand pairs can be detected in the wavelength range from 530 to 940 nm at the same time. And the sensor can also detect three pairs of the antigen antibody simultaneously. Wang et al. [73] applied LSPR sensors based on gold nanorods for the detection of hepatitis B virus (HBV) in buffer, serum, and plasma. Harshala et al. [74] prepared an optical DNA sensor for pathogen diagnosis (Fig. 7.6).

With the development of surface modification technology and nanofabrication technology, by controlling the array distribution, structure, and the surrounding medium of the precious metal nanoparticles and increasing the breadth and depth of LSPR technology, LSPR technology will become a forefront research hotspot in sensing fields.

Compared to other spectral methods, fluorescence analysis is very popular due to its inherent advantages such as high sensitivity, easiness to operate, and the diversity of the measurable parameters. Especially in recent years, some progresses in research of properties of nanomaterial give a new thought for designing chemical and biological fluorescent sensing scheme.

In 2005, Chen et al. [75] found that there are two fluorescence emission belts at 743 nm and 793 nm with different intensity for gold nanorods whose length to diameter ratio is greater than 1:13. The larger the length to diameter ratio is, the higher fluorescent efficiency gold nanorods have. The hybridization between DNA probe and target molecules can be detected through the changing of fluorescence intensity of gold nanorods which is labeled by DNA molecule.

In 2010, Gouet et al. [76] detected the human single-stranded DNA, double-stranded DNA, and G-quadruplex by using the positively charged gold nanorods as optical labels. The conformation changes of telomere DNA are converted to multiple optical signals such as fluorescence quenching, near-infrared absorption, plasma resonance light scattering, and dynamic light scattering. The formation of double-stranded DNA and G-quadruplex, accompanied by strength decreases, leads to the fluorescence quenching and blue shift of longitudinal absorption peak of gold nanorods.

Surface-enhanced Raman scattering (SERS) is an important tool for studying surface molecules. Raman spectrum analysis method is based on the Raman scattering effect found by Indian scientists C.V. Raman. By analyzing the scattering spectrum which is different with incident light, we can get the information of molecular vibration and rotation. And the information is helpful for us to study the structure of molecules. While using normal Raman spectrometry to detect the sample adsorbed on colloidal metal particles such as silver, gold, or copper or on the rough surface of metal, the Raman spectrum intensity of the sample can be improved by 10^3 – 10^6 times. This phenomenon is called surface-enhanced Raman effect.

SERS overcomes the drawback of low sensitivity of Raman spectrum, so it can get the structural information which cannot be easily retrieved from the conventional Raman spectrum, resulting its wide use in the study of biological molecular interface orientation, configuration, structure, etc. Relative information in vivo about biomacromolecule such as protein, nucleic acid, and phospholipid can be accurately obtained by stimulating the near-infrared light which has less damage to organisms. Surface-enhanced Raman scattering can be used in biological research of complex system.

Due to its nanoscale rough surface and good water solubility of AuNPs, it has been widely used as SERS-active substrate which can be applied to study soluble adsorption molecules in solution system in the fields of biochemistry, trace analysis, etc. The study found that the size, shape, and surface topography of gold nanoparticles will greatly influence the SERS enhancement effect. Also, although the SERS enhancement factor of a single gold particle is small, the enhancement of AuNP aggregation is obvious. The aggregation of AuNPs can be obtained by adding some inorganic salts (KCl, NaCl, NaNO_3) and organic amine into the AuNP solution. Nie et al. [77] designed a new kind of SERS AuNP probe. Through

the specific hybridization of DNA, the distance between AuNPs was adjusted, which would change SERS signal and then realize molecular recognition and signal amplification. Besides being used as SERS substrate in the solution system, AuNPs can also be used in the preparation of solid base. Porter et al. [78] proved that the gold substrate assembled by AuNPs can become good solid-phase SERS substrate. Meanwhile they found that SERS substrate with gold basement and gold nanoparticles has strong SERS effect and proved that coupled vibration of these two kinds of surface electron cloud of the basement and gold nanoparticles played an important role in SERS effect.

7.3.2.2 The Application of Semiconductor Nanoparticles in Optical Biosensor

Quantum dots, QD for short, also called the semiconductor nanoparticles or semiconductor nanocrystals, are a kind of nanoparticles composed of VI and V elements [79]. With high fluorescence quantum productivity and better fluorescence lifetime, core-shell type quantum dots have many advantages as fluorescent probes compared with traditional fluorescent dyes and are often used for the detection of biological macromolecules [80].

QD assay labeling uses QDs for in vitro assay detection of DNA, proteins, and other biomolecules. DNA-coated QDs have been shown as sensitive and specific DNA labels for in situ hybridizations, as probes for human metaphase chromosomes, and in single-nucleotide polymorphism and multi-allele DNA detection. Conversely, DNA linked to QD surfaces has been used to code and sort the nanocrystals. These results demonstrate that DNA-conjugated QDs specifically bind their complements both in fixed cells and in vitro. For proteins, our group uses a self-assembled electrostatic protein QD functionalization strategy to create QD-based fluoro-immunoassay reagents. Using antibodies conjugated to QDs by an adaptor protein, we have carried out multiple demonstrations of analyte detection in numerous immunoassays including a four-color multiplex toxin analysis [81, 82]. For bioassays, the ability to excite QDs at almost any wavelength below the band edge combined with high photo bleaching resistance and “multiplexing” capabilities highlights the unique combination of spectral properties that make QD fluorophores of interest to biologist.

Luminescent quantum dots have many applications in nucleic acid detection. DNA molecules labeled with QDs can be used as a fluorescent probe of oligonucleotide which could hybrid specifically with its complementary oligonucleotide. After DNA molecules labeled with QDs match with its complementary DNA, the fluorescence spectrum of QDs will change. It's the arrangement change of QDs that cause changes of their fluorescence [83]. Han et al. [84] assembled QDs which emit different fluorescence on the surface of polymer microspheres and encode according to the type of QDs and the ratio of fluorescence intensity through hybridization reaction, which can provide corresponding fluorescence information

for each of the DNA sequence with 99.99 % accuracy. Patolsky et al. monitored the dynamics of DNA replication by using resonance energy transfer between CdSe/ZnS quantum dots and organic fluorescence [85].

7.3.2.3 The Application of Carbon Nanomaterial in Optical Biosensor

Resonance energy transfer, a kind of non-radiation energy transfer process, is when the donor on excited state transfers energy to receptors on the ground state. When the donor is a kind of fluorescent substance, whether the receptor is a kind of fluorescent substance or not, this process is called fluorescence resonance energy transfer (FRET). The efficiency of FRET is affected by many factors, such as the degree of spectrum overlapping, the orientation of quadrupole on transition state, the distance between the donor and receptor molecules, etc. Donor and receptor are usually two kinds of dye molecules whose charge is limited to the zero dimension in traditional FRET, in which the energy transfer rate (k) is inversely proportional to the six powers of molecule distance. The effective distance of energy transfer is between 1 and 10 nm. When the distance is more than 10 nm, effective FRET process will not happen. According to the report of Sebastian et al. [86, 87], when either receptor or donor has off-domain charge, the rate of energy transfer will deviate from the traditional law of FRET. For example, when the donor is dye molecule and the receptor is CNTs with one-dimensional structure, the rate of energy transfer is inversely proportional to five powers of distance. When the receptor is graphene, the rate of energy transfer is inversely proportional to four powers of the distance. In these systems, even when effective energy transfer distance is far greater than 10 nm, the transfer efficiency is much higher than that of the traditional energy transfer efficiency between dye molecules. Therefore, donor-recipient type based on nanomaterials such as CNTs and graphene has broad application prospects in the chemical and biological sensing.

In terms of CNTs, in recent years, research about the interaction between some biological molecules such as nucleic acids, proteins, and CNTs is very attractive which can be used for the separation of CNTs, biological sensing, and drug transmission. Among them, the ssDNA was proved to have the ability to interact with CNTs non-covalently. ssDNA could wrap around the surface of CNTs to form stable compounds through the π - π stack between its nucleotide bases and sidewall of CNTs. However, the affinity of double-stranded DNA (dsDNA) to CNTs is very small, so it cannot wrap on the surface of CNTs [88, 89]. The differences of affinity properties to CNTs between single- and double-stranded DNA combine with specificity of aptamer to interact its target resulting to the CNTs wide use in chemical biology sensing. For example, Yang et al. built donor-receptor type nanohybrid through the interaction between CNTs and SWCNTs both labeled by fluorophore. As shown in Fig. 7.7, after the formation of dsDNA based on hybridization with the target sequence, fluorescence recovery can be detected. In addition, the detection range of this sensing strategy to detecting target material molecules can expand by utilizing aptamer. For example, Yang et al. [90] detected adenosine

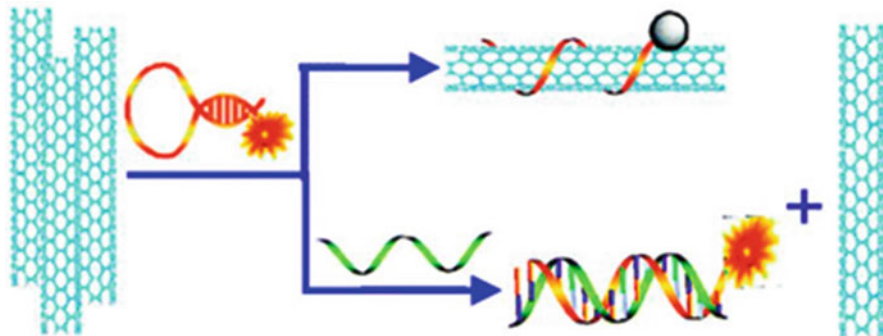


Fig. 7.7 Scheme of the SWCNTs-based fluorescence sensor for DNA detection (Reproduced with permission from [90]. Copyright 2008 American Chemical Society)

triphosphate by assembling aptamer molecular beacon (MAB) and MWCNTs. Not only the problem of high fluorescent background signal and low efficiency of energy transfer between the dye of the traditional molecular beacon can be overcome; the cost was also reduced greatly by using MWCNTs as energy receptors. In addition, by using different Aptamer, the detection range of molecules increased greatly.

Similarly, FRET sensor based on graphene especially graphene oxide (GO) is widely used. They are usually consisted of three parts:

1. Recognition probe, such as ssDNA or aptamer which can respectively combine with the corresponding target sequence or specific molecular targets
2. The report fluorophore which usually coupled on the probe
3. GO

First of all, the DNA probes labeled with fluorescent molecules interact with GO plane through nucleotide bases, forming donor-receptor type GO hybridization and inducing fluorescence quenching. While target molecules were combined with DNA probe, the conformation of DNA probe changed, inducing the dissociation of DNA probe. Finally, the FRET process is terminated, so is the fluorescence recovery of quenching fluorophore led by. Tang et al. [91] reported such kind of sensor system based on the recovery of quenching fluorophore to detect specific ssDNA sequences, whose detection limit can reach nanomole level. The author also discovered that DNA adsorbed on the surface of GO can protect it from nuclease degradation, confirmed that specific detection of target sequences in the presence of nuclease can also be realized. Similarly, the other two DNA sensing methods were further proved that they can be used to detect the mismatch of single base.

In order to further improve the sensitivity of the method, Li et al. [92] built double-quenching fluorescence sensing system by combining GO and molecular beacon (MS), which greatly improves the SNR, and the detection limit can reach 0.1 nM. Dong et al. [93] took CdTe QDs as fluorescent report group and detected target molecules by building the GO/MB-QDs energy transfer quenching system.

Similarly, this method can be used to detect proteins and metal ions by using aptamer as the recognition probe. Lu et al. [94] designed a fluorescent sensor based on the GO/aptamer for thrombin detection whose performance is obviously better than that of purely fluorescent sensor based on traditional fluorescent dye [95] and optical sensing method based on aptamer-CNT hatch. In addition, Chang et al. [96] also developed fluorescence sensing method by using surfactant decentralized reduced graphene oxide (RGO) rather than GO, which improve the detection sensitivity up to picomole. And Wen also developed a FRET method for sensitive detection of Ag by using specific aptamer of Ag labeled by fluorescence and GO (Fig. 7.8).

7.3.3 Piezoelectric Sensor and FET Biosensor

7.3.3.1 The Application of Nanomaterial in Piezoelectric Sensor

The term “piezoelectric” derived from the Greek word *piezen* meaning “to press.” The first investigation on the piezoelectricity was performed in 1880 by Jacques and Pierre Curie [98], who observed that a mechanical stress is applied to the surfaces of various crystals, caused by a corresponding electrical potential across the crystal, whose magnitude was proportional to the applied stress. Many types of materials (quartz, tourmaline, lithium niobate or tantalate, oriented zinc oxide, or aluminum nitride) exhibit the piezoelectric effect, but the properties of quartz make it the most common crystal type used in analytical applications. The most used devices in biosensors are generally bulk acoustic wave-based and surface acoustic wave-based biosensor.

Surface acoustic wave (SAW) is a kind of acoustic wave that spreads along the surface of medium, and its amplitude decreases rapidly with depth. As early as 100 years ago, people had known the properties of this wave theoretically, but until 1965 the technology of generating surface wave had been broken through by using interdigital transducer. Since then, SAW device obtained rapid development. Now it has been widely used in fields such as radar, satellite, communication, electronic countermeasure, and sensor technology, becoming a new kind of promising device.

Typical quartz crystal SAW devices mainly include delay lines, resonators, oscillators, band-pass filter, etc. And the main component of SAW is piezoelectric substrate and interdigital transducer (IDT). IDT is one kind of ultrasonic transducer that could directly excite surface acoustic wave. Because of its low dissipation of electroacoustic conversion, design flexibility, simple fabrication, and certain working frequency range, it has become one of mostly used technologies for the excitation and detection of surface acoustic wave. Firstly, a layer of metal film was fabricated on piezoelectric substrate by vacuum vapor deposition. Then the interdigital fork electrodes were made by photolithography method.

Nanomaterials have incomparable excellent properties, so they are often used for signal amplification of piezoelectric biosensor. Wang et al. [99] deposited amino

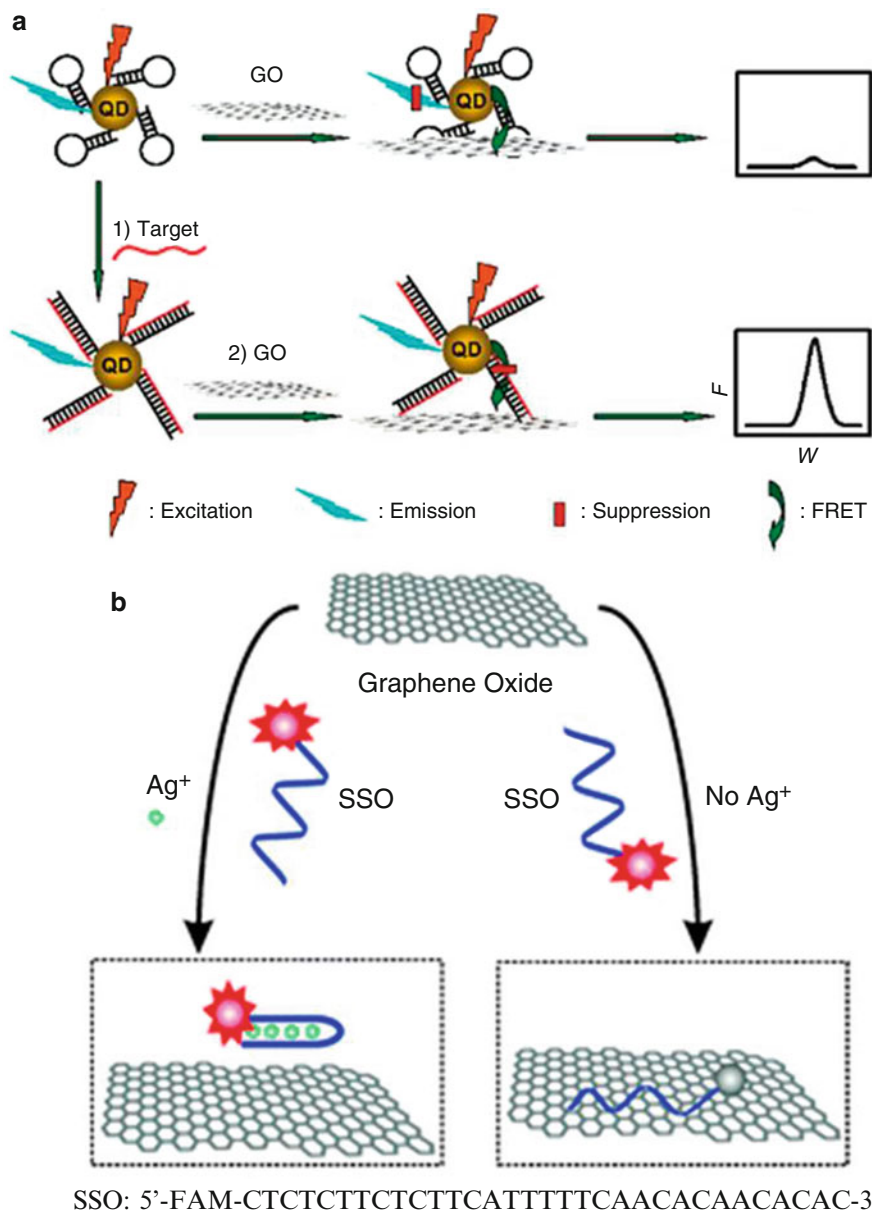


Fig. 7.8 (a) Schematic illustration of DNA hybridization using a double-quenching system consisting of GO and molecular beacon (MB) (Reproduced with permission from [93]. Copyright 2015 2010, American Chemical Society). (b) Schematic illustration of the fluorescence sensor for Ag(I) ions based on the target-induced conformational change of a silver-specific cytosine-rich oligonucleotide (SSO) and the interactions between the fluorogenic SSO probe and graphene oxide (Reproduced with permission from [97]. Copyright 2010 Royal Society of Chemistry)

plasma polymer film on the gold electrode surface of a piezoelectric crystal and then assembled nanoparticles on film to develop an immobilization technology based on the multiplication of gold nanoparticles.

Jin et al. [100] labeled antihuman IgG antibody with nanoscale SiO₂ particles instead of traditional latex and established an improved liquid piezoelectric immune agglutination technology based on piezoelectric immune agglutination sensing for direct and rapid detection for human IgG. Meanwhile, they investigate immune agglutinate phenomenon between nanogold-labeled antibody and IgG and compared its result with that of antibody labeled with SiO₂ nanoparticles. Hua Wang et al. [101] covalently fixed the predominant antigens of schistosome on the surface of functional SiO₂ particles and rapidly detected the concentration of schistosome antibody in infectious rabbit serum by sensitive sensing the changes of non-quality parameters such as density, viscosity induced by agglutination action events of immune particle using piezoelectric crystal, and then successfully developed a piezoelectric sensor-based assay for the diagnosis of schistosomiasis. Compared with normal immune sensing method, this kind of non-mass effect piezoelectric sensor has the advantage of fast and sensitive detection, simple operation, low cost, portable detection instrument, real-time detection, etc.

7.3.3.2 The Application of Carbon Nanomaterial in Field-Effect Transistor-Based Biosensors

Among diverse electrical biosensing architectures, devices based on field-effect transistors (FETs) have attracted great attention because they are an ideal biosensor that can directly translate the interactions between target biological molecules and the FET surface into readable electrical signals. There has been an explosion of interest in using carbon nanomaterials in FET-based biosensors. Owing to their unique physicochemical properties, SWNTs and graphene are in the forefront of this explosion. The major advantages of SWNTs and graphenes are their high conductivity, well biocompatibility, molecule compatibility size, high chemical stability, and easy availability, which suggest that carbon nanomaterial-based FETs have promised as paradigms for device architecture, yielding devices that are capable of converting biological information to easily detectable electrical signals.

The properties of SWNTs can be leveraged by combining them with other functional materials, such as small molecules, lipid membrane, or aptamers, to enhance the biosensing performance and install new functionalities. Kim et al. coated SWNT surfaces with human olfactory receptor 2AG1 and reported the real-time detection of specific odorant molecules (such as amyl butyrate, butyl butyrate, propyl butyrate, and pentyl valerate) [102]. The observed femtomolar sensitivity resulted from the deformation of the human olfactory receptor 2AG1 protein when the WNT-bound protein and the odorants docked.

Previous reports indicated that single-stranded DNA can form a stable complex with individual SWNTs by wrapping around them, the interaction being driven by

the aromatic interactions between nucleotide bases and SWNT sidewalls. Label-free electrical detection of DNA hybridization utilizing SWNT FET-based biosensors suggests a new generation of DNA chips that can give direct electrical readouts. This could provide fast and inexpensive analyses of nucleic acid samples. In 2006, Star et al. [103] used SWNT FETs to study interactions between single-stranded DNA oligonucleotides and SWNTs and the subsequent DNA hybridization processes that occurred on the device surface. They showed that SWNT FETs could be selectively modified with DNA oligonucleotides and maintain hybridization specificity.

Recently, Goldsmith et al. have developed an electrochemical method to create single-point defects in SWNTs in a controllable manner and then covalently bind biomolecules at this scattering site [104]. Using these devices, they also demonstrated continuous, multihour monitoring of the binding of a single molecule with high sensitivity (a conductance change of >100 nS for binding of a reactive carbodiimide). Sorgenfrei et al. [105] covalently attached a single-stranded probe DNA sequence, which was terminated with an amine group, to a carboxylic acid functionalized point defect in a carbon nanotube using a standard amide-formation coupling reaction.

Recently, the increasing interest in graphene for biosensing applications seems to have overtaken the corresponding interest in SWNTs. The first graphene FET-based electrical biosensors were demonstrated by Mohanty et al. [106]. They immobilized GO and GA sheets on silica substrates with either predeposited or postdeposited gold electrodes and used silicon as a back gate for electrical measurements. They exploited the functional groups on GOs or GAs to fabricate three CMG-based biosensors:

1. A single-bacterium biodevice
2. A label-free DNA sensor
3. A bacterial DNA/protein and a polyelectrolyte chemical transistor

The bacteria biodevice was highly sensitive; just a single-bacterium attachment caused a sharp (42 %) increase in conductivity of CMG sheets. To improve the detection sensitivity, Dong et al. [107] used large, CVD-grown graphene films to fabricate biosensors, thereby achieving DNA sensing with a concentration as low as 0.01 nM. They attribute this marked increase in sensitivity to the electronic n-doping to the devices. They further found that adding AuNPs on the surface of graphene devices could extend the upper limit of DNA detection from 10 to 500 nM due to the increase in loading of probe DNA molecules onto the AuNPs. This AuNP strategy is apparently widely applicable.

Ohno et al. [108] developed label-free immunosensing based on an aptamer-modified graphene FETs. The aptamer-modified graphene FETs showed selective electrical detection of immunoglobulin E protein. From the dependence of the drain current variation on the immunoglobulin E concentration, the dissociation constant was estimated to be 47 nM, indicating good affinity. In a similar way, Agarwal et al. [109] recently demonstrated the biocompatibility of reduced GOs with proteins and further used them after protein functionalization to create biosensors for detecting various metals in real time with high sensitivity [110] (Fig. 7.9).

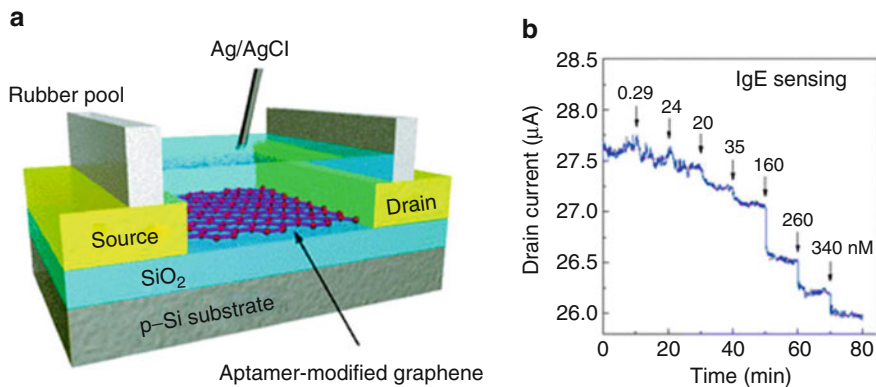


Fig. 7.9 (a) Schematic illustration of aptamer-modified electrolyte-gated graphene FETs. (b) Time course of ID for an aptamer-modified graphene FET. At 10-min intervals, various concentrations of IgE were injected (Reproduced with permission from [108]. Copyright 2010 American Chemical Society)

7.3.3.3 The Application of Silicon Nanowire in Field-Effect Transistor-Based Biosensors

Silicon nanowire field-effect transistors (SiNW-FETs) have recently drawn tremendous attention as a promising tool in biosensor design for their ultrasensitivity, selectivity, and label-free and real-time detection capabilities [111]. In the fields of biological research, SiNW-FETs are employed in the detections of proteins, DNA sequences, small molecules, cancer biomarkers, and viruses.

SiNW-FET has been widely used lately for studying protein interaction mechanisms, not only because of its real-time and label-free detection but also due to its high sensitivity and selectivity. An early measurement made by Cui et al. demonstrated the real-time detection of streptavidin binding to biotin-modified SiNWFET [112]. They also explored the ability of biotin-modified SiNW-FET to detect streptavidin at the concentration of 10 pM, which is much lower than the nanomolar-range detection level obtained from other techniques, such as the stochastic sensing of single molecules [113]. Lately, Zheng et al. have also demonstrated a new methodology based on a frequency (f) domain electrical measurement utilizing a SiNW-FET for protein detection [114]. In the presence of the protein (antigen) which can be recognized specifically by the antibody-functionalized SiNW-FET, the frequency spectrum exhibits a Lorentzian shape with a characteristic frequency of several kHz. They observed the shape of the frequency spectrum to monitor the binding events and further to determine the detection limit.

In addition to the protein-protein interactions mentioned above, SiNW-FETs were adapted for the detection of DNA or RNA. Due to the large amount of negative charges in the phosphate backbones of DNA or RNA, SiNW-FETs offer a good candidate for monitoring DNA or RNA hybridizations, because the

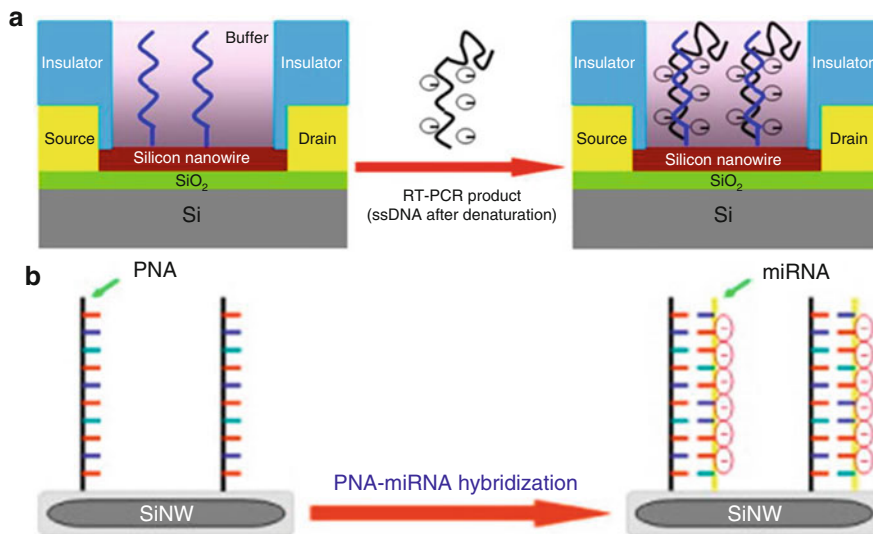


Fig. 7.10 (a) A schematic diagram of the RT-PCR product of DEN-2 hybridized to a PNA-functionalized SiNW-FET sensor (Reproduced with permission from [116]. Copyright 2008 Elsevier B.V.) (b) Label-free direct hybridization for the ultrasensitive detection of miRNA (Reproduced with permission from [117]. Copyright 2008 Elsevier B.V.)

hybridizations cause the accumulation or depletion of charge carriers in the SiNW-FET, leading to a conductance change. Hahm et al. have reported the real-time and label-free detection of DNA with a PNA (peptide nucleic acid)-modified SiNW-FET [115]. In that study, PNA was anchored to the SiNW surface by the strong interaction between avidin and biotin. The successful PNA-DNA duplex formation was demonstrated by the observation of a sizable increase in conductance in the p-type PNA-modified SiNW-FET, because of the negatively charged phosphate backbones of DNA. This ultrasensitive biosensor for sensing DNA is capable of detecting down to 10 fM.

Specific PNA-modified SiNW-FET sensors have recently been established to diagnose dengue virus infection [116]. As represented schematically in Fig. 7.10a, the synthetic PNA receptors were first anchored to the SiNW-FET surface. A specific fragment (69 bp) derived from dengue serotype2 (DEN-2) virus genome sequences was selected as the target DNA and amplified by the reverse transcription polymerase chain reaction (RT-PCR). The detection limit of this biosensor based on SiNW-FET was claimed to be 10 fM. In addition, this promising method allows the detection of microRNAs (miRNAs) for early cancer diagnosis [117]. As illustrated in Fig. 7.10b, a PNA-immobilized SiNW-FET was used to probe miRNA by detecting PNA-miRNA hybridization via base pairing. This approach exhibits an excellent detecting specificity capable of discriminating a single-base mismatch in miRNA.

The SiNW-FET system has also been applied to disease screening. Recently, Zheng et al. utilized a SiNW-FET array for the detection of multiple cancer markers [117]. This SiNW-FET array, shown in Fig. 7.10, is composed of three independent SiNW-FET devices on which different antibodies were immobilized. The conductance vs. time measurements showed the simultaneous recording of the PSA, CEA, and mucin-1 solutions, which were delivered sequentially to the SiNW-FET arrays. By virtue of the ultrasensitive SiNW-FET, the detection limit for these three cancer markers has advanced to the pg/mL scale.

Using nano- and neuro-technologies to couple electrical interfacing with neural systems has great potential to unveil many details of neuron studies [118]. In the past few years, SiNW-FETs and CNT-FETs have been applied for electrophysiological measurements by recording signals from neuron cells and tissues, e.g., recording the electrical signal from a single neuron and cardiomyocyte cells and detecting the released neurotransmitter of CgA from living neurons.

7.4 Summary and Outlooks

Nanomaterial, especially the combination of nanomaterial with other materials or different nanomaterial (such as carbon nanotubes and nanoparticles, quantum dots and nanoparticles) combinations increasing the immobilization amount of bioactive substances and surface active sites, provides good reaction microenvironment, accelerates the electron transfer process, and shortens the response time of signal. The sensitivity, detection range, and repeatability of biosensor have been dramatically enhanced, which broaden the application fields of biosensors. As more and more nanomaterials were discovered and used, biosensors have become a new detection technology. It has broad application prospect in the field of life science and engineering technology.

In the future, the study of novel biosensor design based on nanomaterial can focus on the following aspects:

1. The synthesis of nanomaterial with uniform distribution and obvious physiochemical response, which may play a role in the multi-protein and multi-gene detection simultaneously.
2. The use of nanomaterial for constructing bionic interface, which can be applied to the study of artificial simulation of neural activity.
3. The combination of nanomaterial, microfluidic chip, and ultramicroelectrode technology, which can be applied for real-time monitoring chemical activity of single cell.
4. The combination of nanomaterial and biosensing technology and MEMS technology, which can be utilized to develop biosensors for real-time and on-site detection of complicated real samples. With the deepening of the study, the nanotechnology-based biosensor will play a more important role on the research and exploring of life process and will be widely applied in the areas closely related to people's daily life such as clinical diagnosis, environmental monitoring, and food safety.

References

1. Updike S, Hicks G. The enzyme electrode. *Nature*. 1967;214:986–8.
2. Mohanty SP, Kougiannos E. Biosensors: a tutorial review. *Potentials IEEE*. 2006;25(2):35–40.
3. Higson SP, Reddy SM, Vadgama P. Enzyme and other biosensors: evolution of a technology. *Eng Sci Educ J*. 1994;3(1):41–8.
4. Fraser D. Glucose biosensors: the sweet smell of success. *Med Device Technol*. 1994;5:44–7.
5. Cullum BM, Griffin GD, Miller GH, Vo-Dinh T. Intracellular measurements in mammary carcinoma cells using fiber-optic nanosensors. *Anal Biochem*. 2000;277(1):25–32.
6. Haruyama T. Micro-and nanobiotechnology for biosensing cellular responses. *Adv Drug Deliv Rev*. 2003;55(3):393–401.
7. Chou LY, Ming K, Chan WC. Strategies for the intracellular delivery of nanoparticles. *Chem Soc Rev*. 2011;40(1):233–45.
8. Guo S, Wang E. Noble metal nanomaterials: controllable synthesis and application in fuel cells and analytical sensors. *Nano Today*. 2011;6(3):240–64.
9. Burda C, Chen X, Narayanan R, El-Sayed MA. Chemistry and properties of nanocrystals of different shapes. *Chem Rev*. 2005;105(4):1025–102.
10. Jain PK, Huang X, El-Sayed IH, El-Sayed MA. Noble metals on the nanoscale: optical and photothermal properties and some applications in imaging, sensing, biology, and medicine. *Acc Chem Res*. 2008;41(12):1578–86.
11. Giljohann DA, Seferos DS, Daniel WL, Massich MD, Patel PC, Mirkin CA. Gold nanoparticles for biology and medicine. *Angew Chem Int Ed*. 2010;49(19):3280–94.
12. Kelly KL, Coronado E, Zhao LL, Schatz GC. The optical properties of metal nanoparticles: the influence of size, shape, and dielectric environment. *J Phys Chem B*. 2003;107(3):668–77.
13. Murphy CJ, Sau TK, Gole AM, Orendorff CJ, Gao J, Gou L, Hunyadi SE, Li T. Anisotropic metal nanoparticles: synthesis, assembly, and optical applications. *J Phys Chem B*. 2005;109(29):13857–70.
14. Huang X-J, Choi Y-K. Chemical sensors based on nanostructured materials. *Sensors Actuators B Chem*. 2007;122(2):659–71.
15. Cao YC, Jin R, Mirkin CA. Nanoparticles with Raman spectroscopic fingerprints for DNA and RNA detection. *Science*. 2002;297(5586):1536–40.
16. Nam J-M, Thaxton CS, Mirkin CA. Nanoparticle-based bio-bar codes for the ultrasensitive detection of proteins. *Science*. 2003;301(5641):1884–6.
17. Zhang J, Wang L, Zhang H, Boey F, Song S, Fan C. Aptamer-based multicolor fluorescent gold nanoprobe for multiplex detection in homogeneous solution. *Small*. 2010;6(2):201–4.
18. Jain PK, Huang X, El-Sayed IH, El-Sayed MA. Review of some interesting surface plasmon resonance-enhanced properties of noble metal nanoparticles and their applications to biosystems. *Plasmonics*. 2007;2(3):107–18.
19. Link S, El-Sayed MA. Optical properties and ultrafast dynamics of metallic nanocrystals. *Annu Rev Phys Chem*. 2003;54(1):331–66.
20. Kreibitz U, Vollmer M. *Optical properties of metal clusters*. Berlin: Springer; 1995.
21. Eustis S, El-Sayed MA. Why gold nanoparticles are more precious than pretty gold: noble metal surface plasmon resonance and its enhancement of the radiative and nonradiative properties of nanocrystals of different shapes. *Chem Soc Rev*. 2006;35(3):209–17.
22. Cogley CM, Chen J, Cho EC, Wang LV, Xia Y. Gold nanostructures: a class of multifunctional materials for biomedical applications. *Chem Soc Rev*. 2011;40(1):44–56.
23. Dreaden EC, Alkilany AM, Huang X, Murphy CJ, El-Sayed MA. The golden age: gold nanoparticles for biomedicine. *Chem Soc Rev*. 2012;41(7):2740–79.
24. Daniel M-C, Astruc D. Gold nanoparticles: assembly, supramolecular chemistry, quantum-size-related properties, and applications toward biology, catalysis, and nanotechnology. *Chem Rev*. 2004;104(1):293–346.

25. Ruoff R, Beach D, Cuomo J, McGuire T, Whetten R, Diederich F. Confirmation of a vanishingly small ring-current magnetic susceptibility of icosahedral buckminsterfullerene. *J Phys Chem.* 1991;95(9):3457–9.
26. Hebard A, Rosseinsky M, Haddon R, Murphy D, Glarum S, Palstra T, Ramirez A, Kerton A. Potassium-doped c60. *Nature.* 1991;350:600–1.
27. Geim AK, Novoselov KS. The rise of graphene. *Nat Mater.* 2007;6(3):183–91.
28. Neto AC, Guinea F, Peres N, Novoselov KS, Geim AK. The electronic properties of graphene. *Rev Mod Phys.* 2009;81(1):109.
29. Ferrari AC. Raman spectroscopy of graphene and graphite: disorder, electron–phonon coupling, doping and nonadiabatic effects. *Solid State Commun.* 2007;143(1):47–57.
30. Tombros N, Jozsa C, Popinciuc M, Jonkman HT, Van Wees BJ. Electronic spin transport and spin precession in single graphene layers at room temperature. *Nature.* 2007;448(7153):571–4.
31. Lee C, Wei X, Kysar JW, Hone J. Measurement of the elastic properties and intrinsic strength of monolayer graphene. *Science.* 2008;321(5887):385–8.
32. Balandin AA, Ghosh S, Bao W, Calizo I, Teweldebrhan D, Miao F, Lau CN. Superior thermal conductivity of single-layer graphene. *Nano Lett.* 2008;8(3):902–7.
33. Pereira VM, Neto AC, Peres N. Tight-binding approach to uniaxial strain in graphene. *Phys Rev B.* 2009;80(4):045401.
34. Booth TJ, Blake P, Nair RR, Jiang D, Hill EW, Bangert U, Bleloch A, Gass M, Novoselov KS, Katsnelson MI. Macroscopic graphene membranes and their extraordinary stiffness. *Nano Lett.* 2008;8(8):2442–6.
35. Bunch JS, Verbridge SS, Alden JS, van der Zande AM, Parpia JM, Craighead HG, McEuen PL. Impermeable atomic membranes from graphene sheets. *Nano Lett.* 2008;8(8):2458–62.
36. Leslie-Pelecky DL, Rieke RD. Magnetic properties of nanostructured materials. *Chem Mater.* 1996;8(8):1770–83.
37. Woo K, Hong J, Choi S, Lee H-W, Ahn J-P, Kim CS, Lee SW. Easy synthesis and magnetic properties of iron oxide nanoparticles. *Chem Mater.* 2004;16(14):2814–8.
38. Dias A, Hussain A, Marcos A, Roque A. A biotechnological perspective on the application of iron oxide magnetic colloids modified with polysaccharides. *Biotechnol Adv.* 2011;29(1):142–55.
39. Xiao Y, Patolsky F, Katz E, Hainfeld JF, Willner I. “Plugging into enzymes”: nanowiring of redox enzymes by a gold nanoparticle. *Science.* 2003;299(5614):1877–81.
40. Patolsky F, Weizmann Y, Willner I. Long-range electrical contacting of redox enzymes by SWCNT connectors. *Angew Chem Int Ed.* 2004;43(16):2113–7.
41. Zhao W, Xu J-J, Chen H-Y. Extended-range glucose biosensor via layer-by-layer assembly incorporating gold nanoparticles. *Front Biosci.* 2005;10:1060–9.
42. Zong S, Cao Y, Zhou Y, Ju H. Reagentless biosensor for hydrogen peroxide based on immobilization of protein in zirconia nanoparticles enhanced grafted collagen matrix. *Biosens Bioelectron.* 2007;22(8):1776–82.
43. Zhao J, Zhu X, Li T, Li G. Self-assembled multilayer of gold nanoparticles for amplified electrochemical detection of cytochrome c. *Analyst.* 2008;133(9):1242–5.
44. Pandey P, Upadhyay S. Bioelectrochemistry of glucose oxidase immobilized on ferrocene encapsulated ormosil modified electrode. *Sensors Actuators B Chem.* 2001;76(1):193–8.
45. RetnaáRaj C, KumaráJena B. Efficient electrocatalytic oxidation of NADH at gold nanoparticles self-assembled on three-dimensional sol-gel network, *Chem Commun.* 2005, (15):2005–2007.
46. Welch C, Banks C, Simm A, Compton R. Silver nanoparticle assemblies supported on glassy-carbon electrodes for the electro-analytical detection of hydrogen peroxide. *Anal Bioanal Chem.* 2005;382(1):12–21.
47. Karam P, Halaoui LI. Sensing of H₂O₂ at low surface density assemblies of Pt nanoparticles in polyelectrolyte. *Anal Chem.* 2008;80(14):5441–8.

48. Zhang M, Smith A, Gorski W. Carbon nanotube-chitosan system for electrochemical sensing based on dehydrogenase enzymes. *Anal Chem.* 2004;76(17):5045–50.
49. Luo X-L, Xu J-J, Wang J-L, Chen H-Y. Electrochemically deposited nanocomposite of chitosan and carbon nanotubes for biosensor application. *Chem Commun.* 2005;16:2169–71.
50. Luo X-L, Xu J-J, Du Y, Chen H-Y. A glucose biosensor based on chitosan–glucose oxidase–gold nanoparticles biocomposite formed by one-step electrodeposition. *Anal Biochem.* 2004;334(2):284–9.
51. Zhao W, Sun S-X, Xu J-J, Chen H-Y, Cao X-J, Guan X-H. Electrochemical identification of the property of peripheral nerve fiber based on a biocompatible polymer film via in situ incorporating gold nanoparticles. *Anal Chem.* 2008;80(10):3769–76.
52. Chen X, Wang Y, Zhou J, Yan W, Li X, Zhu J-J. Electrochemical impedance immunosensor based on three-dimensionally ordered macroporous gold film. *Anal Chem.* 2008;80(6):2133–40.
53. Ding L, Hao C, Xue Y, Ju H. A bio-inspired support of gold nanoparticles-chitosan nanocomposites gel for immobilization and electrochemical study of K562 leukemia cells. *Biomacromolecules.* 2007;8(4):1341–6.
54. He P, Hu N. Electrocatalytic properties of heme proteins in layer-by-layer films assembled with SiO₂ nanoparticles. *Electroanalysis.* 2004;16(13-14):1122–31.
55. He P, Hu N, Rusling JF. Driving forces for layer-by-layer self-assembly of films of SiO₂ nanoparticles and heme proteins. *Langmuir.* 2004;20(3):722–9.
56. Luo X-L, Xu J-J, Zhao W, Chen H-Y. Glucose biosensor based on ENFET doped with SiO₂ nanoparticles. *Sensors Actuators B Chem.* 2004;97(2):249–55.
57. Dequaire M, Degrand C, Limoges B. An electrochemical metalloimmunoassay based on a colloidal gold label. *Anal Chem.* 2000;72(22):5521–8.
58. Dai Z, Kawde A-N, Xiang Y, La Belle JT, Gerlach J, Bhavanandan VP, Joshi L, Wang J. Nanoparticle-based sensing of glycan-lectin interactions. *J Am Chem Soc.* 2006;128(31):10018–9.
59. Wang J, Liu G, Merkoçi A. Electrochemical coding technology for simultaneous detection of multiple DNA targets. *J Am Chem Soc.* 2003;125(11):3214–5.
60. Ding L, Cheng W, Wang X, Ding S, Ju H. Carbohydrate monolayer strategy for electrochemical assay of cell surface carbohydrate. *J Am Chem Soc.* 2008;130(23):7224–5.
61. Xu J-J, Zhao W, Luo X-L, Chen H-Y. A sensitive biosensor for lactate based on layer-by-layer assembling MnO₂ nanoparticles and lactate oxidase on ion-sensitive field-effect transistors. *Chem Commun.* 2005;6:792–4.
62. Luo X-L, Xu J-J, Zhao W, Chen H-Y. Ascorbic acid sensor based on ion-sensitive field-effect transistor modified with MnO₂ nanoparticles. *Anal Chim Acta.* 2004;512(1):57–61.
63. Xu J-J, Luo X-L, Du Y, Chen H-Y. Application of MnO₂ nanoparticles as an eliminator of ascorbate interference to amperometric glucose biosensors. *Electrochem Commun.* 2004;6(11):1169–73.
64. Bai Y-H, Du Y, Xu J-J, Chen H-Y. Choline biosensors based on a bi-electrocatalytic property of MnO₂ nanoparticles modified electrodes to H₂O₂. *Electrochem Commun.* 2007;9(10):2611–6.
65. Mirkin CA, Letsinger RL, Mucic RC, Storhoff JJ. A DNA-based method for rationally assembling nanoparticles into macroscopic materials. *Nature.* 1996;382:607–9.
66. Elghanian R, Storhoff JJ, Mucic RC, Letsinger RL, Mirkin CA. Selective colorimetric detection of polynucleotides based on the distance-dependent optical properties of gold nanoparticles. *Science.* 1997;277(5329):1078–81.
67. Rosi NL, Mirkin CA. Nanostructures in biodiagnostics. *Chem Rev.* 2005;105(4):1547–62.
68. Xia F, Zuo X, Yang R, Xiao Y, Kang D, Vallée-Bélisle A, Gong X, Yuen JD, Hsu BB, Heeger AJ. Colorimetric detection of DNA, small molecules, proteins, and ions using unmodified gold nanoparticles and conjugated polyelectrolytes. *Proc Natl Acad Sci.* 2010;107:10837–41.
69. Nie S, Emory SR. Probing single molecules and single nanoparticles by surface-enhanced Raman scattering. *Science.* 1997;275(5303):1102–6.

70. Li H, Rothberg L. Colorimetric detection of DNA sequences based on electrostatic interactions with unmodified gold nanoparticles. *Proc Natl Acad Sci U S A*. 2004;101(39):14036–9.
71. Thanh NTK, Rosenzweig Z. Development of an aggregation-based immunoassay for anti-protein A using gold nanoparticles. *Anal Chem*. 2002;74(7):1624–8.
72. Huang H, He C, Zeng Y, Xia X, Yu X, Yi P, Chen Z. A novel label-free multi-throughput optical biosensor based on localized surface plasmon resonance. *Biosens Bioelectron*. 2009;24(7):2255–9.
73. Wang X, Li Y, Wang H, Fu Q, Peng J, Wang Y, Du J, Zhou Y, Zhan L. Gold nanorod-based localized surface plasmon resonance biosensor for sensitive detection of hepatitis B virus in buffer, blood serum and plasma. *Biosens Bioelectron*. 2010;26(2):404–10.
74. Parab HJ, Jung C, Lee J-H, Park HG. A gold nanorod-based optical DNA biosensor for the diagnosis of pathogens. *Biosens Bioelectron*. 2010;26(2):667–73.
75. John H. Fluorescence properties of gold nanorods and their application for DNA biosensing. *Chem Commun*. 2005;31:3924–6.
76. Gou X-C, Liu J, Zhang H-L. Monitoring human telomere DNA hybridization and G-quadruplex formation using gold nanorods. *Anal Chim Acta*. 2010;668(2):208–14.
77. Qian X, Zhou X, Nie S. Surface-enhanced Raman nanoparticle beacons based on bioconjugated gold nanocrystals and long range plasmonic coupling. *J Am Chem Soc*. 2008;130(45):14934–5.
78. Driskell JD, Lipert RJ, Porter MD. Labeled gold nanoparticles immobilized at smooth metallic substrates: systematic investigation of surface plasmon resonance and surface-enhanced Raman scattering. *J Phys Chem B*. 2006;110(35):17444–51.
79. Klimov V, Mikhailovsky A, Xu S, Malko A, Hollingsworth J, Leatherdale C, Eisler H-J, Bawendi M. Optical gain and stimulated emission in nanocrystal quantum dots. *Science*. 2000;290(5490):314–7.
80. Bruchez M, Moronne M, Gin P, Weiss S, Alivisatos AP. Semiconductor nanocrystals as fluorescent biological labels. *Science*. 1998;281(5385):2013–6.
81. Goldman ER, Balighian ED, Mattoussi H, Kuno MK, Mauro JM, Tran PT, Anderson GP. Avidin: a natural bridge for quantum dot-antibody conjugates. *J Am Chem Soc*. 2002;124(22):6378–82.
82. Goldman ER, Clapp AR, Anderson GP, Uyeda HT, Mauro JM, Medintz IL, Mattoussi H. Multiplexed toxin analysis using four colors of quantum dot fluororeagents. *Anal Chem*. 2004;76(3):684–8.
83. Mitchell GP, Mirkin CA, Letsinger RL. Programmed assembly of DNA functionalized quantum dots. *J Am Chem Soc*. 1999;121(35):8122–3.
84. Han M, Gao X, Su JZ, Nie S. Quantum-dot-tagged microbeads for multiplexed optical coding of biomolecules. *Nat Biotechnol*. 2001;19(7):631–5.
85. Patolsky F, Gill R, Weizmann Y, Mokari T, Banin U, Willner I. Lighting-up the dynamics of telomerization and DNA replication by CdSe-ZnS quantum dots. *J Am Chem Soc*. 2003;125(46):13918–9.
86. Swathi R, Sebastian K. Distance dependence of fluorescence resonance energy transfer. *J Chem Sci*. 2009;121(5):777–87.
87. Swathi R, Sebastian K. Excitation energy transfer from a fluorophore to single-walled carbon nanotubes. *J Chem Phys*. 2010;132(10):104502.
88. Wang S, Humphreys ES, Chung S-Y, Delduco DF, Lustig SR, Wang H, Parker KN, Rizzo NW, Subramoney S, Chiang Y-M. Peptides with selective affinity for carbon nanotubes. *Nat Mater*. 2003;2(3):196–200.
89. Zheng M, Jagota A, Semke ED, Diner BA, McLean RS, Lustig SR, Richardson RE, Tassi NG. DNA-assisted dispersion and separation of carbon nanotubes. *Nat Mater*. 2003;2(5):338–42.
90. Yang R, Jin J, Chen Y, Shao N, Kang H, Xiao Z, Tang Z, Wu Y, Zhu Z, Tan W. Carbon nanotube-quenched fluorescent oligonucleotides: probes that fluoresce upon hybridization. *J Am Chem Soc*. 2008;130(26):8351–8.

91. Sutton DJ, Tchounwou PB, Ninashvili N, Shen E. Mercury induces cytotoxicity and transcriptionally activates stress genes in human liver carcinoma (HepG2) cells. *Int J Mol Sci.* 2002;3(9):965–84.
92. Li F, Huang Y, Yang Q, Zhong Z, Li D, Wang L, Song S, Fan C. A graphene-enhanced molecular beacon for homogeneous DNA detection. *Nanoscale.* 2010;2(6):1021–6.
93. Dong H, Gao W, Yan F, Ji H, Ju H. Fluorescence resonance energy transfer between quantum dots and graphene oxide for sensing biomolecules. *Anal Chem.* 2010;82(13):5511–7.
94. Lu CH, Yang HH, Zhu CL, Chen X, Chen GN. A graphene platform for sensing biomolecules. *Angew Chem.* 2009;121(26):4879–81.
95. Jhaveri SD, Kirby R, Conrad R, Maglott EJ, Bowser M, Kennedy RT, Glick G, Ellington AD. Designed signaling aptamers that transduce molecular recognition to changes in fluorescence intensity. *J Am Chem Soc.* 2000;122(11):2469–73.
96. Chang H, Tang L, Wang Y, Jiang J, Li J. Graphene fluorescence resonance energy transfer aptasensor for the thrombin detection. *Anal Chem.* 2010;82(6):2341–6.
97. Wen Y, Xing F, He S, Song S, Wang L, Long Y, Li D, Fan C. A graphene-based fluorescent nanoprobe for silver (I) ions detection by using graphene oxide and a silver-specific oligonucleotide. *Chem Commun.* 2010;46(15):2596–8.
98. Curie J, Curie P. An oscillating quartz crystal mass detector. *Rendu.* 1880;91:294–7.
99. Wang H, Wang C, Lei C, Wu Z, Shen G, Yu R. A novel biosensing interfacial design produced by assembling nano-Au particles on amine-terminated plasma-polymerized films. *Anal Bioanal Chem.* 2003;377(4):632–8.
100. Jin XY, Jin XF, Ding YJ, Jiang JH, Shen GL, Yu RQ. A piezoelectric immunosensor based on agglutination reaction with amplification of silica nanoparticles. *Chin J Chem.* 2008;26(12):2191–6.
101. Wang H, Zhang Y, Yan B, Liu L, Wang S, Shen G, Yu R. Rapid, simple, and sensitive immunoagglutination assay with SiO₂ particles and quartz crystal microbalance for quantifying *Schistosoma japonicum* antibodies. *Clin Chem.* 2006;52(11):2065–71.
102. Kim TH, Lee SH, Lee J, Song HS, Oh EH, Park TH, Hong S. Single-carbon-atomic-resolution detection of odorant molecules using a human olfactory receptor-based bioelectronic nose. *Adv Mater.* 2009;21(1):91–4.
103. Star A, Tu E, Niemann J, Gabriel J-CP, Joiner CS, Valcke C. Label-free detection of DNA hybridization using carbon nanotube network field-effect transistors. *Proc Natl Acad Sci U S A.* 2006;103(4):921–6.
104. Goldsmith BR, Coroneus JG, Khalap VR, Kane AA, Weiss GA, Collins PG. Conductance-controlled point functionalization of single-walled carbon nanotubes. *Science.* 2007;315(5808):77–81.
105. Sorgenfrei S, Chiu C-y, Gonzalez Jr RL, Yu Y-J, Kim P, Nuckolls C, Shepard KL. Label-free single-molecule detection of DNA-hybridization kinetics with a carbon nanotube field-effect transistor. *Nat Nanotechnol.* 2011;6(2):126–32.
106. Mohanty N, Berry V. Graphene-based single-bacterium resolution biodevice and DNA transistor: interfacing graphene derivatives with nanoscale and microscale biocomponents. *Nano Lett.* 2008;8(12):4469–76.
107. Dong X, Shi Y, Huang W, Chen P, Li LJ. Electrical detection of DNA hybridization with single-base specificity using transistors based on CVD-grown graphene sheets. *Adv Mater.* 2010;22(14):1649–53.
108. Ohno Y, Maehashi K, Matsumoto K. Label-free biosensors based on aptamer-modified graphene field-effect transistors. *J Am Chem Soc.* 2010;132(51):18012–3.
109. Agarwal S, Zhou X, Ye F, He Q, Chen GC, Soo J, Boey F, Zhang H, Chen P. Interfacing live cells with nanocarbon substrates. *Langmuir.* 2010;26(4):2244–7.
110. Sudibya HG, He Q, Zhang H, Chen P. Electrical detection of metal ions using field-effect transistors based on micropatterned reduced graphene oxide films. *ACS Nano.* 2011;5(3):1990–4.

111. Chen K-I, Li B-R, Chen Y-T. Silicon nanowire field-effect transistor-based biosensors for biomedical diagnosis and cellular recording investigation. *Nano Today*. 2011;6(2):131–54.
112. Cui Y, Wei Q, Park H, Lieber CM. Nanowire nanosensors for highly sensitive and selective detection of biological and chemical species. *Science*. 2001;293(5533):1289–92.
113. Movileanu L, Howorka S, Braha O, Bayley H. Detecting protein analytes that modulate transmembrane movement of a polymer chain within a single protein pore. *Nat Biotechnol*. 2000;18(10):1091–5.
114. Zheng G, Gao XP, Lieber CM. Frequency domain detection of biomolecules using silicon nanowire biosensors. *Nano Lett*. 2010;10(8):3179–83.
115. Hahn J-i, Lieber CM. Direct ultrasensitive electrical detection of DNA and DNA sequence variations using nanowire nanosensors. *Nano Lett*. 2004;4(1):51–4.
116. Zhang G-J, Zhang L, Huang MJ, Luo ZHH, Tay GKI, Lim E-JA, Kang TG, Chen Y. Silicon nanowire biosensor for highly sensitive and rapid detection of Dengue virus. *Sensors Actuators B Chem*. 2010;146(1):138–44.
117. Zhang G-J, Chua JH, Chee R-E, Agarwal A, Wong SM. Label-free direct detection of MiRNAs with silicon nanowire biosensors. *Biosens Bioelectron*. 2009;24(8):2504–8.
118. Rutten WL. Selective electrical interfaces with the nervous system. *Annu Rev Biomed Eng*. 2002;4(1):407–52.