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Optical Biosensors Based on ZnO Nanostructures: Advantages and Perspectives. A Review

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Content

Abstract

This review article highlights the application of beneficial physico-chemical properties of ZnO nanostructures for the detection of wide range of biological compounds. As the medical diagnostics require accurate, fast and inexpensive biosensors, the advantages inherent optical methods of detection are considered. The crucial points of the immobilization process, responsible for biosensor performance (biomolecule adsorption, surface properties, surface defects role, surface functionalization *etc.*) along with the interaction mechanism between

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biomolecules and ZnO are disclosed. The latest achievements in surface Plasmon resonance (SPR), surface enhanced Raman spectroscopy (SERS) and photoluminescence based biosensors along with novel trends in the development of ZnO biosensor platform are presented.

Keywords: optical biosensors, ZnO, nanostructures, immobilization, photoluminescence based biosensors, interaction mechanism.

1. Introduction

Biosensors are instrumental analytical devices for selective detection of different analytes. The biosensor contains a bioselective layer, which react with a target biomolcule and transducer that transforms the biologic interaction into physical signal (optical, chemical, electrical, thermal *etc.*) [1-6]. Type of transducer defines a methodology of biosensor measurements, conditions of the measurements and limitations.

In recent times, the researcher's attention has been paid to the nanomaterials since their dimensions are the same scale as the dimensions of the bio-components, which form a bioselective layer. The surface effects in nanostructures are caused by their low size, surface structure, charge, wettability and high surface-to-volume ratio impose the challenges for biosensing applications [3-28].

Nowadays, the most developed and used biosensor platforms are electrical [29, 32, 33, 37, 42, 46, 47, 56, 87] and electrochemical [16, 30, 31, 38, 39, 55, 88, 90, 92-94]. Electrical in particular are based on their Field Effect Transistor (FET) platforms demonstrate high level of detection but require additional input of lithography and nanotechnology, what makes these devices more complex and expensive. Electrochemical platforms demonstrate high level of detection and are compatible for mass production. However, they demand involvement of additional reducing/oxidizing agent (a dye or enzyme labels) which makes these devices a bit complicated. Moreover, some of them are based on the registration of potentio- or conductometrical parameters that depend on the indexes of pH, ionic-strength and capacity of the analyzed samples.

Recently, optical biosensor platforms have attracted the attention. The main advantages of the optical methods of detection are high precision and label free concept. Optical biosensor platforms, based on absorbance, photoluminescence and surface plasmon resonance are the next generation of sensing devices for everyday use.

The biosensor platform requires specific material with advanced structure, electrical and optical properties for the effective signal transformation of biological interaction into physical signal. Among different materials, metal oxides are quite attractive for biosensor applications as they possess all required physical properties (conductivity, luminescence and absorbance) as well as biocompatibility.

One of the most interesting metal oxide material is ZnO – n-type semiconductor with wide band gap (3.37 eV), high isoelectric point (pH=9-9.5) and intense room temperature photoluminescence [34, 35, 43, 62, 66-69, 74-77, 82]. Regarding to a progress in nanotechnology and material science, new technological methods have been developed to fabricate nanostructured ZnO templates with high surface area and advanced properties for biosensors. Nanostructured ZnO films have been widely used as template in electrochemical biosensors before demonstrating good sensitivity and low detection limit [30, 39, 55, 88, 90, 92-

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94], while ZnO nanowires, nanorods *etc*. have been widely used recently as the sensing element in FET biosensors [29, 33, 87].

Particularly, optical properties of ZnO open great possibility to be used for biosensor applications. This paper is dedicated to analysis of ZnO approaches in optical biosensors and future prospects of this research field. Application of ZnO optical properties and enhancement of biosensing properties via combination of ZnO with metal nanoparticles are analysed. The question of ZnO surface functionalization and the possible ways of forming the bioselective layer are disclosed. The crucial points of immobilization process, responsible for biosensor performance (biomolecule adsorption, surface properties, surface defects role, application of assisted layers etc.) are revealed. The mechanism of interaction between ZnO and biomolecules is discussed. New trends, affecting the development of ZnO based optical biosensors are presented.

2. The detection principle and types of optical biosensor

In optical biosensors, the biological sensing element is connected to an optical transducer system and the signal is based on absorption, luminescence or reflectance [10].

Generally, optical biosensors can be divided into two main groups: direct optical detection and indirect optical detection.

Biosensors based on SPR, ellipsometry, reflectometry, interferometry or photoluminescence present direct detection systems. Indirect biosensors (labeled systems) are based on fluorescence, SERS, photoluminescence quantum dots (used as labels) and others. The difference between these groups is that in the case of direct optical detection the one measures the properties of the transducer, in the second case – the attached tags (such as fluorescent dye) are used to detect the target analyte.

The biosensitive layer is formed by immobilization of biological recognition element (enzyme, receptor protein, probe molecule, cell-receptor *etc.*) on the surface of transducer. This bio-recognition layer serves as a basis to capture the target analyte (low molecular compound (LMC), protein, nucleic acid or cell). Surface parameters of the transducer (effective surface area, roughness, porosity), physical and chemical properties (surface charge, energy, valence/conductance states, physical states, functional groups, hydroscopic nature) affect the formation of the biointerface [11, 22]. The main goal of biosensing is to obtain a precise and rapid detection of the target biomolecules with high sensitivity and selectivity. The general scheme of such optical biosensor is shown in Fig. 1.

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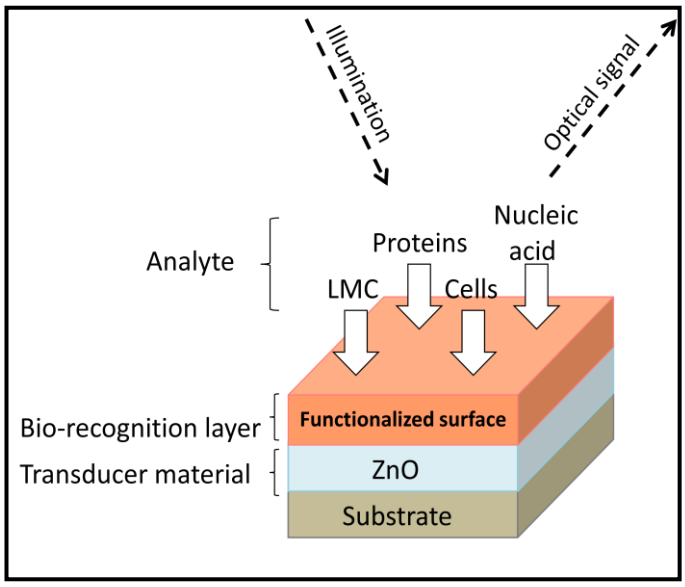

Fig.1. ZnO-based optical biosensor scheme

The light from the source reaches the surface and optical signal (luminescence or reflectance) is recorded before and after immobilization of biosensitive layer, as well as after interaction with analytes. The changes in optical signal, resulted by adsorption of the target analyte, allow plotting a dependence of the biosensor signal *vs* analyte concentration.

2.1. Application of ZnO nanostructures in biosensors

The advances in ZnO crystal growth technology allow obtaining nanostructures of various sizes, shapes and properties which play a crucial role for the effective immobilization of biomolecules. Some types of ZnO nanostructures used as a biosensor platform are shown on Fig. 2.

Mostly 10 types of ZnO nanostructures (nanorods, nanowires, nanoparticles, quantum dots, thin films *etc.*) are reported in biosensors as transducers. Due to their high surface/volume ratio, surface tailoring ability, novel electron transport properties and electronic conductance multifunctionality it is possible to successfully immobilize various types of biomolecules. Mostly nanorods (NRs) [36,38,40,43,45,47,48,53,56,74,82,86] and nanowires (NWs) [29,33,42,46,49,75,77,94] were used as a biosensor platform. Nanoparticles (NPs) [35,37,50,83,84,97,98], quantum dots [34,44], other low-dimentional structures [30,32,39,52,70,71,76,79,80,81,85,89,93,95,99] and thin films [31,51,72,73] were also applied.

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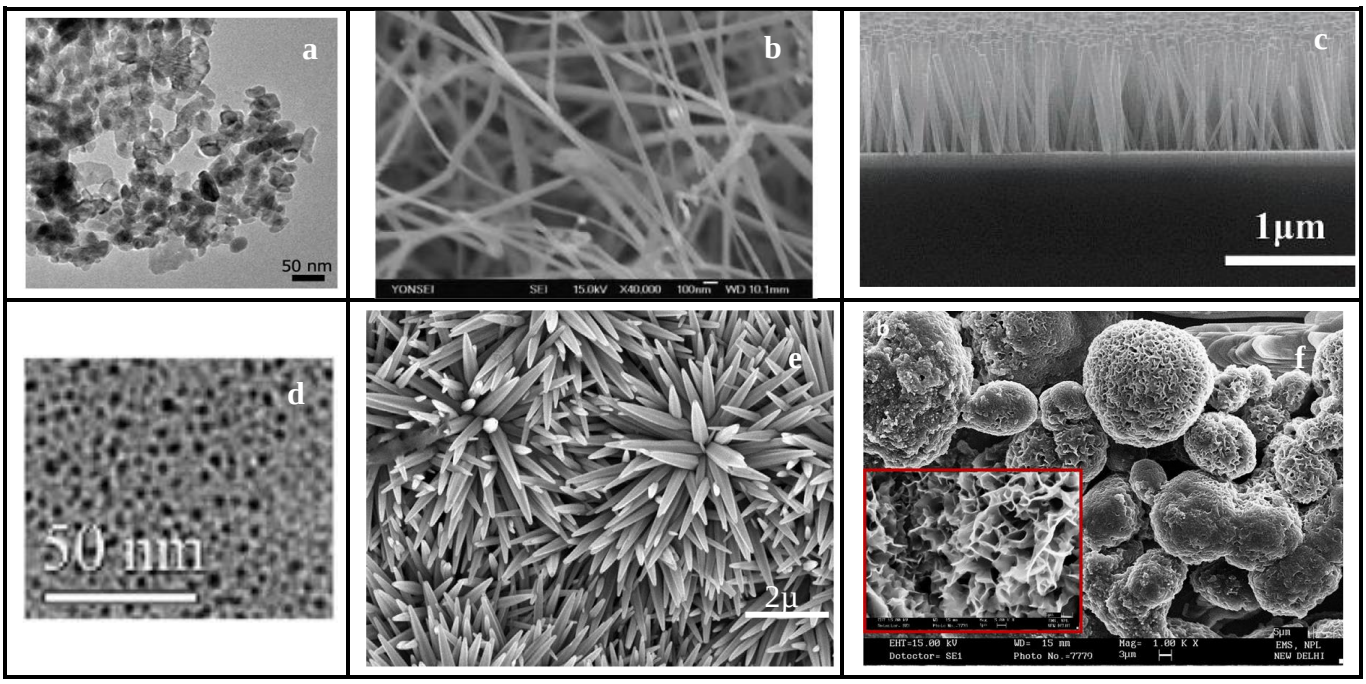

Fig.2. Electron microscopy images of some types of ZnO nanostructures. a) TEM image of ZnO nanoparticles[1] [57], b) SEM images of ZnO nanowires grown on sapphire (0001) substrate[2] [46], c) SEM image of uniform ZnO nanorods[3] [43], d) TEM image of ZnO QDs[4] [19], e) SEM images of ZnO NRs grown on T_Si[5] [48], and f) SEM image of r-IgGs/Nano-ZnO/ITO electrode[6][39]

The most extensively used biological detecting material (analyte) can be classified into the next groups:

- Low-molecular compounds: uric acid, urea, riboflavin, dopamine, ochratoxin (OTA), 4-amino-benzenethiol (4-ABT), and 1,3,5-trinitro-perhydro-1,3,5- triazine (RDX);
- Proteins: bovine serum albumin (BSA), human serum albumine (HSA), streptavidin and rabbit imunoglobilin (IgG);
- Nucleic acid: deoxyribonucleic acid (DNA) and ribonucleic acid (RNA);
- Cells: cancer cells.

For each type of the above mentioned analytes, an individual bio-recognition layer is immobilized on the nanostructured surface. To achieve the most efficient biomolecule immobilization, the assisting layers are widely used for ZnO surface functionalization.

Self-assembled monolayers (SAM) - *Silanes and Alkyl phosphates/phosphonates* were used to modify metal oxide surfaces because of their ease of use and commercial availability. SAM can bear different functional or reactive groups capable of subsequent covalent coupling reactions. SAM on the basis silane can promote surfaces with well-defined topographic features for enhanced bioimmobilization and to control the distance between the sensor surface and the immobilized biomolecule to localize binding events within the region of highest optical intensity [5]. APTES (amino-propyl-triethoxysilane) is used as SAM and reacts with –OH group (hydroxyl) [29]. EDC (1-ethyl-3-(3-dimethylaminopropyl) is used as crosslink agent between APTES and antibodies. A bovine serum albumin (BSA) performs bifunctional role as a cross-

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linker between substrate and proteins and as a blocker inhibiting a nonspecific protein adsorption [28, 39, 43, 44]. Poly(amic acid) (PAA) is used to improve the immobilization process. Wu *et al.* have used the PAA particles in the range of 133 – 213 nm, coated with small ZnO nanoparticles (3 – 13 nm) to obtain high coverage of the ZnO nanoparticles over the Poly(amic acid) microsphere of the raspberry-like particle [36]. Various self-assembly and nanolithography techniques have been employed to fabricate 3D surface enhanced Raman spectroscopy (SERS) substrates, which involves a compromise among sensitivity, scalable area, reproducibility and cost [48].

Recent results about application of ZnO nanostructures in optical biosensors are shown in Table 1.

Table1. Summarized data about the application of ZnO nanostructures in biosensors

Target analyte	**Type biosensitive layer**	**ZnO micro-/nanostruc-ture**	**Charac-teristic size**	**Detection method**	**Perfomance (detection limit / concentration (range)**
FITC (fluorescein-conjugated antibovine)-anti-IgG	Protein G BBSA (biotinylated bovine serum albumin) – DTAF-streptavidin	ZnO Nanorods [45]	d ~ 4.1 ± 0.3 μm l~ 313.3 ± 68.3 nm	Fluorescence	200 μg/ml^{-1}
DNA	FITC-antiIgG (fluorescein-conjugated antibovine IgG)	ZnO Nanorods [15]	677 ± 65 nm 547 ± 17 nm	Fluorescence	2 ng/ml
dye-marked DNA molecules	GOPS (organosilane glycidyloxypropyltrimetho-xysilane), amino-modified capture molecule oligonucleotides	ZnO Nanowires [49]	d ~ 70 nm l up to 5 μm	Fluorescence	Successful attachment of DNA target molecules on the nanowire surface
DNA of dengue virus specific serotypes	Sequence specific probe strand	ZnO thin film [72]	Thickness ~ 150–200 nm	Flourescence	1×10^{-15} moles of DNA
BSA	ZnO	ZnO nanosphere [70]	d ~ 30 nm	Absorbance FTIR I-V	Limit of detection 10 pM
Pancreatic cancer(carbohydrate antigen 19-9)	Carbohydrate antibodies 19-9 (CA 19-9) BSA	ZnO Quantum Dots [44]	4.5 – 5-5 nm	Electrochemical Fluorescence	0.1–180 U/ml, detection limit 0.04 U/ml. 1–180 U/ml, detection limit 0.25 U/ml
Dopamine	APTES (3-aminopropyl triethoxysilane)	Quantum Dots [34]	3-5 nm	Photo-luminescence	0.05-10 μM Detection limit down to 12 nM

Glucose	Glucose oxidase	MUA capped nanoparticles [35]	3-4 nm	Photo-luminescence	Linear detection range 1.6 – 33.3 mM detection limit < 0.33 mM
aProteins (BSA HSA)	DMSA (dimercaptosuccinic acid) BSA,HSA	ZnO Nanorods [43]	d ~70 nm l ~500-520 nm	Photo-luminescence	Successful conjugation of proteins on flexible substrates
Salmonella Ag	Salmonella Ab	ZnO Nanorods [62]	d~57 ± 9 nm l~470 ± 30 nm	Photo-luminescence	102 – 105 cell/ml
Glucose	glucose oxidase	ZnO nanoparticles [68]	70±15 nm	Photo-luminescence	10 mM - 130 mM
Glucose	Non-enzymatic	ZnO NRs [74]	d ~ 70-80 nm l ~ 0.8-0.9μm	Photolumines-cence	0.5 – 30 mM
Avidin-Horseradish peroxidase	Biotinylated-protein A	ZnO NWs [75]	d ~ 200 nm l ~ 1 μm	Photolumines-cence	Sensitivity in therange of tenths of ng/mL per counts
Glucose	Glucose oxidase	ZnO nanostructures [76]		Photolumines-cence I-V	10^{-6} to 10^{-2} M
Protein A	APTES – BS^3	ZnO NWs [77]	d ~ 200 nm l ~ 1 μm	FTIR Photolumines-cence	
Rabbit IgG	antibodies of rabbit IgG	ZnO–Au nanocompo-sites [41]	ZnO–Au ~ 10 nm	SPR	0.15–20.00 μg/ml−1
Cancer cells	Tumor marker carbohydrate antigen 15-3 (CA15-3)	Au coated ZnO Nanorods [54]		SPR	Linear detection 2.5-20 U/mL The cut-off point in cancer patients ~ 4 U/mL Linear range 40-300 U/mL
Glucose	glucose oxidase	ZnO modified gold disc [51]	Au size ~20 nm	SPR	50–250 ng/ml
Cholesterol	Cholesterol oxidase	ZnO/Au/prism [100]	ZnO crystallite size 20 nm	SPR	0.12 - 10.23 mM
Neisseria meningitidis DNA		ZnO thin film of [101]	Film thickness 200nm	SPR	Detection range from 10 to 180 ng/μl Limit of detection of 5ng/μl
Rhodamine 6G	Poly(amic acid)/ ZnO composite	ZnO NPs [36]	d ~ 3 - 13 nm	SERS	Up to 10^{-4} M
4-ABT,RDX	Hexamethylenetetramine (HMT) as habit-control reagent	Nanorods ZnO /Si(100) ZnO/ITO Au coated Nanorods [40]	d~ 60 nm, l ~ 3 μm, d~ 70 nm; l ~ 500 nm d~ 60 - 70nm l ~ 1 - 3 μm	SERS	1.1×10^{-16}g (for 4-ABT) 9×10^{-19} g (for RDX)
Rhodamine 6G	Ag decorated ZnO NRs "3D SERS substrates"	ZnO nanorod array [48]	Number of NRs: 90 in. per μm2	SERS	Up to 10 -8 M
4-ATP probe molecules	4-ATP probe molecules	ZnO–Au nanocomposi-tes [65]	ZnO NRs: d ~ 25–50 nm, l ~ 200–300 nm Au NPs: d ~ 10 nm, thickness of Au on ZnO NRs: from 10 nm	SERS	10−5 M

			to 70 nm		
Malachite green		Ag nanoclusters deposited on ZnO nanodome (ND) [95]	ND diameter up to 120 nm	SERS	Down to 10^{-17} M
Apomorphine		3D ZnO tetrapods [97]	d ~ 500 nm l ~ 100 nm	SERS	Down to 1 μM (0.27 μg /mL)
Rhodamine 6G		3D hierarchical ZnO/Si nanomace [98]	Branch length 300-700 nm	SERS	Down to 10^{-16} M
4-amino-thiophenol (4-ATP)		3D Ag@ZnO nanostructures [99]	d ~ 100 nm l ~ 1-1.5 μm	SERS	Enhancement factor is estimated as ~$2*10^6$

Apparently, the biosensor performance (detection limit or concentration range) indirectly depends on the size and shape of nanostructures, their growth method as well as on the detection principle. Optical biosensors are selective and sensitive devices for the detection of very low levels of chemicals and biological substances including possibility to make real-time analysis without the need for extensive sample pretreatment or large sample volumes [4, 24-27]. The main advantage of optical sensor is the fact, that it allows to measure properties of materials (*inc.* liquids) contactless, without using of electrical fields and therefore it is indifferent to external electromagnetic and electrical fields.

Among optical techniques for ZnO-based biosensors, the most developed are SERS, SPR and fluorescence. However these methods are quite expensive and mostly use labels for analyte detection which complicates the experiment and takes a lot of time. Less often it was used method like photoluminescence. This method does not yet demonstrate a high sensitivity, however it is easier to be applied and it is a very prospective method.

ZnO nanoparticles have been used by themselves as transducers and in the combination with other types of structures and in each case the efficiency of performing analysis was demonstrated. The advantages offered by optical biosensors according to data are the selectivity, specificity, the remote sensing, isolation from electromagnetic fields along with fast, real-time measurements, multi parameters detection and compact design. Unfortunately, ZnO nanoparticles have demonstrated some toxic effects on the living cells according to Starodub *et al* [21, 22]. However there is a hope for the possibility of minimizing such effect and using these structures for the invasive application and for the in vivo measurements by choosing of optical components for biocompatibility and giving detailed chemical information on analytes.

2.2 Functionalization of ZnO surface and forming of the biosensitive layer

Forming of stable bioselective layer is very important task for development of biosensor with good performance. It is known, that ZnO has high value of isoelectric point (pH~9.1) [34, 35, 43, 62, 66-69, 74-77] which makes ZnO nanostructures attractive for immobilization of biomolecules on its surface. It is important to note that the morphology and the structure properties of ZnO play important role in the process of immobilization.

Saha *et al*. reported that the ZnO thin films with higher internal stress, used in electrochemical biosensors, showed higher sensitivity to glucose due to better charge transfer [59]. Cao *et al*. reported that ZnO nanostructures with higher surface area (nanorods and thin films with high roughness) provide better immobilization of DNA on their surface [79]. Sang *et*

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al. reported on the influence of ZnO morphology to biosensor response of ZnO nanorods, grown in the microfluidic system. The ZnO nanorods had different surface-to-volume aspect ratio. It was reported that the samples with the highest surface-to-volume aspect ratio demonstrated the highest protein adsorption rate [89]. Wang *et al*. studied the effect of the ZnO surface topography (nanoparticles (NPs), nanorods (NRs), nanosheets (NSs) and nanobeams (NBs)) on the protein adsorption process. The main results of the work showed the highest protein quantity, adsorbed on ZnO NRs (Table 2). The obtained results relate to higher number of adsorption sites on ZnO NRs surface in comparison with other ZnO nanostructures [85].

Thus, the morphology of ZnO nanostructure defines transport properties, number of adsorption sites and the protein adsorption rate [79,85,89].

There are two main methodologies of forming of bioselective layer on ZnO surface: direct immobilization of biomolecules and covalent binding [47,52,59,78,79,81,82,83,85,86,88,89,90].

2.2.1. Direct immobilization of biomolecules on top of ZnO nanostructures

A number of works published on the direct immobilization of biomolecules on the ZnO surface refers to the electrostatic interaction between ZnO and the adsorbed species [86,90,32,52]. Klaumünzer *et al*. reported on BSA adsorption on the ZnO nanostructures. Investigation of the structure properties with Transmission Electron Microscopy (TEM) showed the formation of an amorphous phase of BSA over well crystalline ZnO structure [82]. The study of the fundamental properties of ZnO nanostructures dipped into buffer solution with pH 7-7.3, showed a positive value of Z-potential for ZnO [83]. The sign and the value of Z-potential have been changed from positive to negative after adsorption of BSA on the ZnO surface [83].
The binding mechanism between ZnO and BSA has been reported as well by Bhogale *et al.* [84]. The changes in entropy (ΔS) and enthalpy (ΔH) of binding reaction between ZnO and BSA have been evaluated. It was reported that the negative values of ΔS (−242 J/mol K) and ΔH (−100.85 kJ/mol) suggested that the hydrogen bond and the Van der Waals forces play major roles in the binding process between ZnO and BSA and the reaction is mainly enthalpy-driven. Xie *et al.* reported on theoretical calculations of proteins on differently oriented ZnO surfaces. The binding energies between aminoacids of the proteins and the ZnO surface have been calculated. It was found that within different ZnO facets, ((0001), (0001), (1010), and (1120)) the highest protein coverage was observed for (1010) facets [80].

The adsorption kinetics of bovine serum albumin (BSA) and fibrinogen (Fg) adsorbed on the four different surface topographies (nanoparticles (NPs), nanorods (NRs), nanosheets (NSs) and nanobeams (NBs)) of Zinc oxide (ZnO) have been studied by Wang *et al.* [85]. It was found that adsorption of proteins depends on their IEP (4.5 for BSA and 5.5 for Fg) and molecular weight (M_W) (66 kDa for BSA and 340 kDa for Fg). It was shown that proteins with lower IEP and M_W had higher adsorption rate on ZnO surface (Table 2).

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Table2. Adsorption of different proteins on ZnO surface [85]

Morphology	BSA equilibrium adsorption quantities	BSA adsorption rate (first stage of adsorption) μg/(mg min)	Fg equilibrium adsorption quantities	Fg adsorption rate (first stage of adsorption) μg/(mg min)
NRs	69.06 ± 3.45	7	13.12 ± 0.66	1.2
NBs	23.04 ± 1.15	2.9	8.85 ± 0.44	0.8
NSs	18.91 ± 0.95	2	4.65 ± 0.23	0.6
NPs	3.67 ± 0.18	0.8	3.50 ± 0.18	0.5

2.2.2. Covalent binding between ZnO surface and biomolecules

The covalent binding involves a cross-linking agent between ZnO surface and molecules of the bioselective layer, which provides a significant structure of the bioselective layer and improves the biorecognition [89,47,78, 81, 87,88].

1. Silanization

As metal oxides have hydroxyl groups on their surfaces, their interaction with silanes (APTES (3-aminopropyl)-triethoxysilane, MPTMS (3-mercaptopropyl)-trimethoxysilane, etc) leads to the formation of covalent –O-Si-Si-O- groups between the surface and the cross-linking agent. Amine ($-NH_2$) and thiol ($-SH_2$) groups are formed after treatment with aminosilanes and mercaptosilanes, respectively [47, 89]. The direct immobilization of protein through the interaction between amine and carboxyl groups after APTES silanization of ZnO surface has been investigated as well [78, 81]. In the case of thiol-based silanizated ZnO surface, one more step needed for a protein immobilization [47, 89]. Sang *et al*. provided the protein adsorption after washing the silanizated ZnO with disuccinimidyl suberate (DSS), solved in dimethyl sulfoxide (DMSO) [89]. Sanguino *et al*. used a crosslinker (N-Hydroxysuccinimide (NHS) ester) to provide a binding with the protein [47]. Politi *et al.* have used standard silanization chemistry to functionalize ZnO NWs surface with a biotinylated-protein A in order to obtain a homogeneous functional surface that covalently binds the cross-linker spacer BS^3 (Bis[sulfosuccinimidyl] suberate) and the bioprobe (biotinylated-protein A). Such a biomolecular complex is able to specifically interact with IgG, through protein A, and with avidin, through biotin. The interaction between the biotinylated protein A and an avidin-IgG like biomolecule as Avidin-Horseradish peroxidase (avidin-HRP) was monitored. The functionalization of ZnO surface and the molecular biorecognition experiment have been monitored by FTIR. The monitoring of the biomolecular recognition of avidin-HRP at different concentrations was performed using the photoluminescence of ZnO NWs. A simple normalization of the emission peaks provided a quantitative monitoring of the biomolecular interaction, revealing an affinity constant in the range of μg/mL per counts and a sensitivity in the range of tenths of ng/mL per counts [75].

2. Thiol-cross linking agents

The use of thiol compounds to form Zn-S surface bonds was reported by Jacobs *et al*. [88] and Munje *et al.* [87]. The cross-linking agent (dithiobis succinimydyl propionate (DSP) dissolved in dimethyl sulfoxide (DMSO)) forms amine groups which are further occupied by proteins [88,87].

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3. Phosphoric cross linking agents

Cross linking between ZnO surface and protein via phosphoric groups is rarely used [91, 57]. Comparing to silanization, it is more complicated process.

3. Crucial points for biosensing

3.1. The role of biomolecules adsorption on the sample surface

Considering the interaction of biomolecules with nanostructures "on the solid surface", the crucial role belongs to the adsorption process [6, 8, 57, 58, 70-77]. The latter depends on the number of factors such as surface properties of the film (roughness, porosity *etc*), its thickness, the amount and types of surface defects and general physico-chemical compatibility of biomolecules and ZnO thin film.

According to Nakanishi *et al.* [58] the amount of adsorbed proteins is affected by properties of the biological molecules, solid substrate surface and environmental conditions. With respect to properties of proteins, charge, size, stability of structure (hard or soft), amino acid composition, and steric conformation may affect the adsorbed amount. The important factors affecting protein adsorption are the structural stability of the protein and the hydrophilic/hydrophobic state of surface. The structurally stable "hard" proteins (chymotrypsin, ribonuclease, lysozyme, 3-Lg) could adsorb on a surface with a charge opposite that of the proteins, while they adsorb on a surface of the same charge only when there is hydrophobic interaction. The structurally unstable "soft proteins" (BSA, HSA, IgG, lactoalbumin, /3-casein, hemoglobin, catalase, phytase) seem to adsorb on any surfaces owing to a gain in conformational entropy. In the case of adsorption of proteins on surfaces of metal oxides, non-electrostatic interactions might be realized through others than hydrophobic interactions. Unfortunately, the changes of the protein conformation at the immobilization process present a complicate problem [58].

The adsorption process can be controlled by defects level and type. Nanostructured metal oxides like ZnO, TiO_2, Al_2O_3, *etc.* are known to be as materials with narrow set of defects that can serve as adsorption centers. For example, if the adsorption center is a defect caused by unsaturated bond on the metal surface due to the presence of oxygen vacancies, then the question arises: what is the adsorption center - unsaturated bond or a metal atom which can be in the interstices? If, for example, it is proved that the basic defects that promote adsorption processes are free metal atoms, then it is possible to dope the sample so that metal atoms were discretely deposited on the surface. Then, it is necessary to check out how it will affect its optical characteristics and the biomolecules adsorption. On the other hand, if we talk about the oxygen vacancies or metal in the interstices then why cannot be the reverse - deficit metal atoms playing the role of adsorption centers? If so, it is necessary to create a deficiency of metal atoms caused for example by defects in the structure of the cell. In this case, the one need to control such growth parameters of the samples, to arise metal vacancies on the surface. Titane, for example, being very electronegative, and not very active, having a positive atom, will simply "eat" a biomolecule, selecting its electrons to saturate outer Ti orbitals.

Correlation between the biosensing characteristics and the presence of defects in the ZnO films was studied by Saha *et al*. ZnO thin films deposited by radiofrequency (RF) magnetron sputtering under varying processing pressure in a reactive gas mixture of argon and oxygen were studied for glucose oxidase detection. The biosensing response characteristics have seen to be strongly influenced by the physical properties of the ZnO matrix, which in turn depends on the

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growth kinetics. The roughness of the film is also seen to be directly related to the processing pressure and increases with increasing deposition pressure. Higher surface roughness of ZnO film leads to the availability of larger surface area for the effective immobilization of biomolecules presence of point defects in the form of interstitial Zn is found to play a major role in improving the electrical conductivity and charge transfer capability of the ZnO thin film matrix. To characterize the influence of grows process to the presence of defects in the ZnO films, room temperature photoluminescence measurements were applied [59].

In general, it is necessary to underline again that the problem of ZnO surface functionalization and providing its physical reaction on the formed specific interaction is very important and complicate. The possible ways of its solving depend on a number of factors, first of all determined by nature of biological materials, their physico-chemical abilities, their ways of the orientation of active binding sites toward solution and many others. The key to this complex problem has its own specific character in each case. For example, to immobilize the one type of biological molecules it is necessary not only to design a specific intermediate layer but to determine how it will effect on the characteristics of the transducer surface compared to when the specific interaction is recorded. First of all it is necessary to consider each time obtaining not only high sensitive level of analysis but, in principle, the possibility of specific signal registration.

3.2. Photoluminescence based biosensors

Photoluminescence (PL) from nanomaterials is a suitable and simple method for characterizing sample surfaces, especially surface defects [23, 25, 59-62, 66-69, 74-77, 82]. PL of metal oxide nanostructures is well studied to date (although not completely), namely, it was established what kind of defects are responsible for certain PL bands. From the change in the luminescence bands as a result of biological impact, the one can conclude what defects act as adsorption centers and work on, improving the sample material. As it can be seen from the Table1, PL is not common to use comparably to other methods although it is an excellent way of nanomaterial characterization and consiquently, the nanobiointerface. Its combination with latter advances in growth technology, which allow to change optical properties, make this method very perspective for future small integrated biosensor devices. It is known two types of optical biosensors connected with PL measurements: used PL quantum dots (QD) as labels and biosensors based on PL signal from the sample surface.

In the first case, it is used labels for biomolecules. Due to the small scales (1–10 nm) of QD they retain new properties that directly depend on their size and they are characterized by large absorption spectra, narrow and symmetric emission bands as well as an excellent photostability and quantum yield (up to 90%). QDs have large absorption cross sections and long fluorescence lifetimes (410 ns). With all these features, QDs have rapidly emerged as potential new fluorescent probes for the imaging of biological samples and widely used for this purposes [15, 17-19, 44]. Gu *et al.* used ZnO QDs as electrochemical and fluorescent labels [44]. A sandwich-type sensitive immunoassay was developed to detect carbohydrate antigen 19-9 (CA 19-9) which is a preferred label for pancreatic cancer. The immobilization process was mainly carried out through the electrostatic adsorption based on the high isoelectric point of ZnO, and the sandwich structure was built through the immunoreaction of CA 19-9 antibodies and antigens. The immunological recognition of CA 19-9 was converted into detection of the amplified signals of

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the square wave stripping voltammetry and intrinsic PL of the labeled QD. ZnO QD excitonic PL increased and the peak position was UV shifted with the increase of the biomolecules concentration. The electrochemical assay demonstrated a dynamic range of 0.1–180 U/ml with detection limit of 0.04 U/ml, while the optical spectral detection revealed 1–180 U/ml with detection limit of 0.25 U/ml [44].

In the second case, the changes in PL signal of some nanostructured material is used for the detection of analyte and its amount by characterization of PL spectra that corresponds to changes in the properties of biointerface. The principle of PL biosensor action is based on the changing of PL signal before and after immobilization of biological selective sites as well as after interaction with analyte. Recently, novel immune PL biosensors based on TiO_2 nanoparticles or nanowires have been developed, for the diagnostics of retroviral leucosis [60] and for the detection of *sallmonella spp.* [61] which were highly sensitive and selective.

The intensity of PL signal from ZnO nanorods (57 ± 9 nm in diameter and 470 ± 30 nm long) was used to detect *sallmonella spp* antigens by Viter *et al.* [62]. Two emission bands of PL from ZnO nanorods observed at room temperature are centered at 376 and 520 nm. A biosensitive layer was prepared by immobilization of anti-*salmonella* antibodies from liquid solutions on the ZnO surface. Immobilization of the biosensitive layer onto ZnO nanorods was found to increase the intensity of PL. After further reaction with *salmonella* antigens, the PL intensity was found to decrease proportional to their concentrations in the range of 10^2~10^5 cell/ml. The oxygen vacancies on ZnO surface are found to act as adsorption sites forming bridging bounds with organic molecules. Organic molecules can be anchored to the ZnO surface via phosphonic acid and carboxilate groups. Due to this results, PL considers to be a highly sensitive and selective method of biomolecules detection.

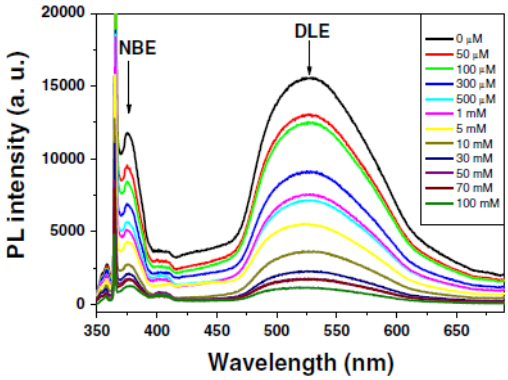

Fig.3. ZnO NPs PL spectra dependence on the hydrogen peroxide concentration[7][68].

For instance, the change of both PL peaks intensities (near band emission (NBE) and deep level emission (DLE)) from ZnO nanoparticles was used separately as a signal for hydrogen peroxide (H_2O_2) and glucose detection by Sodzel *et al.* [68]. Furthermore, an indirect optical determination of glucose via the detection of H_2O_2 generated during the glucose oxidase (GO_x) catalyzed oxidation of glucose was performed. The method was based on the finding that ultraviolet (~374 nm) and visible (~525 nm) photoluminescence of pristine ZnO nanoparticles strongly depends on the concentration of H_2O_2 in water solution. It was found that photoluminescence is quenched by up to 90 % at a 100 mM level of H_2O_2 (Fig. 3). By measuring the decrease of the PL intensity, one can estimate the glucose concentration in solution. The sensor constructed by immobilizing GO_x on ZnO nanoparticles enabled glucose to be

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continuously monitored in the 10 mM to 130 mM concentration range with the limit of detection 10 mM [68].

In enzymatic sensors, the glucose oxidase is needed as a catalyst to oxidize the glucose and produce gluconic acid and H_2O_2. However, in the case when glucose oxidase is absent, ZnO nanorods themselves may act as a catalyst to oxidize the glucose [74]. Sarangi *et al.* have developed non-enzymatic PL-based glucose biosensor with detection range of 0.5–30 mM.

Considering PL based biosensors, it is worth noting that the quenching of PL signal after target adsorption is being observed [62, 68, 74-77]. The mechanism of PL quenching may be due to several reasons. The surface reaction with a quencher may introduce non-radiative surface defects. In addition, the charge transfer from a radiative material to a quencher can also be one of the main mechanism of the PL quenching [74]. Sodzel *et al.* have suggested the following collisional PL quenching mechanism, responsible for the recognition of H_2O_2. Under laser excitation, the carriers in ZnO are separated into conduction band (CB - electrons) and valence band (VB - holes). The carriers aim to recombine radiatively while emitting a photon with energy close to the ZnO band gap (~3.3 eV) for NBE emission or alternatively through deep level defects with energies ~2.5 eV for DLE emission (Fig. 4). H_2O_2, being in proximity, decomposes catalytically on the ZnO surface into H_2O and O_2. This reaction is followed by acceptance of electrons from the conduction band of ZnO, thus preventing its radiative recombination, and resulting in quenching of the light emission intensity [68].

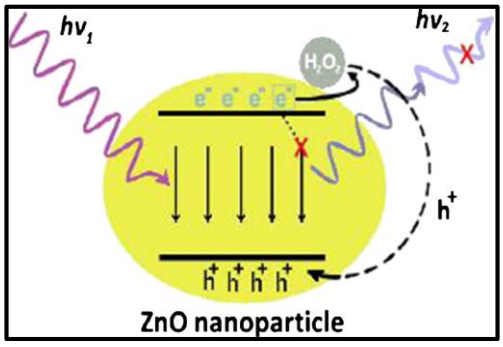

Fig.4. Mechanism of ZnO NPs PL sensitivity for H_2O_2 [68]

Results reported in references [59 – 62, 68, 74-77, 82] show that the photoluminescence signal is very sensitive to target concentration changes. PL based biosensors demonstrate high competitiveness along with ease of use as non-labeled direct detection of analytes, comparably to biosensors based on PL quantum dots used as labels. The control of PL from ZnO nanostructures is very effective and productive way to register the formation of specific complex. This index may be registered and realized with the application of special labels and without them and it may be accomplished by different physical approaches that widened the application and increased the efficiency of biosensors based on the ZnO nanostructures.

4. Novel trends in ZnO biosensor platforms

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Various types of ZnO platforms that used as a biosensor basis have been developed [40, 41, 43, 57, 63, 64, 69,73, 89,90,92, 94-99]. The growth-controlled synthesis of ZnO nanorods in the aqueous phase has been investigated by Balaguera-Gelves *et al.* [40]. The rods were grown on ZnO films previously deposited onto Si (100) and indium tin oxide (ITO) substrates. The formation of the rods took place in the presence of HMT as habit-control reagent. The grains in the base ZnO film acted as seeds that promoted the longitudinal growth of the oxide. As-synthesized based films and rods were characterized by X-ray diffraction, Scanning Electron Microscopy (SEM), field emission, optical absorption and PL spectroscopy techniques. Subsequently, a wet chemistry procedure was performed to achieve ZnO nanorods growth. This methodology was conducive to the formation of rods of a relatively narrow distribution of diameters (60 - 70 nm) with lengths in the 1 - 3 μm range. Au coating on ZnO nanorods was used to evaluate the detection capability by SERS of different analytes such as: 4-aminobenzenethiol (4-ABT) and 1,3,5-trinitroperhydro-1,3,5- triazine (RDX) at low levels. A strong SERS Raman spectrum was observed for 4-ABT. A detection limit (DL) of 1×10^{-8} M for 4-ABT was achieved corresponding to a minimum of 5.4×10^{5} molecules under the experimental conditions at excitation wavelength of 785 nm with a sensitivity of the ZnO nanorods in the range of 1.1×10^{-16} g under the laser spot. The pH solution was tailored by adding of Cl^{-} ions to increase the ionic strength effect, which induces electrostatic charge changes on the Au coated ZnO nanorods surface [40].

SPR based biosensor with the using ZnO-Au nanocomposites was developed for the detection of rabbit IgG by Wang *et al*. [41]. The ZnO-Au nanocomposites can bind proteins by covalent attachment to construct a probe for target analyte. The probe with unique optical properties and good biocompatibility could enhance the sensitivity of SPR biosensor. Under the optimized conditions, the biosensor based on ZnO-Au nanocomposites exhibits a satisfactory response to rabbit IgG in the concentration range of 0.15-20.00 μg ml^{-1}. Also it was compared the sensitivities of the biosensors based on Au film and on Au nanoparticles. It was revealed that first one shows a response to rabbit IgG in the concentration range of 2.50–20.00 μg ml^{-1}. Second type shows a response in the concentration range of 0.30–20.00 μg ml^{-1}. The biosensor based on ZnO-Au nanocomposites was therefore found to be the most sensitive among of the three types of the above mentioned biosensors. The lowest concentration of rabbit IgG that can be determined by the proposed biosensor is about 16-folds lowers than that of the biosensor based on Au film alone.

A square pattern of thioctic acid self-assembled ZnO nanorods arrays was grown on a large 4-in thermoplastic polyurethane (TPU) flexible substrate via an in-situ process at low temperature (348 K) by Liu *et al*. [43]. With the addition of DMSA, the surface chemistry forms a disordered ZnO phase, and the morphology of the ZnO-DMSA nanorods changes with various DMSA addition times. As evidenced by the $Zn_{2p3/2}$, C_{1s}, O_{1s}, S_{2p}, and N_{-1s} scans of X-ray photoelectron spectroscopy (XPS) and X-ray diffraction (XRD), DMSA and proteins were conjugated on the single crystalline ZnO nanorods. The PL spectra indicated that the optical properties of ZnO nanorod arrays were changed while the DMSA was inserted, and the proteins were conjugated. Furthermore, a control test found that the ZnO nanorods show a significant improvement in sensitive characterization over the ZnO film.

It was found that an enhanced of UV emission was detected for the ZnO nanorods with the binding of DMSA and BSA. The ZnO-BSA nanorods have a stronger UV emission intensity than the ZnO-DMSA nanorods due to higher grafting density. Furthermore, the UV emission intensity increases is about 2-folds and exhibits a slight red shift (from 376.52 to 378.17) when HSA was bound to ZnO-BSA nanorod arrays. The enhanced UV intensity and red shift of these

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ZnO nanorods in PL might be attributed to both defect passivation and modification on the surface region of ZnO nanorods due to the conjugation of biomolecules (Fig. 5). In addition, a control test found that the ZnO nanorods show more enhanced PL intensity than a flat-ZnO film. In this experiment, a simple trial was made by bonding HSA onto a flat-ZnO surface. A weaker PL intensity was detected for the ZnO-buffered HSA layer, which can be attributed to the poor crystallinity of flat-ZnO (ZnO-buffered layer), leading to poor sensitivity.

The ZnO nanorods showed a significant improvement in sensitive characterization over the flat-ZnO. Therefore, the conjugation of specific biomolecules on the patterned region of arrayed ZnO nanorods can be anticipated to detect the complementary biomolecules on the acceptor side, such as antibody-antigen bioconjugation by PL spectra. As another protein (e.g. HSA) was bound onto the ZnO-BSA nanorod arrays, an enhanced UV emission intensity was detected. On the basis of these results, one might be expected to conjugate specific biomolecules [43].

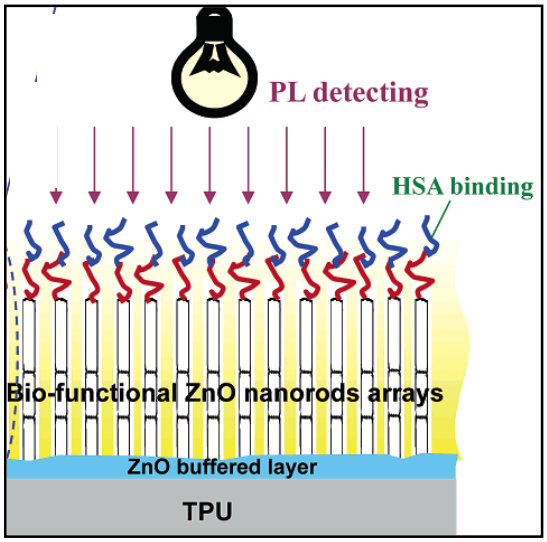

Fig.5. Chemical scheme of DMSA-self-assembled and protein-conjugated ZnO nanorods, and the sensitive optical quality observed by PL detecting while proteins bound[3] [43].

Nowadays, new approaches are targeted to combine ZnO nanostructures with carbon based nanomaterials such as graphene [63], carbon nanotubes [64], etc. in order to obtain new improved of electrical and optical properties, which can be used for biosensor applications. Yan Yue *et al.* reported on easy route of ZnO fabrication in 3D graphene foam for biomarker detection. High active surface area has been achieved [94]. This recent reports have proved the concept of good charge transfer and good biomolecule binding through π-π interaction in ZnO/graphene nanocomposites [55, 92, 94]. Despite that only electrochemical biosensors have been developed at the moment, [55, 92, 94] new published works on ZnO/graphene optical biosensors are foreseen.

4.1. New targets and integrates systems

It is known, that ZnO nanostructures can be formed using different techniques [62, 67, 89, 90]. Among these methods, Atomic layer deposition and hydrothermal growth allow depositing ZnO

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nanostructues at low temperatures [66,89,92] simplifying the actual problem of miniaturization and integration with testing systems. Xie *et al.* reported on 3D Ag@ZnO structures, formed into a microfluidic system for Surface Enhanced Raman Spectroscopy (SERS). Sang *et al*. [89] and Han *et al.* [90] reported on hydrothermal growth of ZnO nanostructures into microfluidic channels. After biofuncionalization, the developed nanostructures provided good fluorescence response for captured dye-labeled target protein molecules [89]. Non enzymatic glucose detection was proposed by Tarlani *et al.* [93]. The ZnO surface functionalization was achieved through special growth conditions in the presence of aminoacids. The developed biosensor showed good electrochemical response and selectivity to fructose and uric acid. This methodology of non-enzyme ZnO template has a great potential and can be transferred to optical methods of detection. Sarangi *et al.* proposed a direct optical (ZnO photoluminescence quenching) non enzymatic glucose sensing, based on photodegradation of glucose on ZnO surface [74]. Although the sensors showed good sensitivity and, as reported,
selectivity, the question of selective capture of glucose is raised by the authors. Meanwhile, the non-enzymatic glucose detection with ZnO optical biosensors remains to be an actual topic.

4.2. Combination of ZnO with metal nanoparticles

Combination of ZnO with metal particles of Au and Ag allows forming the most efficient biosensors based on SPR [41, 52 54, 69, 73, 100, 101] and SERS [36, 40 48, 65, 95, 97, 98, 99] methods. The doping of ZnO with Fe, Ni, Co, Sn *etc* improves the electrical characteristics and allows obtaining highly efficient electrical and electrochemical biosensors. This doping effect with these metals is not considered in this review as it is dedicated to optical biosensors.

4.2.1. SPR based biosensors

Basically, Au and Ag nanostructures are used in surface Plasmon resonance (SPR) devices due to absorption in visible range. Combining of ZnO with metal nanoparticles induces modification on structure, electrical and optical properties of the nanocomposites [69]. As reported in by Viter *et al.,* the deposition of ZnO nanolayers over Au nanoclusters has influenced PL of ZnO and the position and the intensity of SPR peaks. Due to Schottky barrier at the interface between ZnO and Au, the charge transfer of electrons from the conductive band of ZnO to Au occurs. It might increase the number of electrons which will participate in the interaction with biomolecules in the SPR device [69].

ZnO can be used as a passive component and an active component of SPR biosensor. García-Marín *et al.* used Al-doped ZnO (AZO) nanolayers in Au SPR biosensor as a passive component to reduce losses in optical signal and provide better adhesion of Au nanostructures [102]. Previously, Au nanoparticles were used as biosensitive layer and ZnO was applied to increase the active surface area and to amplify the SPR signal [41, 54]. However, new approaches in ZnO-Au based SPR biosensors have been proposed [100, 101]. Kaur *et al.* reported on ZnO/Au SPR biosensor for detection of Neisseria meningitidis. ZnO thin film was deposited on glass prism, coated with Au nanolayers. Single strain (ss) DNA was immobilized on ZnO layer due to electrostatic interaction. The SPR reflectance curves showed an IR shift of SPR peak after ZnO deposition and ssDNA biofunctionalization. The SPR tests to different concentrations of complementary DNA (target DNA) showed linear IR shift of SPR peak vs target DNA concentration. The developed SPR biosensors showed good sensitivity in the range of target DNA concentration 10-180 ng/µl [101].

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Kaur *et al.* reported as well on ZnO/Au SPR biosensor for detection of cholesterol. Cholesterol oxidize (ChOx) was used as a bioselective layer, electrostatically deposited on ZnO. The SPR biosensor system made of ChOx/ZnO/Au/prism was tested to different concentrations of cholesterol. The developed biosensor showed linear response in the range of 0.1-10 mM of cholesterol [100]. Finaly, Shukl *et al.* have provided analysis of structural and optical properties of SiO_2/Au/ZnO sensing elements. The optimization of the sensor sensitivity within varying of the Au and ZnO thicknesses was performed. The achieved results could be appied to Au/ZnO SPR based biosensors [73].

4.2.2. SERS based biosensors

ZnO and ZnO/metal nanostructures are widely used as templates for surface enhanced Raman spectroscopy (SERS) [95, 96, 97, 98, 99]. Enhancement of the Raman intensity signal from organic molecules, adsorbed on ZnO surface has been observed due to the charge transfer between ZnO and the adsorbed species as reported by Sivashanmugan *et al.* [95] and Wang *et al.* [96]. Application of SERS nanostructures with 3D architecture (Au NP coated ZnO tertapods) has been reported as well [97-99]. The developed SERS sensors were studied for apomorphine detection (a component, used for treatment of Parkinson disease) and demonstrated the linear response in the range of concentrations of 1 μM to 100 nM. The tests to cancer cells detection have been also performed. The enhancement of the Raman signal was obtained due to cell-ZnO/Au interaction. The methodology for cell analysis was discussed.

Prospects for SERS detection of melamine in milk reported on 3D ZnO nanostructures, decorated with Ag nanoparticles for environmental monitoring and food control have been discussed [95, 96, 97, 98, 99]. It was proposed integration of SERS ZnO/Ag 3D nanostructures with microfluidic system. The detection of biomolecules in liquid phase has been developed. The SERS tests were performed in the range of 10^{-10}-10^{-4} M.

Although good working principle of SERS biomolecules detection with ZnO nanostructures, the question of selectivity is still remaining. In all reported works, [96-99] no bioselective layer was formed for SERS biomolecules detection. Perhaps in the future, the detection principle could be modified in order to provide selective detection of the biomolecules by SERS devices.

5. Mechanisms of interaction between ZnO with biomolecules

It is known that the consumer is not interested in what is happening in the biosensor, *i.e.* physical and chemical processes that lie at the basis of its work. The end user is primarily interested in such characteristics as accuracy, stability, reversibility (disposable or reusable biosensor), as well as the price, which takes into account all the previous features. But only if we understand the mechanism of what happens between biomolecules and nano-objects in nanobiointerface, we could control all of these features and improve the process of the biosensing.

Mechanisms of interaction between ZnO based biosensors and biomolecules have been widely analyzed [32, 34, 39, 52, 53, 55, 60, 62, 74-77, 82, 85]. There were proposed several possible mechanisms of interaction: charge transfer [34], resonance energy transfer [53], hydrophobic and electrostatic interaction [32, 39, 60, 62].

The charge transfer mechanism was offered by Zhao *et al.* to explain ZnO PL quenching after dopamine (DA) adsorption (Fig. 6). The study of UV absorption and FTIR confirmed DA-QD non covalent interaction (shift of the peak position in the range 230-300 nm and FTIR shifts,

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pointing to DA-QD interaction). As DA adsorption and ZnO emission (~530 nm) lied in different spectral ranges, so via no quenching and energy transfer could happen. DA can be in a reduced (donor) and an oxidized (acceptor) forms in liquid solutions. The DA form depends on pH of the solution. As pH of the solution during measurement was in the range of 7-8, the DA was oxidized by O_2 in basic solution. Thus, the possible PL quenching effect may be caused by the electron transfer from ZnO QDs to oxidize dopamine–quinone [34].

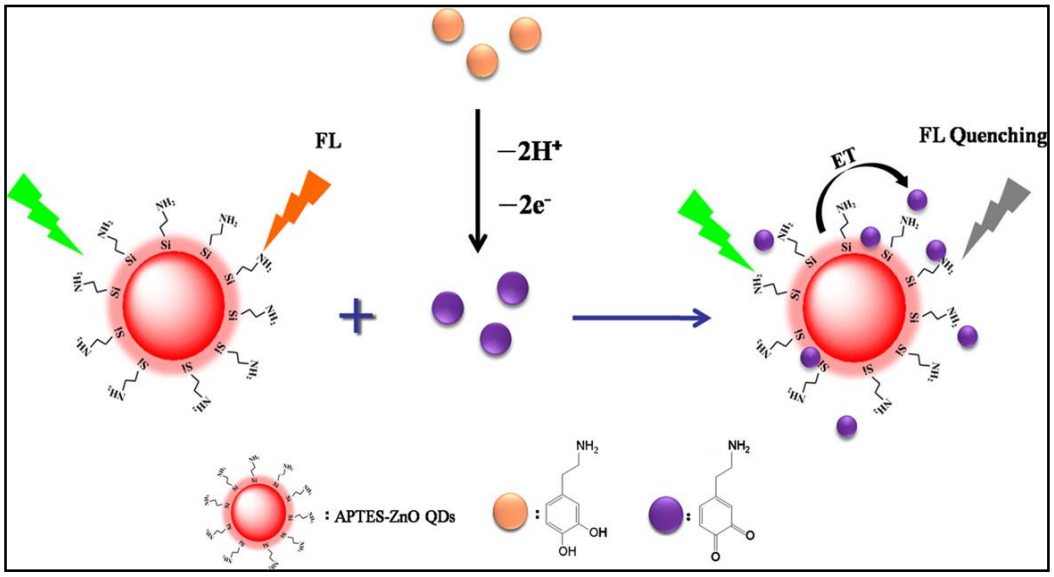

Fig.6. Schematic illustration of the developed ZnO QDs-based fluorescent probe for DA detection[8] [34]

For the effective analyte detection as well as to set the interaction mechanism, there is need to realize more than one method of registration. In different studies [30, 33, 39] it was investigated the sensitivity mechanism within the combination of optical and electrical methods.

Energy transfer mechanism was proposed to explain sensitivity mechanism [53]. The absorption of UV light by later adsorbed cancer cells decreased the UV peak of PL. Alternative method was described by Ansari *et al.* [30] where I-V tests were performed before and after immobilization of enzyme (urease) on ZnO nanostructures. Immobilization led to the increase of ZnO conductivity. ZnO was good for immobilization of negatively charged urease and provided fast electron transfer. The authors proposed following sensing mechanism to urea. During redox process, NH^{4+} and CO^{3-} can form as products of urea-urease reaction. NH^{4+} reacts with oxygen O_2^-, previously adsorbed on ZnO surface and produces electrons into the conductance band of ZnO. Concentration range of urea detection was in range of 1-100 mM (6-600 mg/ml).

ZnO field effect transistors (ZnO-FET), covalently functionalized with single stranded DNA aptamers, provide a highly selective platform for label-free small molecule sensing [33]. The selective detection of riboflavin down to the pM level in aqueous solution using the electrical current response of the ZnO-FET by covalently attaching a riboflavin binding aptamer to the surface was demonstrated. The response of the biofunctionalized ZnO-FET was tuned by attaching a redox tag (ferrocene) to the 3’ terminus of the aptamer, resulting in positive current modulation upon exposure to riboflavin down to pM levels.

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Ansari *et al.* [39] have studied nanostructured ZnO film deposited onto ITO glass plate for co-immobilization of rabbit IgG and BSA for OTA detection (Fig. 7). It was shown that IgG was adsorbed with F_c complex on ZnO surface and F_{ab} fragments of IgG was over ZnO surface. Immobilization was monitored with SEM. FTIR and electrochemical impedance spectroscopy (EIS) was used to detect mechanisms of interaction. FTIR showed that rabbit IgG was bound to Zn-O-Zn via hydrogen bonds and via electrostatic interaction. EIS showed decrease of electron transfer resistance after IgG immobilization due to accelerated electron communication between protein and the ZnO/ITO electrode. OTA adsorption led to the increase of the resistance. This may be attributed to the increased number of OTA molecules bound to the immobilized antibodies that perhaps provide a kinetic barrier for the electron transfer. IgG adsorption creates conductive layer over ZnO/ITO. When OTA is attached to the surface on IgG sites, the radius increases and blocked the channel for electron transfer. Electrochemical impedance spectroscopy measurements showed a good linear relationship between the electron transfer resistance and OTA concentration in the range, 0.006–0.01 nM/dm^3. It was proposed a special energy transfer mechanism to explain sensitivity effect. The lowest concentration of rabbit IgG that can be determined by the proposed biosensor is about 16-folds lowers than that of the biosensor based on Au film alone [39].

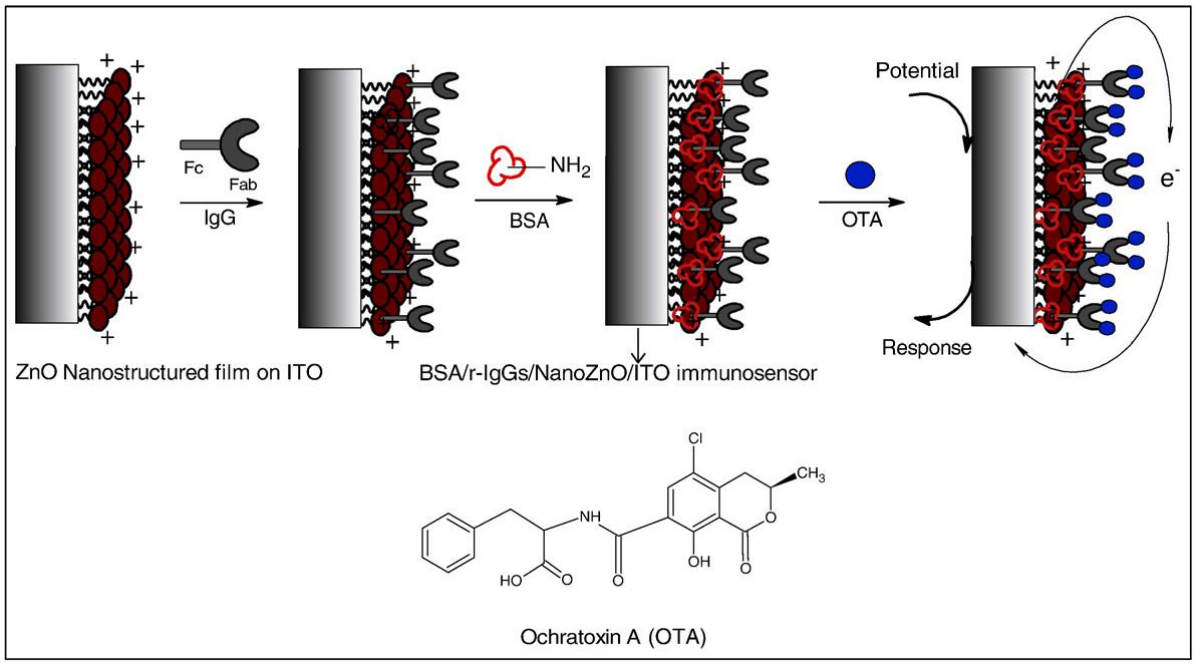

Fig.7. Schematic of fabrication of BSA/r-IgGs/Nano-ZnO/ITO immunosensor along with the biochemical reaction between OTA and immunosensor[6] [39]

Theoretical assumptions of interaction between ZnO and nucleic acid bases (adenine (A), guanine (G), cytosine (C), thymine (T) and uracil (U)) were calculated by Iyera *et al.* [50]. Interaction strength was highest at the nitrogen ring site. The interaction strength expressed in terms of the binding energy is found to be highest at the ring nitrogen site for all nucleobases, the sequence being G < T < U < A < C. The interaction between the ZnO-cluster and nucleobases is dominated by the covalent and weak Van der Waals forces, where the degree of hybridization

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between Zn-d with N-p orbitals determines the relative interaction strength of individual nucleobases and $(ZnO)_{12}$ with a marginal contribution from the ionic forces [50].
Viter *et al.* [62] suggested Van der Vaals and hydrophobic bonds as the mechanism of interaction between ZnO surface and *salmonella* particles. Immobilization of anti-*salmonella* antibodies (Ab) was assisted by charge transfer from protein to ZnO surface. It was assumed that lone electron pair density moves from the ring nitrogen atom of anti-salmonella Ab molecules to uncoordinated surface ZnO atoms (oxygen vacancies). As a result, the concentration of free carrier density increases, which in its turn, diminishes the depletion layer and stimulates the increase of the near band emission luminescence. Adsorbed anti-*salmonella* antibodies stimulate exiton-phonon interaction in ZnO nanorods, resulted in the increase of first exciton replica emission. Specific selective interactions between immobilized antibodies and antigens of *salmonella* (key-lock principle) can be monitored by photoluminescence of ZnO. The detection range of the fabricated biosensor is observed at concentration of $10^2 – 10^6$ cells/ml. Thus, for the analyte detection there is a need to realize more than one method of registration. Combination of optical and electronic parameters of ZnO based biosensors is a good method for biomolecules detection.

FTIR analysis of bare ZnO and ZnO with proteins has been performed by Wang *et al*. It was found that amide I (1550-1750 cm^{-1}) and amide II (1050-1200 cm^{-1}) bands of the proteins were weakened and shifted after their deposition on the ZnO surface. The interaction mechanism between ZnO and the proteins was based on the ZnO surface binding with amino acid resides of the main polypeptide chain of proteins. Protein adsorption models with reversible and irreversible adsorption were discussed. Conformal changes and orientation of the irreversibly adsorbed protein on ZnO surface are expected [85]. Klaumünzer *et al*. observed a decrease of visible band of ZnO PL after BSA adsorption, which was explained by the interaction of BSA with ZnO via aminoacids. Red shift of the ZnO visible emission was registered after BSA adsorption on the ZnO surface. It was proposed that BSA can interact electrostatically via its carboxylate anions with the positively charged ZnO surface. It was mentioned that oxygen vacancies could be the adsorption sites. In particular, electrostatic interactions between carboxylate anions of BSA and monovalent oxygen vacancies in the ZnO surface should induce the formation of doubly positively charged oxygen vacancies. It results in the reduction of the intensity of the green defect-center emission in ZnO [82].

Politi *et al*. reported on the ZnO photoluminescence biosensors for avidin-HRP detection. ZnO NWs were functionalized with protein A via covalent binding. Defect and exciton bands of ZnO PL have been studied at every step of ZnO functionalization and biosensor testing. The decrease of PL was observed during the forming of the bioselective layer and the enhanced PL was found after avidin-HRP adsorption. It was proposed that the transfer of electrons to biomolecules is possible via bioselective layer formation and the change of surface band banding resulted from avidin-HRP adsorption [75].

Interaction mechanisms are not completely identified and are of interest to researchers in order to effectively immobilize the sensitive layer, to get high level of analyte detection, as well as to make it easier the use of the biosensor and its economic utilization. Interaction mechanisms need more than one method to be determined. Correlation between optical and electronic parameters of ZnO based biosensors is a good method for detection of biomolecules.

6. Conclusions

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The wide application of ZnO nanostructures in biosensors is caused by combination of specific optical and electrical properties that can be controlled due to the availability of many techniques. ZnO nanostructures may be successfully combined with other materials by doping, generating nanocomposites, heterostructures and hybrid structures *etc*. allow obtaining the necessary parameters of nanobiointerface. One-dimentional ZnO nanostructures (nanorods, nanowires, nanotubes and other structures) are primarily used in optical biosensors. Their high surface/volume ratio, surface tailoring ability, novel electron transport properties and electronic conductance makes them extrasensitive for surface functionalization.

There are two main methodologies of forming of bioselective layer on ZnO surface: the direct immobilization of biomolecules and the covalent binding. In the case of direct immobilization the hydrogen bonds and the Van der Waals forces are suggested to play major roles in the binding process between ZnO and biomolecules. The covalent binding involves a cross-linking agent between ZnO surface and molecules of the bioselective layer, which provides a significant structure of the bioselective layer and improves a biorecognition thus it requires more complicated procedure. The mechanism of biomolecules absorption by ZnO is based on the interaction of biomolecules with ZnO via amino acids reside of the main polypeptide chain of protein. Protein adsorption models with reversible and irreversible adsorption are discussed. Conformal changes and orientation of the irreversibly adsorbed protein on ZnO surface can take place.

Crucial role in the biomolecule immobilization process plays the surface adsorption, which in turn depends significantly on the surface morphology and topography as well as the type and the number of defects on ZnO surface. Among the variety of ZnO surface topography nanorods claims to be as the most efficient for the immobilization of biomolecules. Using optical methods such as PL, it is also possible to determine the type of defect(s) playing the role of adsorption centers of biomolecules, and to increase the efficiency of immobilization, respectively. Although the PL biosensors are not the most commonly used with respect to other ones, but PL is noteworthy as simple, highly sensitive and selective method for establishing defects responsible for adsorption of biomolecules.

Combination of ZnO with metal particles of Au and Ag allows to form the most efficient biosensors based on SPR and SERS optical methods. New approaches are targeted to combine ZnO nanostructures with carbon based nanomaterials such as graphene, carbon nanotubes, *etc*. in order to obtain new improved electrical and optical properties, which can be used for biosensor applications.

According to the analysed data, there are all reasons to underline that the sensitivity of analysis based on the ZnO nanostructures with the different approaches for registration of formed specific signal, achieves high level which is, as a rule, correspond to practice demands. Nevertheless, there is a one very important question - what kind of biosensor based on the ZnO construction and concrete algorithm of the analysis fulfillment are the most effective for practical application? The answer to it depends on many factors which first of all include such characteristics as: selectivity, sensitivity, expressivity, simplicity, reproducibility, cheapness as well as possibility of analysis fulfillment in on-line regime and in field conditions.

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Footnotes

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