Trends in Analytical Chemistry 79 (2016) 286–296

Contents lists available at ScienceDirect

Trends in Analytical Chemistry

journal homepage: www.elsevier.com/locate/trac

# Recent advances in layer-by-layer strategies for biosensors incorporating metal nanoparticles

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ARTICLE INFO

*Keywords:*
Metal nanoparticles
Metal nanoparticle hybrids
Layer-by-layer
Self-assembly
Biosensor

ABSTRACT

This review focuses on the recent advances in biosensors based on metal nanoparticles (MeNP) incorporated in layer-by-layer (LbL) or self-assembled layers. LbL methodology has been widely used to immobilize biomolecules without affecting their native conformation, and enables at the same time the incorporation of metallic nanomaterials with controlled molecular architecture, in order to improve electronic communication between the biomolecule and the electrode substrate. The methodologies employed for LbL build up will be reported, with depiction of the procedures used to investigate the size, morphology and distribution of the synthesized MeNP. The benefits conveyed by incorporating MeNP into LbL multilayers will be critically examined. Finally, biosensors based on MeNP-LbL architectures will be described and compared, stressing the analytical parameters and applicability to real sample analysis.


**Contents**



*Abbreviations:* AChE, acetyl choline esterase; AFM, atomic force microscopy; AgNP, silver nanoparticles; APS, aminopropyl triethylene silane; ATP, 4-aminothiphenol; AuNP, gold nanoparticles; BSA, bovine serum albumin; CBU, cyclic bisureas; chit, chitosan; ChlsOx, cholesterol oxidase; ChOx, choline oxidase; CMM, carbon mesoporous material; CNT, carbon nanotubes; con A, concanavalin A; CTAB, hexadecyltrimethylammonium bromide; CV, cyclic voltammetry; Cys, cysteine; EIS, electrochemical impedance spectroscopy; FTO, fluorine doped tin oxide; G, graphene; GNs, graphene nanosheets; Hb, hemoglobin; HRP, horseradish peroxidase; IL, ionic liquid; ITO, indium tin oxide; $k_s$, apparent electron transfer rate constant of immobilized species; LOx, lactate oxidase; MeNP, metal nanoparticles; MPA, mercaptopropionic acid; MPTMOS, (3-mercaptopropyl)-trimethoxysilane; PAH, poly(allylamine hydrochloride); PAMAM, polyamidoamine; PB, Prussian blue; PDDA, poly(diallyl dimethylammonium) chloride; PdNP, palladium nanoparticles; PEI, polyethyleneimine; PS, polystyrene; PSS, polystyrene sulfonate; PtNP, platinum nanoparticles; $R_{ct}$, charge transfer resistance; SEM, scanning electron microscopy; SP1, stable protein 1; TEM, transmission electron microscopy; TvL, Trametes versicolor Laccase.

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http://dx.doi.org/10.1016/j.trac.2015.11.019
0165-9936/

## 1. Introduction

The layer-by-layer (LbL) methodology enables the preparation of structurally well-defined materials, interconnected through weak interactions, such as hydrogen bonding, biospecific recognition, coordination, electrostatic, hydrophobic and dipole–dipole interactions. The methodology is especially valuable for the immobilization of enzymes, since it enables the preservation of their native structure and activity. Therefore, an increasing number of LbL architectures, with precise control of the composition and film thickness at the molecular level, are being developed for use in new bioanalytical devices [1]. The molecular organisation resulting from the self-assembly process should lead to more efficient sensor platforms as well as requiring smaller quantities of the chemical and biological modifier components than in conventional sensor construction.

The build-up of LbL structures on solid substrates requires the presence of a functional group, usually introduced by deposition of a self-assembled monolayer (SAM), which is a highly organized adsorbed layer. SAM can be formed by chemisorption of amphiphilic organic molecules, containing groups such as thiols, disulphides, amines, acids or silanes [2,3], on top of which LbL assemblies are easily constructed by electrostatic attraction, with precise control of their properties by carefully choosing the configuration, type of polyelectrolytes, number of layers, etc. Moreover, the use of nanomaterials in such LbL architectures, such as metal nanoparticles, carbon nanotubes, graphene etc., has been shown to increase the electronic conductivity and improve the communication between the immobilized biomolecule and the electrode surface. Among the nanoscale materials, metal nanoparticles (MeNP) have been extensively used to improve LbL biosensor performance, especially noble metal nanoparticles (NP), e.g. AuNP, AgNP, PtNP and PdNP, since they possess high stability, conductivity, biocompatibility and size-related electronic, magnetic and optical properties [4]. Beside noble metal NP, iron and iron-oxide magnetic NP have been widely employed in electrochemical biosensors, offering the same advantages as the noble NP such as an increase in enzymatic enzyme apparent activity and reduction of the difficulties associated with mass transfer, but with lower cost and easier preparation [5]. A new trend is the adsorption of metal nanoparticles on porous micro-capsules of controllable size, which can be synthesized using a combination of polyelectrolytes [6,7]. Other nanoparticles used in LbL biosensors are titanium nitride (TiN_NP), $MnO_2$ and ZnO_NP.

The overall performance of the biosensor is directly influenced by the MeNP size, distribution and immobilization/dispersion, the first of these being controllable by the synthesis protocol. Chemical techniques are by far the most used for MeNP synthesis, usually involving the reduction of the noble metal ion by chemical reducing agents, such as sodium borohydride, sodium citrate, etc. The MeNP synthesised chemically are very unstable and prone to form aggregates, thus requiring the addition of a stabilizer, which can functionalize the MeNP at the same time, facilitating their use in LbL assemblies and/or their dispersion in organic/inorganic media [8]. In general, chemical synthesis has little control on the MeNP size, electrochemical and photochemical techniques emerging as a new alternative since they allow precise control of the particle size, morphology and density, by controlling the deposition parameters, such as the applied potential, the pulse time for both nucleation and growth processes, etc. [9–12].

The aim of this review is to examine the ways in which electrochemical and optical biosensors incorporating MeNP, constructed using the LbL technique, have evolved during the past 5 years (2010–2015). The methodology behind the MeNP_LbL assemblies will be discussed, focussing on the functionalization of MeNP, and the influence of the metal nanoparticles employed on the overall performance of the biosensor. The strategies used to explore the MeNP size, distribution and morphology will be presented, with emphasis on the structure of hybrid materials containing MeNP. Finally, the analytical performance of the biosensors developed using the MeNP are discussed, including electrochemical biosensors based on enzymes, protein, DNA/RNA and aptamers, and optical biosensors.

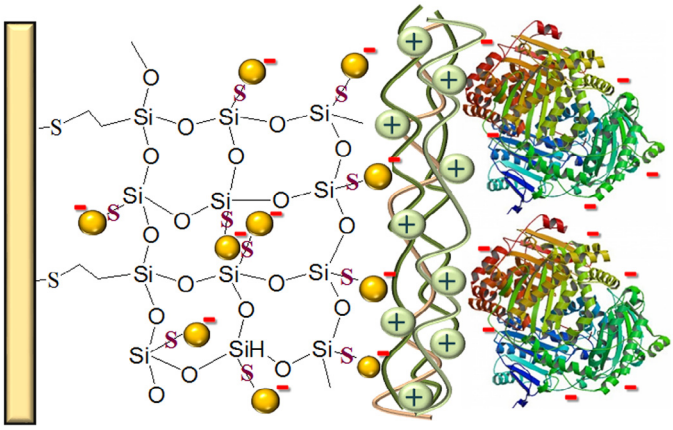
## 2. Methodology for the self assembly of metal nanoparticles

The methodologies employed in LbL self-assembly containing metal nanoparticles, have usually involved thiolated compounds, or amino-terminated compounds including PAMAM dendrimer, chitosan etc., and are exemplified in Schemes 1 and 2. Commonly used precursors for synthesizing noble metal MeNP are chloroauric acid ($HAuCl_4$), hexachloroplatinic acid ($H_2PtCl_6$), potassium tetrachloroplatinate or tetrachloropalladate ($K_2PtCl_4$, $K_2PdCl_4$), and for the chemical reduction the most used are sodium citrate and borohydride, hydrazine, hydrogen, and ascorbic acid. These will now be surveyed in more detail.

### 2.1. Procedures based on thiolated compounds

Thiolated sol gels were extensively used for enabling the self-assembly of AuNP in different electrode architectures, and is illustrated in Scheme 1. A self-assembled monolayer of 3-mercaptopropyltrimethoxysilane (MPTMOS) on ITO was modified with adsorbed AuNP using two methods: 1) pre-concentration of copper followed by its galvanic replacement with Au or 2) chemical adsorption of AuNP through the thiolate (-SH) terminal functionality from the monolayer, to obtain ITO/MPTMOS-$AuNP_{1,2}$ [13], the latter also being used in [14,15]. In [15], a Pt electrode modified with MPTMOS, containing CNT and ChOx, was immersed in AuNP and used as substrate for stepwise deposition of oppositely charged $PDDA^+$ and $AChE^-$ to get the final biosensor Pt/MPTMOS(CNT_$PDDA^+$+$ChOx^-$+$AuNP^-$)/{$PDDA^+$/$AChE^-$}$_n$. The sol gel network entrapped the CNT and ChOx and the –SH of the sol-gel enabled covalent attachment of AuNP. In other work, a GCE/G modified with ZnO dispersed in a sol-gel of aminopropyl triethylene silane (APS), served to anchor AuNP in order to obtain GCE/G/$TiO_2$/AuNP [16].

Other thiolated compounds were also used for the self-assembly of AuNP. A hybrid material of graphene nanosheets_AuNP (GNs_AuNP) was obtained using sulfur-modified GNs, which was able to bind gold ion precursors from the solution phase onto the GNs, these being reduced by an Ar/$H_2$ stream to form GNs_AuNP hybrids. A film of GNs_AuNP hybrid was then deposited on GCE using



**Scheme 1.** Schematic representation of self-assembly of thiolated sol-gel, AuNP, cationic polymer and enzyme.