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To cite this version:
Octavio Graniel, Matthieu Weber, Sebastien Balme, Philippe Miele, Mikhael Bechelany. Atomic layer deposition for biosensing applications. Biosensors and Bioelectronics, Elsevier, 2018, 122, pp.147 - 159. 10.1016/j.bios.2018.09.038 . hal-01919095

HAL Id: hal-01919095
https://hal.umontpellier.fr/hal-01919095
Submitted on 4 Jun 2021

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Atomic Layer Deposition for Biosensing Applications

Octavio Graniel¹, Matthieu Weber¹, Sébastien Balme¹, Philippe Miele¹,² and Mikhael Bechelany*¹

¹Institut Européen des Membranes IEM, UMR-5635, Université de Montpellier, ENSCM, CNRS, Place Eugène Bataillon, F-34095 Montpellier Cedex 5, France

²Institut Universitaire de France (IUF), MESRI, 1 rue Descartes, 75231 Paris cedex 05, France

* Corresponding author: mikhael.bechelany@umontpellier.fr

Abstract

Atomic layer deposition (ALD) is a thin film deposition technique currently used in various nanofabrication processes for microelectronic applications. The ability to coat high aspect ratio structures with a wide range of materials, the excellent conformality, and the exquisite thickness control have made ALD an essential tool for the fabrication of many devices, including biosensors. This mini-review aims to provide a summary of the different ways ALD has been used to prepare biosensor devices. The materials that have been deposited by ALD, the use of the ALD layers prepared and the different types of biosensors fabricated are presented. A selected list of studies will be used to illustrate how the ALD route can be implemented to improve the operational performance of biosensors. This work comprehensively shows the benefits of ALD and its application in various facets of biosensing and will help in exploiting the numerous prospects of this emerging and growing field.

1. Introduction

Atomic layer deposition (ALD) is a vapor deposition technique enabling the preparation of thin film materials with high conformality and excellent control over the thickness (Chalker, 2016; George and Steven M. George, 2010; Ritala and Leskelä, 2002). Its origins can be traced back to two different places (Puurunen, 2014). ALD was first developed by Aleskovskii during the 1960s in Russia and was referred to as molecular layering (ML) (Malygin et al., 2015). Later on, at the beginning of the 1970s in Finland, Suntola developed the atomic layer epitaxy (ALE) technique to deposit ZnS for electroluminescent displays (Puurunen, 2014; Suntola and Antson, 1977). In the next decades and up to today, the route was then referred to as ALD.

ALD is based on self-limiting reactions between two gaseous precursors and allows the deposition of thin films in a layer-by-layer fashion. The ability to deposit conformal films on high aspect ratio structures, with high uniformity over large areas, at (relatively) low temperatures, has made ALD a technique of choice for the preparation of ultrathin films and a key enabling technology (George and Steven M. George, 2010; Leskelä and Ritala, 2003; Ritala and Leskelä, 2002). ALD allows the deposition of a wide range of materials such as oxides (George and Steven M. George, 2010; Hämäläinen et al., 2014), nitrides (Kim, 2003; Weber et al., 2017), sulfides (Meng et al., 2017; Peters et al., 2015), and pure elements (Aaltonen et al., 2004; Johnson et al., 2014; Lim et al., 2003; Weber et al., 2014). These features
have made ALD a relevant technique for many applications such as fuel cells (Gong et al., 2013), metal oxide semiconductor field effect transistors (MOSFETs) (Hong et al., 2016), water splitting (Ho et al., 2018) and purification (Weber et al., 2018), encapsulation (Black et al., 2018), membranes (Weber et al., 2017), solar cells (Elias et al., 2012; Van Delft et al., 2012), and batteries (Liu et al., 2014). Also, the possibility of controlling the composition of the deposited layer by making nanolaminated or alloyed structures allows the tailoring of the chemical and physical properties of the ALD prepared materials (Chaaya et al., 2014; Gu et al., 2013).

ALD is a cycle based process involving four steps (depicted schematically in Figure 1). In the first step, a precursor is introduced into the reactor chamber and is left enough time to react with the surface groups of the substrate. Next, the unreacted precursor molecules and the by-products are removed by purging/pumping the system with an inert gas (usually N₂ or Ar). The third step involves the introduction of a second precursor (the co-reactant) to react with the adsorbed molecules. The final step consists in purging/pumping again the system to remove unreacted precursor and by-products molecules. As a result, one (sub) monolayer of the desired material is deposited on the substrate surface. This cycle is then repeated until the desired thickness is obtained.

![Figure 1. Schematic representation of the ALD process](image)

The use of ALD in the fabrication of nanomaterials for biological and medical applications has proliferated and is now widely spread (Guo et al., 2010; Marichy et al., 2012; Pessoa et al., 2017; Vähä-Nissi et al., 2014; K. Zhang et al., 2017). The compatibility of ALD with the nanoscale of the components found in biomedical devices, the biocompatibility of the materials that can be deposited, and the tuning of the chemical reactivity are some of the reasons why there is a keen interest for using ALD for biomedical applications. Among these applications, biosensing has recently benefited from ALD as a tool for the fabrication of biosensors. Figure 2 shows a histogram of the number of publications (using the Web of Science platform) referring to “biosens*” and either “atomic layer deposition” or “ALD”. The survey clearly shows that the number of works relating both terms has been steadily increasing in the last decade.
Generally speaking, biosensors are devices that allow the selective detection of a target molecule (Turner, 2013), making them useful for many applications such as clinical diagnosis (Fu et al., 2016), food safety (Luo et al., 2009), environmental monitoring (Mishra et al., 2017), security and bioterrorism (Liu et al., 2018; Muhammad-Tahir and Alocilja, 2003). A typical biosensor configuration is presented in Figure 3. Biosensors have two main components: a biorecognition layer and a physical transducer. When the analyte of interest (e.g., low molecular compound, (bio) macromolecules, protein, virus, cell) is captured by the biorecognition element, a biochemical signal is produced, and the transducer element transforms it into a signal that can be measured and correlated (in some cases) to the concentration of it. Biosensors are selective thanks to the bioselective layer that interacts only with the analyte of interest. They can be classified either by the type of biorecognition element (e.g., enzyme, antibodies, cell, nucleic acids) or by the nature of transducer signal (i.e., electrochemical, optical, electrical, mass-sensitive, magnetic).
In the field of biosensing, ALD has been used mainly as a fabrication tool, or as a way to enhance the biosensing performance. In the next sections, a review of the different applications of ALD for these purposes is given. The type of materials deposited by ALD, the use of the ALD layers prepared, the different types of biosensors fabricated, and the detection methods used for sensing are reported.

2. ALD for biosensing applications

ALD has proven to be a versatile technique to fabricate nanostructures (Hong and Kim, 2016; Li et al., 2017; Viter et al., 2015; Xiong et al., 2015). The precise thickness control and excellent conformality allowed by the technique enables to fabricate complex nanostructures with a high surface area that can be used for the design of biosensors. Some of the films deposited by ALD can function as sacrificial layers that can be removed later by chemical or physical etching to produce the desired nanostructure. Other films can be used to fabricate the transducer element of a biosensor, which requires biocompatible materials that can interact with the bio-recognition elements. Additionally, they can be used to protect biosensors and improve their stability towards harsh environments. In this section, ALD films deposited by ALD used in combination with other techniques for nanofabrication will be discussed.

2.1 ALD to produce nanostructured biosensors
Recently, the interest in fabricating biosensors with nano-sized features has grown because their dimensions are in the same range as the biological components of the bio-recognition layer. A selection of nanostructured biosensors fabricated using ALD is presented in Table 1.

Table 1. Examples of films deposited by ALD for the fabrication of nanostructured biosensors

<table>
<thead>
<tr>
<th>Material deposited by ALD</th>
<th>Application of the ALD coating</th>
<th>Substrate/Probe</th>
<th>Analyte</th>
<th>Detection Method</th>
<th>Concentration range/Detection Limit</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al₂O₃</td>
<td>Sacrificial layer for nanogap fabrication</td>
<td>Gold nanogap/Biotin</td>
<td>Streptavidin</td>
<td>Electrochemical (current)</td>
<td>Detection down to 1.5 nM</td>
<td>(Jang et al., 2007)</td>
</tr>
<tr>
<td>Al₂O₃</td>
<td>Layer for nanoring fabrication</td>
<td>Plasmonic nanoring cavities</td>
<td>Adenine</td>
<td>Optical (surface enhanced Raman spectroscopy [SERS])</td>
<td>Limit of detection 76 nM</td>
<td>(Im et al., 2013)</td>
</tr>
<tr>
<td>Al₂O₃</td>
<td>Layer for coax fabrication</td>
<td>Nanoscale coaxial electrodes/anti-cholera toxin antibody</td>
<td>Cholera toxin</td>
<td>Electrochemical (DPV [differential pulse voltammetry] or SWV [square wave voltammetry])</td>
<td>Linear dynamic range of detection of 10 ng/ml – 1 µg/ml Limit of detection 2 ng/ml</td>
<td>(Arab et al., 2015)</td>
</tr>
<tr>
<td>Al₂O₃</td>
<td>Nano-spacer for metal-enhanced fluorescence (MEF)</td>
<td>Microarray platform on Ag film/split DNA aptamer</td>
<td>17-β-estradiol</td>
<td>Optical (fluorescence)</td>
<td>Detection limit of 1 pg/ml</td>
<td>(Lee et al., 2017)</td>
</tr>
<tr>
<td>Al₂O₃/ZnO</td>
<td>Nanopore modification, enhancement of sensing capabilities</td>
<td>poly(ethylene terephthalate) (PET) nanochannel/Al₂O₃/ZnO/biotin</td>
<td>Avidin/streptavidin/bovine serum albumin (BSA)/IgG (Immunoglobulin G) /anti-BSA</td>
<td>Electrochemical (current)</td>
<td>Avidin and streptavidin discrimination/BSA, IgG and anti-BSA detection</td>
<td>(Lee et al., 2017)</td>
</tr>
<tr>
<td>ZnO</td>
<td>Decoration of silicon nanowires (SiNWs) with ZnO nanoparticles</td>
<td>SiNWs FETs (field effect transistor)/chemical receptors</td>
<td>Explosive chemical species</td>
<td>Electrochemical (FET)</td>
<td>Detection down to the parts-per-quadrillion range</td>
<td>(Lichtlein et al., 2014)</td>
</tr>
<tr>
<td>ZnO</td>
<td>Electrode body</td>
<td>ZnO/ZnS core/shell nanotubes/GOx (glucose oxidase)</td>
<td>Glucose</td>
<td>Electrochemical (current)</td>
<td>Linear range of 2.39 x 10⁻³ – 2.66 x 10⁴ mM</td>
<td>(Tirish et al., 2017)</td>
</tr>
</tbody>
</table>
Jang et al. deposited a film of Al₂O₃ by ALD to be used as a sacrificial layer for the fabrication of a vertical nanogap in a label-free electrical biosensor for the detection of streptavidin (Jang et al., 2007). Al₂O₃ films of 5, 10 and 15 nm were deposited to explore the effect on sensitivity from different gap sizes. Figure 4 shows the vertical nanogap fabricated with 5 nm of Al₂O₃ and 2 nm of Ti. By comparing the ratio of the current before and after binding of streptavidin to biotin, they found out that the 7 nm gap is too small for the binding to take place (no change in current). However, they observed significant current increases for the 12 and 17 nm gaps.

Similarly, Im et al. combined nanosphere lithography (NSL) and ALD for the fabrication of a periodic array of ring-shaped nanocavities with 10 nm gap size to be used as a SERS substrate (Im et al., 2013). Figure 5 presents a schematic representation of the fabrication process. The number of ALD cycles controls the thickness of the Al₂O₃ layer and this, in turn, controls the resulting gap size. Adenine was...
used as an analyte to test the film-over-nanospheres (FON) substrate “FON-gap.” SERS measurements revealed a strong 731 cm\(^{-1}\) purine stretch coming from adenine that was used to test the detection limits of the SERS substrate. A 76 nM detection limit was obtained, which makes the FON-gap an excellent bioanalytical platform for SERS biosensing.

**Figure 5.** (a) A schematic representation of the fabrication process for plasmonic nanoring cavities based biosensors, using NSL and ALD. First ALD is used to conformally deposit an Al\(_2\)O\(_3\) layer on the FON substrate prepared by depositing Ag films on polystyrene nanospheres dispersed on a glass slide. Another Ag layer is then deposited on the Al\(_2\)O\(_3\) layer, forming Ag/Al\(_2\)O\(_3\)/Ag layers stacked on the nanospheres. Anisotropic etching of the top Ag layer using ion-milling reveals the underlying Al\(_2\)O\(_3\) layer with a bowl-shaped Ag residual layer on the nanospheres. Partially removing the Al\(_2\)O\(_3\) layer reveals Ag/air/Ag nanoring cavities with a nanogap size defined by the thickness of the ALD-grown Al\(_2\)O\(_3\) film. (b) Cross-sectional schematics of the fabrication process. (c) Scanning electron micrograph (SEM) of the nanoring cavities on the FON substrates. Scale bar: 200 nm. (d) SEM image of the nanoring cavity array formed over a 16 \(\mu\)m \(\times\) 10 \(\mu\)m area. (e) Photograph of the nanoring cavity (FON-gap) sample. On the standard glass slide, the FON-gap structures are made in a 2 cm-wide circular area. Reprinted with permission from (Im et al., 2013). Copyright 2013 John Wiley and Sons.

In order to fabricate materials with low dimensions, ALD has been used to fill high aspect ratio nanoholes of porous materials (Elam et al., 2006) such as anodic aluminum oxide (AAO) (Banerjee et al., 2009; Yao et al., 2015). Thanks to its controllable pore diameter, periodicity, and density distribution (Poinern et al., 2011), AAO has been incorporated successfully as a template for
biosensors fabrication. As an example, Tarish et al. obtained highly ordered ZnO/ZnS core/shell nanotube arrays by using ALD of ZnO and rapid thermal deposition with AAO as a template (Tarish et al., 2017).

ALD has also been used as a tool for tuning single solid-state nanopore sensors. Such sensors, inspired by the pioneering work of Kasianowicz et al. on α-hemolysin (Kasianowicz et al., 1996), are nanometer-sized apertures fabricated in thin films that allow label-free detection of single molecules (Dekker, 2007; Lepoitevin et al., 2017; Miles et al., 2013). Thanks to its conformal deposition, ALD has been able to coat nanopores with high aspect ratio (Spande et al., 2015). For example, Cabello-Aguilar et al. deposited Al2O3/ZnO nanolaminates to reduce the size of a hydrophobic nanopore (Cabello-Aguilar et al., 2013). The diameter of the nanopore was fine-tuned for α-hemolysin insertions. Al2O3/ZnO nanolaminates were chosen due to their low surface roughness, which prevents the colinear growth of layers that can clog the nanopore (Balme et al., 2015). The ability of ALD to homogeneously coat the internal surface of a nanopore allows an excellent surface functionalization (Lepoitevin et al., 2015). This capability limits the non-controlled adsorption of proteins outside the track-etched nanopore and is useful for biosensing (Lepoitevin et al., 2016). ALD has also been used to reduce electrical noise in SiN (Chen et al., 2004) and PET (Thangaraj et al., 2014) nanopores by coating them with a thin layer of Al2O3.

These examples show the relevance of ALD for the fabrication of nanostructured biosensors and its versatility to be used in combination with other fabrication techniques. The advantages of using ALD to deposit thin films can be seen in the nanometer scale features achieved in the different types of biosensors and the various morphologies that can be coated.

2.2 ALD for the fabrication of transducers

In order to produce a signal that can be measured and correlated to the presence and concentration (in some cases) of a specific analyte, the transducer element of a biosensor requires well-defined morphologies with optimal optical, electrical, chemical, mechanical, and structural properties (Tereshchenko et al., 2016; Velasco-Garcia and Mottram, 2003; Zhang et al., 2000). It is crucial that the interaction between the analyte and the biorecognition element on the transducer material produces a change in one or more of its physicochemical properties (e.g., mass change, photoluminescence change, pH change, photocurrent change). Also, the biocompatibility of the transducer element is necessary to facilitate the immobilization of the biorecognition elements (Guo et al., 2010; Im et al., 2012; Schindler et al., 2008). The capabilities offered by ALD can effectively meet these challenges and examples of oxide transducers fabricated with ALD are presented in Table 2.

Table 2. Examples of materials deposited by ALD for the fabrication of transducers

<table>
<thead>
<tr>
<th>Material deposited by ALD</th>
<th>Application of the ALD coating</th>
<th>Substrate/Probe</th>
<th>Analyte</th>
<th>Detection method</th>
<th>Concentration range/Detection Limit</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>TiOx</td>
<td>Transducer layer</td>
<td>Long-period grating (LPG)/TiOx/Biotin</td>
<td>Avidin</td>
<td>Optical (refractive index)</td>
<td>Detection of avidin-biotin complex</td>
<td>(Dominik et al., 2017)</td>
</tr>
<tr>
<td>ZnO</td>
<td>Transducer layer</td>
<td>ZnO nanorods/GOx</td>
<td>Glucose</td>
<td>Electrochemical (current)</td>
<td>Sensitivity of 69.8 (nA/µM-</td>
<td>(Kim et al., 2014)</td>
</tr>
</tbody>
</table>
Recently, Tereshchenko et al. used a ZnO film deposited by ALD as an optical biosensor platform to detect Grapevine virus A-type (GVA) proteins (GVA-antigens) for the first time (Tereshchenko et al., 2017). ZnO was chosen due to its high isoelectric point (∼9.5), biocompatibility for the immobilization of GVA-antibodies, and ability to change its photoluminescence (PL) emission when interacting with biomolecules. In their work, they deposited a 110 nm thick ZnO layer on a Si substrate at a low deposition temperature of 100 °C. By looking at the PL signal of the biosensor while adding different concentrations of GVA-antigen, they detected the protein with sensitivity in the range from 1 pg/ml to 10 ng/ml.

Kim et al. grew ZnO nanorods using a hydrothermal method and a seed layer deposited by ALD (Kim et al., 2014). A 20 nm ZnO was deposited and used to promote the growth of high aspect ratio ZnO nanorods in a layer-by-layer fashion. GOx was immobilized on the ZnO nanorods to detect glucose over a range of different concentrations by cyclic voltammetry. The biosensor showed a sensitivity of 69.8 nA/(µM·cm²) for the ZnO nanorods with the highest surface area.

Transducer materials can also be functionalized chemically to immobilize the biorecognition element (Arya et al., 2012; Brétangol et al., 2006). The chemical grafting of biomolecules shows an increase of the surface coverage when compared to physical adsorption and improves the general stability and performance of the biosensor (Gervais et al., 2007). TiOx was deposited on a LPG induced in an optical fiber as a transducer for the recognition of biotin-avidin interactions (Dominik et al., 2017). The transducer layer was functionalized with amine groups to form a peptide bond with the carboxyl group of biotin. X-ray photoelectron spectroscopy (XPS) was used to confirm the successful functionalization with APTES as well as biotin on the TiOx layer. A 13.2 nm shift in the resonance wavelength was obtained when binding of avidin to biotin took place, proving the biosensor’s ability to detect this event. The possibility of fabricating the transducer element in biosensors with ALD has been demonstrated in the examples mentioned above. With the growing need to fabricate biosensors prepared by ALD will become more common and will play a key role in the development of these devices.

2.3 ALD layers to protect biosensors from their environment

ALD deposited films have been used as protective coatings, for example, to limit corrosion processes (Diaz et al., 2013; Shan et al., 2008; Standridge et al., 2009). The ability to coat large areas with substantial uniformity and excellent conformality are some of the advantages of ALD over other thin film deposition techniques. Also, ALD provides dense, pinhole-free films with outstanding adhesion properties (Matero et al., 1999). These benefits have made ALD an excellent choice to protect the surface of biosensors from aqueous environments and render them stable and, in some cases, reusable. To illustrate this, a list of biosensors with protective coatings deposited by ALD is presented in Table 3.

<table>
<thead>
<tr>
<th>Material</th>
<th>Application of Substrate/Probe</th>
<th>Analyte</th>
<th>Detection</th>
<th>Concentration</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZnO</td>
<td>Transducer layer</td>
<td>ZnO film/GVA antibody</td>
<td>GVA antigen</td>
<td>Optical (photoluminescence)</td>
<td>Sensitivity in the range from 1 pg/ml to 10 ng/ml</td>
</tr>
</tbody>
</table>

Table 3. Examples of films deposited by ALD for biosensor protection purposes
<table>
<thead>
<tr>
<th>Deposited by ALD coating</th>
<th>ALD coating</th>
<th>ALD coating</th>
<th>Method</th>
<th>Detection Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al₂O₃</td>
<td>Protection against oxidation of Ag</td>
<td>Silver film-over-nanosphere (AgFON)</td>
<td>Anthrax biomarker CaDPA (calcium dipicolinate)</td>
<td>Optical (SERS)</td>
</tr>
<tr>
<td>Al₂O₃</td>
<td>Protection of ZnO</td>
<td>ZnO passivated with Al₂O₃</td>
<td>Biofilm</td>
<td>Mass-sensitive (surface acoustic wave [SAW])</td>
</tr>
<tr>
<td>SiO₂</td>
<td>Protection of nanolaser structure</td>
<td>InGaAsP multiple quantum well (MQW)/biotin</td>
<td>Streptavidin tagged with phosphor (SAPE)</td>
<td>Optical (air-bridge-type Γ-band-edge laser (BEL) lasing wavelength shift)</td>
</tr>
</tbody>
</table>

Cha et al. used ALD of SiO₂ to protect the surface of a photonic crystal (PC) BEL structure (Cha et al., 2015). These In-P based structures are subject to chemical attack, and their surface is full of defects that can cause rapid carrier annihilation. By depositing a 5 nm SiO₂ film, they successfully protected the nanolaser device from harsh chemicals and used it as a layer for biotin functionalization. Figure 6 shows the overall functionalization process schematically. Regions with PC pattern show a stronger fluorescence thanks to their higher surface to volume ratio when compared to planar regions. Also, they detected streptavidin with a figure of merit of $\sim 800$, which showed the high sensibility of the BEL biosensor.

**Figure 6.** (a) Schematic representation of the surface functionalization steps for biosensing test. From top-left: 2D PC BEL fabrication; conformal deposition of ALD-SiO₂ layer; biotinylation of silica-terminated surface; and chemical interaction between biotin and streptavidin molecules tagged with...
SAPE. (b) Fluorescence microscopy image taken after the dye-conjugated streptavidin was bound onto the biotin-functionalized PC surface. Reprinted with permission from (Cha et al., 2015). Copyright 2015 Royal Society of Chemistry.

The sensitivity of a biosensor can be lowered when a protective layer is applied. The properties of the material to be deposited, as well as its thickness, must be optimized to reduce the possible detrimental effects on the performance of the biosensor. Kim et al. used an ALD Al$_2$O$_3$ film as a passivation layer to protect the ZnO piezoelectric layer from bacterial growth media or animal serum of a SAW biofilm sensor (Kim et al., 2012). They calculated the normalized theoretical sensitivity of the SAW sensor after applying a protective layer of different materials (Al$_2$O$_3$, Si$_3$N$_4$, SiO$_2$, and Teflon) and observed that Al$_2$O$_3$ was the one that provided the lowest degradation in sensitivity. A 45 nm Al$_2$O$_3$ film was deposited by e-beam evaporation, RF sputtering and ALD to assess the performance of each of the deposition techniques. After two days in Lysogeny Broth (LB) media bacterial suspension, the films prepared by e-beam evaporation and RF sputtering led to some damage and did not protect the ZnO layer successfully as compared to the ALD film (Figure 7). E. coli was cultured to test the SAW sensor for biofilm growth monitoring, and a detection limit of 5.3 pg was achieved.

Zhang et al. deposited an ultrathin Al$_2$O$_3$ layer on a AgFON substrate for SERS detection (Zhang et al., 2006). The Al$_2$O$_3$ layer was used to protect Ag against oxidation and allows preserving an excellent sensitivity. Also, the adsorption affinity of the anthrax biomarker detected in this study for the Al$_2$O$_3$ is five times stronger than that for the AgFON alone. The SERS substrate was later used for bacillus spores detection, and a limit of detection (LOD) of $\sim 1.4 \times 10^3$ spores was attained. The SERS substrate remained functional over a period of 9 months and opens up the possibility to be used for biomedical, homeland security, and environmental applications.
These examples illustrate the feasibility of ALD to deposit highly dense and conformal films for the protection of biosensors. The improved long-term stability of ALD coated devices allows them to be reused without having a detrimental impact on their sensing performance and opens up the possibility for biosensors to be used for in vivo monitoring.

3. Materials deposited by ALD

The properties of materials at the nanoscale strongly depend on their size, shape, chemical composition, and surface area (Bechelany et al., 2015; S. Zhang et al., 2017). Thus, ALD has been extensively used to modify and enhance different electrical, optical, catalytical, and structural properties of biosensor materials (Chen et al., 2004; Cheun et al., 2010; O’Neill et al., 2015; Wang et al., 2012). In order to be used in biosensing applications, the materials need to be biocompatible with the biological components of the biosensor and shouldn’t compromise the sensitivity and overall performance of these devices.

Due to the popularity of FET type biosensors (Li et al., 2018; Sarkar et al., 2014; Zafar et al., 2018), most of the applications of ALD in biosensing have been focused so far on depositing high dielectric materials. Nonetheless, materials with high refractive index and high catalytic activity have also been reported. In this section, the materials deposited by ALD to fabricate biosensors have been classified by their relevant property and are showcased with a few examples.

3.1 Materials with high dielectric constant

ALD has been used to deposit thin films of materials with high dielectric constant to reduce leakage current, passivate surfaces and, in some cases, serve as gate dielectrics (Kim et al., 2009; Ponraj et al., 2013). Examples of high $k$ oxides films deposited by ALD for the fabrication of biosensors are presented in Table 4.

<table>
<thead>
<tr>
<th>Material deposited by ALD</th>
<th>Application of the ALD coating</th>
<th>Substrate/Probe</th>
<th>Analyte</th>
<th>Detection method</th>
<th>Concentration range/Detection Limit</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al$_2$O$_3$</td>
<td>Current leakage prevention</td>
<td>Silicon wire</td>
<td>Urea</td>
<td>Electrochemical (FET)</td>
<td>0.1 – 0.68 mM</td>
<td>(Chen et al., 2008)</td>
</tr>
<tr>
<td>Al$_2$O$_3$/TiO$_2$</td>
<td>Buffer layer during photolithographic process and current leakage decrease/film for ZnO stability</td>
<td>Three-dimensional (3D) bioelectronics-FET (bio-FET)/prostate specific antigen – 1-antichymotrypsin (PSA–ACT) antibody</td>
<td>PSA–ACT antigen</td>
<td>Electrochemical (FET)</td>
<td>Dynamic range of 10$^7$ and detection down to the fM level</td>
<td>(Kim et al., 2015)</td>
</tr>
<tr>
<td>Al$_2$O$_3$</td>
<td>Current</td>
<td>Magnetic</td>
<td>Influenza</td>
<td>Magnetoelectric</td>
<td>Detection range</td>
<td>(Kri</td>
</tr>
</tbody>
</table>

Table 4. Examples of materials with high dielectric constant deposited by ALD for the fabrication of biosensors
<table>
<thead>
<tr>
<th>Material</th>
<th>Function</th>
<th>Sensing Layer</th>
<th>Other Components</th>
<th>Sensing Mechanism</th>
<th>Sensitivity</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al₂O₃</td>
<td>Insulating layer, current leakage prevention</td>
<td>Nickel interdigitated electrodes/Al₂O₃/GSH</td>
<td>Glutathione S-transferase (GST)</td>
<td>Electrochemical (impedance)</td>
<td>Lowest detectable value (LDV) $2 \times 10^{-10}$ M, Highest detectable value (HDV) $2 \times 10^{-6}$ M</td>
<td>(Velllo et al., 2017)</td>
</tr>
<tr>
<td>Al₂O₃</td>
<td>Insulating layer, current leakage prevention</td>
<td>SnO₂ NW-FET/streptavidin</td>
<td>Biotinylated tetracycline repressor protein (bTetR)</td>
<td>Electrochemical (FET)</td>
<td>Successful detection of $600 \mu$g/ml</td>
<td>(Jakob et al., 2017)</td>
</tr>
<tr>
<td>Al₂O₃</td>
<td>Insulating layer, gate dielectric, adhesion improvement</td>
<td>MoS₂/anti-PSA antibody</td>
<td>PSA</td>
<td>Electrochemical (FET)</td>
<td>Down to 1 pg/ml</td>
<td>(Yoo et al., 2017)</td>
</tr>
<tr>
<td>HfO₂</td>
<td>Gate dielectric material</td>
<td>Bio-FET</td>
<td>Biotin</td>
<td>Electrochemical (FET)</td>
<td>Biotin functionalization</td>
<td>(Chen et al., 2010)</td>
</tr>
<tr>
<td>HfO₂</td>
<td>Passivation film and gate insulator</td>
<td>Carbon nanotube (CNT) FET/3-Mercaptopropionic acid</td>
<td>Cytochrome C and beta lactoglobulin A</td>
<td>Electrochemical (FET)</td>
<td>Detection range for cytochrome C: $350$ pM – $3$ µM, Detection range for beta lactoglobulin A: $2.5$ pM – $21$ nM</td>
<td>(Nakasima et al., 2010)</td>
</tr>
<tr>
<td>HfO₂</td>
<td>Reduction of current leakage and enhancer of gate capacitance</td>
<td>Silicon wafer/HfO₂/anti-human interleukin-10 (IL-10) monoclonal antibody (mAb)</td>
<td>Recombinant human (rH) IL-10</td>
<td>Electrochemical (impedance)</td>
<td>Linear range of detection of $0.1$ – $20$ pg/ml</td>
<td>(Lee et al., 2012)</td>
</tr>
<tr>
<td>HfO₂</td>
<td>Gate dielectric layer, protection of electrodes from liquid environment</td>
<td>MoS₂ nanosheet FET/monoclonal PSA</td>
<td>Cancer marker protein PSA</td>
<td>Electrochemical (FET)</td>
<td>Detection down to $375$ fM</td>
<td>(Wang et al., 2014)</td>
</tr>
<tr>
<td>HfO₂</td>
<td>Reduction of leakage current</td>
<td>MoS₂ FET/anti-human TNF-α</td>
<td>Human TNF-α</td>
<td>Electrochemical (FET)</td>
<td>Detection down to the fM level</td>
<td>(Nam et al., 2014)</td>
</tr>
</tbody>
</table>
One of the most used high-κ oxides is Al₂O₃ (Knez et al., 2007; Yan Zhao et al., 2013). Due to its high breakdown field and high thermal stability, Al₂O₃ deposited by ALD can successfully reduce surface leakage current (Ye et al., 2003). Another positive aspect of this material for biosensing is the fact that Al₂O₃ films by ALD present good biocompatibility (Finch et al., 2008). Chen et al. deposited a 10 nm film of Al₂O₃ to prevent current leakage between silicon nanowires and analyte solution in a FET biosensor for urea detection (Chen et al., 2008). The Al₂O₃ film was later treated with oxygen plasma (to clean it and render its surface hydrophilic) before functionalization with 3-aminopropyltriethoxysilane (APTES) and urease enzyme. Krishna et al. developed a GMR biosensor based on magnetic nanoparticles for detection of influenza A virus (Krishna et al., 2016). In their work, they deposited an 18 nm thick Al₂O₃ film onto a GMR chip to prevent current leakage. Vello et al. deposited a 3.3 nm Al₂O₃ insulating film on nickel interdigitated electrodes to prevent leakage current and enable them to operate in an aqueous buffered medium (Vello et al., 2017). To obtain this, they immersed the interdigitated electrodes coated with Al₂O₃ in a phosphate buffered saline (PBS) solution while applying 1 V to the pads of the device. The Al₂O₃ coated electrodes were functionalized with tripeptide reduced glutathione (GSH) to detect the target enzyme GST by evaluating variations on the overall capacitance values. The biosensor could detect GST at concentrations as low as 200 pmol/L (the lowest value reported according to references) and could be easily regenerated to be used several times. Yoo et al. deposited an Al₂O₃ layer on an SU-8 organic layer to fabricate a hybrid gate dielectric in a MoS₂ FET biosensor (Yoo et al., 2017). The 30 nm Al₂O₃ layer provided high insulating properties and improved the adhesion between the organic SU-8 layer and the MoS₂/source-drain electrodes multilayer. Figure 8 shows the different layers of the epidermal skin-type MoS₂ biosensor. The flexible biochip was used for real-time detection of PSA with concentrations as low as 1 pg/ml, which is much lower than the value needed for clinical trials (~4 ng/ml). In addition, they introduced a commercial light-emitting diode (LED) to provide a direct diagnostic result, which opens up the possibility to use this type of biosensors in point-of-care (POC) and forensic applications.
Another high $k$ material that has been deposited by ALD for biosensing applications is HfO$_2$. HfO$_2$ is known for its thermodynamic stability when deposited on Si, which effectively reduces leakage current and makes it suitable as a gate material for metal-oxide-semiconductor (MOS) devices (Aarik et al., 2004; Green et al., 2002; Gusev et al., 2003; Gutowski et al., 2002). Also, HfO$_2$ has an isoelectric point around pH 7 that makes it uncharged in many biological solutions and can be functionalized with biomolecules (Chen et al., 2010). Lee et al. developed a biosensor based on HfO$_2$ for human IL-10 detection by electrochemical impedance spectroscopy (EIS) (Lee et al., 2012). They deposited a 10.7 nm film of HfO$_2$ on a silicon wafer which was later functionalized with self-assembled monolayers (SAMs) of an aldehyde-silane (11-(triethoxysilyl) undecanal (TESUD)) and anti-human IL-10 mAb. By following the EIS of the modified HfO$_2$ with increasing human-IL10 concentrations, they demonstrated that the biosensor had a working linear range of 0.1–20 pg/ml and a sensitivity of 0.49 (ng/ml)$^{-1}$. Wang et al. reported a label-free MoS$_2$ nanosheet-based FET biosensor covered with a 7–8 nm layer of HfO$_2$ (Wang et al., 2014). Figure 9 shows the homebuilt microfluidic channel system where sample solutions were flowed to be detected by the active area. Figure 6c shows the different layers that make up the FET biosensor. They used HfO$_2$ to serve as the gate dielectric layer, to protect the metal electrodes from a fluidic environment, and to serve as a starting layer for functionalization with silanes. The FET biosensor showed good sensitivity (down to the femtomolar level) to cancer marker protein PSA and high selectivity by not responding to BSA protein.
Besides Al₂O₃ and HfO₂, ALD of ZrO₂ has also been reported for biosensors fabrication. Thanks to its large energy bandgap and thermodynamic stability, ZrO₂ has been considered as an alternative gate dielectric material (Kukli et al., 2002; Nam and Rhee, 2004; Niinistö et al., 2004). Barik et al. deposited ZrO₂ by ALD as gate insulator on an enzyme FET (ENFET) biosensor (Barik et al., 2014). They deposited ZrO₂ on the channel region of the biosensor to increase its capacitance and thus gain sensitivity. Sticker et al. deposited a 15 nm thick ZrO₂ insulation layer on interdigitated microelectrodes for nanotoxicological cell analysis (Sticker et al., 2015). By running computational simulations, they showed that insulated interdigitated electrode structures had an improved electrical current distribution when compared to bare electrodes. They successfully evaluated the toxicity of silica nanoparticles (with and without protein coatings) on H441 cells by monitoring the impedance signal over time.

The numerous examples of biosensors that employ high $k$ oxides films prepared by ALD highlight the importance of this technique for the fabrication of these devices. Moreover, the conformal and pinhole free thin films of metal oxides successfully passivate the surface of the biosensors without loss of sensitivity and make them promising for miniaturized POC diagnostics.

### 3.2 Materials with high refractive index

Materials deposited by ALD with high refractive index have been used in optical applications (Alasaarela et al., 2010; Zhu et al., 2016) and microelectromechanical systems (Rissanen et al., 2012). Owing to the smoothness of the films and the ability to coat complex-shaped substrates with a precise thickness control, ALD has been preferred over other thin film deposition techniques like dip coating and self-assembly (Sobel and Hess, 2015; Ying Zhao et al., 2013). Examples of high refractive index films deposited by ALD for the fabrication of biosensors are presented in Table 5. Recently, Oubaha et al. deposited a Ta₂O₅ layer by ALD to increase the sensing properties of a multianalyte biosensor (Oubaha et al., 2015). Ta₂O₅ was chosen due to its high refractive index, which increases the intensity of the evanescent field (EF) by 440 times when compared to the system without the high refractive
index layer (HRIL). The thickness of the HRIL chosen for this work was 35 nm because it provides the maximum evanescent wave enhancement. Cy5-labeled mouse IgG antibody was detected through fluorescence detection, and a limit of detection of 0.25 µg/ml was achieved. Smietana et al. deposited an ALD layer of TiO2 on LPGs for a label-free optical biosensor (Smietana et al., 2015). TiO2 was used for improving the refractive index sensitivity of the biosensor and as a biocompatible material for endotoxin binding protein (adhesin) functionalization. The deposition of a 70 nm thick TiO2 layer on the LPG increased the refractive index sensitivity 2.8 times when compared to the bare LPG. Binding of E. coli B lipopolysaccharide (LPS) to bacteria adhesion was confirmed thanks to an increase in the spectral separation of resonances.

<table>
<thead>
<tr>
<th>Material deposited by ALD</th>
<th>Application of the ALD coating</th>
<th>Substrate/Probe</th>
<th>Analyte</th>
<th>Detection Method</th>
<th>Concentration range/Detection Limit</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ta2O5</td>
<td>EF enhancement</td>
<td>Waveguide-based photonic platform/Cy5-labelled anti-mouse IgG antibody</td>
<td>Mouse IgG antigen</td>
<td>Optical (fluorescence)</td>
<td>Limit of detection of 0.25 µg/ml</td>
<td>(Oubah a et al., 2015)</td>
</tr>
<tr>
<td>TiO2</td>
<td>Improvement of refractive index sensitivity</td>
<td>LPG/TiO2/E. coli B bacteriophage g37 adhesin</td>
<td>E. coli B LPS</td>
<td>Optical (refractive index)</td>
<td>Positive test for adhesin-LPS binding</td>
<td>(Smietana et al., 2015)</td>
</tr>
</tbody>
</table>

3.3 Materials with catalytic activity

ALD has been used to improve the catalytic activity of materials thanks to its ability to precisely control the catalyst particles properties and uniform dispersion on the surface of the support (Sun et al., 2013; Weber et al., 2015, 2012). Examples of materials with catalytic activity deposited by ALD for the fabrication of biosensors are presented in Table 6. Recently, Choi et al. reported the decoration of CNTs with Ni nanoparticles by ALD for non-enzymatic glucose sensing (Choi et al., 2015). The presence of Ni nanoparticles on CNTs was confirmed by TEM, high-angle annular dark-field (HAADF), and energy-dispersive X-ray spectrometry (EDX). The ~8 nm Ni nanoparticles were uniformly distributed on the walls of the CNTs. The selectivity of the sensor was confirmed by showing minimum changes in the oxidation current when adding ascorbic acid or uric acid, whereas, in the case of glucose, the value of the oxidation current was far more important. The sensor presented a detection limit of 2 µM and a linear range of 0.005 – 2 mM. Furthermore, it showed a rapid response and repeatability for non-enzymatic detection of glucose.

At the nanoscale, both mass transport and electron transport play a crucial role in obtaining excellent electrocatalytic properties. Nanoporous metals decorated with transition-metal oxides have been proposed as structures that can serve as electrochemical biosensors. Zhang et al. deposited a CoO layer on nanoporous gold (NPG) for the detection of glucose and H2O2 (Zhang et al., 2016). The Au nanopores with a size ranging between 40-100 nm were covered with 100-800 cycles of CoO. When less than 200 cycles were used, the amorphous CoO formed a discontinuous layer. On the other hand,
a continuous layer was observed at higher cycles. The NPG/CoO heterostructure showed an excellent
electrocatalytic activity of glucose oxidation owing to the interconnected Au skeletons and the
synergistic effect between Au and CoO. Furthermore, the electrochemical biosensor could detect
centations of $\text{H}_2\text{O}_2$ as low as 0.1 mM and its sensitivity was comparable to other types of
composite electrodes based on graphene sheets, noble metals, and metal oxide nanoparticles.

**Table 6.** Examples of materials with catalytic activity deposited by ALD for the fabrication of biosensors

<table>
<thead>
<tr>
<th>Material deposited by ALD</th>
<th>Application of the ALD coating</th>
<th>Substrate/Probe</th>
<th>Analyte</th>
<th>Detection Method</th>
<th>Concentration range/Detection Limit</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>CoO</td>
<td>Electrode body, catalytic activity</td>
<td>NPG/CoO</td>
<td>$\text{H}_2\text{O}_2$</td>
<td>Electrochemical (current)</td>
<td>Linear range of detection 0.1 – 92.9 mM</td>
<td>(Zh et al., 2016)</td>
</tr>
<tr>
<td>Ni</td>
<td>Electrocatalyst for glucose oxidation</td>
<td>CNT-Ni nanocomposites</td>
<td>Glucose</td>
<td>Electrochemical (current)</td>
<td>Linear range of detection 0.005 – 2 mM Detection limit of 2 µM</td>
<td>(Ch et al., 2015)</td>
</tr>
<tr>
<td>NiO</td>
<td>Electrocatalyst for glucose oxidation</td>
<td>NiO/SiC nanocomposite</td>
<td>Glucose</td>
<td>Electrochemical (current)</td>
<td>Linear range of detection 0.004 – 7.5 mM Detection limit of 0.32 µM</td>
<td>(Ye ng et al., 2015)</td>
</tr>
</tbody>
</table>

4. Conclusions and future perspectives

ALD has rapidly become a valuable technique for the fabrication of biosensors thanks to its ability to
deposit a wide range of materials with precise thickness control and excellent conformality. In this
minireview, the crucial role of ALD for the fabrication of different types of nanostructured biosensors
and its capability to improve their sensing properties has been shown. Immobilization of bio-
recognition elements has also been demonstrated on layers deposited by ALD thanks to their
biocompatibility and controlled chemical composition. The continuous miniaturization trend of
biosensor devices and the complexity of the structures to coat demand high conformality, uniformity,
and film quality that conventional thin film deposition techniques such as chemical vapor deposition
(CVD) and physical vapor deposition (PVD) cannot achieve (Crowell, 2003). However, there are still
some challenges to overcome. ALD remains a slow technique and requires vacuum conditions that
make the processes somewhat expensive and difficult to scale up (Muñoz-Rojas and MacManus-
Driscol, 2014). Different approaches such as spatial atmospheric ALD (Hoffmann et al., 2018; Hoye et al., 2015; Poodt et al., 2012), roll-to-roll systems (Ali et al., 2014; Maydannik et al., 2014), and rotating reactors (Longrie et al., 2014; Sharma et al., 2015) are being developed to make the process (even) more scalable and compatible with industrial requirements. Additionally, the design and synthesis of new
and affordable precursors are needed to further increase the availability of materials that can be
deposited. This is a difficult task given the constraints that the ALD reaction imposes on the
precursors. The chemicals must be volatile at room temperature (or by being slightly heated) and
thermally stable. Also, they must react quickly with the substrate to allow surface saturation and provide a fast growth (Johnson et al., 2014; Leskelä and Ritala, 2002; Marichy et al., 2012).

Also, while some biosensors have passed the testing phase and have become available in healthcare applications as handheld devices or portable units (Ronkainen et al., 2010), their design must still be improved so they can diversify their applications in fields such as environmental monitoring, security and bioterrorism, and food safety. Finally, it is safe to say that ALD (in combination with other fabrication techniques) will push biosensors past the limitations they currently face by providing robust, sensible, and selective platforms that will become part of our everyday lives.

5. Acknowledgment

Octavio Graniel would like to thank CONACYT for funding. This work was supported by the European project “CanBioSe, Project ID: 778157”.

List of acronyms

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Full name</th>
</tr>
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<tbody>
<tr>
<td>3D</td>
<td>three dimensional</td>
</tr>
<tr>
<td>AAO</td>
<td>anodic aluminum oxide</td>
</tr>
<tr>
<td>AgFON</td>
<td>silver film-over-nanosphere</td>
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<tr>
<td>ALD</td>
<td>atomic layer deposition</td>
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<tr>
<td>ALE</td>
<td>atomic layer epitaxy</td>
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<tr>
<td>APTES</td>
<td>3-aminopropyltriethoxy silane</td>
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<tr>
<td>BEL</td>
<td>band edge laser</td>
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<tr>
<td>Bio-FET</td>
<td>bioelectronics field effect transistor</td>
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<tr>
<td>BSA</td>
<td>bovine serum albumin</td>
</tr>
<tr>
<td>bTetR</td>
<td>biotinylated tetracycline repressor protein</td>
</tr>
<tr>
<td>CaDPA</td>
<td>calcium dipicolinate</td>
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<td>ChOx</td>
<td>cholesterol oxidase</td>
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<tr>
<td>CNT</td>
<td>carbon nanotube</td>
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<tr>
<td>CVD</td>
<td>chemical vapor deposition</td>
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<tr>
<td>DPV</td>
<td>differential pulse voltammetry</td>
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<tr>
<td>EDX</td>
<td>energy-dispersive X-ray spectrometry</td>
</tr>
<tr>
<td>EF</td>
<td>evanescent field</td>
</tr>
<tr>
<td>EIS</td>
<td>electrochemical impedance spectroscopy</td>
</tr>
<tr>
<td>ENFET</td>
<td>enzyme field effect transistor</td>
</tr>
<tr>
<td>FET</td>
<td>field effect transistor</td>
</tr>
<tr>
<td>GMR</td>
<td>giant magnetoresistance</td>
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<td>GOx</td>
<td>glucose oxidase</td>
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<td>GSH</td>
<td>tripeptide reduced glutathione</td>
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<td>GST</td>
<td>glutathione-S-transferase</td>
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<td>GVA</td>
<td>Grapevine virus A-type</td>
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<tr>
<td>H441</td>
<td>human lung adenocarcinoma epithelial</td>
</tr>
<tr>
<td>HAADF</td>
<td>high-angle annular dark-field</td>
</tr>
<tr>
<td>HDV</td>
<td>highest detectable value</td>
</tr>
<tr>
<td>HRIL</td>
<td>high refractive index layer</td>
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<td>IAVs</td>
<td>Influenza A viruses</td>
</tr>
<tr>
<td>IgG</td>
<td>immunoglobulin G</td>
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<td>IL-10</td>
<td>interleukin-10</td>
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https://doi.org/10.1038/srep10135

https://doi.org/10.1038/nnano.2009.37


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https://doi.org/10.1063/1.1590743


