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Design, Preparation, and Evaluation of a Fixed-Orientation Antibody/Gold-Nanoparticle Conjugate as an Immunosensing Label

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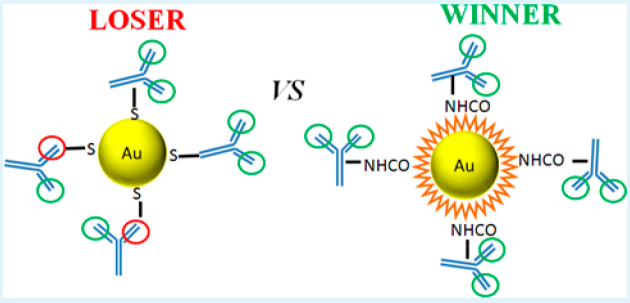
Supporting Information

ABSTRACT: Herein, we describe the development of a new, highly efficient label for immunosensing comprising an antibody/PEGylated gold-nanoparticle (AuNP) conjugate in which the antibody molecules are bound to the AuNP surface in a fixed orientation. Our method exploits the high density of positive charges on the major plane of antibodies that exists when the pH of the solution is lower than the isoelectric point of the antibody; the antibody molecules interact with the negatively charged AuNP surface through their major plane, enabling the antigen binding sites to move freely and therefore to reach maximum accessibility. This directed ionic interaction is reinforced by the formation of a peptide bond between the amino group of the Lys residues in the antibodies and the carboxylic groups of the PEGylated-AuNP surface via EDC chemistry. Electrochemical analyses revealed that the fixed-orientation conjugate offers a limit of detection that is 1 order of magnitude lower than that of a randomly oriented label. The performance of the new conjugate as an immunosensing label was assessed for the quantitative detection of IgG in human serum.



KEYWORDS: *gold nanoparticles, antibodies, oriented functionalization, electrochemical biosensing, immunosensor*

1. INTRODUCTION

During the past 2 decades, a myriad nanomaterials (e.g., gold nanoparticles [AuNPs], carbon nanotubes, quantum dots, etc.) have been integrated in different biosensing platforms[1,2] with the aim of exploiting their unique nanoscale properties to either improve already existing biosensing strategies or to develop new sensing methods. Gold nanoparticles are among the most widely used nanomaterials owing to their low toxicity,[3] facile preparation and modification, and amenability to different sensing methods.[4,5] They can be prepared in different sizes and shapes that give rise to properties such as plasmon resonance and catalytic activity, which are relevant for the use of AuNPs as labels in bioassays (e.g., electrochemical sensing of DNA,[6] proteins,[7] or cells[8−10] and even optical sensing[11,12]).[13−17] However, these properties alone are not sufficient for labeling:[18] the specificity of a given affinity biosensor (immunosensors or DNA biosensor) is dictated by the biomolecules that are attached to the label that should recognize the analyte. For instance, achieving high performance in immunosensors require the use of a label/antibody conjugate that maintains the antigen-binding sites available for antigen capture.[19,20]

Here, we report that the limit of detection (LoD) of an antibody/AuNP conjugate used as an immunosensor label is dictated by the orientation of the surface-bound antibody molecules. To control the functionalization in order to leave the antigen-binding sites of the antibody molecules accessible, we adapted a previously developed two-step coupling reaction[21,22] in which an oriented ionic attraction is coupled to strong covalent-bond formation via carbodiimide chemistry.[23] The technique is based on the fact that when the pH of an antibody solution is lower than the isoelectric point of the antibody the concentration of positive charges in the major plane of the antibody is high (Figure 1A). Under these conditions, the NH_2 groups of the antibody's Lys residues are protonated, thereby generating NH_3^+. Thus, if the antibodies are in solution with negatively charged AuNPs, then they most likely interact through their major plane with the surface of the AuNPs. This controlled approach guarantees that the antigen-binding sites remain accessible.

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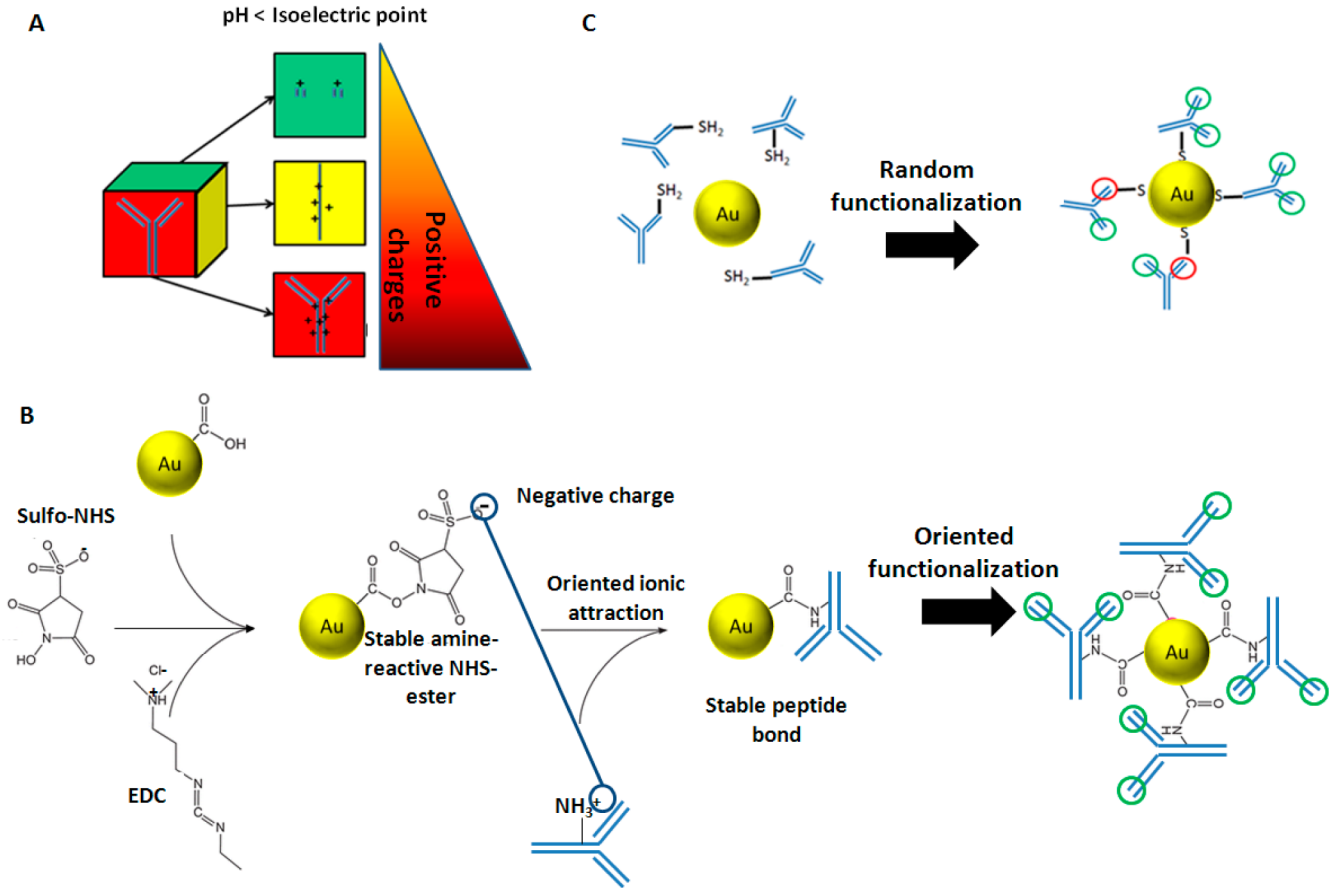

Figure 1. (A) Density of the positive charges on the antibody structure for pH lower than the isoelectric point of the antibody. The major plane (corresponding to the red square at the bottom) has the highest density. (B) Scheme of the oriented functionalization proposed based on the oriented ionic attraction and the formation of the peptide bond. (C) Scheme of the random adsorption of antibody onto the surface of AuNPs. NOTE: the schemes are not in scale.

We prepared an antibody/PEGylated gold-nanoparticle (AuNP) conjugate in which with the antibody molecules are bound to the AuNP surface in a fixed orientation. We then compared its performance for antigen detection to that of a corresponding random-orientation conjugate in which the antibody molecules are bound in random orientations. Finally, we assessed the performance of the fixed-orientation conjugate as an immunosensing label for the quantitative detection of IgG in human serum.

To cover the AuNP surface with negative charges and to enable the formation of the covalent bond, we functionalized the NP surfaces with carboxylated poly(ethylene glycol) (PEG) using the aforementioned carbodiimide chemistry, which generates a stable intermediate. This negatively charged group catalyzes the peptide coupling of the PEG carboxylic groups to the amino groups of the Lys residues (Figure 1B).

To ascertain the performance of our new label relative to that of a randomly functionalized AuNP (Figure 1C), we tested both using two different antibodies: αHRP and αHIgG. The former was used only as an optical tool to verify that for a saturating amount of antigen the fixed-orientation conjugate would bind more HRP molecules than would the random label, whereas the latter was used in an electrochemical magneto-sandwich immunoassay. Having confirmed that our new fixed-orientation label works better than the randomly oriented one, we then proceeded to assess its performance for the detection of analytes in human serum.

2. METHODS

Reagents and Instruments. A Jeol JEM-2011 (Jeol Ltd., Japan) transmission electron microscope (TEM) was used to characterize the AuNPs. An ultrasonic bath (JP Selecta, Spain) was used to prevent agglomeration in the AuNP suspensions. The isoelectric focusing of the Abs was done using the Pharmacia Phast System and PhastGel® IEF 3-9. Bradford reagent (B6916) was purchased from Sigma-Aldrich (Spain). A Sigma 2-16 PK thermostatic centrifuge (Fisher Bioblock Scientific, France) was used to purify the conjugates of the AuNPs with antibodies. Spectrophotometric measurements were taken using a Spectramax_M2^e multimode microplate reader (Molecular Devices Inc., U.K.). A DEK248 semiautomatic screen-printing machine (DEK International, Switzerland) was used for the fabrication of the screen-printed carbon electrodes (SPCEs) (Figure S1 of the Supporting Information). The reagents used for this process were an Autostat HT5 polyester sheet (McDermid Autotype, U.K.), Electrodag 423SS carbon ink, Electrodag 6037SS silver/silver chloride ink, and Minico 7000 Blue insulating ink (Acheson Industries, The Netherlands). The detailed fabrication procedure is provided in the Supporting Information. Electrochemical measurements were performed at room temperature with an Autolab 20 (Ecochemie, The Netherlands) connected to a computer. Streptavidin-coated magnetic beads (M-280; 2.8 μm size) were purchased from Dynal Biotech (Invitrogen, Spain) and were used in the magnetosandwich immunoassay. Hydrogen tetrachloroaurate (III) trihydrate ($HAuCl_4 \cdot 3H_2O$, 99.9%) and trisodium citrate ($Na_3C_6H_5O_7$) were purchased from Sigma-Aldrich (Spain) and were used in the synthesis of AuNPs. Bifunctional poly(ethylene glycol) SH-EG(8)-$(CH_2)_2$-COOH was purchased from Iris Biotech. ABTS, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt, was purchased from Sigma-Aldrich and used

as the HRP substrate. Human IgG (whole molecule; I2511), goat anti-human IgG (whole molecule; I1886), γ-chain-specific biotinylated goat anti-human IgG (B1140), rabbit anti-HRP (P7899), and horseradish peroxidase (HRP) from horseradish type VI (P8375) were purchased from Sigma-Aldrich (Spain). All buffer reagents and other inorganic chemicals were purchased from Sigma, Aldrich, or Fluka unless otherwise stated. All of the solutions were prepared using milli-Q water, which was produced using Milli-Q system (>18.2 MΩ cm^{-1}) purchased from Millipore (Sweden).

Gold Nanoparticle Synthesis and PEGylation. Gold nanoparticles (diameter, 16 nm) were synthesized following the Turkevich[24] procedure and analyzed by TEM (Figure 2A). Briefly, a solution of 0.508 mL $HAuCl_4 \cdot 3H_2O$ [254 μM] in 49.492 mL H_2O mQ was heated at 150° C and stirred. Once the solution reached boiling, 5 mL of aqueous sodium citrate [40 mM] was added rapidly. Over the following 10 min of heating and stirring, the solution changed color from pale yellow to red. After that, it was stirred for another 15 min at 25° C. The reflux was used all of the time to prevent a loss of volume. The AuNPs were protected from light and stored at 4° C.

We covered the AuNPs with a dense layer of in situ formed bifunctional poly(ethylene glycol) SH-EG(8)-$(CH_2)_2$-COOH, which we chose for its terminal thiol group to enable subsequent covalent linkage to the gold surface and for its carboxylate group to enable functionalization using carbodiimide chemistry.[20] Thus, an aqueous solution of AuNP (0.5 mg/mL) was mixed with SDS (0.025%) and PEG (0.024 mg/mL) under basic conditions for 16 h. Purification by centrifugation (13 400 rpm, 30 minutes) afforded the desired PEGylated AuNPs.[25]

Random Conjugation of Antibodies to AuNPs. We prepared the randomly oriented antibody/AuNPs by random adsorption of antibodies onto the AuNP surface via reaction of the thiol groups in the antibody (Cys residues) with the gold atoms (Figure 1C). First, a gold aggregation test (GAT), in which antibodies are incubated with gold nanoparticles under various conditions, was done to find the optimum pH and concentrations.[11] This entailed incubating (in a 96-well microplate, at 25 °C, for 20 min, and 650 rpm) a 10 μL aqueous antibody solution (at 7.5, 10, 12.5, 15, or 20 μg/mL in mQ water) with 150 μL of aqueous AuNP solution (3 nM) at different pH (7, 8, or 9; the pH was corrected with borate buffer [10 mM], pH 9.2). After the incubation, 25 μL of aqueous NaCl (10% in mQ water) was added, and the solutions were incubated for 5 min at 650 rpm and 25 °C. To identify the optimal pH, meaning the pH at which the least amount of antibody is required to stabilize the AuNPs (i.e., to minimize aggregation of the AuNPs), the absorbance spectrum of each solution was measured (from 300 to 700 nm) after incubation.

We then prepared the random-orientation conjugates αHIgG/AuNP and αHRP/AuNP by incubation using the GAT test conditions, which for αHIgG were 20 μg/mL and pH 9 and for αHRP were 15 μg/mL and pH 7. After incubation, the conjugates were blocked by incubation of each solution with 100 mL of aqueous BSA (1 mg/mL in mQ water) at 25 °C for 20 min. Finally, to purify the conjugates, each solution was centrifuged (14 000*g*, 4 °C, 20 min), and each resulting pellet was resuspended in mQ water.

Measurement of the Isoelectric Points of the Antibodies. We measured the isoelectric points of the αHRP and αHIgG antibodies using the Pharmacia Phast System and PhastGel® IEF 3-9. Two gradient gels were run (one in which the samples were dispensed at pH 3 and the other at pH 7) according to Phast System Technique File no. 100: 2 μL of antibody solution (100 μg/mL) was separated under 2000 V potential. An isoelectric focusing calibration kit was used to determine the isoelectric point of each protein in the pH range of 3 to 10 and to generate standard curves from which the isoelectric points of different antibodies were read. The proteins were fixed into the gels in 10% trichloroacetic acid immediately after the run, and gels were stained with silver according to the Phast System instructions.

Fixed-Orientation Conjugation of Antibodies to AuNPs. All of the steps of the oriented conjugation were performed in MES buffer (10 mM, pH 5) unless otherwise stated. A solution of 20 μL EDC (10 mg/mL) was mixed with 3.8 μL of sulfo-NHS (100 mg/mL) at room temperature for 10 min. Then, 23.8 μL of this solution was mixed with 0.5 mg of PEGylated AuNPs, and the total volume was increased to 500 μL with MES buffer. The resulting solution was incubated at 37 °C and 650 rpm for 30 min to activate the carboxylic groups of the PEGylated AuNPs. After the incubation, the excess of EDC-sulfo-NHS was eliminated by centrifuging the solution in Amicon Ultra 0.5 filters (14 000 rpm; cut off, 50 K) for 5 min. The resulting conjugate solution (volume, ca. 30 μL) was mixed with 10 μg of antibody and diluted to a final volume of 500 μL in MES buffer. The diluted solution was then incubated at 37 °C and 650 rpm for 60 min. The obtained conjugates were centrifuged at 14 000 rpm at 4 °C for 20 min and then washed with 500 μL of bicarbonate buffer (10 mM, pH 8) at 37 °C and 650 rpm for 30 min. The conjugates were then centrifuged (14 000 rpm) at 4° C for 20 min and blocked with 2% BSA in MES buffer (10 mM, pH 6). The resulting solution was ultrasonicated for 5 s to separate the aggregated complexes and was then incubated at 37 °C and 650 rpm for 60 min. Finally, the resulting oriented-antibody/AuNP conjugates were resuspended in mQ water.

Characterization of the Oriented Conjugates. First, we verified that αHRP (as a representative antibody) is attracted to the surfaces of negatively charged AuNPs at a pH lower than its isoelectric point. We used PEGgylated AuNPs that had not been treated with any activating agent. The PEGgylated AuNPs were incubated with 500 μL of antibody solution (26 μg/mL) at 37 °C for 30 min. The resulting solution was centrifuged, and the supernatant was stored. Then, 500 μL of carbonate buffer (pH 8) was added to break the ionic bonds between the antibodies and the AuNPs (for a comparison of four different buffers, see the Supporting Information). After a second centrifugation, all of the supernatants were checked by a Bradford assay to verify the amount of Abs that had been ionically adsorbed.

We then sought to prove that the NH_2 groups of the antibodies had covalently bonded to the COOH groups of the PEG. Thus, the above procedure was repeated using only the oriented conjugates. In this case, the nature of the bond was checked by SDS-PAGE rather than with a Bradford assay.

Finally, using optical tests, we sought to confirm our hypothesis that the oriented αHRP/AuNP conjugate would capture a greater amount of antigen than would the random αHRP/AuNP conjugate at a saturating concentration of antigen and nearly the same surface concentration of antibody. Briefly, 150 μL of each conjugate were separately mixed with 12 μg of HRP in sodium phosphate buffer (10 mM, pH 7), and the resulting solutions were incubated at 37 °C for 30 min. After centrifugation (14 000 rpm, 4 °C, 20 min), the pellets were resuspended in 1.5 mL of sodium phosphate buffer. Then, in a 96-well plate, 10 μL of each sample was mixed with 300 μL of a solution of ABTS (1 mM + H_2O_2 1 mM). As soon as the ABTS solution was added, the reaction kinetics of the reaction were measured at 1 min intervals for 1 h.

Magnetosandwich Immunoassay and Electrochemical Detection of HIgG. The magnetosandwich immunoassay was run following a method that we had previously optimized.[26] Briefly, 120 μg of streptavidin-coated MBs were washed twice with 120 μL of PBS with 0.05% Tween-20, and the beads were then used to immobilize 30 μL of the biotinylated αHIgG γ-chain-specific antibody (0.5 μg/mL in PBS) by incubating for 30 min at 25 °C at 650 rpm. After two washing steps in PBS, the MBs were blocked with 120 μL of BSA (5% in PBS) at 4 °C overnight. They were then washed twice in PBS and incubated with 120 μL of HIgG (in PBS) at 25 °C and 650 rpm for 30 min. After three washes with PBS, the magneto−immuno sandwich was formed by incubating the MBs with 120 μL of αHIgG/AuNP conjugate at 25 °C and 650 rpm for 30 min, washing four times, and resuspending in 120 μL of mQ water. In the real human serum measurements, we diluted the sample by a factor of 10^6 in PBS.

We then performed electrochemical detection of AuNPs by measuring their catalytic activity in the hydrogen-evolution reaction (HER). A drop (25 μL) of the magnetosandwich complex was deposited onto an SPCE, and the complexes were collected onto the working electrode by a magnet. Finally, 25 μL of HCl (2M) was added. The AuNPs of the immune complexes catalyzed the formation of hydrogen in a 1 M HCl solution.[27] By holding the working electrode at a potential of +1.35 V for 1 min followed by a negative

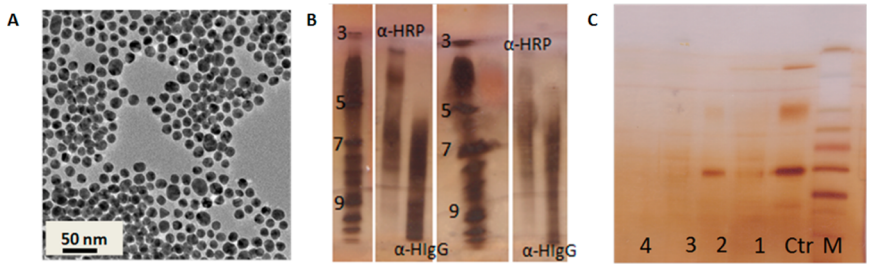

Figure 2. (A) Transmission electron microscopy image of the synthesized AuNPs. (B) Isoelectric point measurements: the antibodies αHRP and αHIgG both have an isoelectric point of 7. The extra bands at 5 and 9 correspond to other serum proteins. (C) SDS gel of supernatants after conjugate centrifugation. From right to left: M, marker; Ctr, αHRP; 1, ionic absorption without EDC; 2, ionic desorption without EDC in carbonate buffer (pH 8); 3, αHRP/AuNP conjugate; and 4, αHRP/AuNP conjugate in carbonate buffer (pH 8). Even in the presence of carbonate buffer (pH 8) there are no bands in raw 4, proving the formation of the peptide bond.

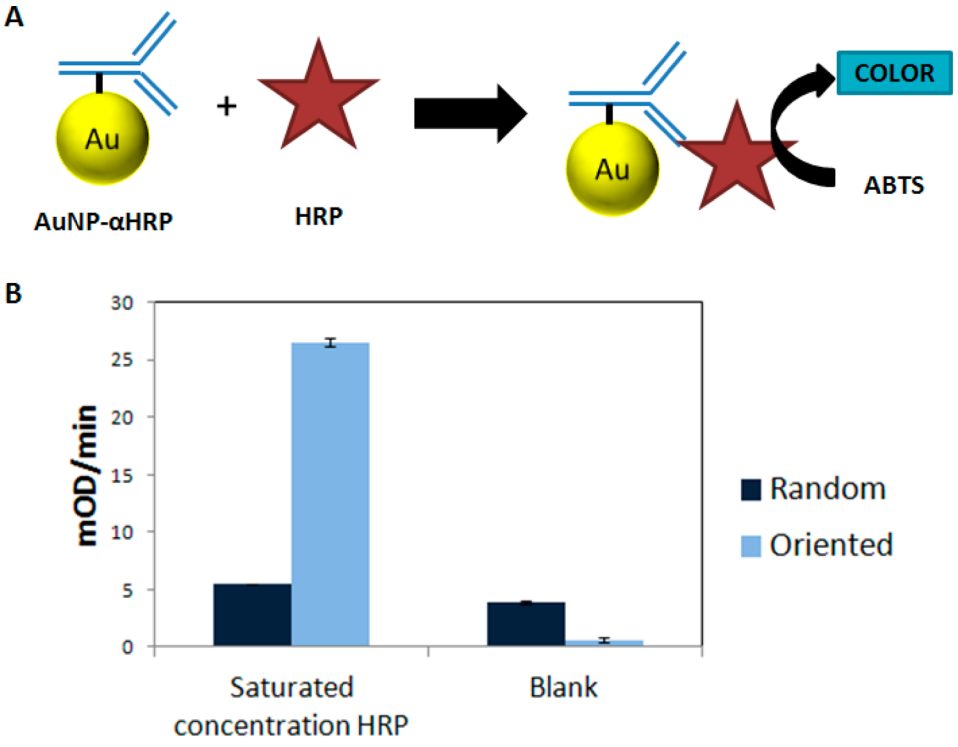

Figure 3. (A) Schematic of the optical method used to qualitatively evaluate the orientation of the αHRP antibody on the AuNP surface. (B) Comparison of the results obtained using the random-orientation and fixed-oriented conjugation for a blank sample (without HRP) and for a saturated concentration of HRP.

potential of −1.00 V for 100 s, the hydrogen evolution was monitored by measuring the current in chronoamperometric mode. A fixed time of 100 s was chosen for measuring the value of the current, whose absolute value is considered to be an analytical signal. Consequently, this value is directly related to the amount of AuNPs and therefore to the amount of analyte captured by the immunocomplexes. For measurements made after shorter times, a certain irreproducibility in the current was observed, which is probably due to the fact that the accommodation of the AuNPs on the electrode surface conducted through the MBs under the magnetic field exerted by the magnet requires some stabilization time. A new electrode was employed for each measurement.

3. RESULTS AND DISCUSSION

Gold Aggregation Test. As previously mentioned, we used the GAT test to establish the best conditions (in terms of avoiding nanoparticle aggregation) for the random functionalization of the AuNPs with the antibodies: 20 μg/mL αHIgG at pH 9 or αHRP 15 μg/mL at pH 7. The function of the NaCl is to displace the charges on the AuNP surface; this leads to aggregation of the NPs, which can be monitored by measuring the absorbance of AuNP. In the presence of aggregates, the wavelength of the plasmon peak of the AuNPs shifts to higher values. However, this phenomenon cannot happen if the surface of the AuNPs is covered with antibodies because the NaCl cannot break the Au−S bond.

Isoelectric Point Measurements. The measurements of the isoelectric points of αHRP and of αHIgG produced several bands. This was because we were using antisera and therefore other serum proteins were present in the solutions, generating extra bands (Figure 2B). However, comparing the two different gels, we estimated that the isoelectric points of each antibody is ca. 7 because the most intense bands in each solution, corresponding to αHRP or αHIgG, are at pH 7. On the basis of this result, MES buffer (pH 5) was chosen for all of the oriented functionalization reactions.

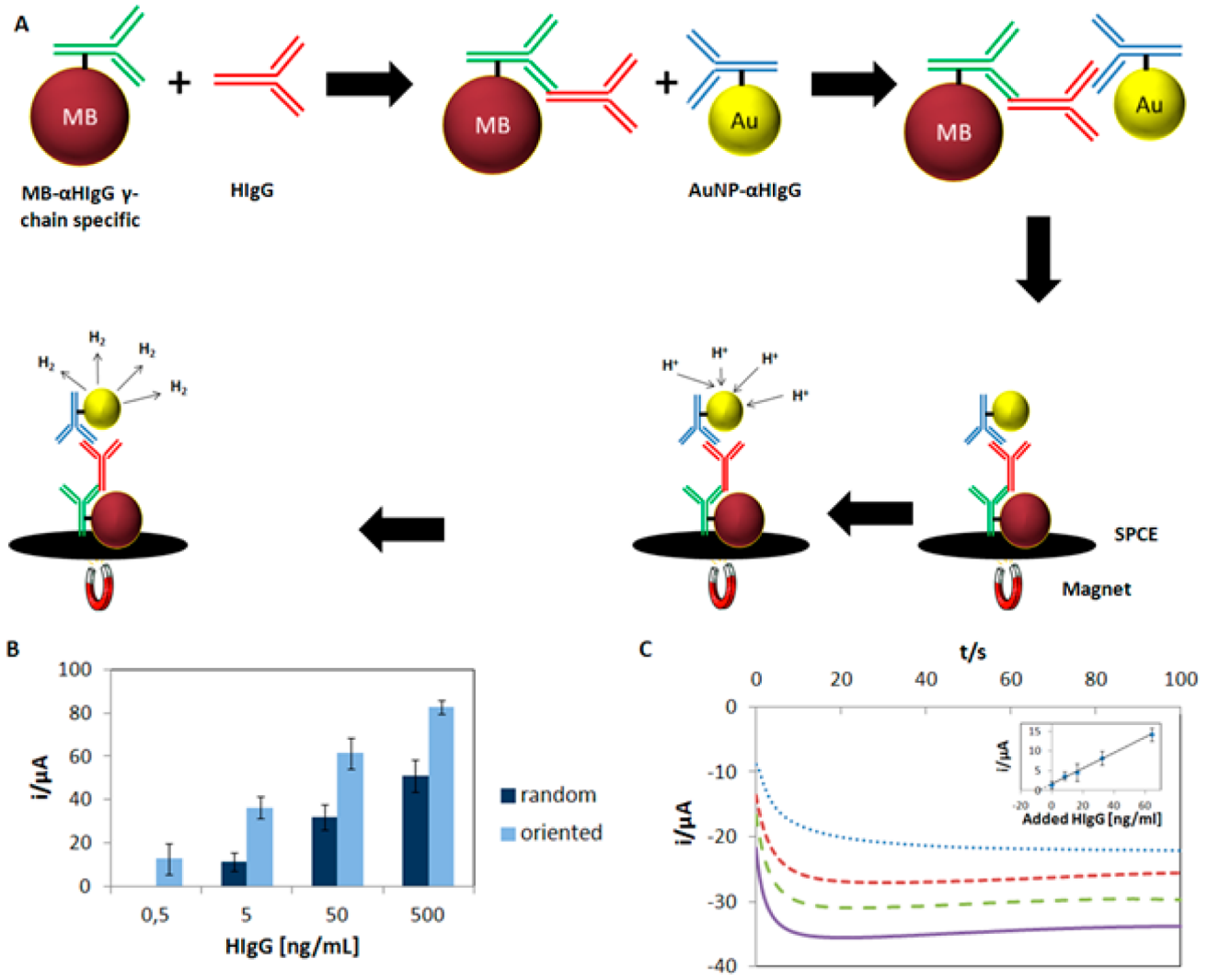

Figure 4. (A) Scheme of the electrochemical magnetosandwich immunoassay used to detect HIgG. (B) Results obtained for the random-orientation and fixed-orientation αHIgG/AuNP conjugates used as labels after subtracting the blank in 1 M HCl. (C) Representative chronoamperometric results obtained using the fixed-orientation conjugate as a label. The working electrode was held at a potential of +1.35 V for 1 min followed by a negative potential of −1.00 V for 100 s. From top to bottom: 0 (blue dotted line), 8 (red dashed line), 32 (green dashed line), 64 (purple solid line) ng/mL of HIgG. The inset shows the correlation curve obtained from the addition of HIgG in human serum by the standard addition method.

Characterization of the Oriented Conjugates. Our Bradford assay measurements indicated that at pH 5 there was ionic attraction between the negatively charged PEGylated-AuNPs and the antibodies. These measurements were taken on PEGylated-AuNPs whose surface carboxylic groups had not been activated; therefore, formation of the peptide bond between the carboxylic groups of the PEG and the amino groups of the antibody was not catalyzed. As such, we assumed that the interaction between the PEGylated-AuNPs and the antibodies was mainly due to ionic interactions. In the supernatant of the first sample, the concentration of antibodies, which had not been ionically adsorbed onto the AuNP, was 13.87 ± 5.52 μg/mL, whereas in the second supernatant, the concentration of the antibodies, which had been ionically adsorbed onto the AuNP and subsequently released by the carbonate buffer (pH 8), was 12.74 ± 2.21 μg/mL. The sum of the values is ca. 26 μg/mL, which is equal to the concentration of antibody that we used for the incubation. Additionally, the concentration of the ionically adsorbed antibody is close to those of the GAT (15 μg/mL), indicating that the surface of the AuNPs had been fully covered with antibodies.

We then performed control experiments to prove that the conjugation was covalent. Thus, the fixed-orientation conjugates were incubated in carbonate buffer (pH 8) because it would desorb all of the ionically adsorbed antibodies (as indicated by the previously mentioned Bradford results), but it would not desorb those that were covalently attached. In this case, the supernatants were analyzed by SDS-PAGE. All of the antibodies in the fixed-orientation conjugate were covalently attached to the surface of the AuNPs, as indicated by the absence of visible bands in the raw 3 (Figure 2C) of the gel corresponding to this sample. However, the previous supernatants, which were obtained without the activation of the carboxylic groups, revealed the two typical antibody bands: one corresponding to the light chain and the other, the heavy chain (Figure 2C, raw 2). These two experiments confirmed the ionic adsorption and peptide-bond formation.

Antigen Capturing: Oriented Conjugate versus Random-Orientation Conjugate. The advantage of using the

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αHRP antibody is that the enzymatic activity of the antigen, horseradish peroxidase, generates color in the presence of the proper substrate (Figure 3A), a feature that can be exploited to calculate the amount of captured antigen. In fact, the kinetics of the enzymatic reaction can be correlated to the amount of enzyme present. This experiment gives qualitative information on the orientation of the antibody molecules bound to the AuNP surface: the amount of captured antigen correlates to the orientation of the antigen-binding sites. The aim of this experiment was to determine which conjugate could capture a greater amount of antigen. This experiment was done using a saturated concentration of horseradish peroxidase and equal amounts of antibodies and AuNP conjugates. The oriented conjugate captured approximately 5 times the amount of antigen than did the random-orientation conjugate (Figure 3B).

Signal-Amplification Efficiency of AuNP Labels in a Magnetosandwich Immunoassay. The fixed-orientation conjugate and the random-orientation conjugate were also compared for their performance as labels in a magnetosandwich immunoassay for the electrochemical detection of HIgG in PBS buffer (Figure 4A). The detection is based on the ability of AuNPs to catalyze the formation of hydrogen gas from hydrogen ions, a reaction that generates a current that can be chronoamperometrically measured. The value of the catalytic current at a fixed time (as described in the Methods section) can be correlated to the concentration of AuNPs in solution and therefore to the concentration of antigen captured by the magnetosandwich. Typical chronoamperograms are shown in Figure 4C. The rapid increase in the absolute value of the catalytic current followed by a certain instability observed during the first seconds can be attributed to the accommodation of the AuNPs on the electrode surface conducted through the MBs under the magnetic field exerted by the magnet (placed on the opposite side of the electrode), which requires some stabilization time. For this reason, the 100s was the time chosen for measuring the current that constitutes the analytical signal.

The obtained results are plotted in Figure 4B. In each test, a logarithmic relationship was observed (correlation coefficient of 0.999) between the current intensity and the HIgG concentration (0.5 to 500 ng/mL for the fixed-orientation conjugate and 5 to 500 ng/mL for the random-orientation conjugate). The reproducibility of the method shows a relative standard deviation (RSD) of 7% for the oriented label and 5% for the random label ([HIgG] = 5 ng/mL, n = 6). The estimated limit of detection (LoD) for each conjugate, calculated as the concentration of HIgG corresponding to three times the standard deviation of the estimate, was 0.19 (fixed-orientation) and 1.9 ng/mL (random). In other words, the fixed-orientation conjugate offered a nearly 1 order of magnitude better LOD than did the random-orientation conjugate. This advantage stems from the fact that the fixed-orientation conjugate has a greater number of accessible antigen-binding sites, which translates to a higher probability of recognition between the antibody and antigen. Furthermore, the LOD achieved is comparable with those of standard techniques such as ELISA, with the advantage that the proposed method is faster and cheaper. It is also possible to improve the LOD by using some amplification techniques, such as silver enhancement, or by using secondary antibodies.

Having demonstrated that the fixed-orientation conjugate has a lower LOD, we then ascertained its performance as a label for the detection of a real biological analyte: HIgG in human serum sample. The standard addition method was used to estimate the concentration of the sample (Figure 4C, inset), minimizing the matrix effects. In the IgG concentration range of 10 to 70 ng/mL, a linear relationship between the standard addition of HIgG and the voltammetric peak current was found (correlation coefficient = 0.998). It was adjusted to the following equation: peak current (μA) = −0.1958[added HIgG (ng/mL)] + 1.7359. Thus, the estimated concentration of HIgG in the serum was 8.86 ± 0.4 mg/mL (standard addition = 0 ng/mL, n = 3), which is within the reported range for IgG in human serum (6 to 11 mg/mL).[28,29]

4. CONCLUSIONS

We have created a new antibody/AuNP conjugate for use as a label in immunosensing, which is characterized by the fixed orientation of its surface antibody groups (as opposed to the random orientation that is used traditionally) and was achieved through ionic and covalent interactions. The conjugate exploits the fact that when the pH of a solution is lower than the isoelectric point of the antibody the high density of positive charges on the antibody will be on its major plane. To ionically orient the antibodies, the AuNPs were modified with COOH-PEG, which enabled the formation of a stable negative intermediate that subsequently interacted with the major plane of the antibody to form a covalent (peptide) bond. The formation, nature, and orientation of the fixed-orientation conjugate were confirmed by analyses using the antibody αHRP. It was then evaluated as a label in a magnetosandwich immunoassay, exhibiting an LOD of 160 pg/mL, which is nearly 1 order of magnitude better than that of a similar random-orientation conjugate. Finally, the conjugate was used as a label to detect the concentration of IgG in human serum, providing values consistent with the reported range of concentrations for IgG in serum.

We expect that this fixed-orientation antibody-immobilization approach developed for AuNPs, the most widely used nanoparticles in biosensing, will have a great impact on several other electrochemical and optical biosensing applications of interest for proteins as well as for whole cells (e.g., cancer cells) and even for plasmonic detection of biomarkers.[30]

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

SPCE electrode production and Bradford assay and SDS procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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