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Biosensors elaborated on gold nanoparticles, a PM-IRRAS characterisation of the IgG binding efficiency

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ABSTRACT

This work is focused on studying the grafting of gold nanoparticles (Np) on a cystamine self-assembled monolayer on gold, in order to build sensitive immunosensors. The synthesis and deposition of gold nanoparticles, 13 and 55 nm sizes, were characterised by combining Polarisation Modulation Infrared Reflection–Absorption Spectroscopy (PM-IRRAS), X-ray Photoelectron Spectroscopy (XPS) Surface Enhanced Raman Scattering (SERS), and Atomic Force Microscopy (AFM) which all indicated the formation of a dispersed layer of nanoparticles. This observation is explained by the compromise between the high reactivity of amine-terminated layers towards gold, and interparticle repulsions. Nps were then functionalised with antibody probes, and the recognition by an anti-rIgG was assayed both on planar and Np gold surfaces.

The important result is that nanoparticles of 55 nm are preferable for the following reasons: they enable to build a denser and well dispersed layer and they increase both the number of receptors (IgGs) and their accessibility. Beside these geometric improvements, a net enhancement of the Raman signal was observed on the 55 nm nanoparticle layer, making this new platform promising for optical detection based biosensors.

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1. Introduction

The use of gold nanoparticles (AuNps) is particularly attractive in biological and medical fields for imaging or optical detection enhancement thanks to the unique properties of surface plasmon resonance (SPR) and resonance light scattering, while they are biocompatible and easily chemically modified [1]. After recalling the principle of bioconjugate chemistry on gold, as well as of AuNp-based techniques like SPR and SERS, Astruc and Boisselier describe, in a recent review, the fantastic interest of AuNps for medical diagnosis and therapeutic applications [2]. As an example, AuNps, conjugated to antibodies, may be used as cancer biomarkers; alternatively, they may be stabilized by dendrimers thus leading to supramolecular properties applicable to encapsulation or specific substrate-specific interactions [3]. Recent developments in the synthesis and optical properties of gold nanoparticles are given in reference [4].

Gold nanoparticles have been used for improving the sensitivity of biosensors thanks to their optical properties or because they enable easy electrochemical detection of biomolecular recognition phenomena [5]. Various procedures to modify electrodes by gold NPs have been implemented and compared, showing the interest of immobilising AuNp on thiol monolayers [6,7], or using Nps assembled on a polymer grafted on an electrode [8]. Another example reports the construction of an amperometric immunosensor on a mixed NH_3^+/SH-terminated SAM layer, modified with gold Nps, taking advantage of the high affinity of gold to either of these two functions [9]. Antibodies are subsequently directly immobilised on the gold Nps, by simple incubation in an antibody solution, resulting in an efficient immunosensor for α-fetoprotein; the enhanced number and accessibility of probes were mentioned but not measured in this case [6].

In a very pioneer work, Natan and co-workers demonstrated the possibility to take advantage of the electronic coupling between gold planar films and gold nanoparticles to obtain larger changes in reflectivity and, thus, amplified SPR signals [10]. More recently, gold nanoparticles were immobilised on a dithiol layer or within a protein–polymer mixture, both methods resulting in the enhanced sensitivity of surface plasmon resonance biosensors [11,12]. The interest of building an immunosensor on gold nanoparticles, in particular the increase of the number of molecular receptors

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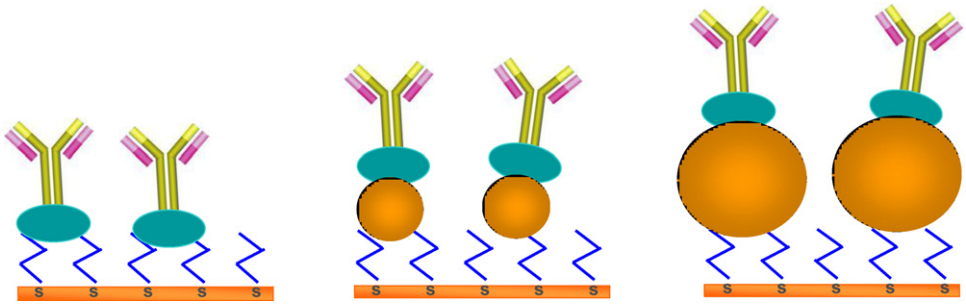

Scheme 1. Schematic representation of the 3D immunosensor built on a cystamine layer or gold nanoparticles; for sake of clarity, the respective sizes of the nanoparticles and chemicals are not at the right scale.

and of their accessibility, was also mentioned in reference [13].

Gold Nps, combined with a SiO_2 interface were shown to enhance plasmon coupling and thus SPR sensor sensitivity [14]. Even the gold Np colour change, when aggregated, was used for assaying DNA hybridisation [15].

Eventually, surfaces modified by gold nanoparticles have been applied to bioanalysis taking advantage of the high sensitivity of Surface Enhanced Raman Scattering (SERS) on such structures [16]. Recent papers made clear the complex interplay of the particle size, shape, as well as distance to the substrate, upon SERS intensity [17,18].

Though not exhaustive, this short overview of some AuNp-based immunosensors shows the huge interest of such nanostructured sensing platforms. Among the multiple interest of using AuNps, it is difficult to isolate, and thus optimise, the possible causes of signal enhancement, increase of the receptor number, of their accessibility, or gain of sensitivity due to the remarkable physical/optical properties of gold Nps for example.

In the present paper, we thus focus on the preparation and grafting of gold nanoparticles of various sizes on an amine-terminated Self-Assembled Monolayers (SAMs), and their applications as new platforms for building sensitive immunosensors following Scheme 1. The number and accessibility of the immobilised molecular probes, rabbit immunoglobins (rIgG), will be estimated on three different systems, (i) a planar gold surface, (ii) 13 nm or (iii) 55 nm nanoparticles immobilised on a gold surface. To do so, the Np layers will be characterised by combining Polarisation Modulation InfraRed Reflection Absorption Spectroscopy (PM-IRRAS), Photoelectron Spectroscopy (XPS), Atomic Force microscopy (AFM) and SERS; the anti-rIgG recognition reaction will be measured by PM-IRRAS a technique which enables a strict comparison of the numbers of immobilised probes or target molecules without any optical or electrical effect.

2. Experimental

2.1. Materials

$HAuCl_4$ (99.5%) was purchased from Merck (Darmstadt, Germany). 1-Mercapto-11-undecanoic acid (MUA), *N*-hydroxysuccinimide (NHS), *N*-1-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC), Ethanolamine, trisodium citrate, cystamine dihydrochloride (98%) (CA) were obtained from were purchased from Aldrich (France). Rabbit IgG (rIgG), goat anti-rabbit IgG (anti-rIgG), goat serum, bovine serum albumin, (BSA) and recombinant protein A (PrA) were purchased from Sigma. All solvents were reagent-grade and used without any further purification.

Planar gold samples were glass substrates (11 mm × 11 mm) coated with a 2.5 nm thick chromium layer and a 200 nm thick gold layer (Arrandee, Werther, Germany). The clean gold chips were annealed in a butane flame to ensure a good crystallinity of the topmost layers, as recommended by the company.

2.2. Gold nanoparticle synthesis and characterisation

The gold nanoparticles were prepared by reducing $HAuCl_4$ in a trisodium citrate aqueous solution according to a previously published route [19,20]. The two following stock solutions were prepared from pure water, 1 mM $HAuCl_4$ and 38.8 mM trisodium citrate in pure water; 50 mL of 1 mM $HAuCl_4$ solution was heated to boiling with vigorous stirring, and 5 mL of the Na_3-citrate solution were added rapidly resulting in a colour change from pale yellow to red-wine. The nanoparticle size was tuned by changing the citrate/Au molar ratio as first described by Frens [21]. As an example, 1.3 mL of 38.8 mM Na_3-citrate solution was used to obtain nanoparticles of ca 60 nm diameter. After boiling for 15 min, the heating mantle was removed, and stirring was continued for an additional 15 min. After the solution reached room temperature, it was kept at +4 °C.

The resulting colloidal solution was characterised by UV–visible spectroscopy, and by Transmission Electron Microscopy.

- Gold Np sizes were visualised using a JEOL transmission electron microscope operating at 100 kV. Samples were prepared by evaporating microdrops of nanoparticle solution (25 °C) on Formvar-coated copper grids

2.3. Gold surface functionalisation and protein immobilisation

Before gold Np grafting, gold samples were immersed in a 10 mM aqueous CA solution during 12 h, then washed three times in pure water and dried under nitrogen flow. After that, substrates were immersed in 10 mL freshly prepared colloidal solution for 1 h, then washed once in pure water, and dried under nitrogen flow. Planar gold surfaces and gold nanoparticles-derived platforms were then submitted to an identical protocol in order to build immunosensing surfaces; they were first immersed in a 10 mM mercapto-11-undecanoic (MUA) solution in ethanol, in order to graft accessible acidic functions. On gold nanoparticles, the objective was to replace citrate ligands by acid-terminated thiols. After 3 h, the substrates were washed 3 times in the same volume of EtOH and dried under nitrogen flow. The acidic functions were activated with a solution of NHS (60 mM) and EDC (30 mM) in water for 1 h 30 then, 150 μL of a solution of PrA (50 and 100 mg/L) in PBS (pH 7.4) was deposited. After 1 h, substrates were washed three times with PBS. Then a drop of 1 M ethanolamine in water was deposited onto the samples in order to deactivate residual ester functions. After 20 min, they were rinsed once in water and dried under nitrogen flow. To block non specific binding sites, a microdrop of a 50 mg L^{-1} solution of BSA in PBS was deposited for 30 min. After rinsing 3

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