Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org
Review

# Nano-biosensors in cellular and molecular biology

Sajad Moradi[1], Salar Khaledian[1, 2], Mohadese Abdoli[1], Mohsen Shahlaei[1], Danial Kahrizi[1, 2*]

[1] Nano Drug Delivery Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran
[2] Department of Nano Biotechnology, Razi University, Kermanshah, Iran

**Correspondence to:** dkahrizi@yahoo.com

**Received** February 26, 2018; **Accepted** April 18, 2018; **Published** April 30, 2018

**Doi:** http://dx.doi.org/10.14715/cmb/2018.64.5.14



**Abstract:** Detection and quantification of various biological and non-biological species today is one of the most important pillars of all experimental sciences, especially sciences related to human health. This may apply to a chemical in the factory wastewater or to identify a cancer cell in a person's body, it may be apply to trace a useful industrial microorganism or human or plant pathogenic microorganisms. In this regard, scientists from various sciences have always striven to design and provide tools and techniques for identifying and quantifying as accurately as possible to trace various analyte types with greater precision and specificity. Nano science, which has flourished in recent years and is nowadays widely used in all fields of science, also has a unique place in the design and manufacture of sensors and this, in addition to the new and special characteristics of nanoparticles, is due to the ability of nano-devices to penetrate into very tiny places to track the species. On the other hand, due to the high specificity of biological molecules in identifying and connecting to their receptors that have evolved over millions of years, Scientists are now trying to design hybrid devices using nano science and biology, called Nano-biosensors So that they can trace and quantify target molecules in very small amounts and in inaccessible places, such as within the organs and even the cells.

***Key words:*** Electrochemical sensor; Bioimaging piezoelectric; Surface Plasmon Resonance; Fluorescence sensor.

## Background

A Nano-biosensor is a Nano-scale device that can accurately determine the presence and quantity of an analyte by mediating a biological molecule. The biomaterial is attached to the target molecule, and this connection produces a signal depending on the type of sensor. These signals are then received by sensors with a high sensitivity and transmitted to output after being converted into understandable signals for humans. Depending on the biological material used, sensor type, and their mechanisms of detection, Nano-biosensors are categorized in different ways (1-3). The biomaterial can be a nucleic acid aptamer to introduce a kind of biosensors called aptasensor (4), or an antibody to be called as immunosensor (5). Nano-biosensors with enzymes have also been studied in abundance and some of them have been commercialized to detect glucose (6).

The production signal can also be electrical or optical (7, 8). Electrochemical sensors combine oxidation reactions at the electrode surface with changes in the current passing through the electrode, and these electrical signals, generate current or resistance variations. These redox reactions, in turn, can be produced by own analyte redox activity, or by the user's added electro active molecule (9).

There are several types of optical sensors (7): In fluorosensors, changes in the intensity of light emission from an existing fluorophore molecule at the presence of quencher or enhancer analyte, are recorded and investigated quantitatively. Various molecules, biologic or chemical, which can be light-emitting-light-stimulated, can be used as a fluorophore in these types of sensors (10). Surface Plasmon Resonance (SPR) sensors, which are the result of hybrid electron and optical properties of some metallic nanostructures such as gold or silver, have also recently been used extensively in various Nano-biosensors. In this category of sensors, the high sensitivity of the surface frequency Plasmon's of nanostructures to environmental changes in mass or refractive index is used to generate optical signals (11).

A combination of mechanic and electric is used in sensors based on the piezoelectric properties of some materials for diagnostic sciences. In these sensors, the presence of the analyte causes very fine changes in the piezoelectric cantilever, and these changes cause pressure and deformation of the cantilever in a particular direction. This change leads to alteration in frequencies or the generation of traceable electrical signals (12). Research in cellular and molecular biology requires specials tools and information around all components in this area includes analyte, receptor, sensor, signal conversion-transduction and so on (13, 14). This review discusses the latest works on the provision of Nano-biosensors for diagnostic use in cellular and molecular science.

## Electrical nano-biosensors

In this type of sensor, the interaction between the analyte and the receptor produces a very weak electrical signal which the signal amplifies, and converts by the detector. Two major types of these biosensors are commonly used: electrochemical and piezoelectric sensors

and here will be described in details.

***Electrochemical sensors***

The basis of the production of an electrical signal in these types of sensors is to carry out redox-electron transfer reactions at a distance of up to about 200 angstrom from the surface of the electrode (9). Types of electrodes used in these sensors include gold, carbon and glassy carbon, which are capable of varying surface modifications and can be used to detect various types of the analyte. The receptors are attached to the electrode surface and, due to the binding of the analyte molecule to the receptor; the electro active material comes to the surface of the electrode and based on the method of analyzing is measured by changes in current, voltage or impedance (15).

Antibodies are very precise and specific biological structures that are made in animals against foreign antigens and are able to detect them with great care (16). This unique strength and specificity of antibodies in binding to target molecule has made them one of the most widely used qualitative and quantitative diagnostic tools, and today the vast majority of reliable and commercially available diagnostic kits are immunosensor (17). Electrochemical-based immunosensors can be very sensitive than those based on optical detectors. It is easy to attach a specific antibody against the desired analyte on the electrode surface and quantify the analyte using each one of the amperometry, voltammetry or impedance methods (18). As an example of such sensors, we can point to the work were done by Rebecca et al. for detection of cancer cells by targeting the fibroblastic growth factor4 in the cell extract. In this research, the sandwich method has been used to enhance the specificity of analyte identification. The hydroquinone-hydrogen peroxide-peroxidase has been used for conducting a redox reaction, and on the other hand, magnetic nanoparticles have been used to Bring the system to the electrode surface. One of the antibodies is added to the surface of the magnetic nanoparticles. After binding of the target protein, another antibody, -labelled with the enzyme peroxidase- attaches to the other side of the protein so that it can be swirled by a sandwich. Then, by applying the magnetic field, the whole system is brought to the electrode surface so that the redox reactions are carried out at the electrode surface and generate the desired electrical signal (Fig 1A). This method is capable of detecting concentrations of 48 pg/ml of the protein (19).

Some of the aptamers, like the AS1411, have the potential to form G-quarter, and these structures have a lot of tending to nucleolin protein. The nucleolin protein in most cancers is over expressed on the surface of the plasma membrane. This difference in nucleolin expression can be used as the basis for diagnosis and differentiation between normal and cancerous cells. The aptamer is connected to the surface of electrodes using covalent interfaces and links, and in the presence of potassium ions, it forms the G-quarter structures. Then, by connecting the cancer cells to the surface of the electrode with an aptamer manner, the electrical conductivity of the surface is reduced and this reduction is investigated using the impedance method (Fig 1B). Due to its simplicity and accessibility and its good penetration coefficient, the ferrocyanide considered electro active species (20).

The hybridization of two complementary elements of nucleic acids to each other is also highly specific (21). The desire for some electro active molecules such as Hoechst 3224, methylene blue, ethidium bromide, or some drugs such as daunorubicin and doxorubicin for electrostatic bonding, grooved vision, or intercalation into nucleic acid can be used well to design label free Nano-biosensors (21). Micro-RNAs are sequences of 17 to 25 nucleotides that cells use as one of the ways to regulate gene expression. Depending on the genes under control, each micro-RNA can be closely related to a specific category of diseases and genetic abnormalities, or some cancers. Due to the low concentration of these molecules in the cell, their identification and quantification with the usual methods such as Northern-Blot, Reverse-PCR etc. are extremely difficult or even impossible. Yi et al. using the hemin molecule tendency to DNA-RNA hybrids prepared an electrochemical biosensor to quantify the micro-RNA by the Voltammetric method. The complementary micro-array sequence is connected to the gold electrode surface. This sequence is connected in the presence of micro-RNA, and this coupling induces electromechanical hemin to the electrode surface to generate the desired electrical signal. This high-resolution sensor has been able to quantify concentrations of 0.1 mM of the micro-RNA (22).

The tendency of the electro active methylene blue to DNA G-quarters formed by the activity of the telomerase enzyme is used to detect the proper activity of this enzyme. The enzyme is capable of transforming the G rich DNA sequence into G-quarter structures in the presence of potassium ions. In the presence of active and healthy telomerase, the enzyme is attached to the target sequence and converts the terminal DNA to G-quarter. In the absence of enzyme, methylene blue has a slight tendency to DNA and greatly penetrates the surface of indium tin oxide electrode and produces a strong Voltammetric signal. As the telomerase activity begins, G-quarter structures develop rapidly and, due to the high methylene blue tendency to this structure, its penetration to the electrode surface is greatly reduced. This results in a sharp decrease in the Voltammetric current of the electrode (Fig 1C). This method requires surface modifications at neither the electrode surface nor the target molecule, and is very fast and efficient. The detection limit of this method is up to 3 cancerous cells (23).

***Piezoelectric nano-biosensors***

The basis of the signal generation in these types of sensors is the change in the frequency created by the change in the mass due to the connection of the analyte to the receptor in the surface of piezoelectric Nano-crystalline. This change in frequency makes it possible to detect voltage changes (12).

Piezoelectric biosensors have been used to detect breast cancer cells. In this method, a gold layer was deposited on a quartz crystal, and then a low molecular weight chitosan, functionalized by folic acid, was fixed onto the gold layer. Folic acid can specifically absorb the breast cancer cells that contain folate receptors to the electrode surface. The minimum detection rate for MCF-7 cancer cells was 430 cells per ml in this method

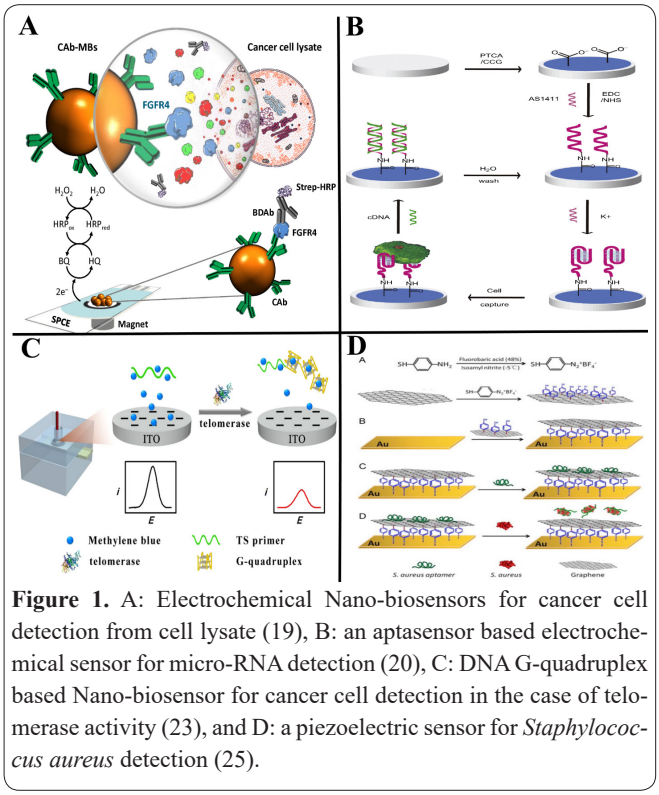


**Figure 1.** A: Electrochemical Nano-biosensors for cancer cell detection from cell lysate (19), B: an aptasensor based electrochemical sensor for micro-RNA detection (20), C: DNA G-quadruplex based Nano-biosensor for cancer cell detection in the case of telomerase activity (23), and D: a piezoelectric sensor for *Staphylococcus aureus* detection (25).

(24).

Also, *Staphylococcus aureus* has been detected by piezoelectric biosensors. Graphene was bonded to the gold electrode surface using chemical methods in this biosensor. Gold electrodes are connected to quartz crystal electrodes. The specific aptamer of *Staphylococcus aureus* was bound to the surface of graphene using Pi-stacking forces. Because of the high specificity between the aptamer and *Staphylococcus aureus* bacteria, when the bacterium is present, they are attached to the aptamers and separated it from the graphene surface (Fig 1D). By doing this, the electrical parameters of the electrode surface are changed and the piezoelectric biosensor frequency is changed. The diagnostic limit of bacteria in this method was 41 CFU per ml (25).

## Optical nano-biosensors

This category of sensors is characterized by changing the wavelength or light intensity due to the stable interaction of the ligand and receptor. The light used may be in ultraviolet, visible or infrared, and the generated signal may be emitting, absorbent or reflective and so on.

### *Sensors based on absorption*

In this type of sensor, a marker molecule called the chromophore plays a major role. The chromophore is usually a part of the receptor, or an external molecule that links to the receptor (7). Determining the amount of amino acids as building blocks of proteins, as well as other functions in vital systems such as the nervous system is very important. Shang et al. used different derivatives of the azo group to track and quantify the amount of L-arginine based on location shift and intensity changes of ultraviolet light absorption. In this study, using 5 different 5-X-Salicylaldehyde including: 2-Azo-Naphthalene, O-Nitrophenylazo, m-Carboxyl-phenylazo, p-Nitro-phenylazo and p-Sulfonic-Phenylazo, Nanoparticles were synthesized and their specificity and sensitivity in the identification and quantification of L-arginine were studied. The nanoparticles derived from the carboxyl azo compound exhibited the best results, and were able to separate the 5 mM concentrations of this amino acid from other natural amino acids with high specificity (26).

Changes in the infrared peak location for some functional groups have been used for detection of *Escherichia coli* bacteria. Pal Singh and co-workers were detected the binding of *E. coli* to the surface of the Nano-biosensor with high sensitivity and specificity using a specific anti-beta-galactosidase antibody coated to the graphene oxides plates (27).

Raman-based biosensors are very important due to the easy preparation steps and also the high sensitivity due to the use of plasmonic nanostructures in their design. This biosensor has been used for the label free detection of glucose. In this method, the gold Nano-stares / $SiO_2$ were functionalized with the glucose oxidase enzyme (this enzyme oxidize glucose and produce $H_2O_2$). Under the influence of laser radiation, $H_2O_2$ molecules can produce a strong Surface Enhanced Raman Scattering (SERS) signal. Using this method, it is possible to detect 25 µM to 25 mM of glucose with a limit detection of 16 µM. In addition, it has been shown that the presence of ascorbic acid and uric acid with glucose is not a disturbance in the detection of glucose, and it has been suggested that this biosensor can also be used for salivary specimens (28).

### *Sensors based on the light emission*

This type of Nano-biosensors has been studied extensively in various reports due to its high sensitivity and ease of doing work. The generated signal in this Nano-biosensor is electromagnetic waves that are due to optical stimulation, which may be exacerbated or reduced by binding of the analyte to the receiver. The fluorophore is a light transmitter in this type of sensor and may be organic or inorganic (29). Accordingly, Nano-biosensors based on light diffusion have been used to detect intracellular micro-RNA. A structure called nano-flare is designed to detect intracellular micro-RNA. Nanoflare contains an antisense DNA (a diagnostic sequence) that is attached to the surface of gold nanoparticles using thiol groups. The reporter flare sequence that coupled to a fluorophore material (cyanine 5) is hybridization to a part of the diagnostic sequence; in this case. the fluorescence of the fluorophore material is turned off. When micro-RNA targets exist in the environment and are hybridized with the diagnostic sequence, the reporter flare is isolated and its fluorescence is detectable (Fig 2A). It is also known that using this system, Circulating Tumour Cells (CTCs) are detectable (30).

Recently, two-dimensional nanostructures such as graphene oxide, molybdenum disulfide, and black phosphorus have been used in the design of biosensors based on light emission (31-35). Molybdenum disulfide Nano-sheets has a higher ability to quenching fluorescence than nanoparticles of graphene oxide. With this ability of molybdenum disulfide Nano-sheets a biosensor was designed that is used for detection of Prostate Specific Antigen (PSA). In this method, when the aptamer

containing the fluorophore molecule (FAM) is exposed to samples containing PSA, it forms a rigid shape and provides a poor interaction with molybdenum disulfide Nano sheets, and the fluorescence does not quench and the sensor is turned ON. But when the PSA is absence in the sample, the aptamers contain a fluorophore molecule is flexible and attached to molybdenum disulfide Nano sheets and the fluorescence quenched and the sensor is turned OFF. This method has a high sensitivity and the limit of detection of PSA is 0.2 ng/ml (36).

Black phosphorus nanoparticles have been used as a novel fluorophore agent in the design of biosensors to detect a nucleic acid. In this biosensor, a fluorescence quenching agent (dabcil) connects to the end of the 3 molecules of the probe. In the absence of the target molecule, the probe-containing dabcil molecule attaches with hydrophobic forces to the surface of the black phosphorus nanoparticles and turns off fluorescence. However, in the presence of the target molecule, it has a hybrid with a probe molecule containing a dabcil, a double stranded DNA was formed and the tendency of binding to black phosphorus nanoparticles is reduced, in which case the fluorescence of the black phosphorus nanoparticles can be measured. The minimum amount of DNA detected by this method is 5.9 pMoles and it has been proposed that in future, black phosphorus nanoparticles can be used to detect proteins, enzymes and etc. based on light emission (37).

Quantum dots are semiconductor crystals that are typically below 10 nm, which have unique optical properties, and are therefore used for detection of the different biomolecule. Shamsipour and colleagues designed biosensors using Thioglycolic Acid-CdTe quantum dots to detect human papilloma virus. An oligo nucleotide probe complementing to the human papillomavirus DNA is attached to the quantum dot and another probe is attached to the cyanine fluorophore molecule. When the target DNA is present, it forms a hybrid whit probe and the fluorophore is placed next to the quantum dot. By quantum dot stimulation as a donor, the Fluorescence Resonance Energy Transfer (FRET) signal of the fluorophore molecule is measurable as the acceptor (Fig 2B). The limit of virus DNA detection with this biosensor is 200 pM and the detection range is between 0.2 and 50 nM (38).

The molecular beacons are the stem-loop oligonucleotides that are attached to the fluorophore in one end and the quencher in another end that can be used as a fluorescence probe to detect biomolecules. The molecular beacons are used to design biosensors for detection of micro-RNA. In this biosensor, the molecules beacons, with a fluorescence dye in one end and the pyrine in other end, were immobilized on graphene quantum dot, in which case there is a biosensor in ON state because of the pyrine and the fluorophore molecule are in the close proximity (pyrine is a fluorescence stimulating molecule). But when the micro-RNA target is present, the pyrine molecule and the fluorophore molecule are separated and the biosensor becomes OFF state (Fig 2C). The biosensor was capable of detecting micro-RNA in the range of 0.1 to 200 nM, with a minimum trace of 100 pMoles (39).

### *Sensors based on surface plasmon resonance*

Changes in the optical refractive index of the surrounding surface plasmons can lead to a change in their frequency. This frequency change is the basis for producing a signal in this type of biosensor (11). In metallic nanoparticles such as gold, silver, platinum, etc., the electrons of surface-layer atoms do not belong to their atoms, and there is an electron cloud around the entire nanoparticle. This electron cloud is called Surface plasmons and their density variations on the surface of the nanoparticle, depending on the type of metal, have their own frequency (40). These plasmons can be intensified by absorbing electromagnetic waves of their own frequency and this absorption either in the form of a change in wavelength, or as a change in the angle of radiation, generates the desired signal (41). Surface plasmon resonance biosensors have been used to detect a prostate specific antigen (PSA). In this method, first, on a gold chip, the PSA specific antibodies molecules (capture antibody) were fixed. On the other hand, gold nanoparticles in sizes of 20 and 40 nm were functionalized by reporter antibodies, which are specific to the PSA also. By attaching the PSA molecule to the receptor antibodies, the gold nanoparticles functionalized with the secondary antibody attached to PSA and increased the refractive index of the light at the biosensor surface, thereby securing the PSA molecules in the blood serum is diagnosed (Fig 2D). The limit of detection with this method is 0.39 ng per ml in total serum, which is much lower than the threshold for detecting prostate cancer of 2.5 ng per ml ( (42).

To further increase the susceptibility of SPR-based biosensors, silver is used alongside gold. For this purpose, for detection of mouse IgG a biosensor was designed that combine two element (gold and silver). First, a thin film of gold is placed, and then nanocubes of silver-chitosan composites are coated on the golden film. After that, the antibody against the IgG of the mouse is stabilized on the desired structure and the biosensor is made. Using this biosensor, the concentration range between 0.6 to 40 µg/ml of mouse IgG is detectable, which is four times lower than other such SPR-based biosensors (43).

SPR-based biosensors have been used to detect

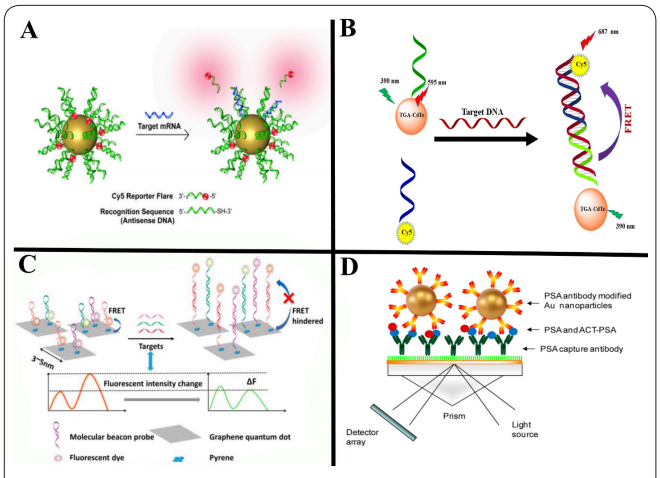


**Figure 2.** A: Nanoflare for Mi-RNA detection (30), B: fluorescence Nano-biosensor based on FRET phenomenon for papilloma virus detection (38), C: molecular beacon used for Mi-RNA detection (39), and D: SPR based Nano-biosensor for PSA (42).

human and plant pathogenic bacteria. Khaledian and co-workers used this method to detect *Ralstonia solanacearum*. At first, gold nanoparticles (with the size of 20 nm) were functionalized by using a thiolated bacterial specific probe, and then the extracted DNA from the bacteria was detected. By adding DNA target of the bacteria, gold nanoparticles are attached to a DNA molecule by a probe, which prevents the accumulation of nanoparticles and the degradation of their plasmonic properties by adding acid. The limit of detection for a designed biosensor was 15 ng DNA extracted from the target bacteria. The results also showed that this high-specific biosensor was able to detect *Ralstonia solanacearum* versus other soil-borne bacteria. One of the main advantages of this SPR based biosensor is that it can be apply visually without needing to advanced instruments and may be applicable for tracking different biomolecules (44).

## Conclusion

In this review, we attempt to introduce some of the most recent diagnostic methods used to detect and identify cells and biological molecules. Many diagnostic methods, depending on the type of detector, receptor or signaling, have been studied by researchers from different research groups aver the world. Different types of sensors and bio-sensors are available for each target molecule. The choice of method for tracking any analyte may vary depending on its physical and chemical properties. So the first step in providing a sensor for detection of any molecule involves a detailed study of the target molecule. The target molecule may have one of the optical or electrical properties and it is more logical and easy to design and prepare a sensor that matches that feature. Also Depending on the capabilities exist in each laboratory, it may also be possible to provide a specific type of Nano-sensor. Finally, using the information provided in this paper, anyone can gain initial idea of design, and prepare the Nano-biosensor to detect the desired analyte and use it in the construction of the sensor.

## References

1. Salvati E, Stellacci F, Krol S. Nanosensors for early cancer detection and for therapeutic drug monitoring. Nanomedicine 2015; 10(23): 3495-3512.
2. Carneiro KM, Greschner AA. Recent advances in self-assembled DNA nanosensors. American Journal of Nano Research and Application 2015; 3(1-1): 1-7.
3. Mehrotra P. Biosensors and their applications–A review. Journal of oral biology and craniofacial research 2016; 6(2): 153-159.
4. Zhao W-W, Xu J-J, Chen H-Y. Photoelectrochemical aptasensing. TrAC Trends in Analytical Chemistry 2016; 82: 307-315.
5. Lim SA, Ahmed MU. Electrochemical immunosensors and their recent nanomaterial-based signal amplification strategies: a review. RSC Advances 2016; 6(30): 24995-25014.
6. Tian K, Prestgard M, Tiwari A. A review of recent advances in nonenzymatic glucose sensors. Materials Science and Engineering: C 2014; 41: 100-118.
7. Kneipp J, Kneipp H, Wittig B, Kneipp K. Novel optical nanosensors for probing and imaging live cells. Nanomedicine: Nanotechnology, Biology and Medicine 2010; 6(2): 214-226.
8. Windmiller JR, Wang J. Wearable electrochemical sensors and biosensors: a review. Electroanalysis 2013; 25(1): 29-46.
9. Grieshaber D, MacKenzie R, Voeroes J, Reimhult E. Electrochemical biosensors-sensor principles and architectures. Sensors 2008; 8(3): 1400-1458.
10. Shcherbakova DM, Shemetov AA, Kaberniuk AA, Verkhusha VV. Natural photoreceptors as a source of fluorescent proteins, biosensors, and optogenetic tools. Annual review of biochemistry 2015; 84: 519-550.
11. Singh P. SPR biosensors: Historical perspectives and current challenges. Sensors and Actuators B: Chemical 2016; 229: 110-130.
12. Skládal P. Piezoelectric biosensors. TrAC Trends in Analytical Chemistry 2016; 79: 127-133.
13. Esmaeili F, Ghaheri M, Kahrizi D et al. Effects of various glutamine concentrations on gene expression and steviol glycosides accumulation in Stevia rebaudiana Bertoni. Cellular and molecular biology (Noisy-le-Grand, France) 2018; 64(2): 1-5.
14. Beheshti AAA, Kahrizi D, Ahmadvand A, Bashiri H, Fakhri R. Development of PCR primer systems for amplification of 16S-rDNA to detect of Thiobacillus spp. Cellular and molecular biology (Noisy-le-Grand, France) 2017; 63(11): 37-41.
15. Zhu C, Yang G, Li H, Du D, Lin Y. Electrochemical sensors and biosensors based on nanomaterials and nanostructures. Analytical chemistry 2014; 87(1): 230-249.
16. Mozes E, McDevitt HO, Jaton J-C, Sela M. The genetic control of antibody specificity. Journal of Experimental Medicine 1969; 130(6): 1263-1278.
17. Liu BL, Saltman MA. Immunosensor Technology: Historical Perspective and Future Outlook. Laboratory medicine 2015; 27(2): 109-115.
18. Kokkinos C, Economou A, Prodromidis MI. Electrochemical immunosensors: Critical survey of different architectures and transduction strategies. TrAC Trends in Analytical Chemistry 2016; 79: 88-105.
19. Torrente-Rodríguez RM, Montiel VR-V, Campuzano S et al. Electrochemical sensor for rapid determination of fibroblast growth factor receptor 4 in raw cancer cell lysates. PloS one 2017; 12(4): e0175056.
20. Feng L, Chen Y, Ren J, Qu X. A graphene functionalized electrochemical aptasensor for selective label-free detection of cancer cells. Biomaterials 2011; 32(11): 2930-2937.
21. Naef F, Lim DA, Patil N, Magnasco M. DNA hybridization to mismatched templates: a chip study. Physical Review E 2002; 65(4): 040902.
22. Yi X, Lu Z, Kong Y, Chen Z. Label-Free Electrochemical Detection of MicroRNAs via Intercalation of Hemin into the DNA/RNA Hybridization. Int J Electrochem Sci 2017; 12: 2813-2821.
23. Liu X, Wei M, Xu E et al. A sensitive, label-free electrochemical detection of telomerase activity without modification or immobilization. Biosensors and Bioelectronics 2017; 91: 347-353.
24. Zhang S, Bai H, Luo J, Yang P, Cai J. A recyclable chitosan-based QCM biosensor for sensitive and selective detection of breast cancer cells in real time. Analyst 2014; 139(23): 6259-6265.
25. Lian Y, He F, Wang H, Tong F. A new aptamer/graphene interdigitated gold electrode piezoelectric sensor for rapid and specific detection of Staphylococcus aureus. Biosensors and Bioelectronics 2015; 65: 314-319.
26. Shang X, Luo L, Ren K et al. Synthesis and cytotoxicity of azo nano-materials as new biosensors for l-Arginine determination. Materials Science and Engineering: C 2015; 51: 279-286.
27. Singh KP, Dhek NS, Nehra A, Ahlawat S, Puri A. Applying graphene oxide nano-film over a polycarbonate nanoporous membrane to monitor E. coli by infrared spectroscopy. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 2017; 170: 14-18.

28. Al-Ogaidi I, Gou H, Al-Kazaz AKA et al. A gold@ silica core–shell nanoparticle-based surface-enhanced Raman scattering biosensor for label-free glucose detection. Analytica chimica acta 2014; 811: 76-80.
29. Thompson RB. Fluorescence sensors and biosensors: CRC Press; 2005.
30. Halo TL, McMahon KM, Angeloni NL et al. NanoFlares for the detection, isolation, and culture of live tumor cells from human blood. Proceedings of the National Academy of Sciences 2014; 111(48): 17104-17109.
31. Shao Y, Wang J, Wu H, Liu J, Aksay IA, Lin Y. Graphene based electrochemical sensors and biosensors: a review. Electroanalysis 2010; 22(10): 1027-1036.
32. Jung JH, Cheon DS, Liu F, Lee KB, Seo TS. A graphene oxide based immuno-biosensor for pathogen detection. Angewandte Chemie International Edition 2010; 49(33): 5708-5711.
33. Liu Y, Yu D, Zeng C, Miao Z, Dai L. Biocompatible graphene oxide-based glucose biosensors. Langmuir 2010; 26(9): 6158-6160.
34. Mayorga-Martinez CC, Sofer Z, Pumera M. Layered black phosphorus as a selective vapor sensor. Angewandte Chemie 2015; 127(48): 14525-14528.
35. Chen Y, Ren R, Pu H, Chang J, Mao S, Chen J. Field-effect transistor biosensors with two-dimensional black phosphorus nanosheets. Biosensors and Bioelectronics 2017; 89: 505-510.
36. Kong R-M, Ding L, Wang Z, You J, Qu F. A novel aptamer-functionalized MoS2 nanosheet fluorescent biosensor for sensitive detection of prostate specific antigen. Analytical and bioanalytical chemistry 2015; 407(2): 369-377.
37. Yew YT, Sofer Z, Mayorga-Martinez CC, Pumera M. Black phosphorus nanoparticles as a novel fluorescent sensing platform for nucleic acid detection. Materials Chemistry Frontiers 2017; 1(6): 1130-1136.
38. Shamsipur M, Nasirian V, Mansouri K, Barati A, Veisi-Raygani A, Kashanian S. A highly sensitive quantum dots-DNA nanobiosensor based on fluorescence resonance energy transfer for rapid detection of nanomolar amounts of human papillomavirus 18. Journal of pharmaceutical and biomedical analysis 2017; 136: 140-147.
39. Zhang H, Wang Y, Zhao D et al. Universal fluorescence biosensor platform based on graphene quantum dots and pyrene-functionalized molecular beacons for detection of microRNAs. ACS applied materials & interfaces 2015; 7(30): 16152-16156.
40. Sadowski JW, Lekkala J, Vikholm I. Biosensors based on surface plasmons excited in non-noble metals. Biosensors and Bioelectronics 1991; 6(5): 439-444.
41. Nishijima Y, Hashimoto Y, Balčytis A, Seniutinas G, Juodkazis S. Alloy Materials for Plasmonic Refractive Index Sensing. Sensors and Materials 2017; 29(9): 1233-1239.
42. Uludag Y, Tothill IE. Cancer biomarker detection in serum samples using surface plasmon resonance and quartz crystal microbalance sensors with nanoparticle signal amplification. Analytical chemistry 2012; 84(14): 5898-5904.
43. Zhang D, Sun Y, Wu Q et al. Enhancing sensitivity of surface plasmon resonance biosensor by Ag nanocubes/chitosan composite for the detection of mouse IgG. Talanta 2016; 146: 364-368.
44. Khaledian S, Nikkhah M, Shams-bakhsh M, Hoseinzadeh S. A sensitive biosensor based on gold nanoparticles to detect Ralstonia solanacearum in soil. Journal of General Plant Pathology 2017; 83(4): 231-239.