

## Nanostructured metal oxide-based biosensors

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Nanostructured metal oxides (NMOs) have recently become important as materials that provide an effective surface for biomolecule immobilization with desired orientation, better conformation and high biological activity resulting in enhanced sensing characteristics. Nanostructured metal oxides with unique optical, electrical and molecular properties along with desired functionalities and surface charge properties provide interesting platforms for interfacing biorecognition elements with transducers for signal amplification. In this review, we discuss the various approaches that have been adopted for improving the performance of NMO-based biosensors for clinical and non-clinical applications. The performance of an NMO-based biosensor can be improved by tailoring the properties of the metal oxide–biomolecule interface through engineering of morphology, particle size, effective surface area, functionality, adsorption capability and electron-transfer properties. These interesting NMOs are expected to find applications in a new generation of miniaturized, smart biosensing devices.

Among the various types of nanomaterials that have been developed, nanostructured metal oxides (NMOs) have recently aroused much interest as immobilizing matrices for biosensor development (Figure 1) [1–9]. Nanostructured oxides of metals such as zinc, iron, cerium, tin, zirconium, titanium, metal and magnesium have been found to exhibit interesting nanomorphological, functional biocompatible, non-toxic and catalytic properties. These materials also exhibit enhanced electron-transfer kinetics and strong adsorption capability, providing suitable microenvironments for the immobilization of biomolecules and resulting in enhanced electron transfer and improved biosensing characteristics. Various morphologies of NMOs have been obtained using a variety of methods, including soft templating for the preparation of nanorods and nanofibers [10], sol–gel methods for the production of three-dimensional (3D) ordered rough nanostructures [6,11], radiofrequency sputtering for rough nanostructures [4] and hydrothermal deposition for nanoparticles with controlled shape [12]. All of these NMOs have been reported to have potential applications in biosensors. Recently, the optical, electrical and magnetic properties of NMOs have been reported to be enhanced through the incorporation of nanoparticles of conducting or semiconducting materials such as carbon nanotubes, graphene, gold and silver, as well as quantum dots of various semiconductors, with advantages for improved biosensor characteristics [13–16]. It is expected that the judicious application of an NMO may lead to the fabrication of novel biosensing devices with enhanced signal amplification and coding strategies for bioaffinity assays and efficient electrical communication with redox biomolecules/enzymes that may address future diagnostic needs.

The unique properties of NMOs offer excellent prospects for interfacing biological recognition events with electronic signal transduction and for designing a new generation of bioelectronics devices that may exhibit novel functions. The controlled preparation of an NMO is considered to play a significant role in the development of biosensors. Efforts are being made to explore the prospects and future challenges of NMOs for the development of biosensing devices and the evolution of new strategies for bioaffinity assays and efficient electrical

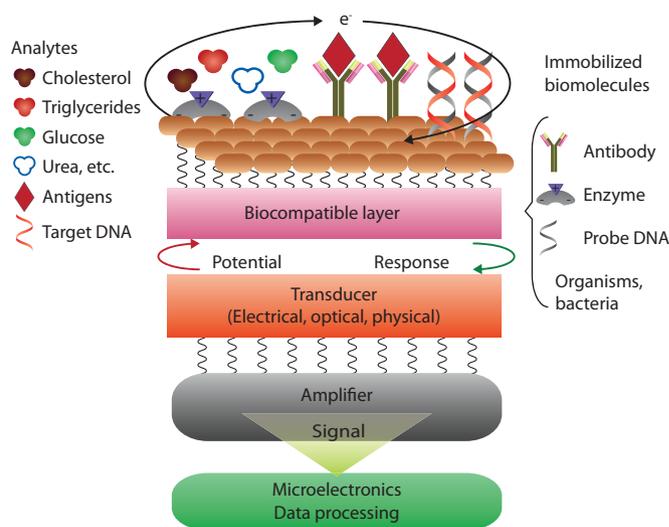


Figure 1. Schematic structure and operating principle of a biosensor

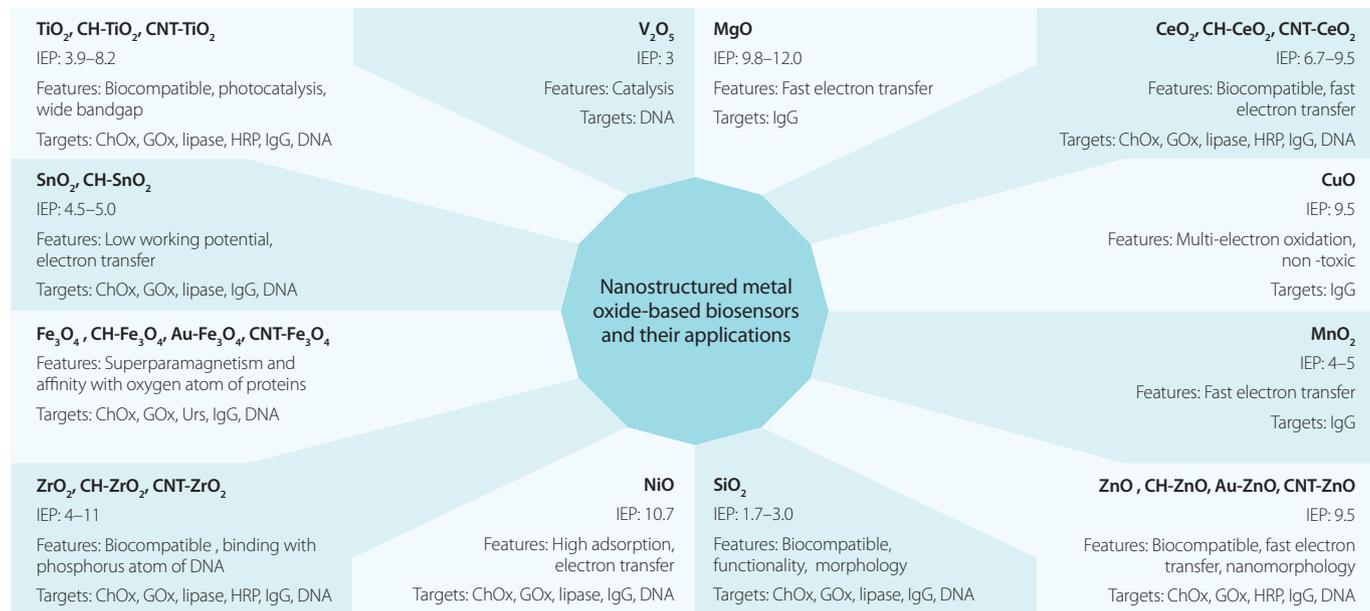
communication. In this review, we focus on developments over the past five years in NMO-based biosensors for clinical and non-clinical applications (Figure 2).

### Fundamentals of nanostructured metal oxides for biosensing

Among the various immobilizing matrices that have been developed, NMOs have exceptional optical and electrical properties due to electron and phonon confinement, high surface-to-volume ratios, modified surface work function, high surface reaction activity, high catalytic efficiency and strong adsorption ability. For these reasons, NMOs have been utilized to increase the loading of biomolecules per

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**Figure 2.** Representative NMOs and their biosensing applications (IEP, isoelectric point; ChOx, cholesterol oxidase; GOx, glucose oxidase; HRP, horseradish peroxidase; IgG, immunoglobulin G; Urs, urease).

unit mass of particles. The use of an NMO allows for the possibility of many new signal transduction technologies in biosensors arising from the sub-micrometer dimensions that can be utilized for simple and rapid *in vivo* analysis [17].

A biosensor is an integrated miniaturized device that employs a biological element (antibody, enzyme, receptor protein, nucleic acid, whole cell or tissue section) as a sensing element coupled to a transducer for signal detection. A biosensor utilizes the selectivity of the biomolecule and the processing power of modern microelectronics and optoelectronics and is hence a powerful analytical tool with applications in medical diagnostics and other areas. Due to their specificity, portability, rapid response time and low cost, biosensors are projected to play a critical role in both clinical and non-clinical applications. The prevailing miniaturization tools allow packing of numerous microscopic electrodes along with transducers into a small footprint of a biochip device, leading to the design of high-density bioarrays [18].

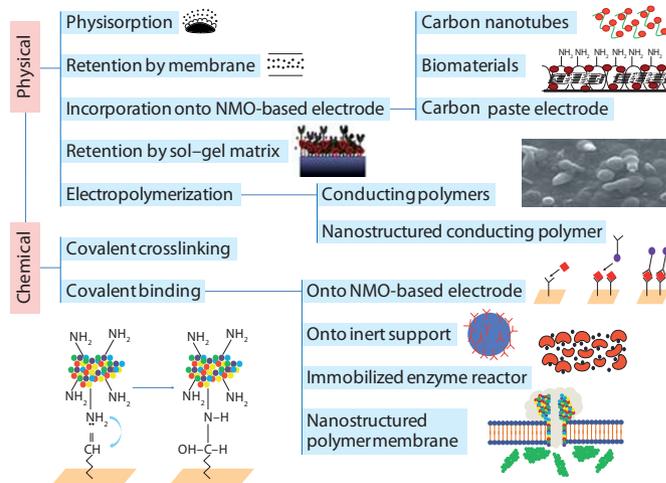
Investigations relating to the effect of the optical and electrochemical properties of NMOs, suspended media, solid–liquid interfaces and nanobiointerfaces are being conducted for biosensor applications. To fabricate an efficient biosensor, it is crucial to select an NMO that is suitable for immobilization of the desired biomolecule (Figure 3). The interface formed due to binding between an NMO and a biomolecule is known to affect the performance of a biosensor. The formation and properties of a nanobiointerface depend on the nature of the NMO; parameters like effective surface area, surface charge, energy, roughness and porosity, valence/conductance states, functional groups, physical states and hygroscopic nature all affect the formation of a biointerface. In general, biomolecules bind with metal oxide nanoparticles via physical adsorption or chemical binding. The physical adsorption of a biomolecule arising due to weak interactions (*e.g.* van der Waals, electrostatic, physisorption) depends on the surface morphology, reaction medium and net surface charge. Short-range forces arising via charge, steric, depletion and solvent interactions also play an important role in the preparation of a nanobiointerface. Covalent binding of a biomolecule to an NMO depends on the availability of functional groups, which can be prepared by appropriate engineering of chemical reactions. An effective NMO biointerface can help a biomolecule retain its biological activity with high stability by establishing a biocompatible microenvironment. The rate of electron transfer depends on the characteristics of the NMO–biomolecule interactions [19]. There is thus considerable opportunity for the development of biosensors with

improved sensitivity and detection limits, as well as lower cost and extended shelf life, through the use of NMOs. Various biomolecules, including enzymes, nucleic acids and antibodies, can be immobilized on NMOs (Figure 3), and some of the most promising biomolecular systems are reviewed below.

### Enzyme-immobilized NMO-based biosensors

An enzyme biosensor is based on a biochemical reaction that occurs between an analyte and a biocatalyst immobilized on a suitable substrate. Nanostructured metal oxides have excellent prospects for interfacing biological recognition events with electronic signal transduction as a new generation of biosensors in which active enzyme sites are coupled directly with an NMO electrode resulting in direct electron transfer between the enzyme and NMO with improved biosensing properties [17].

Nanostructured metal oxide-based electrodes provide a biocompatible electroactive surface for enzyme immobilization with improved conformation, orientation and biological activity [17]. An important challenge in the development of amperometric bioelectrodes is the establishment of satisfactory electrical communication between the



**Figure 3.** Various immobilization schemes for biosensor fabrication

active sites of biomolecules and the NMO electrode surface. For example, the redox centers of most oxidoreductases are electrically insulated by protein shells. As a result, enzymes cannot be oxidized or reduced at the electrode surface at any potential. The possibility of direct electron transfer between enzymes and the NMO-based electrode surface could pave the way for the development of superior reagentless biosensing devices, as it may obviate the need for co-substrates or mediators, allowing efficient transduction of biorecognition events. The close approach of a redox site to an electrode is often impossible without the application of a large overpotential that could lead to poisoning of the electrode surface and the denaturation of enzymes. Advances in achieving direct electron transfer can be made by modification of an enzyme or electrode surface with a mediator or a nanoparticle.

Many approaches have been followed for the engineering of biosensor components, particularly in the area of NMO-based immobilizing matrices and transducers (electrochemical and optical), to obtain enhanced biosensing performance in various applications. Nanostructured metal oxides with useful optical, electrochemical and molecular properties have been prepared by various syntheses routes, as well as through the doping of nanomaterials and fabrication of organic/inorganic nanocomposites. In particular, NMOs with engineered morphology, size (for biocompatibility), functionality, adsorption capability and high biomolecule loading capacity have been found to provide enhanced electron transport between biomolecules and electrodes. The microenvironment provided by an NMO helps an enzyme to retain its conformation with maximum biological activity, resulting in enhanced signal transduction and biosensor stability. The performance of a biosensor can also be affected by the choice of transducer (Figure 4). Sensitive and selective biosensors have been fabricated using the effect of surface plasmon resonance. However, such systems are complex and require considerable time, expense and expertise to develop. Electrochemical techniques such as cyclic voltammetry, on the other hand, are simple and can be utilized for the rapid and cost-effective development of biosensors. The characteristics of an electrochemical biosensor can be improved through the use of differential pulse voltammetry (DPV). Electrochemical impedance spectroscopy (EIS) is a non-destructive electrochemical analytical technique that has recently been used for signal transduction and which has been particularly useful for clarifying the mechanisms of charge transfer resistance arising due to electron transport between a biomolecule and the electrode surface. Electrochemical devices have advantages in terms of size, cost, detection limit, response time, long-term stability and power requirements, and therefore have great promise for a wide range of biomedical and environmental applications. Nanostructured metal oxides with various surface architectures have been used to produce a number of electrochemical biosensing devices with improved sensitivity and selectivity.

### Glucose oxidase

There have been many attempts to immobilize glucose oxidase (GOx) on NMOs for the fabrication of a glucose biosensor. Metal oxide-based nanomaterials with high isoelectric point (IEP) have been found to be suitable for the immobilization of biomolecules with low IEP via electrostatic interactions. Improving the sensitivity and linearity of glucose biosensors using other NMOs and their composites with desired biopolymers has been the focus of many studies in this area.

A single-crystal NMO consisting of ZnO nanocombs prepared in bulk by vapor-phase deposition provides a 3D porous network with high specific surface area and good biocompatibility for GOx, allowing GOx to be loaded at high densities [20]. This favorable NMO-based microenvironment offers high sensitivity ( $15.33 \mu\text{A mM}^{-1}\text{cm}^{-2}$ ) and high affinity of GOx to glucose, achieving a Michaelis–Menten constant ( $K_m$ ) of 2.19 mM. An NMO-based glucose biosensor based on ZnO nanotube arrays synthesized by chemical etching of ZnO nanorods and electrochemical deposition onto a gold surface to immobilize GOx also displays improved characteristics [21]. The

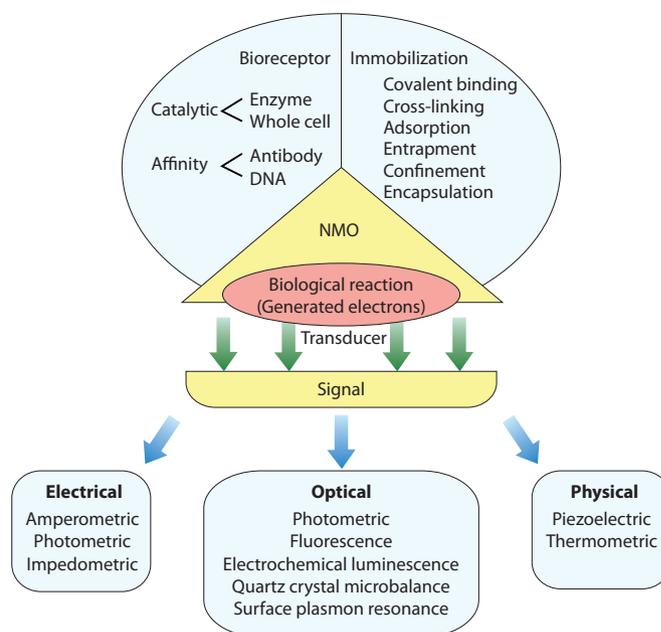


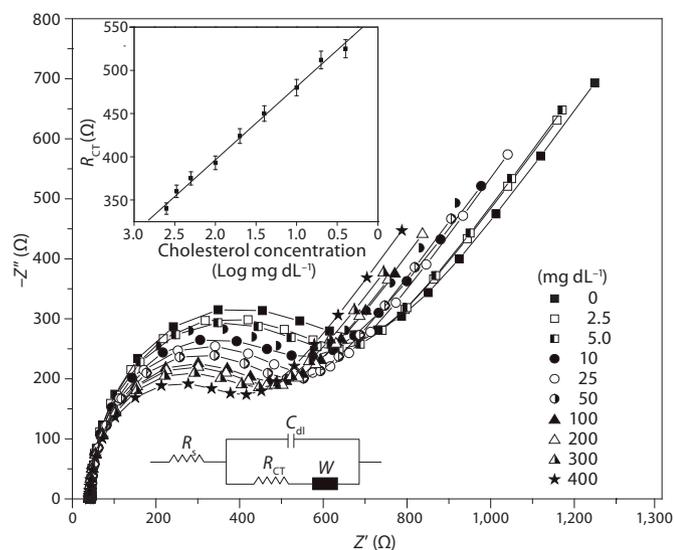
Figure 4. Various types of transducers for biosensing

intrinsic nanotubular structure of ZnO provides good response without a mediator, wide linear range, low detection limit ( $1 \mu\text{M}$ ), sensitivity of  $21.7 \mu\text{A mM}^{-1}\text{cm}^{-2}$  and  $K_m$  of 21.7 mM.

A highly sensitive amperometric biosensor for continuous glucose monitoring based on a single ZnO nanofiber (195–350 nm in diameter) synthesized by electrospinning has been shown to have high and reproducible sensitivity of  $70.2 \mu\text{A mM}^{-1}\text{cm}^{-2}$  due to its high specific surface area and higher capacity for enzyme loading, providing a microenvironment that helps GOx retain its bioactivity and enhanced thermal stability over a temperature range of 20–85 °C [22]. A recently fabricated mediator-free glucose biosensor based on GOx and horseradish peroxidase (HRP) immobilized on a carbon-decorated ZnO (C–ZnO) nanowire array electrode provides high sensitivity and a low detection limit for glucose [23]. The C–ZnO nanowire array is a fast, direct electrochemical platform for GOx. Glucose oxidase has also been adsorbed onto hexagonal ZnO nanorods on the tip of a silver-coated borosilicate glass capillary (0.7  $\mu\text{m}$  in diameter) for intracellular glucose measurement in human adipocytes and frog oocytes [24]. ZnO nanowires have been found to be biosafe with high ionic bonding (60%), and to be insoluble at biological pH. This intracellular biosensor exhibits fast response times and wide linear range.

Nanostructured  $\text{CeO}_2$ , with a high IEP of 9.4, exhibits a high electron-transfer rate constant and good surface coverage, allowing high enzyme loading and fast electron transfer between the active GOx site and the electrode. Saha *et al.* [25] deposited nanoporous thin  $\text{CeO}_2$  films onto platinum-coated glass plates using pulsed laser deposition for the immobilization of GOx, producing a glucose biosensor with a linear response to glucose and a  $K_m$  value as low as 1.01 mM. However, this glucose biosensor is expensive to produce, requires considerable expertise to realize, and has only a limited glucose detection range and poor detection limit. A simple sol–gel method can instead be used to produce a nanostructured  $\text{CeO}_2$  film for the fabrication of a glucose biosensor [6].

Wen *et al.* [26] used a novel approach to fabricate a biofunctional nanocatalyst comprising evenly dispersed Au/Pt hybrid nanoparticles assembled onto  $\text{NH}_2$ -functionalized  $\text{TiO}_2$  colloid spheres via electrostatic interactions. The nanoparticulate  $\text{TiO}_2$ -Au/Pt has been reported to be an excellent performance-enhancing material, providing superior catalytic reduction of  $\text{H}_2\text{O}_2$ .  $\text{NH}_2$ -functionalized nanoporous  $\text{TiO}_2$  assembled with platinum nanoparticles has also been utilized for glucose biosensing



**Figure 5.** Electrochemical impedance spectroscopy response of a ChOx/nano-Fe<sub>3</sub>O<sub>4</sub>/ITO bioelectrode as a function of cholesterol concentration at bias potential of 0.06V. Inset shows a calibration curve between charge-transfer resistance ( $R_{CT}$ ) value and the logarithm of cholesterol concentration.

via the immobilization of GOx [27]. Such CH/TiO<sub>2</sub>-Pt nanoparticle films provide a low detection limit (0.25 μM) due to enhanced electron transfer between GOx and the electrode surface. The results of these studies have implications regarding the development of other enzyme electrodes based on these NMOs.

A nanocomposite film consisting of GOx immobilized on Fe<sub>3</sub>O<sub>4</sub> nanoparticles (~22 nm in diameter) well-dispersed in CH for mediator-free glucose detection has been shown to provide enhanced electron transfer resulting in improved sensing characteristics including better linearity, higher sensitivity and lower  $K_m$  (0.141 mM) [28]. The Fe<sub>3</sub>O<sub>4</sub> nanoparticles in this nanocomposite system provide a high electroactive surface area that contributes to the oriented immobilization of GOx. MgO-based polyhedral nanocages and nanocrystals prepared by simple non-catalytic thermal evaporation have been utilized for the fabrication of glucose biosensors with high and reproducible sensitivity of 31.6 μA μM<sup>-1</sup> cm<sup>-2</sup> with a response time of less than 5 s, a linear dynamic range of 1.0–9.0 μM and a detection limit as low as 68.3 ± 0.02 nM [29]. A mediator-free glucose biosensor based on the electrochemical co-deposition of nanoporous NiO<sub>x</sub> nanoparticles and GOx onto a glassy carbon electrode (GCE) has been reported [30]. The surface coverage and heterogeneous electron transfer rate constant ( $k_s$ ) of GOx immobilized on NiO<sub>x</sub> film are 0.945 pmol cm<sup>-2</sup> and 25.2 ± 0.5 s<sup>-1</sup>, indicating that the NiO nanoparticles have high enzyme loading ability and fast electron transfer between GOx via conducting tunnels. Such a sensor could be useful for real-time monitoring of glucose and the research opens a new direction for the utilization of NiO and MgO nanostructures as an electron mediator for efficient biosensors.

### Cholesterol oxidase

Many NMOs have been used to fabricate cholesterol biosensors using cholesterol oxidase (ChOx). A nanostructured ZnO film with a wide bandgap (3.37 eV) and large excitation binding energy (60 eV), prepared using sol-gel process, has been used for the immobilization of ChOx (IEP 4.7) to fabricate a cholesterol biosensor with a wide linear range (5.0–400 mg dL<sup>-1</sup>), low  $K_m$  (0.98 mg dL<sup>-1</sup>), and high sensitivity (59 nA mg<sup>-1</sup> dL cm<sup>-2</sup>) [31]. A thin porous ZnO film obtained by radiofrequency magnetron sputtering under high pressure (50 mTorr) has been utilized to fabricate a cholesterol biosensor with a linear range from 0.65 to 10.34 mM and  $K_m$  values as low as 2.1 mM [4]. These cholesterol biosensors could be used to estimate cholesterol concentration in serum/blood samples.

A highly sensitive amperometric cholesterol biosensor based on platinum-incorporated fullerene-like ZnO hybrid nanospheres (50–200 nm) has been fabricated by electrodeposition of the nanospheres onto a GCE [32]. This biosensor exhibits improved sensitivity of 1.886 mA mM<sup>-1</sup> cm<sup>-2</sup> resulting in increased enzyme activity. It has been found that the combination of ZnO and platinum nanoparticles facilitates low-potential (0.2V) amperometric detection of cholesterol with enhanced anti-interference ability. Improved sensitivity and a low detection limit have been achieved for a cholesterol biosensor prepared using a modified ZnO nanostructure with a gold electrode [33]. This bioelectrode shows high and reproducible sensitivity of 23.7 μA mM<sup>-1</sup> cm<sup>-2</sup>, detection limit of 0.37 ± 0.02 nM and linear range of 1.0–500 nM. However, the observed higher value of  $K_m$  (4.7 mM) indicates poor interaction between ZnO and ChOx.

A cholesterol biosensor based on a nanocomposite film consisting of SnO<sub>2</sub> nanoparticles and CH has been reported by Ansari *et al.* [34]. The high sensitivity of this sensor has been attributed to the increased adsorption ability, high electrocatalytic activity and good biocompatibility of the CH-SnO<sub>2</sub>/ITO nanocomposite for ChOx. It appears that the hydrogen bonds in this nanocomposite facilitate maintenance of the native configuration of ChOx. The lower  $K_m$  (3.8 mM) obtained for this nanocomposite is due to enhanced affinity of ChOx towards cholesterol, indicating strong binding of ChOx to the CH-SnO<sub>2</sub> surface. Electrodeposited CoO<sub>x</sub> nanoparticles have been used both for entrapment of ChOx and as a mediator for faster oxidation [35]. The porous surface of CoO<sub>x</sub> acts as a transport channel for the substrate, reducing the mass transfer resistance and enhancing the efficiency of biocatalysis. This sensor shows linearity up to 50 mM, sensitivity of 43.5 nA mM<sup>-1</sup> cm<sup>-2</sup> and a detection limit of 4.2 mM. The apparent  $K_m$  and the response time of this biosensor are 0.49 mM and 15 s.

A nanostructured Fe<sub>3</sub>O<sub>4</sub>-based EIS platform has been utilized for cholesterol detection through the immobilization of ChOx [36]. In this system, the charge-transfer resistance decreases with increasing cholesterol concentration (Figure 5). This biosensor exhibits improved linearity, a low detection limit and low  $K_m$  (0.8 mg dL<sup>-1</sup>). In spite of these interesting developments, there remains considerable room for improvement of NMO-based cholesterol biosensors. For example, no NMO-based biosensor has been developed for the estimation of total cholesterol concentrations.

### Horseradish peroxidase

A waxberry-like ZnO microstructure with 8–10 nm-diameter nanorods on a GCE has been used for the immobilization of HRP [37]. This matrix has been found to be an excellent material for the immobilization of HRP, providing rapid electron transfer and allowing the fabrication of efficient biosensors with high electrical conductivity, large surface area, abundant oxygen vacancies and good biocompatibility. A flower-like ZnO-gold nanoparticle-Nafion nanocomposite bearing immobilized HRP has been reported for H<sub>2</sub>O<sub>2</sub> detection via direct electron transfer [38]. This sensor has a linear response over a wide range of H<sub>2</sub>O<sub>2</sub> concentration (15–1,100 μM) and a detection limit of 9.0 μM, indicating that this matrix provides a favorable microenvironment for the enzyme to retain its activity.

ZnO nanorods have also been fabricated on gold wires by a hydrothermal reaction without a surfactant [39]. In this system, the ends of the gold wires were coated with a thin layer of Zn-Au alloy to improve the nucleation and growth of ZnO nanostructures. This approach improved the performance of the biosensor by allowing the alternate immobilization of poly(sodium-4-styrenesulfonate) and HRP onto ZnO for H<sub>2</sub>O<sub>2</sub> detection without the need for a mediator. This multi-layered HRP sensor exhibits a wide linear range and low detection limit, and its sensitivity increases with the thickness of the immobilized HRP layer from 36.28 μA mM<sup>-1</sup> cm<sup>-2</sup> for a monolayer. Using a physisorption technique, HRP has been immobilized on nanostructured CeO<sub>2</sub> [40]. The bioelectrode thus prepared exhibits low  $K_m$  (2.21 μM) and linearity for H<sub>2</sub>O<sub>2</sub> detection over the range 1.0–170 μM.

Various morphologies of nanostructured MgO, including flower-like nanostructures and nanotubes, have recently been reported. For example, a composite of MgO nanoparticles and CH has been developed for the immobilization of HRP for H<sub>2</sub>O<sub>2</sub> detection in the presence of hydroquinone as a mediator [41]. This biosensor has a rapid response to H<sub>2</sub>O<sub>2</sub> due to the excellent electrocatalytic behavior of MgO. A linear relationship is obtained in the concentration range of 0.1–1,300 mM with a detection limit of 50 μM. However, this sensor can be utilized for only a few weeks before degrading.

An SnO<sub>2</sub> nanorod array-based biosensor produced by Liu *et al.* [42] has high sensitivity (0.379 mA mM<sup>-1</sup> cm<sup>-2</sup>), a low detection limit (0.2 μM), high selectivity and low K<sub>m</sub> (33.9 μM). These single-crystalline SnO<sub>2</sub> nanorods were grown on an alloy substrate and provide a large number of void spaces for HRP immobilization.

### Urease and glutamate dehydrogenase

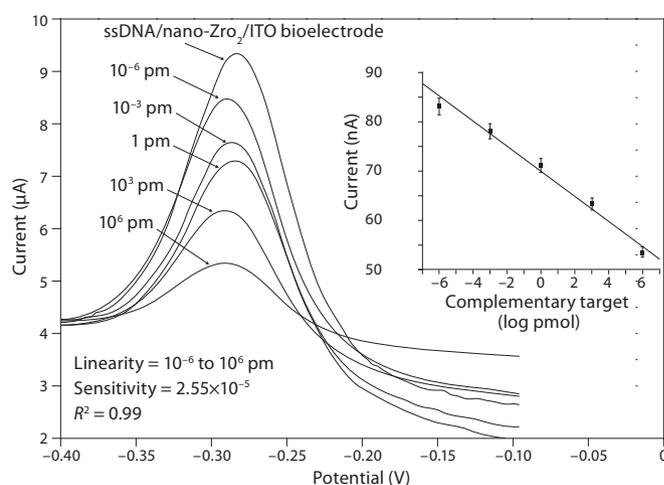
Solanki *et al.* [43] have co-immobilized urease (Urs) and glutamate dehydrogenase (GLDH) on a nanocomposite consisting of nanostructured ZnO for application as a urea biosensor. This bioelectrode shows high sensitivity and a detection limit of 13.5 mg dL<sup>-1</sup> with K<sub>m</sub> of 6.1 mg dL<sup>-1</sup>, indicating good affinity of Urs-GLDH for urea. A nanocomposite of ZnO and CH [44] has been shown to provide improved biosensing characteristics due to its high electroactive surface area and the combined effect of CH and ZnO nanoparticles resulting in increased loading of Urs-GLDH and improved charge transport. This biosensor exhibits high linearity and low K<sub>m</sub> (4.92 mg dL<sup>-1</sup>) due to enhanced affinity of enzymes toward the nanobiocomposite. The affinities of Urs and GLDH are also high for a nanobiocomposite film of superparamagnetic Fe<sub>3</sub>O<sub>4</sub> nanoparticles and CH due to a low K<sub>m</sub> of 0.56 mM [45]. The Fe<sub>3</sub>O<sub>4</sub> nanoparticles increase the active surface area for the immobilization of enzymes, enhanced electron transfer and increased the shelf-life of the electrode. It will be interesting to utilize these bioelectrodes for estimation of urea concentrations in clinical samples.

### Lipase

Lipase immobilized on magnetic nickel-ferrite nanoparticles at the gate of an ion-selective field-effect transistor has been used to detect tributyrin, trioctanoate and triolein concentrations in the range of 0.1–1.5% [46]. Nanostructured CeO<sub>2</sub> films (~35 nm thick) have also been used for the fabrication of a lipase-based tributyrin biosensor. This biosensor exhibits good linearity, low detection limit, long shelf-life and low K<sub>m</sub> (22.27 mg dL<sup>-1</sup>). A lipase-based bioelectrode of nanostructured CeO<sub>2</sub> and indium tin oxide (ITO) prepared by Solanki *et al.* [47] similarly shows high affinity for tributyrin [47]. Efforts should be made to utilize these electrodes in NMO-based biosensing devices for estimation of triglyceride concentrations in blood.

### Other enzymes

MnO<sub>2</sub> nanoparticles with various structures, including amorphous MnO<sub>2</sub>, α-MnO<sub>2</sub> nanoparticles and β-MnO<sub>2</sub> nanowires, electrochemically deposited on a GCE with CH hydrogel and choline oxidase have been used for the detection of choline [48]. Biosensors based on crystalline MnO<sub>2</sub> respond to choline far more quickly than those based on amorphous MnO<sub>2</sub> under amperometric measurement conditions. β-MnO<sub>2</sub>-based biosensors show a particularly fast response time. It has been found that the specific surface area, the amount of MnO<sub>2</sub> trapped on the electrodes, the crystalline structure and even dimensionality play critical roles in determining the biosensing behavior. Such biosensors exhibit a linear range of 2.0–580 μM for α-MnO<sub>2</sub> nanoparticles and 1.0–790 μM for β-MnO<sub>2</sub> nanowires with detection limits of 1.0 and 0.3 μM, respectively [48]. Luo *et al.* [49] fabricated a highly conductive TiO<sub>2</sub>-nanoneedle film as a support for the immobilization of cytochrome c to facilitate electron transfer between redox enzymes and electrodes. In this system, they found inherent enzymatic activity towards H<sub>2</sub>O<sub>2</sub> released from human liver cancer cells.



**Figure 6.** Differential pulse voltammetry response of a DNA/nano-ZrO<sub>2</sub>/ITO bioelectrode after hybridization with complementary target probe. Inset shows the calibration curve between current and the logarithm of target concentration.

### Nucleic acid-immobilized NMO-based biosensors

Nanostructured metal oxide-based biosensors for DNA detection have recently aroused much interest due to recent revelations these could be used to convert a hybridization event into an analytical signal. Wang *et al.* [50] reported a DNA sensor based on ZnO nanowires, multiwalled carbon nanotubes (MWCNTs) and gold nanoparticles for nucleic acid detection. In their system, a single-stranded DNA (ssDNA) probe with a thiol group is covalently immobilized on the gold nanoparticle surface through Au–S bonding. This DNA biosensor can detect target DNA at concentrations of 0.10 to 10<sup>5</sup> pM with a detection limit of 0.35 pM using [Ru(NH<sub>3</sub>)<sub>6</sub>]<sup>3+</sup> as an intercalator.

A DNA biosensor based on a composite of ZnO nanoparticles, MWCNTs and CH on a GCE has been used for hybridization detection by DPV using methylene blue as an indicator [51]. This sensor can discriminate among different DNA sequences related to the phosphinothricin acetyl transferase gene in transgenic corn, with a detection limit of 2.8 pM of target sequence. Well-dispersed Cu<sub>2</sub>O hollow microspheres of Cu<sub>2</sub>O nanoparticles synthesized in aqueous solution at 25 °C with polyvinylpyrrolidone as a surfactant have been used for hepatitis B virus detection [52]. Efforts should be made to improve the sensitivity of nucleic acid sensors.

Zuo *et al.* [53] fabricated a DNA biosensor by immobilizing ssDNA with a terminal 5'-phosphate group to a ZrO<sub>2</sub> surface utilizing the strong affinity between the oxygen atoms of the phosphoric group and zirconium. This biosensor was found to have high sensitivity and selectivity for hybridization detection (≤20 nM complementary DNA). A nanostructured ZrO<sub>2</sub> film electrochemically deposited on gold has been used for the immobilization of 21-mer ssDNA specific to *Mycobacterium tuberculosis*, achieving a detection limit of 65 ng mL<sup>-1</sup> and a response time of less than 60 s [54]. An improvement in sensitivity and detection limit (0.1 fM) for *M. tuberculosis* detection has been reported through the use of a nanocomposite film of 3-glycidoxypropyltrimethoxysilane and Fe<sub>3</sub>O<sub>4</sub> nanoparticles for the covalent immobilization of 24-mer peptide nucleic acid [55]. This bioelectrode can be used for the detection of hybridization with the complementary sequence in sonicated *M. tuberculosis* genomic DNA within 90 s.

A sol-gel-derived nanostructured ZrO<sub>2</sub> film has similarly been used for the immobilization of 17-base ssDNA identified from the 16s rRNA coding region of *Escherichia coli* [56]. This bioelectrode exhibits a high selectivity and sensitivity for hybridization, with linearity in the range of 10<sup>-6</sup> to 10<sup>6</sup> pM of complementary DNA (Figure 6). An interesting sensor for *E. coli* cell detection in drinking water has been developed based on the photodeposition of nanoparticulate silver on a TiO<sub>2</sub>-coated

piezoelectric quartz crystal electrode [57]. The silver coating enhances the binding of complementary DNA, allowing the sensor to detect a single *E. coli* cell in 100 mL of water.

A nanocomposite of CeO<sub>2</sub> and CH has been used to immobilize an ssDNA probe for colorectal cancer genes using methylene blue as an indicator [58]. This NMO biocomposite shows excellent electronic conductivity and enhanced loading of the ssDNA probe. Sun *et al.* [59] have developed a novel and stable biosensor using V<sub>2</sub>O<sub>5</sub> nanobelts, MWCNTs and a CH nanocomposite for the immobilization of ssDNA probes for the detection of *Yersinia enterocolitica* using DPV with methylene blue. This sensor can detect specific complementary DNA at concentrations of 0.01–1,000 nM with a detection limit of 1.76 pM.

A DNA sensor based on a composite of MWCNTs, nanostructured ZrO<sub>2</sub> and CH on GCE reported by Yang *et al.* [60] has high surface area and good charge-transport characteristics, providing increased DNA loading capacity and enhanced sensitivity. This DNA sensor shows a linear response to the logarithm of the target DNA concentration in the range of 0.149–93.2 nM with a detection limit of 75 pM.

A nanocomposite of nanostructured shuttle-shaped CeO<sub>2</sub>, single-walled nanotubes and hydrophobic room-temperature ionic liquid (1-butyl-3-methylimidazolium hexafluorophosphate) on a GCE has been used for electrochemical DNA hybridization detection [61]. The electron-transfer resistance of the electrode surface in this system increases after the immobilization of probe ssDNA. The biosensor has been used for the sequence-specific DNA detection of phosphoenolpyruvate carboxylase gene.

An electrochemical DNA biosensor based on a thin gold film sputtered onto anodic porous NbO<sub>x</sub> has recently been reported [62]. This interesting biosensor shows enhanced biosensing performance with resolution 2.4 times higher than that of conventional bulk gold electrodes and a sensitivity three times higher. This sensor can be used for the detection of single-base mismatch DNA as well as for the discrimination of mismatch positions. Efforts should be made to improve the sensitivity of the nucleic acid sensor.

### Antibody-immobilized NMO-based immunosensors

Electrochemical immunosensors have recently aroused much interest for the detection of proteins, biomarkers, biological toxins and biowarfare agents in critical situations such as food security, environmental assessment, pharmaceutical chemistry and clinical diagnostics. The immunosensors employ either antibodies or their complementary binding partners as biorecognition elements in combination with electrochemical transducers [63].

The surface-charged nanoporous morphology and efficient electron-transfer catalytic properties of NMOs make these materials useful as solid supports for antibody immobilization. In this architecture, the Fc terminal of an antibody binds with the NMO, and the Fab terminal is free to bind with the antigen with high specificity. To fabricate an NMO-based immunosensor, the desired antibodies are immobilized onto an electronically conducting surface such as ITO. The sensing performance then depends on the density of antibodies immobilized on an appropriate surface. There is considerable scope for improving the functionality, electrical properties and morphology of biocompatible NMOs using novel chemical/physical routes for immunosensor fabrication [63–64].

A film of IrO<sub>2</sub> grown electrochemically on a parent metal has been used as a substrate for an immunosensing electrode used in immunoassays for immunoglobulin G (IgG) [65]. The IrO<sub>2</sub> film in this case behaves as a hydrophilic and porous 3D matrix with high surface area. The stability of this immunosensor, however, has yet to be investigated. A pyrolytic graphite electrode bearing single-walled nanotubes and a self-assembled Nafion/Fe<sub>3</sub>O<sub>3</sub> coating has been used to immobilize anti-biotin antibodies to detect HRP-labeled and unlabeled biotin [66]. This sensor has a low detection limit due to the high surface area of SWCNT assemblies and the substrate's patternable conductivity. However, the sensing characteristics of this sensor have not been investigated. Hafaid *et al.* [67] reported

an immunosensor based on magnetic functionalized Fe<sub>3</sub>O<sub>4</sub> nanoparticles as a platform for biotin–streptavidin interaction. Surface plasmon resonance analysis has revealed a deviation of the measured angle during antigen–antibody recognition. Label-free detection based on EIS, on the other hand, allows variations in polarization resistance to be measured.

To investigate immunological interactions, Mantzila *et al.* fabricated anodic Ti/TiO<sub>2</sub> (anatase) electrodes at different potentials as an impedimetric measurement platform [68]. Wang *et al.* [69] fabricated an immunosensor based on hydrothermally prepared TiO<sub>2</sub> nanowire bundles connected to a gold microelectrode by mask welding. Monoclonal antibodies immobilized on this platform provide sensitive, specific and rapid detection of *Listeria monocytogenes* at concentrations as low as 10<sup>2</sup> cfu mL<sup>-1</sup> in just 1 h without significant interference from other food-borne pathogens. TiO<sub>2</sub> arrays have also been utilized for label-free optical interferometric biosensing using proteins [70]. Immobilization in this case is achieved via electrostatic interactions between the negatively charged TiO<sub>2</sub> surface and an IgG analyte for trinitrotoluene detection in the pH range 2–12. Antibodies for prostate-specific antigens have been immobilized on modified TiO<sub>2</sub> substrates [71]. The efficiency of this optical immunoassay-based biosensor is dependent on the silanization of the hydroxylated TiO<sub>2</sub> substrate. The density of hydroxyl groups has been found to increase on exposure to oxygen plasma, resulting in high antibody loading and making this system suitable for label-free optical transduction.

A nanocomposite film of TiO<sub>2</sub> and CH has been used for the preparation of an anti- $\alpha$ -fetoprotein (AFP) sensor [72]. By immobilizing AFP, this sensor detects AFP in serum amperometrically with a linear range of 1.0–160 ng mL<sup>-1</sup> and a low detection limit of 0.1 ng mL<sup>-1</sup>. An AFP sensor has also been prepared by immobilizing anti-AFP on the surface of gold nanoparticles electrodeposited on a CH-MnO<sub>2</sub>/MWNT-Ag composite on a GCE [73]. This immunosensor exhibits enhanced sensing characteristics, including a detection range of 0.25–250 ng mL<sup>-1</sup> and detection limit of 0.08 ng mL<sup>-1</sup>, due to the high surface area and conductivity of MWCNT-Ag. The excellent film-forming ability of the CH-MnO<sub>2</sub> composite in this system prevents leakage and with gold nanoparticles provides a congenial microenvironment for anti-AFP.

A sol-gel-derived composite of a CeO<sub>2</sub> film and nanostructured ZnO with a particle size of ~5.0 nm on ITO glass has been used for co-immobilization of rabbit IgG (r-IgG) and bovine serum albumin (BSA) for ochratoxin A (OTA) detection by EIS [64]. This immunosensor exhibits linearity in the concentration range of 6–10  $\mu$ M, a detection limit of 6  $\mu$ M, a response time of 25 s and sensitivity of 189  $\Omega$  nM<sup>-1</sup> cm<sup>-2</sup> with a regression coefficient of 0.997. A sol-gel derived nanostructured CeO<sub>2</sub> film has also been used for the co-immobilization of r-IgG and BSA to detect OTA [63]. A BSA/r-IgG/nano-CeO<sub>2</sub>/ITO immunoelectrode developed by Kaushik *et al.* [74] exhibits interesting characteristics such as a wide linear range (0.5–6.0 ng dL<sup>-1</sup>), low detection limit (0.25 ng dL<sup>-1</sup>), high sensitivity (1.27  $\mu$ A ng<sup>-1</sup> dL<sup>-1</sup> cm<sup>-2</sup>) and a high association constant ( $K_a$ , 0.9  $\times 10^{11}$  M<sup>-1</sup>), indicating the high affinity of the immunoelectrode for OTA. The improved sensitivity and detection limit of CeO<sub>2</sub>/CH-based immunosensor have been attributed to the nanostructured surface and electrocatalytic properties of the NMO.

Kaushik *et al.* [75] also developed a nanocomposite in which fumed SiO<sub>2</sub> nanoparticles form a 3D network in a CH matrix via hydrogen bonding. The availability of NH<sub>2</sub>/OH groups and the excellent film-forming ability of CH results in increased electron transport and high effective surface area, making this nanobiocomposite suitable for polyclonal r-IgG immobilization. A nanobiocomposite platform of r-IgG and BSA immobilized on Fe<sub>3</sub>O<sub>4</sub>-CH has been developed for OTA detection [76]. The charged surface Fe<sub>3</sub>O<sub>4</sub> moieties provide a high electroactive surface area for loading of IgG and improved electron transport between IgG and the electrode. This IgG/CH-Fe<sub>3</sub>O<sub>4</sub> nanobiocomposite on ITO immunoelectrode exhibits a fast response time and high sensitivity (36  $\mu$ A ng<sup>-1</sup> dL<sup>-1</sup> cm<sup>-2</sup>).

Au-ZnO nanocomposite films have been employed for enhanced surface plasmon resonance-based detection of tumor markers using

carbohydrate antigen 15.3 [77]. The sensor responds to marker concentrations of 0.0125–160 U mL<sup>-1</sup> in pleural fluid with high sensitivity, and the platform has been used to investigate bimolecular interactions.

Fu *et al.* [78] have developed a potentiometric immunoassay protocol for the determination of carcinoembryonic antigen (CEA) in human serum via immobilization of CEA monoclonal antibodies on Fe<sub>3</sub>O<sub>4</sub> nanorods as a novel transparent immune affinity reactor. In this system, Fe<sub>3</sub>O<sub>4</sub> 'bionanorods' with an average diameter of 40 nm and length of 1.0 μm are used as the affinity support for the conjugation of anti-CEA antibodies. An electrochemical immunosensor based on dumbbell-like Au-Fe<sub>3</sub>O<sub>4</sub> nanoparticles has also been developed for the detection of the prostate-specific antigen (PSA) cancer biomarker [79]. This immunosensor has a sandwich-like structure that is prepared by immobilizing primary anti-PSA antibodies on a graphene surface and secondary anti-PSA antibodies on Au-Fe<sub>3</sub>O<sub>4</sub> nanoparticles. Signal amplification in this case is attributed to the effect of Au-Fe<sub>3</sub>O<sub>4</sub>. There is considerable scope for improving NMO-based immunosensors using monoclonal antibodies.

### Whole cell-immobilized NMO-based biosensors

Gou *et al.* [80] investigated the mechanistic toxicity of nanostructured silver and TiO<sub>2</sub> anatase using a prokaryotic real-time gene expression profiling method. These NMOs were chosen for the study because of their widespread use in the detection of analytes, and were examined in experiments relating to the properties of compound-specific and concentration-sensitive two-dimensional (genes and time) gene expression using nanostructured TiO<sub>2</sub>.

### Conclusions and future prospects

Nanostructured metal oxide-based biosensors provide a new horizon for novel functions with a variety of applications in clinical and non-clinical diagnostics. Various NMO particles have already made a major impact in various applications, ranging from enzyme electrodes to genelectronics. The useful properties of NMOs suggest that future interdisciplinary research is likely to lead to a new generation of electrochemical biosensors. Researchers are now focusing on understanding the various biomolecule–transducer interactions using these interesting nanomaterials. Embedded particle carriers, nanoparticle-based DNA barcodes, nanoparticle-based surface plasmon resonance and chemiluminescence detection, and biocatalytic metallization for amplified assay studies should all be regarded as promising research targets for the development of amplified bioaffinity assays. Highly sensitive biodetection protocols based on NMOs open up the possibility of creating biosensors for disease markers, biological and infectious agents in early-stage detection of disease and threats. DNA-, enzyme- and antibody-based bioassays on nanoparticulate MO platforms are also expected to be useful for multi-analyte detection. In this context, it will be interesting to explore the dependence of size and toxicity of MO nanoparticles in a variety of new applications.

A wide range of new NMOs is expected to further expand the area of biosensors. New strategies for the synthesis of one-dimensional NMOs are likely to result in new bioelectronic sensing applications. The ability to functionalize NMOs with the desired groups to bind target biomolecules, and doping these with electronically active materials to obtain enhanced charge transfer, may pave the way for new methods for biosensor development. The synthesis of MO nanoparticles with various morphologies at the nanodomain level may provide a suitable environment for obtaining the oriented immobilization of desired biomolecules and thereby amplified signals. These NMOs can be fabricated and tested in desired patterns, such as sensor arrays, for the development of functional integrated devices. The interface of NMO-based devices could also be used for parallel real-time monitoring of multiple analytes. It will be interesting to focus on new methods for the fabrication of innovative biosensor arrays with desired properties

for health care, confining different biomolecules onto closely spaced one-dimensional nanostructures based on NMOs.

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