

Gold Coated Nanoparticles Functionalized by Photochemical Immobilization Technique for Immunosensing



B. Della Ventura, R. Campanile, M. Cimafonte, V. Elia, A. Forente,
A. Minopoli, E. Scardapane, C. Schiattarella, and R. Velotta

Abstract Gold nanoparticles (AuNP)s play a fundamental role in biosensing in view of their various applications, each of them exploiting one or more properties of such a system. In any case, to make AuNPs effective as biosensing tools, it is necessary to provide them with high specificity by means of processes able to achieve an efficient gold surface functionalization with antibodies. This issue can be easily addressed by the so-called Photochemical Immobilization Technique (PIT). Herein, we report PIT-functionalized AuNPs applied as ballast for improving the sensitivity of quartz-crystal microbalances, but also as optical transducers (colorimetric biosensor) in which the key role is played by the plasmonic properties of nanostructured gold. Eventually, the role of gold is highlighted in its combination with magnetic nanoparticles, a physical system in which the magnetic behaviour of the core is joined to the optical properties of the surface.

Keywords Biosensors · Nanoparticles · Core-shell nanoparticles · Photochemical immobilization technique · Colorimetric · Localised surface plasmon resonance

1 Introduction

Gold nanoparticles (AuNP)s are being largely used in biosensing thanks to their inertness and plasmonic properties, which make them highly exploitable in manifold ways [1]. One of the key steps to be taken into account when using AuNPs is their functionalization, whereby they specifically interact with the analyte to be detected. Although molecularly imprinted polymers (MIP)s are attracting wide interest in view of their stability [2], “natural” bioreceptors, and particularly antibodies (Ab)s, still represent the most effective choice for their unique capability of selectively

B. Della Ventura · R. Campanile · M. Cimafonte · V. Elia · A. Forente · A. Minopoli ·
E. Scardapane · C. Schiattarella · R. Velotta (✉)

Dipartimento di Fisica Ettore Pancini, Università degli Studi di Napoli Federico II, Via Cintia, 26,
80126 Naples, Italy

e-mail: rvelotta@unina.it

interacting with a single analyte (antigen) thereby providing unparalleled specificity [3].

A typical Ab used in biosensing is the immunoglobulin G (IgG), a protein with a molecular weight of approximately 150 kDa, tens nanometers in size, and a characteristic Y-like shape (Fig. 1a). For biosensing purposes, the most important domain is the so-called Fragment antigen binding (Fab) (depicted in red in Fig. 1a) that constitute the antigen-binding sites of the Ab providing its characteristic high specificity [4]. Of course, when dealing with the design of a biosensing platform that exploits Abs, a spatial orientation that leaves at least one of the two Fab exposed to the solvent is mandatory; alternatively, both Fabs anchored onto the biosensor surface would be highly detrimental for the device sensitivity since no recognition can take place. Moreover, an effective functionalization would require a high Abs surface density so to fully exploit the interacting surface of the biosensor. Currently, even by using complex and time-consuming chemical functionalization procedures, satisfactory results are not warranted [5–7].

In an effort towards the achievement of a simple and effective gold surface functionalization with Abs, we set up the Photochemical Immobilization Technique (PIT), a two-steps procedure that includes the activation of whole IgGs in solution by high intensity UV lamp and their subsequent transfer onto the biosensor surface for straightforward Ab surface binding (Fig. 1a) [8, 9]. PIT is based on the selective photoreduction of the disulphide bridge in some of the cysteine–cysteine/tryptophan (Cys-Cys/Trp) triads [10], which are a typical structural feature of the IgG [11]. The UV excitation of the Trp residue leads to the generation of solvated electrons, which are captured by the nearby disulphide bridge resulting in its destabilization and subsequent breakage of the cysteine–cysteine bond. The as-produced free thiol groups interact with proximal gold surface giving rise to an oriented covalent IgG immobilization (Fig. 1b). This strategy has been shown to ultimately enhance the antigen detection efficiency of immunosensors based on quartz-crystal microbalances [12] as well as on screen-printed electrodes for electrochemical sensing [13]. Herein, we review our recent results that show how PIT can be successfully applied to colloidal AuNPs to provide either a “ballasting” tool for mechanical platforms or colorimetric transducers.

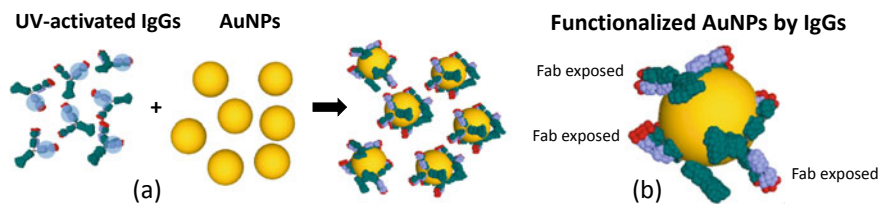


Fig. 1 Schematic representation of the functionalization process. **a** Antibodies (IgG)s are activated by UV irradiation and mixed to a solution containing AuNPs. After short incubation AuNPs are functionalized with Abs. **b** Magnification of a single AuNP highlighting the heavy chain (green), light chain (blue) and Fab (red) domain, the latter being exposed to the solvent for all the three Abs

2 AuNPs Synthesis and Functionalization

2.1 AuNPs Synthesis

Spherical gold nanoparticles were synthesized by modifying an existing protocol [14]. In short, we added sodium citrate (20 mg/mL) to a previously prepared gold salt solution (10 mg/mL of $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$) to achieve particles nucleation. After that, particles growth was induced with continued stirring and boiling until the colour of the suspension turned dark red. The reached optical density was approximately 1.0 at 530 nm, corresponding to a concentration of approximately 10^{10} particles/mL [15].

2.2 Synthesis of Core-Shell Magnetic NPs (Au@MNP)

The synthesis consisted in mixing 75 μL (5 mg/mL) of Fe_3O_4 and 1 mL (10 mg/mL) of sodium citrate dihydrate in 15 mL of milliQ water at 120 °C under a slow stirring. When the solution boiled, 4 additions of 50 μL (10 mg/mL) of HAuCl_4 every 5 min were performed. The first step produced the gold seeds that anchored to the magnetic core, while the second step induced the growth of the gold layer. The formation of the Au shell was signalled by a color change of the solution from brownish to burgundy.

2.3 Functionalization of Nanoparticles by PIT

1 mL of AuNPs (or core-shell NPs) was centrifuged at 3000 g for 10 min. The resulting supernatant was discarded and the AuNP pellet was resuspended in ultrapure water. Simultaneously, a solution of 50 $\mu\text{g/mL}$ IgG was prepared and irradiated for 30 s with the UV lamp (Trylight®) at 254 nm (Fig. 2a). The power of each lamp is 6 W so that the intensity on the quartz cuvette is approximately 0.3 W/cm².

A volume of 20 μL of the irradiated IgG solution was added to 1 mL of AuNPs, and the resulting mixture was incubated for 3 min. The amount of IgG in the mixture was progressively increased by further adding 4 μL of the irradiated Ab solution. The UV-vis absorption spectra showed a red shift of the maximum absorption wavelength as a result of the formation of the protein corona (Fig. 2b). Only few spikes were necessary to stabilize the red shift; thus, a total number of five additions, corresponding to 1 $\mu\text{g/mL}$ IgG concentration, demonstrated to ensure the maximum coverage of the AuNP surfaces. Unbound Abs were removed from the mixture by centrifugation at 3000 g for 10 min (low temperature) and supernatant discarding. The IgG-functionalized AuNP pellet was resuspended in ultrapure water. Finally, 1 mg/mL of bovine serum albumin (BSA) was added to the functionalized colloid to block the AuNP surface from nonspecific adsorption.

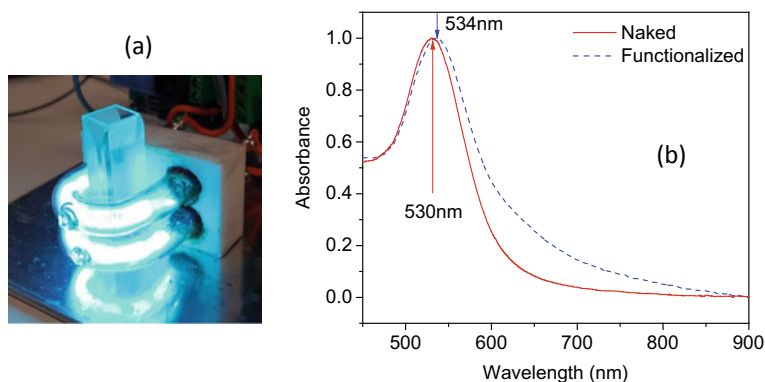


Fig. 2 **a** TryLight[®] lamp used for PIT. The intensity in the cuvette is approximately 0.3 W/cm^2 . **b** Absorption spectrum of a colloidal solution of AuNPs before (red continuous line) and after functionalization (dashed blue line) (color figure online)

3 Results

A direct application of AuNPs exploits the high density of gold in a detection scheme depicted in Fig. 3, which uses a quartz-crystal microbalance as mechanical transducer for small molecules ($<300 \text{ Da}$) detection. The target (violet circle) is detected thanks to the highly specific ballast provided by functionalized AuNPs in the sandwich configuration Ab-analyte-AuNP. In such a way, parathion in drinkable water could

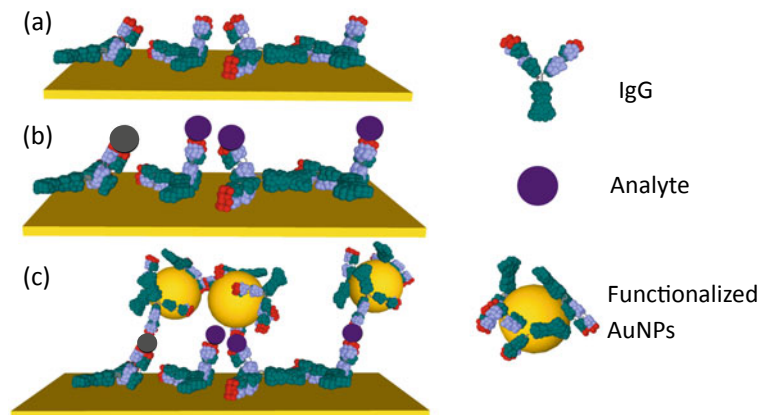


Fig. 3 **a** The gold surface is functionalized with PIT. The IgGs are close packed (high surface density) and expose one Fab to the solvent (upright orientation) so to provide an ideal functionalization. **b** The Abs recognize the analytes with small or even undetectable signal if the latter are small. **c** Strong signal is detected when AuNP bind the analyte leading to a highly specific detection (color figure online)

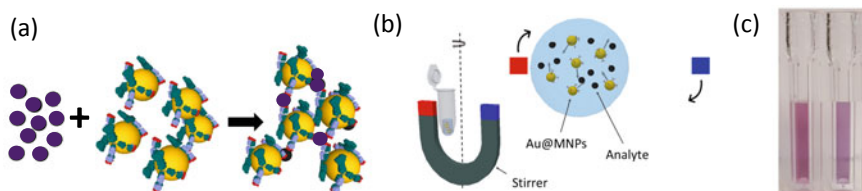


Fig. 4 **a** The presence of the analyte (violet circle) induces aggregation of functionalized Au@MNPs. **b** A rotating magnet improves the mobility of magnetic nanoparticles thereby increasing the yield of aggregate occurrence. **c** A colloidal solution of Au@MNPs (left) changes its color when 2 μL of IgG solution (250 ng/mL) is added (right) (color figure online)

be detected with a limit of detection lower than 1 ppb [16], with an improvement of more than one order of magnitude in comparison with 15 ppb previously measured with a simple sandwich Ab-analyte-Ab [17].

The highly effective gold surface functionalization offered by PIT shows its potential when AuNPs are exploited for their plasmonic properties. The detection scheme is depicted in Fig. 4a and shows AuNP aggregation induced by the presence of the analyte that acts as a linker. A solution of 1 mL of functionalized AuNPs was mixed to a smaller volume of solution containing the analyte (typically 100–200 μL). This is helpful in all the situations, as it is the case for tap or drinking water, in which the analyte solution contains salts, which may induce (non-specific) aggregation. With this approach, we were able to realize a colorimetric biosensor for 17 β -estradiol in tap water with a limit of detection of only few pg/mL [18].

Additional properties can be provided to the user when gold is exploited as coating of a magnetic nanoparticle (MNP), so to realize a core-shell nanoparticle (Au@MNP). Indeed, these nanoparticles can be “steered” by a magnetic field while retaining all the plasmonic properties. In fact, the gold surface of Au@MNPs lends themselves to be easily functionalized with antibodies by PIT, thereby becoming promising “analyte catchers” at the nanoscale with inherent application to biosensing devices. To test the occurrence of both optical and magnetic properties, we used Au@MNPs (size \approx 50 nm with an inner diameter of 30 nm) to reduce the limit of detection of a colorimetric immunosensor previously developed [19].

The rationale of our approach relies on the higher nanoparticle mobility induced by a microstirrer realized in a microtube placed off-axis with respect to a rotating magnet (see Fig. 4b). The rotating magnetic field acts as external force, which pushes the otherwise slow nanoparticles so to increase the collision rate among Au@MNPs and analytes. This, in turn, is directly related to the efficiency with which the aggregates are formed and, hence, to the limit of detection. Such an improvement is demonstrated by the color change that takes place when IgG solution at 250 ng/mL is mixed to a colloidal solution of Au@MNPs (Fig. 4c). Besides, no effect could be observed when simple AuNPs of the same size were used (data not shown). It is worth noticing that the stirring realized by our approach takes place in a volume as small as 50 μL .

4 Conclusion

Surface functionalization is a key step in realizing a biosensor, which we address by adopting the Photochemical Immobilization Technique (PIT), a simple and effective procedure able to tether antibodies upright on a gold surface. In this manuscript, we report PIT application to AuNPs, showing that the resulting functionalized nanostructures are effective in binding antigens; thus, they can be successfully used in a large variety of biosensing applications such as ballasting tool for quartz crystal microbalance or in colorimetric assays. Eventually, we show that gold-coated core-shell magnetic NPs can also be functionalized by PIT, allowing mixing in volumes as small as 50 μL (microstirrer) under application of a proper external field. Such a procedure can improve the limit of detection in colorimetric biosensors.

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