



Review Article

Nanostructures of noble metals as functional materials in biosensors

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**Abstract**

Recent advancement in nanoscience and nanotechnologies inspired a wide spectrum of uses of nanodimensional materials ranging from industrial sector to biomedical applications. Inorganic nanomaterials made of noble metals, which are corrosion-resistant, are often included as electrode modifiers in designing electrochemical chemosensors and biosensors because of their unique catalytic, electric, and surface-related properties. This review summarizes the developments in electrochemical biosensors with integrated in their architecture metal nanostructures reported mainly during the last two years with a summary on some of the commonly used methods for the synthesis of metallic nanostructures. Nanodimensional noble metal structures might be considered as multipurpose electrode modifiers because of their abilities to act at the same time as electrocatalysts, signal amplifiers, and tools for immobilization and spatial orientation of redox proteins/enzymes or other type of bioreceptors.

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Introduction

Among the numerous unique properties of nanomaterials, their ability to largely increase the surface area while expanding insignificantly the volume of the system is considered the most beneficial for heterogeneous reactions, e.g. electrochemical ones. Metallic nanostructures created of noble metals offer some additional advantages of great significance for

electrochemical research in general, and for biosensor technologies, in particular: excellent electroconductivity, corrosion resistance, high catalytic activity in a variety of reactions, biocompatibility, and facile functionalization of their surface with organic molecules [1••].

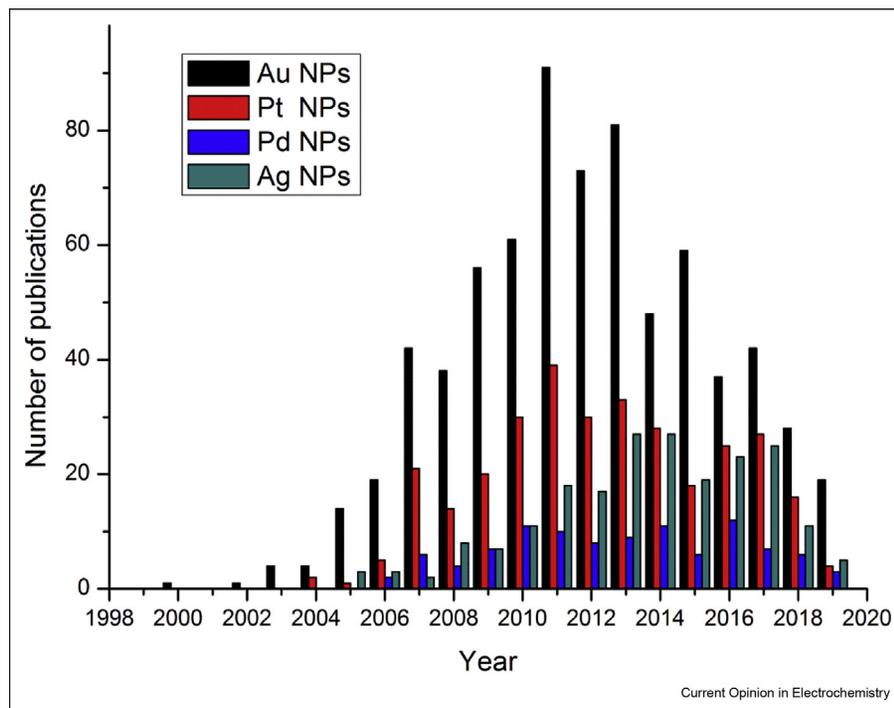
Incorporation of gold nanocrystals in the glucose biosensor design was firstly reported some two decades ago [2•], later Pt nanostructures and nanoparticles made of silver and palladium, were the next to be integrated in biosensor architecture. Since then more than 800 papers have been published, with a peak in publication activity between 2011 and 2013 (Figure 1), pointing that Au, Pt, Pd, and Ag nanostructures became a widespread element of biosensor architecture. Gradually decreasing number of publications during the last 5–6 years suggests a shift of the research focus toward more complex studies related to interactions between inorganic nanomaterials and biorecognition elements.

Commercial availability of noble metals' colloid dispersions and a large variety of protocols for their in-lab synthesis were among the main prerequisites that encouraged such a large volume of publications. Where discussed in the articles subjected to this review, preparative approaches commonly used for the fabrication of both monometallic and bimetallic nanostructures were based on either wet chemical methods or electrochemical ones (electrodeposition).

Gold nanostructures can be produced by treating diluted solutions of tetrachloroaurate with reductants as NaBH₄ to obtain gold seeds (further grown to nanorods [NRs]) [3], citrate [4,5] to produce gold nanoparticles with varying size (attainable through the change of citrate to tetrachloroaurate ratio) [5••], or naturally derived mild reducing agents, e.g. quercetine [6•]. Electrodeposition has also been exploited for the fabrication of gold nanostructures [7,8].

Synthesis of silver nanoparticles was accomplished via reduction of AgNO₃ with NaBH₄ [9], with mild reducing agents such as citrate [10], naturally derived reductants [6•,11], or transition metal carbides [12] that served also as carriers for the Ag NPs. Platinum nanostructures were synthesized by either electrodeposition onto their carrier graphene oxide (GO) with its

Figure 1



Number of publications reporting on biosensors with incorporated nanostructures of the most commonly used noble metals: Au, Pt, Pd, and Ag, over the last two decades (according to Scopus database by end-April, 2019).

coinciding electroreduction [13] or by reduction of hexachloroplatinate with NaBH_4 [14,15] and methylene blue [3]. Nanocomposite material, containing Pd nanostructures was fabricated through high-temperature treatment of PdCl_2 (together with the carrier) in a viscous solvent—ethylene glycol [16]. Bimetallic nanostructures of noble metals have been synthesized by applying a more complex approach, e.g. formation of monometallic and bimetallic NPs onto glassy carbon surface through potentiodynamic electro-deposition from neutral diluted solutions of Pt^{4+} and Ru^{3+} salts [17]. Alternatively, constant potential electroreduction of an aqueous solution containing PtCl_6^- and AuCl_4^- was used to deposit Au–Pt alloy onto reduced GO nanosheets [18], thus forming the working electrode of a wearable glucose biosensor. Wet chemical methods have been used to obtain bimetallic core-shell nanostructures. Au@Ag hollow nanospheres further used as an essential element of a third-generation biosensor [19] were prepared by substitution of the surface atoms of silver hollow nanostructures (produced with silver halide photographic technique) with gold atoms. Nanorods with Pd core and Au shell [20] were created through a similar redox substitution of the surface atoms of Pd NRs with gold, and the prepared Au@Pd nanostructures were applied in a biosensor for pesticides.

All these complex and time-consuming syntheses are worth the effort because metallic nanostructures incorporated in biosensor architecture accomplish at least one of the following functions: catalyse an electrochemical transformation thus generating response, improve conductivity of the matrix for enzyme/protein immobilization, enhance electrode surface area, serve as an anchor for biocatalyst's immobilization or/and as a tool for its spatial orientation, as it is discussed in the following sections.

Metallic nanostructures as electrocatalysts

The simplest way to construct an enzyme electrode working for the first-generation amperometric biosensors is to put a biocatalyst in close contact with a catalytic electrode. The detection of the analyte is based on the monitoring of the current variation because of the redox transformation of either an electroactive product or a reagent of the biocatalyzed process.

Hydrogen peroxide (H_2O_2) is usually generated as a by-product in most of the enzyme-catalysed oxidative reactions, where its quantity is directly proportional to the concentration of the analyte of interest—the enzyme substrate. H_2O_2 is electrochemically active and might be electrocatalytically oxidized over solid platinum (Pt)

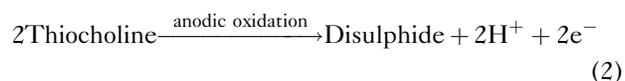
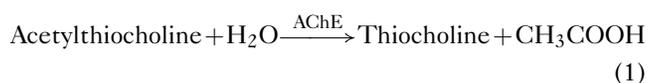
electrodes. Several examples of oxidase-based biosensors have shown, however, that the electro-oxidation of enzymatically generated H₂O₂ onto the electrodes modified with platinum nanostructures used as either a sole inorganic modifier in a glutamate biosensor [21], or a component of more complex systems, such as Pt NPs decorated reduced GO with immobilized glucose oxidase (GOx) or choline oxidase (ChOx) [13]; Pt NPs decorated multiwall carbon nanotubes with immobilized GOx [14] and Pt NPs - zeolite nanocomposite in sarcosine oxidase - based biosensor [15] afford a more sensitive determination than the bulk Pt electrode. Electrocatalytic oxidation of the enzymatically produced H₂O₂ in first-generation glucose biosensors has been reported also for Pd nanostructures—polymer composite material [22]; Ag NPs deposited on composite material [23] as well as bimetallic Au—Pt NPs [18].

The main drawback of this type of sensing is that, at the operating potentials for electro-oxidation of H₂O₂ (i.e. above 0.5 V), organic compounds normally attending the real samples (ascorbic or uric acids, neurotransmitters, antioxidants, pigments, drugs, and even glucose) could be co-oxidised, thereby contributing to the electrode response, which consequently results in overestimated analyte levels. One strategy to overcome this issue is to restrict the access of the potentially interfering compounds by mechanical means—e.g. with a permselective membrane [18] which causes, however, additional diffusion limitations.

A much more successful strategy, however, is to reduce electrochemically the peroxide at operating potentials close to 0 V [24,25]. Electrocatalytic reduction of H₂O₂ over Au NRs resulted in a low background current glucose biosensor [26], while another type of GOx-based biosensor operated at near zero potential due to the incorporation in its architecture of Pt NPs [27]. Under such conditions, it is scarcely conceivable that some of the substances normally present in real samples, e.g. antioxidants, neurotransmitters, phenols, etc., might be electrochemically active and contribute to the electrode response. Other examples of electrocatalysts for the reduction of enzymatically produced H₂O₂ include bimetallic Pt—Rh NPs modifiers in biosensors for primary alcohols and methylamine [17•] and cysteine-modified silver nanoparticles used in the bi-enzyme (acetylcholinesterase, AChE and ChOx) biosensor for pesticides [28].

AChE belongs to the hydrolases group and might readily be inhibited by a variety of toxic compounds: heavy metals, nicotine, neuroparalytic gases, medications, or insecticides. This enzyme catalyses the hydrolysis of acetyl (thio-) choline to (thio-) choline and acetic acid, with the thiolated substrate being preferred for electrochemical studies. Usually, the detection of AChE

enzyme inhibitors proceeds via following steps [9]: i) enzymatic hydrolysis of acetylthiocholine to thiocholine and acetic acid (Eq. (1)); ii) electrochemical oxidation of thiocholine onto the electrode surface (Eq. (2)); and iii) inhibition stage.



Thiocholine electro-oxidation takes place at potentials greater than 0.7 V (vs SCE) over nonmodified electrodes; however, modification with nanostructured gold layer [29], Au NPs - quantum dots [30], or Ag NPs [9], may decrease the operating potentials. Upon the inhibition of the AChE enzyme, the thiocholine oxidation current drops proportionally to the extent of enzyme inhibition.

Urease is also a hydrolase-type enzyme, which catalyses the hydrolysis of urea to ammonium and bicarbonate ions (Eq. (3)):



Ammonia liberated during the hydrolysis can be further oxidized at an operating potential as low as 0.2 V over a composite material with incorporated Au NPs, as it was implemented in a recently reported urea biosensor [31].

Ascorbate oxidase, a member of multicopper oxidase family that oxidises L-ascorbic acid to dehydroascorbic acid and water, has been used jointly with Au NPs in a first-generation ascorbate biosensor [32], which responds to ascorbate diastereomers at one and the same oxidation potential, however, with different current intensities, thus lacking selectivity. By contrast, a clear discrimination between the peaks of catechol and hydroquinone was achieved at their simultaneous determination with a voltammetric laccase sensor based on Au NPs—enriched composite material [33•]. Electrocatalytic activity of Au NPs—containing composite together with tyrosinase—another multicopper enzyme [34]—was applied to sense tyramine in fermented foods and beverages. As reported recently [16,35], nanostructures of noble metals, such as Pt, Au, and Pd, have excellent electrocatalytic performance for the oxidation of acetaminophen, which was found helpful in the development of biosensing platforms for detecting telomerase activity.

Noncatalytic ways to amplify biosensor response

The incorporation of metallic nanostructures is a smart way to increasing the conductivity of organic semiconductors, e.g. polypyrrol layers [4,8,36,37,38], polydopamine [39], or other polymers [40,41], often used as matrix for enzyme immobilization (Figure 2a). Impedance studies have shown considerably decreased charge transfer resistance on electrode modification with Au NPs [42] or Ag NPs [11]. Moreover, when aligned vertically, metallic NRs can bridge electrically a conductive structure bearing immobilized enzyme with the underlying electrode surface (Figure 2b), as it has been shown in Ref. [43]. In addition to greatly improved conductivity, metallic nanostructures provide a considerable increase in the electrode surface area [3,7,39,44–47] which in turn results in greater electrode response. Up to 44 times amplified response has been achieved due to synergetic action of catalysis, enhanced surface area, and functionalization of the electrode surface with tyrosinase enzyme [48] to assay catechol.

Tool for enzyme/protein immobilization and spatial orientation

Besides great catalytic activity, improved conductivity, and enhanced biosensor surface area, metallic nanoparticles are often capable of retaining the recognition bio-element (bioreceptor) in close proximity to the transducer's surface. The strategies to accomplish this are based on i) physical interactions, e.g. electrostatic attraction; ii) chemisorption of SH-terminated proteins onto Au, Ag, and Pt nanostructures; or iii) the formation of covalent [49] or affinity bond [44] with functionalized nanostructures. Physisorption is the simplest immobilization method [10], but resulting in random enzyme orientation (Figure 3a). Proteins containing terminal sulfhydryl groups, e. g. AChE, chemisorb irreversibly onto Ag [12] or Au nanostructures [20,29,45]

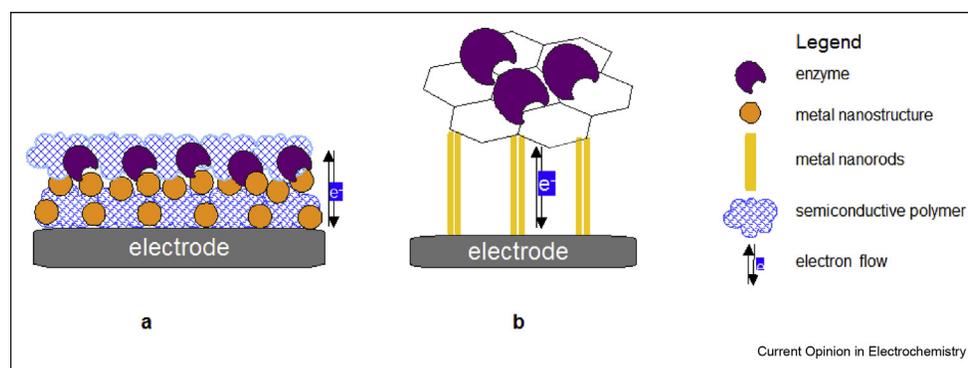
forming self-assembled monolayers. Some other examples of thiolated proteins anchored to Au NPs via the formation of gold-thiol bond include Cys-tagged peptide [50] or sulfhydryl-tagged glucose dehydrogenase [39].

In third-generation biosensors, the immobilised redox enzyme is capable of exchanging electrons between its active site and the underlying electrode directly; thus, the analyte concentration is directly proportional to the redox current generated at the polarised electrode. There are two prerequisites for a DET process to take place: i) the enzyme active site shall be oriented towards the electrode surface, and ii) owing to tunnelling mechanism of ET processes, the distance between the enzyme active site and the electrode surface shall not exceed 2 nm.

The effect of enzyme orientation on the DET processes has been extensively explored by Gorton's group [6,49,51,52]: favourable for DET orientation of the immobilized enzyme has been accomplished through electrostatic attraction between oppositely charged nanoparticles and enzyme active site. Proper enzyme orientation has been achieved by covering the nanostructures with polyethyleneimine [51,52] (Figure 3b), or by their functionalisation with a thiol monolayer [49] (Figure 3c). Similarly, functionalized Au NPs have led to proper orientation of horseradish peroxidase (HRP) [53], fructose dehydrogenase [19], bilirubin oxidase [54], and FAD-dependent glucose dehydrogenase [55].

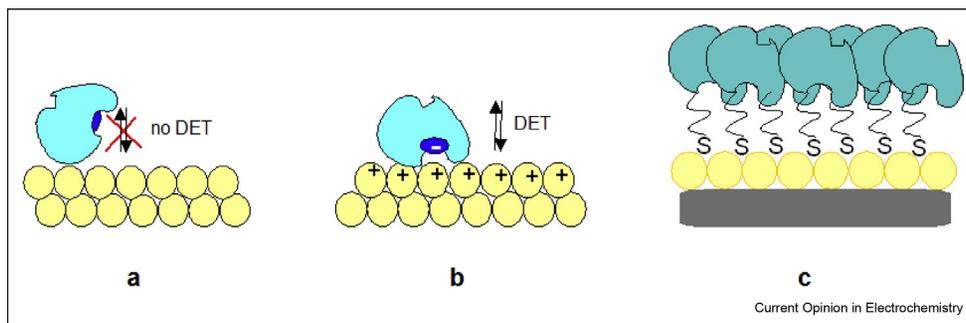
Besides orientation effect, a shortened distance between the active site of the immobilized enzyme and the electrode surface has been achieved [54,56,57]. An enhanced rate of the electron exchange has been noted for HRP enzyme immobilized on Au nanotriangles [58] and for GOx immobilized in Au NPs–polymer composite material [59] due to shortened distance. By

Figure 2



Schematic representation of two possible scenarios for increasing the conductivity of the electrode-modifying layers by means of metallic nanostructures: (a) metallic nanoparticles dispersed in the bulk of the modifier to ensure electron conductivity; (b) metallic nanorods bridging the electrode surface with the enzyme-loaded graphene nanosheets.

Figure 3



Schematic representation of immobilized enzymes on metal nanostructure-modified electrode surface: **(a)** random enzyme orientation, long distance between the active site and electrode surface resulting in lack of electron transfer (ET) process; **(b)** directed enzyme orientation and shortened distance between the active site and electrode surface ensuring successful direct (mediatorless) electron transfer (DET) process; **(c)** formation of self-assembled thiol monolayer onto metallic nanoparticles: enzyme active site is oriented in favourable for DET configuration due to interaction with the terminal groups of thiol.

means of scanning electrochemical microscopy (SECM), it was demonstrated that the rate of the electron exchange between GOx and Au NPs seeded on an insulating surface is strongly dependent on the distance between the enzyme active site and the NPs' surface [50]; the rate of the ET process was found to increase as the distance between the enzyme active site and the NPs' surface decreased, and *vice versa*.

In brief, the design of third-generation biosensors is still a great challenge, as illustrated in the last section; therefore, the choice of electrode modifier is of key importance; metallic nanostructures, via suitable functionalization, may help favourable for DET enzyme orientation [49,51–55], while choosing NPs with the right size [50] may shorten the distance with the electrode surface, thus achieving both direct electrochemistry of the enzyme and bioelectrocatalysis – the necessary prerequisite to construct third-generation biosensors with exclusive selectivity and improved operational characteristics.

Conflict of interest statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

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