

Aptamer–Gold(III) Complex Nanoparticles: A New Way to Detect Cu, Zn SOD Glycoprotein

Rawdha Dekhili, Khaoula Cherni, Hui Liu, Xiaowu Li,* Nadia Djaker, and Jolanda Spadavecchia*



Cite This: *ACS Omega* 2020, 5, 13851–13859



Read Online

ACCESS |



Metrics & More

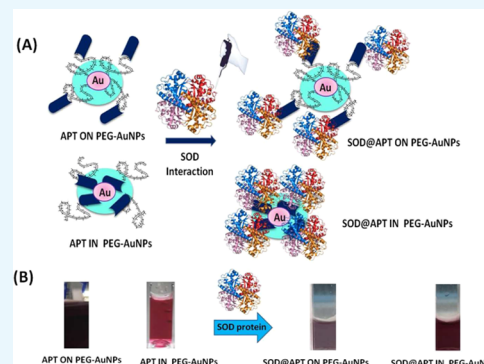


Article Recommendations



Supporting Information

ABSTRACT: Aptamers are small biomolecules composed of 20–100 nucleotides that recognize target molecules in three-dimensional structures. These natural targeting molecules have attracted interest in the biomedical field as biomarkers for cancer diagnostics. In this study, we investigated the interaction of a characteristic aptamer with its target protein, Cu, Zn superoxide dismutase (SOD 4), on a gold nanoparticle (AuNP) surface under experimental conditions. For this purpose, we applied two protocols to coat SOD 4 aptamer (APT) on the nanoparticle surface: carbodiimide chemistry (EDC/NHS) (Method ON) and a complexation methodology (Method IN). The nano-aptamer's interactions with SOD 4 were detected by UV–vis absorption and Raman spectroscopy in a range of protein concentrations (from 1 μ M to 50 nM). We believe that the interaction is heavily dependent on the nature of the biomarker (SOD 4) and also on the steric arrangement of the aptamer on the gold nanoparticle surface. The lowest detectable concentration (limit of detection, LOD) was about 2 nM for APT IN PEG-AuNPs and 8 nM for APT ON PEG-AuNPs. For the first time, we demonstrated a very sensitive detection of SOD 4 in the nanomolar concentration range with new ways of biosensor synthesis (APT IN and ON), providing a very strong tool to understand the effect of aptamer conformation to detect SOD 4.



1. INTRODUCTION

Previously, gold nanoparticles (AuNPs) were extensively applied as biomaterials and many studies have been carried out due to their optical properties.^{1,2} Some characteristics of nanomaterials have been recognized such as targeting a specific disease biomarker for a nanosensor process.³ One of the better biomarkers is the DNA aptamer notably conceived to possess good affinity to proteins⁴ or other biomolecules.^{5,6} Benefiting from their characteristics, aptamers can be employed in biomedical applications such as diagnostics and therapeutics.^{7–9}

At the moment, research studies in nanotechnology are directed toward natural materials for nanomedicine applications.¹⁰ Especially, AuNPs can promptly react with biomolecules to enhance their detection.¹¹ To this end, many parameters were tested and bioconjugation with several aptamer sequences was carried out.^{5,7} Actually, the interaction between aptamers and proteins is fundamental to the development of the nanomedicine field.¹² Many methods have been used to detect biomolecules.¹³ Among these methods, conjugation of biomolecules at the surface of AuNPs as bioreceptors having great affinity to targeted analytes is very effective.¹⁴ One of the best bioreceptors is the DNA aptamer, conceived to possess good affinity to proteins⁴ or other types of biomolecules. For this purpose, the study of the interaction between aptamers and proteins is very important to exploit the biomedical application of nanoparticles.^{12a,b} Among many important analytes, superoxide dismutase (SOD) is known as a significant cancer

biomarker, and the detection of its concentration in body fluids can lead to the diagnosis of this disease.^{15,16} The enzyme works as a therapeutic agent against reactive oxygen species-mediated diseases.¹⁷ On the basis of the active site metal, SOD isoforms are divided into three groups designated to specific cell compartments.¹⁸ Among them, we focused our interest on Cu/Zn-SODs (SOD 4) present in cytosol, peroxisomes, plastids, and extracellular space. It has been proposed that SOD can check cancer progression and can be applied as a novel target for cancer treatment.¹⁹ The aim of this paper is to realize the bioconjugation of SOD 4 aptamer (APT) onto AuNPs through different methodologies to assess their biological interactions with SOD 4 glycoprotein. Especially, two protocols for aptamer functionalization onto pegylated gold nanoparticles (PEG-AuNPs) were carried out: EDC/NHS chemistry²⁰ and complexation. The grafting between SOD 4 and its aptamer will be applied in the diagnostic field to realize specific diagnosis of cancers.¹⁹ In this study, we investigate the aptamer/SOD 4 interaction depending on the chemical strategy applied to

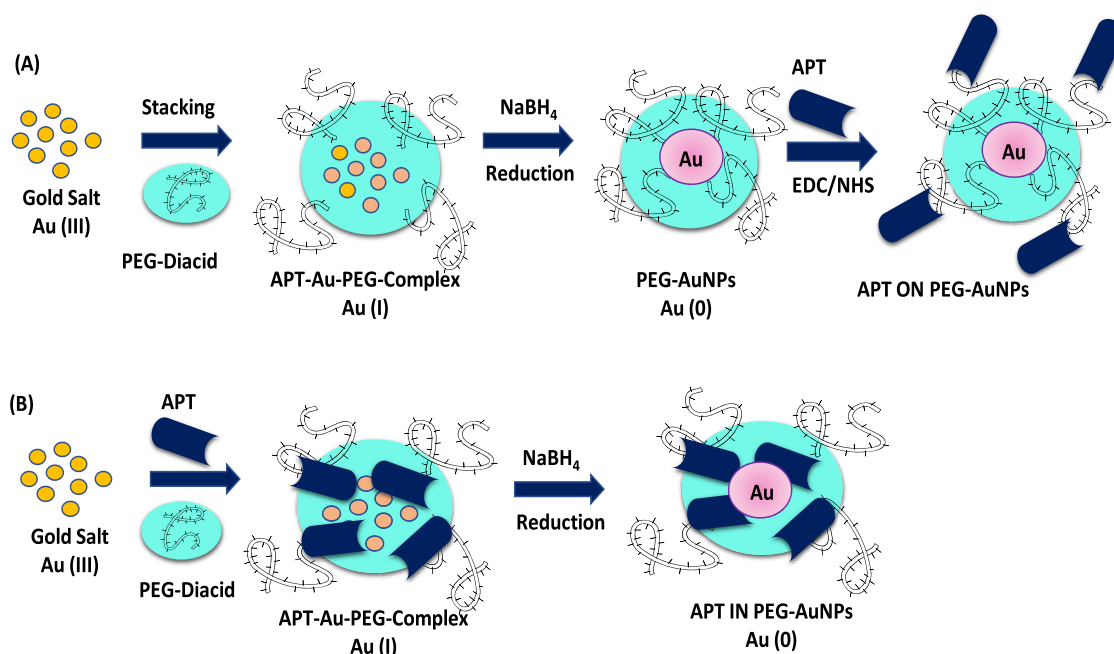
Received: March 19, 2020

Accepted: May 21, 2020

Published: June 4, 2020



Scheme 1. Depiction of the Synthesis of (A) APT ON PEG-AuNPs and (B) APT IN PEG-AuNPs via EDC/NHS (A) and Complexation Reaction (B) (Designs Are Not in Scale)



conjugate the aptamer on PEG-AuNPs. We investigated whether the type of conjugation will modify the interaction and, thus, whether some parameters can improve this interaction. This study will enable fast methodologies to realize aptamer-based nanomaterials with major applications as cancer biomarkers.

2. RESULTS AND DISCUSSION

2.1. Biocoating of SOD 4 Aptamer on Pegylated Gold Nanoparticles (APT ON PEG-AuNPs; APT IN PEG-AuNPs). Previously, many authors have grafted different types of aptamers to detect various biomarkers.²⁵ In the last few years, Spadavecchia et al. have functionalized polymeric gold nanoparticles with macromolecules by several methods of chemical surface functionalization.^{26,27} Some authors have successfully pioneered a simple and ingenious strategy, called “Method IN”, in which the biomolecule is chelated with a gold salt (HAuCl_4) by electrostatic bonding, through a complexation reaction.^{28–32} We started this study with the bioconjugation of the SOD 4 aptamer (APT) on the surface of diacid pegylated gold nanoparticles (PEG-AuNPs) through carbodiimide chemistry (Method ON) and chelation bond (Method IN) methods. In the first case, APT was coated on the surface of PEG-AuNPs via amide bonding between the carboxylic (COOH) groups on the surface of the PEG-AuNPs and the amino (NH_2) groups of the aptamer (APT ON PEG-AuNPs) through previous activation of EDC/NHS by carbodiimide chemistry²³ (Scheme 1 panel A). In the second case, APT takes part actively in the nucleation and growth of PEG-AuNPs via a chelation reaction (APT IN PEG-AuNPs), as previously described for other types of aptamers and biomolecules²⁴ (Scheme 1 panel B).

Each grafting functionalization (Method ON; Method IN) resulted in a specific steric arrangement and consequently a different chemical behavior of APT on the PEG-AuNPs confirmed by UV–vis spectroscopy, transmission electron microscopy (TEM), and Raman spectroscopy, as reported in the following sections.

2.2. Aptamer Bioconjugation: Physicochemical Characterization. The UV–visible spectra of PEG-AuNPs showed a surface plasmon band at 523 nm (Figure 1, black line). After APT conjugation onto PEG-AuNPs (APT ON PEG-AuNPs) via EDC/NHS, the plasmon band is red-shifted to 535 nm (Figure 1, red line). Furthermore, we believe that the red shift depends on the grafting method and the APT model. Regarding the complexation method, the red shift (to 525 nm) is weak with significant broadening (Figure 1, green line). The red shift is attributed to a change in the dielectric environment of the nanoparticles, therefore corroborating the effective conjugation of the AuNPs in both cases. Especially, the red shift observed for APT ON PEG-AuNPs is due to agglomeration through van der Waals interactions³³ and the various steric conformations of APT through the carbodiimide chemistry (Method ON). The fingerprint of the plasmon band indicates that both chemical methodologies (ON and IN) give rise to a characteristic chemical behavior of APT on PEG-AuNPs. This behavior will have remarkable consequences on the steric conformation of the aptamer on the nanoparticle surface under several immobilization kinetics.^{34,35} In fact, the functionalization will impact the position of APT. Through carbodiimide (EDC/NHS) grafting, APT is situated on the gold surface capped with a PEG layer, while for the chelation method, APT will be placed into the core of pegylated nanoparticles. This phenomenon is related to the various degrees of adsorption of APT on the [110] gold facets, due to the steric conformation of chemical groups during the nucleation and growth process of PEG-AuNPs. As a consequence, the resulting colloidal solution remains stable for 3 months at room temperature. This finding is extremely remarkable and confirms that the capping layer on AuNPs plays a crucial role in the achievement of functionalization and comparative kinetics.^{26a} This was estimated by TEM. Figure 1B, panel 1, displays a spherical pegylated gold nanoparticle (i.e., control) with a diameter of 13 ± 2 nm.^{26b,36} After EDC/NHS conjugation, APT ON PEG-AuNPs are formed as spherical agglomerates of nanoparticles of 17 ± 3 nm decorated with a

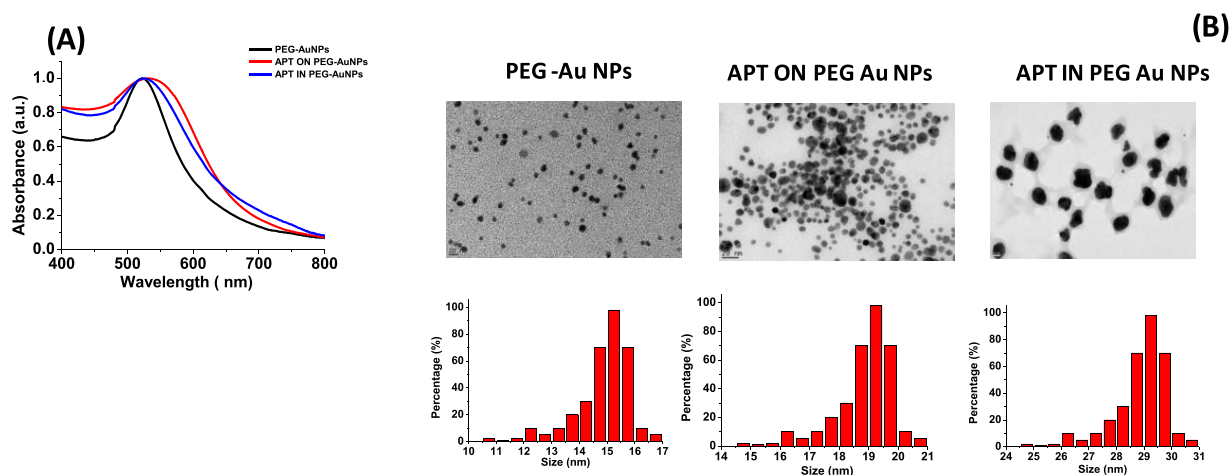


Figure 1. (A) Normalized UV-vis absorption of APT ON PEG-AuNPs (red line), APT IN PEG-AuNPs (blue line), and PEG-AuNPs (black line); (B) TEM images and size distribution of PEG-AuNPs (on the left) before and after functionalization of APT by carbodiimide chemistry (APT ON PEG-AuNPs in the middle) and chelation reaction (APT IN PEG-AuNPs on the right). Scale bars: 20 nm.

Table 1. ζ -Potential and Hydrodynamic Diameter of PEG-AuNPs, APT ON PEG-AuNPs, and APT IN PEG-AuNPs by EDC/NHS and Chelation Chemistry

synthetic product	ζ -potential (mV)	hydrodynamic diameter (nm)	PdI
PEG-AuNPs	-25 ± 1	13 ± 2	0.330
APT ON PEG-AuNPs	-29 ± 1	17 ± 3	0.325
APT IN PEG-AuNPs	-32 ± 1	27 ± 2	0.321

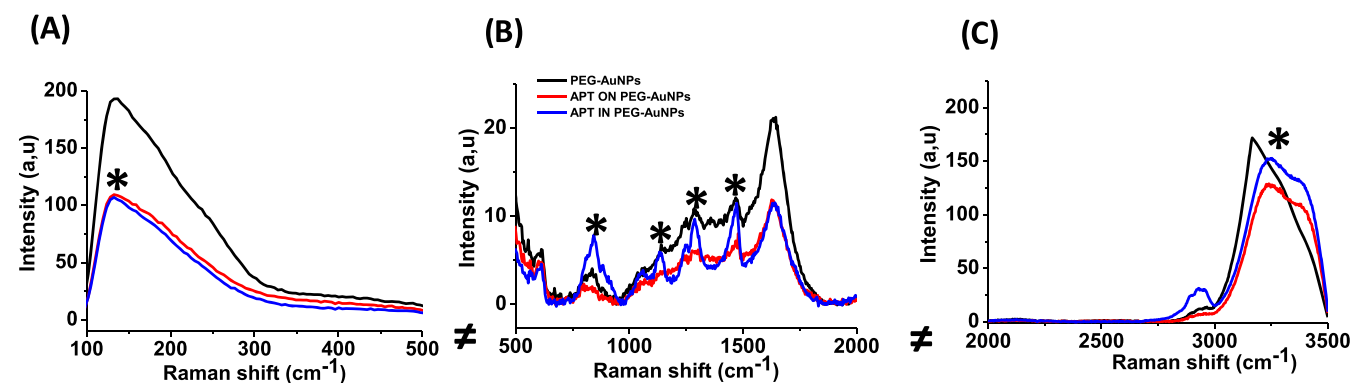


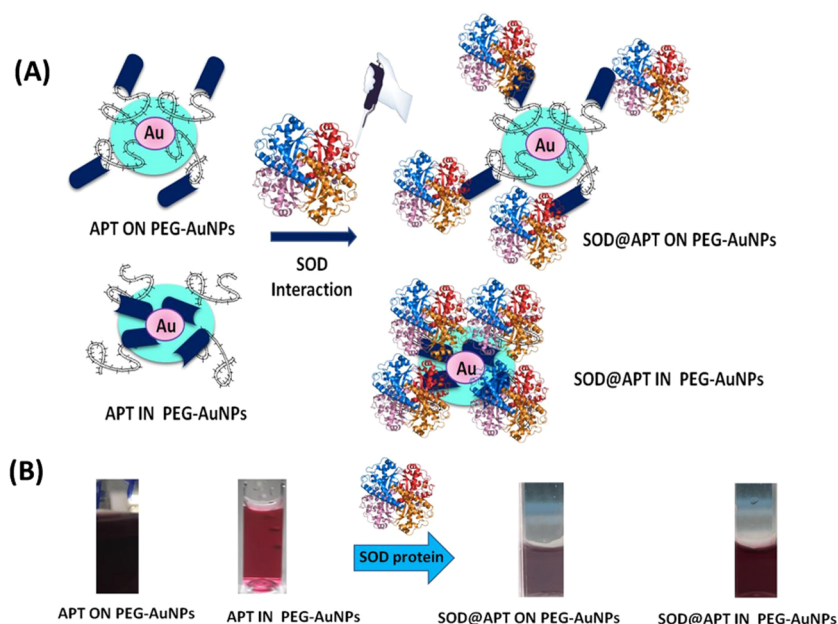
Figure 2. (A–C) Raman analysis in three spectral ranges (from 190 to 3500 cm^{-1}) of APT ON PEG-AuNPs (red line) and APT IN PEG-AuNPs (blue line) compared to free PEG-AuNPs (black line). Experimental conditions: $\lambda_{\text{exc}} = 785 \text{ nm}$; laser power 20 mW; accumulation time 180 s.

thick layer of PEG diacid (Figure 1B, panel 2). Estimating that the size of a single aptamer is about 5 nm,³⁷ we believe that APT occupies the privileged location of a PEG molecule on the particles. In contrast, APT IN PEG-AuNPs show a popcorn shape, stuck in a shell of PEG, with a diameter of $27 \pm 2 \text{ nm}$ (Figure 1B, panel 3). Previously, Spadavecchia et al. have realized similar nanostructures using dicarboxylic PEG^{21,28,38} and macromolecules, while characteristic popcorn shaped nanoparticles were achieved by incorporating a Cu macrocycle in the growth solution of AuNPs.³⁹ In agreement with prior findings,^{21,26a,40} when APT reacts with the gold salt (AuCl_4^-) complex, PEG fits in its chemical conformation. APT conjugation is evaluated by dynamic light scattering (DLS) and ζ -potential techniques (Table 1). We observe an enhancement of the hydrodynamic diameter of about 10 nm and consequently an increase of 5 nm of the radius. This value confirms the aptamer length.²⁴ Moreover, ζ -potential measurements confirm the stability of PEG-AuNPs and APT PEG-

AuNPs at physiological pH (ζ -potential = $-28 \pm 1 \text{ mV}$, -30 mV for APT ON PEG-AuNPs and $-33 \pm 1 \text{ mV}$ for APT IN PEG-AuNPs) (Table 1). This stability was improved by the presence of the PEG coating.^{26b}

The successful functionalization of SOD 4 aptamer onto and/or into PEG-AuNPs was confirmed by Raman spectroscopy (Figure 2). Raman spectra of PEG-AuNPs (as control) show diverse bands assigned to the PEG molecules. The signature of PEG-COOH on the AuNP surface was shown at 1137, 1270, and 1455 cm^{-1} due to the vibration of the C-O-H, C-O-C, and C-O chemical groups, respectively (Figure 2, black line). After the aptamer functionalization, several peaks can be noted for both functionalization methods used (Figure 2, red line, APT ON PEG-AuNPs; Figure 2, blue line, APT IN PEG-AuNPs). On the basis of these findings, we believe that the conformation of the aptamer is correlated with the grafting method. In detail, the peak at 1528 cm^{-1} corresponding to adenine and out-of-plane NH is more pronounced for APT IN

Scheme 2. Schematic Picture of the Interaction Mechanism of SOD 4 with APT ON PEG-AuNPs and APT IN PEG-AuNPs (A); Colorimetric Variation of Colloidal Building Blocks after SOD 4 Interaction (B)



PEG-AuNPs compared to APT ON PEG-AuNPs, confirming a different steric disposition of APT on PEG-AuNPs. SERS signals were observed in the range $100\text{--}300\text{ cm}^{-1}$ corresponding to Au–O–C, Au–Cl, and O–O vibrations and $2500\text{--}3500\text{ cm}^{-1}$ due to the aliphatic C–H and N–H stretching (Figure 2, panel B). Anyway, for each functionalization method, we believe that the aptamer assumes various conformations influenced by experimental parameters. Many bands are specific to APT such as the peak at 1058 cm^{-1} due to the phosphate group (PO_4^{3-})⁴¹ or the intense peak at 1371 cm^{-1} related to thymine base. This enhancement of intensity is due to the better alignment of the aptamer with the Au surface of nanoparticles,⁴² in contrast to the weak signal achieved for thymine DNA in water.⁴³

We also observed a shift of the Raman bands from 3200 cm^{-1} of PEG-AuNPs (Figure 2C black line) to 3400 cm^{-1} due to APT ON PEG-AuNPs (Figure 2C, red line) and APT IN PEG-AuNPs (Figure 2C, blue line). The appearance of the peaks at 2900 , 900 , and 1500 cm^{-1} confirms a different chemical configuration of APT on PEG-AuNPs.

2.3. Aptamer Loading onto Pegylated Gold Nanoparticles (APT ON PEG-AuNPs, APT IN PEG-AuNPs). The use of the aptamer in the biosensing field was evaluated for a novel application in therapeutics due to its targeting skills.⁴⁴

Recently, we have functionalized and characterized a renal aptamer on pegylated gold nanoparticles (PEG-AuNPs) by two chemical conjugations.²⁴

Previously, Dam et al.⁴⁵ showed that loading of aptamer AS1411 onto AuNPs could be regulated through the charge of the ligands and pH. In our case, the loading of APT onto PEG-AuNPs was confirmed by the absorption peak at 260 nm from APT (Figure S2, Supporting Information). The standard absorption of APT is plotted in the inset of the figure showing the UV–vis absorbance spectra of APT at several concentrations. The loading efficiency was calculated to be 86% with $8.4\text{ }\mu\text{g}$ present in $2.4 \times 10^{-8}\text{ mol}$ of NPs.

2.4. Cu, Zn SOD Glycoprotein (SOD 4) Detection. Superoxide dismutases (SODs) are a group of metalloenzymes very important in the biomedical field. They showed good

affinity toward heparin and heparan sulfate through the positive charge of amino acids in the carboxy-terminal ends of the subunits.⁴⁶

In all SODs, the region from His-101 to Gly-199 is highly homologous to the active site of the cytosolic dimeric Cu and Zn-containing SODs. This chemical configuration suggests that the amino acid residues involved in dimer contact with Cu Zn-SODs.⁴⁶ Similarly, tetrameric MnSOD has been shown to have two interacting dimers,⁴⁷ in which the dimer interface is structurally similar to that of the dimeric MnSOD.

For these reasons, SODs are promising candidates in diagnostics for human illnesses.^{48–50}

In our previous work we studied the biomolecular interaction of MnSOD with APT (15T) bound onto PEG-AuNPs by electrostatic interactions and thiol covalent bonds.²³ Herein, we would like to understand how the chemical structure of building blocks influences the spectroscopic detection. For this purpose, APTs cross-linked onto PEG-AuNPs through both methodologies (Method ON; Method IN) were applied as building blocks to realize the detection of SOD 4 (Cu, Zn SOD). To understand the mechanism of interaction between SOD 4 and APT ON PEG-AuNPs and APT IN PEG-AuNPs, SOD 4 recombinant proteins were incubated at different concentrations in the presence of solutions of aptamer–AuNPs (APT ON PEG-AuNPs and APT IN PEG-AuNPs) (Scheme 2).

We demonstrate that SOD 4 interacts with APT in the two different conformations (ON/IN) with high binding affinity as shown by LSPR and Raman measurements.

The interaction of the protein with aptamer–AuNPs (APT ON PEG-AuNPs and APT IN PEG-AuNPs) was evaluated by UV–visible absorption (Figure 3A,B) and Raman spectroscopy (Figure 4A,B). Indeed, after incubation with aptamer–AuNPs, we observed a strong shift of the plasmon band from 535 nm (for APT ON PEG-AuNPs) and 525 nm (for APT IN PEG-AuNPs) to 818 nm due to a modification of the local environment with a consequence of colorimetric variation of the colloidal solution (Scheme 2B). When SOD 4 interacted with APT ON PEG-AuNPs and APT IN PEG-AuNPs at concentrations of $1\text{ }\mu\text{M}$ to

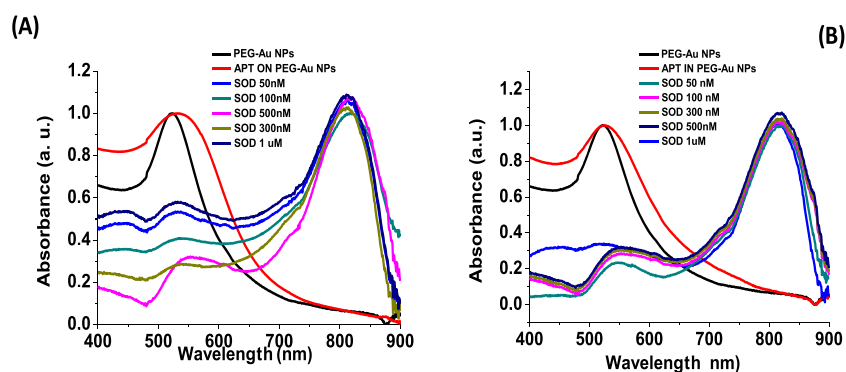


Figure 3. (A, B) Normalized UV-vis absorption spectra in the range 400–900 nm of APT ON PEG-AuNPs and APT IN PEG-AuNPs before (red line) and after SOD 4 interaction (from 1 μ M to 50 nM) under experimental conditions (NaCl 0.9%).

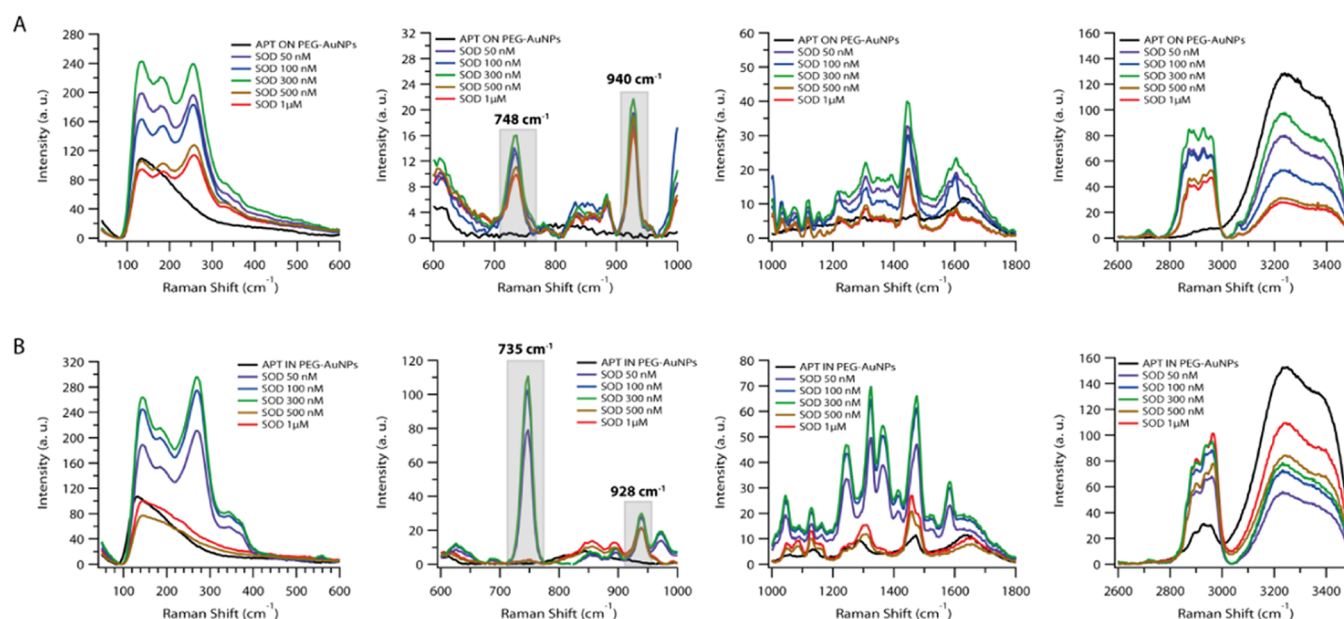


Figure 4. (A, B) Raman spectra of the APT ON PEG-AuNPs and APT IN PEG-AuNPs before (black line) and after SOD 4 interaction (SOD 4 concentration range: 1 μ M to 500 nM). Experimental conditions: $\lambda_{\text{exc}} = 785$ nm; laser power 20 mW; accumulation time 180 s.

50 nM, we noted a strong variation of the plasmon position and width, depending on the amount of SOD 4. Therefore, we assumed that the biochemical interaction of SOD 4 with aptamer–AuNPs (APT ON PEG-AuNPs; APT IN PEG-AuNPs) induced a strong agglomeration and variation of plasmonic and local environments due to the chemical and steric arrangement of SOD 4 on the aptamer building blocks. The strong red shift and the weak decrease of the plasmon band can be interpreted by the lower nanoparticle aggregation and the dissociation of agglomerates after the addition of SOD 4 at a concentration of 1 nM (Scheme 2). This means that at concentrations from 1 μ M to 300 nM, SOD 4 interacts with the aptamer–AuNPs (APT ON PEG-AuNPs; APT IN PEG-AuNPs), inducing very strong changes in the plasmon band (modification of the dielectric constant around the AuNPs with the addition of SOD 4 proteins and more dissociations) (Figure 3A,B). At concentrations from 100 to 50 nM, the number of SOD 4 molecules is enough to prevent interactions between the proteins on aptamer–AuNPs, thus inducing lower dissociation of the nanoparticle agglomerates (Figure 3A,B). The consequent decrease of the plasmon band is due to the formation of a shell of proteins adsorbed on the aptamer–AuNPs that

exchange with the aptamer in both configurations (ON; IN) at high concentrations of SOD 4 in the environment. With the saturation of the surface of the AuNPs, we believe that a better orientation of SOD 4 is due to chemical hindrance.

We assume that the individual aptamer monomer on the probes may act as an intermediate seed for further aggregation of the SOD 4 protein, promoting the formation of probe agglomerates. This behavior generates definite shifts in the surface plasmon resonance associated with the color change of the colloidal solution, detected by UV-vis absorption spectroscopy. In the case of unmodified PEG-AuNPs, we observed no interparticle aggregation induced by further association between APT PEG-AuNPs and SOD 4 agglomerates (Figure S3 in the Supporting Information). In contrast to a previous study,⁵¹ we directly obtain (after 15 min of interaction) a strong red shift of the plasmon peak after SOD 4 interaction with APT PEG-AuNPs with consequent stable agglomerate formation (Figure S4 in the Supporting Information).

The effective interaction of SOD 4 with the APT PEG-AuNP surface was established by Raman spectroscopy at different SOD 4 concentrations (Figure 4A,B).

The fingerprint of PEG-COOH on the AuNP surface was proved through the observation of the Raman bands at 1137, 1270, and 1455 cm^{-1} due to the vibration of C-O-H, C-O-C and C-O chemical groups, as previously described.^{21,22} After SOD 4 interaction, the fingerprint of amide II (1587–1620 cm^{-1}) and amide III (1200–1300 cm^{-1}) confirmed the protein interaction,⁵² while the peak at 934 cm^{-1} corresponded to C-C vibrations of the tripeptide in the SOD 4 molecule (Figure 4A,B).

The peak at 1450 cm^{-1} confirmed the vibration of the amine group and the interaction of protein. In the spectral region corresponding to 2000–3000 cm^{-1} , we observed an enhancement of the bands at 2880, 2936, and 2965 cm^{-1} characteristic of stretching ν C-H and O-H. Some peaks were enhanced when the concentration decreased from 1 μM to 50 nM.

From 100 to 50 nM, the peaks at 941 and 1450 cm^{-1} were enhanced. This behavior was most probably due to the single bond stretching vibrations of the amino acids proline and valine and polysaccharides,⁵³ which confirmed the interaction of the SOD 4 protein.

In the case in which SOD 4 interacts with APT IN PEG-AuNPs (SOD@APT IN PEG-AuNPs), we observe a different chemical behavior of SOD, depending on the steric conformation of APT. In fact, from 50 to 300 nM, we observe a strong modification of Raman spectral bands with the characteristic peak at 269 cm^{-1} due to the Cu-O bond, which means that SOD at 100 nM assumes a different conformation than at 1 μM . In the case of SOD@APT ON PEG-AuNPs, new bands and SERS signals appear: a double peak at 561–625 cm^{-1} characteristic of tryptophan, cytosine, and guanine.⁵³ A strong peak at 748 cm^{-1} is due to symmetric breathing of tryptophan that confirms the different conformation of SOD 4 as a function of concentration and steric arrangement of the aptamer on gold nanoparticles. Indeed according to the literature, two basic amino acids in the heparin-binding cluster, Lys-217 and Arg-219, are replaced by tryptophan. A double peak at 940–972 cm^{-1} is due to the protein arrangement (skeletal), and a double peak at 1323–1364 cm^{-1} is due to the presence of proline.

The peak at 1474 cm^{-1} is due to the amine bond and CH_2 vibrations. In particular, at high concentrations of SOD 4, the peaks at 972, 1242, and 1364 cm^{-1} disappear for APT IN PEG-AuNPs with a decrease in the intensity of other peaks. In the area 2000–3000 cm^{-1} , there is an enhancement of the bands at 2880, 2936, and 2965 cm^{-1} characteristic of the stretching ν C-H and O-H.

By evaluating the Raman results it was possible to confirm that the chemical and steric conformations of SOD 4 depend on its concentration, which consequently influences the interaction with APT PEG-AuNPs in ON and IN conformations.

In fact, the interaction of SOD 4 with APT PEG-AuNPs will take place due to their great packing density as well as the force of repulsion between the negatively charged SOD 4.^{54,55} However, the self-assembly of SOD 4 on APT PEG-AuNPs at concentrations from 1 μM to 50 nM is confirmed by amide bands; in the presence of lower SOD 4 concentrations (from 1 μM to 50 nM) amide bands progressively appear. This phenomenon is due to the swelling of polymers in water that hinders with protein steric conformation at low concentrations.

The two specific Raman peaks of SOD 4 detected at 748 and 940 cm^{-1} for APT ON PEG-AuNPs and at 735 and 928 cm^{-1} for APT IN PEG-AuNPs were integrated giving an intensity evolution of these two peaks to make the calibration curve

presented in Figure 5. In this figure, the Raman intensity increased before reaching a plateau around 100 nM of SOD 4

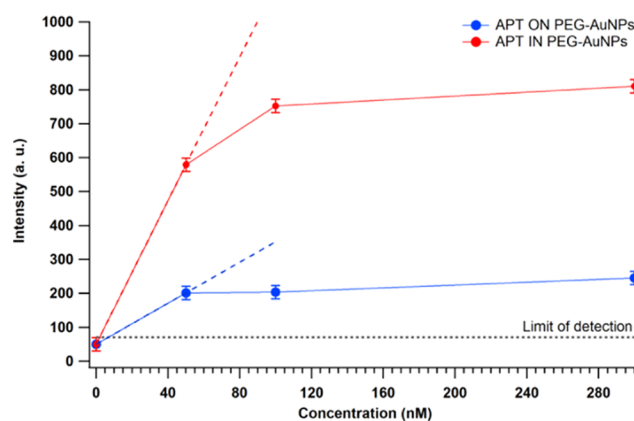


Figure 5. Integrated Raman intensity of SOD 4 specific bands detected at 748 and 940 cm^{-1} for APT ON PEG-AuNPs (blue) and at 735 and 928 cm^{-1} for APT IN PEG-AuNPs (red). The gray dashed line is the calculated limit of detection (LOD = 70 counts). The blue and red dashed lines represent the linear fits of APT ON PEG-AuNPs and APT IN PEG-AuNPs, respectively, of the intensity versus SOD 4 concentration.

concentration. At 50 nM, the intensity was three times stronger with APT IN PEG-AuNPs than with APT ON PEG-AuNPs. Herein, we followed the recommendations of the International Union of Pure and Applied Chemistry (IUPAC) to calculate the limit of detection (LOD) using the different concentrations of SOD 4. In this case, the definition of the limit of detection was given by the formula $\text{LOD} = \langle x_{\text{bl}} \rangle + k\sigma_{\text{bl}}$, where $\langle x_{\text{bl}} \rangle$ is the mean of the blank measure (APT PEG-AuNPs without SOD 4), σ_{bl} is the standard deviation of the blank measure, and k is a numerical factor chosen with a confidence level of 95%.⁵⁶

Based on the results shown in Figure 4, the mean value of the blank, $\langle x_{\text{bl}} \rangle$, (nanoparticles without SOD 4) was around 50 counts and its standard deviation, σ_{bl} , was about 20 counts. Using these values, the LOD level has been estimated as $\langle x_{\text{bl}} \rangle + \sigma_{\text{bl}} = 70$ counts. The LOD is displayed in the Figure 5 by the gray dashed line. To determine the concentration corresponding to this LOD, the calibration curve was fitted using a linear function (blue and red dashed lines for APT ON PEG-AuNPs and APT IN PEG-AuNPs, respectively). The lowest concentration that can be noticeable, which corresponds to the intersection between the data-fitted curves and the LOD line, is about 2 nM for APT IN PEG-AuNPs and 8 nM for APT ON PEG-AuNPs. APT IN PEG-AuNPs showed four times stronger sensitivity than APT ON PEG-AuNPs. Compared to other works, the detection of SOD 4 in solution was demonstrated at millimolar⁵⁷ or micromolar⁵¹ concentrations. Here, we demonstrated a very sensitive detection of SOD in the nanomolar concentration range with new ways of biosensor synthesis (APT IN and ON), providing a very strong tool to understand the effect of aptamer conformation to detect SOD.

3. CONCLUSIONS

We designed a fast detection system for SOD 4 agglomerates. PEG-AuNPs, having peculiar plasmonic properties, can be applied as a colorimetric sensor, after conjugation with SOD 4 aptamer to interact with SOD 4 agglomerates, using optical and Raman spectroscopy. The aptamer monomer grafted on the

PEG-AuNP surface with two different chemistry protocols (Method ON, Method IN) that played an important role in SOD 4 detection. In this work, we evaluated the interaction between the aptamer and SOD 4 glycoprotein under two different conformations. The analysis of the localized plasmon resonance of the nanoparticles functionalized with aptamers enabled us to study the formation of aggregates due to complexation and interaction between the aptamers. We observed some dissociation for specific SOD 4 concentrations giving evidence of the interaction of SOD 4 with the aptamer. We put in evidence that the grafting method has a strong influence on the affinity between SOD 4 and the aptamer. This scientific research permits us to perceive the interaction mechanism to optimize the detection sensitivity and the affinity of the bioreceptor to the analyte.

4. EXPERIMENTAL SECTION

4.1. Materials and Methods. Tetrachloroauric acid (HAuCl_4), sodium borohydride (NaBH_4), dicarboxylic poly(ethylene glycol) (PEG)-600(PEG), *N*-hydroxysuccinimide (NHS), 1-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimidehydrochloride (EDC), and phosphate-buffered saline (PBS, 50 mM Tris–acetate, 100 mM NaCl, 5 mM MgCl_2) were all provided by Sigma-Aldrich of maximum purity grade. Superoxide dismutase 4 (Cu, Zn SOD) was purchased from Eurogentec, France.

4.2. SOD Aptamer (APT). The SOD aptamer was purchased from Eurogentec. The aptamer was dispersed in PBS buffer (pH = 9).

The sequence of the aptamer (APT) is TCT-TCT-CTA-GCT-GAA-TAA-CCG-GAA-GTA-ACT-CAT-CGT-TTC-GAT-GAG-TTA-CTTCCG-GTT-ATT-CAG-CTA-GAG-AAG.

4.3. Synthesis of PEG-AuNPs. The synthesis of COOH-terminated PEG-coated AuNPs (PEG-AuNPs) was achieved according to a previously described procedure²¹ (Scheme 1).

4.4. Determination of PEG-AuNP Concentration. Colloid concentration was determined by standard experimental methods as described previously.²²

4.5. Preparation of SOD Protein Solutions. SOD 4 powder was solubilized in buffer (50 mM Tris–acetate, 100 mM NaCl, 5 mM MgCl_2 ; pH: 8.2) and then diluted to obtain solutions with concentrations in the range 1 μM to 50 nM. Molar concentrations of SOD 4 were determined taking into account that molecular weight of SOD 4 is equal to 15.9 kDa.

4.6. Bioconjugation of PEG-AuNPs with SOD Aptamer (APT ON PEG-AuNPs; APT IN PEG-AuNPs). The PEG-AuNP surface was modified with the SOD 4 aptamer by two different methods of functionalization (Method ON by carbodiimide chemistry and Method IN by chelation bonding), according to the grafting procedures depicted in Scheme 1.

The first binding strategy consists of the conjugation of the SOD 4 aptamer (APT) on the surface of PEG-AuNPs (APT ON PEG-AuNPs) by carbodiimide chemistry as previously described.^{23,24} The second strategy consists of the complexation between the SOD 4 aptamer (50 μL of APT 10 μM ; PBS pH 9) (APT), gold salt ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$) (5 mL, 2.6×10^{-4} M), and 50 μL of PEG diacid (PEG) (1 mM) through a chelation reaction, to form APT IN PEG-AuNPs as described above.²⁴

4.7. Aptamer Loading Efficiency. The amount of the SOD 4 aptamer (APT) grafted onto APT ON PEG-AuNPs and APT IN PEG-AuNPs was characterized by UV–vis absorption spectroscopy. Absorption at 260 nm was used to extrapolate

APT concentrations based on a calibration curve (Figure S2, Supporting Information). The aptamer loading efficiency was calculated by the chemical equation described previously.²⁴

4.8. SOD 4 Interaction with APT ON PEG-AuNPs and APT IN PEG-AuNPs. The interaction of SOD 4 with the surface of APT ON PEG-AuNPs and APT IN PEG-AuNPs was accomplished through the following protocols of incubation: 450 μL of APT ON PEG-AuNPs and APT IN PEG-AuNPs (1 mM) were added into separate tubes containing 50 μL of SOD 4 (from 1 μM to 50 nM; PBS pH 9; NaCl 0.15 M). After 18 h of incubation, the resulting colloid suspension was centrifuged twice at 5000 rpm for 10 min to eliminate the excess of biomolecules and then the pellets were redispersed in 1 mL of Milli-Q water.

4.9. Physicochemical Characterization. All measurements were performed in triplicate to confirm the reproducibility of the experimental analysis described previously.^{21,22}

4.10. UV–Vis Measurements. Absorption spectra were recorded using a PerkinElmer Lambda UV/Vis 950 spectrophotometer in plastic cuvettes with an optical path of 10 mm. The wavelength range was 200–900 nm.

4.11. Transmission Electron Microscopy (TEM). TEM images were acquired with a JEOL JEM 1011 microscope (JEOL) at an accelerating voltage of 100 kV. The particle suspension (2 μL) was placed on a carbon-coated copper grid (Smethurst High-Light Ltd.) and dried at room temperature.

4.12. Raman Spectroscopy. The Raman experiments were performed on an Xplora spectrometer (Horiba Scientific-France). The Raman spectra were recorded using an excitation wavelength of 785 nm (diode laser) at room temperature. For measurements in solution, a macro-objective with a focal length of 40 mm (NA = 0.18) was used in the backscattering configuration. The achieved spectral resolution was close to 2 cm^{-1} .

4.13. Dynamic Light Scattering (DLS) and ζ -Potential Measurements. The size and ζ -potential measurements were performed using a Zetasizer Nano ZS (Malvern Instruments, Malvern, U.K.) equipped with a He–Ne laser (633 nm, fixed scattering angle of 173°) at room temperature.

4.14. Stability of APT AuNPs (APT ON PEG-AuNPs; APT IN PEG-AuNPs) as a Function of pH. For stability studies, APT AuNPs were dispersed in PBS (0.1 M; pH 7 and 5.5) and absorption spectra were collected over 3 months (Figure S1 in the Supporting Information).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsomega.0c01192>.

Stability test (Figure S1); loading efficiency (Figure S2); normalized UV–vis absorption spectra of PEG-AuNPs before (black line) and after interaction with SOD 4 at 1 μM (Figure S3); TEM images of SOD@APT IN PEG-AuNPs under experimental conditions (Figure S4) (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

Xiaowu Li – Department of Hepatobiliary Surgery, Shenzhen University General Hospital & Guangdong Provincial Key Laboratory of Regional Immunity and Diseases & Carson International Cancer Shenzhen University General Hospital &

Shenzhen University Clinical Medical Academy Center,
Shenzhen University, Shenzhen 518000, China; Email: Xiaowu
Li: lixw1966@163.com

Jolanda Spadavecchia – CNRS, UMR 7244, NBD-CSPBAT,
Laboratory of Chemistry, Structures and Properties of
Biomaterials and Therapeutic Agents University Paris13,
Bobigny 93000, France; orcid.org/0000-0001-6697-1174;
Email: jolanda.spadavecchia@univ-paris13.fr

Authors

Rawdha Dekhili – CNRS, UMR 7244, NBD-CSPBAT,
Laboratory of Chemistry, Structures and Properties of
Biomaterials and Therapeutic Agents University Paris13,
Bobigny 93000, France

Khaoula Cherni – CNRS, UMR 7244, NBD-CSPBAT,
Laboratory of Chemistry, Structures and Properties of
Biomaterials and Therapeutic Agents University Paris13,
Bobigny 93000, France

Hui Liu – Department of Hepatobiliary Surgery, Shenzhen
University General Hospital & Guangdong Provincial Key
Laboratory of Regional Immunity and Diseases & Carson
International Cancer Shenzhen University General Hospital &
Shenzhen University Clinical Medical Academy Center,
Shenzhen University, Shenzhen 518000, China

Nadia Djaker – CNRS, UMR 7244, NBD-CSPBAT, Laboratory
of Chemistry, Structures and Properties of Biomaterials and
Therapeutic Agents University Paris13, Bobigny 93000, France;
orcid.org/0000-0001-7912-5436

Complete contact information is available at:

<https://pubs.acs.org/10.1021/acsomega.0c01192>

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We acknowledge the PRISME platform facility (University Paris 13, France) and the National Natural Science Foundation of China under Grant 81430063.

REFERENCES

- (1) Giljohann, D. A.; Seferos, D. S.; Daniel, W. L.; Massich, M. D.; Patel, P. C.; Mirkin, C. A. Gold nanoparticles for biology and medicine. *Angew. Chem., Int. Ed.* **2010**, *49*, 3280–3294.
- (2) Homberger, M.; Simon, U. On the application potential of gold nanoparticles in nanoelectronics and biomedicine. *Philos. Trans. R. Soc., A* **2010**, *368*, 1405–1453.
- (3) Raj, S.; Jose, S.; Sumod, U. S.; Sabitha, M. Nanotechnology in cosmetics: Opportunities and challenges. *J. Pharm. BioAllied Sci.* **2012**, *4*, 186–193.
- (4) Song, K.-M.; Lee, S.; Ban, C. Aptamers and Their Biological Applications. *Sensors* **2012**, *12*, 612–631.
- (5) Lee, S.; Pérez-Luna, V. H. Dextran–Gold Nanoparticle Hybrid Material for Biomolecule Immobilization and Detection. *Anal. Chem.* **2005**, *77*, 7204–7211.
- (6) Priyadarshini, E.; Pradhan, N. Gold nanoparticles as efficient sensors in colorimetric detection of toxic metal ions: A review. *Sens. Actuators, B* **2017**, *238*, 888–902.
- (7) Medley, C. D.; Bamrungsap, S.; Tan, W.; Smith, J. E. Aptamer-Conjugated Nanoparticles for Cancer Cell Detection. *Anal. Chem.* **2011**, *83*, 727–734.
- (8) Oh, S. Y.; Heo, N. S.; Shukla, S.; Cho, H.-J.; Vilian, A. T. E.; Kim, J.; Lee, S. Y.; Han, Y.-K.; Yoo, S. M.; Huh, Y. S. Development of gold nanoparticle-aptamer-based LSPR sensing chips for the rapid detection of Salmonella typhimurium in pork meat. *Sci. Rep.* **2017**, *7*, No. 10130.
- (9) Shevchenko, Y.; Francis, T. J.; Blair, D. A. D.; Walsh, R.; DeRosa, M. C.; Albert, J. In Situ Biosensing with a Surface Plasmon Resonance Fiber Grating Aptasensor. *Anal. Chem.* **2011**, *83*, 7027–7034.
- (10) Bhardwaj, V.; Kaushik, A. Biomedical Applications of Nanotechnology and Nanomaterials. *Micromachines* **2017**, *8*, No. 298.
- (11) Peng, L.; Li, B. L.; Zhou, C. W.; Li, N. B.; Setyawati, M. I.; Zou, H. L. “Naked-eye” recognition: Emerging gold nano-family for visual sensing. *Appl. Mater. Today* **2018**, *11*, 166–188.
- (12) (a) Gwinn, M. R.; Vallyathan, V. Nanoparticles: Health Effects—Pros and Cons. *Environ. Health Perspect.* **2006**, *114*, 1818–1825. (b) Giri, K.; Shameer, K.; Zimmermann, M. T.; Saha, S.; Chakraborty, P. K.; Sharma, A.; Arvizo, R. R.; Madden, B. J.; McCormick, D. J.; Kocher, J.-P. A.; Bhattacharya, R.; Mukherjee, P. Understanding Protein–Nanoparticle Interaction: A New Gateway to Disease Therapeutics. *Bioconjugate Chem.* **2014**, *25*, 1078–1090.
- (13) (a) Huang, C.-C.; Chiu, S.-H.; Huang, Y.-F.; Chang, H.-T. Aptamer-Functionalized Gold Nanoparticles for Turn-On Light Switch Detection of Platelet-Derived Growth Factor. *Anal. Chem.* **2007**, *79*, 4798–4804. (b) Li, F.; Li, J.; Tang, Y.; Wang, C.; Li, X.-F.; Le, X. C. Targeted Enlargement of Aptamer Functionalized Gold Nanoparticles for Quantitative Protein Analysis. *Proteomes* **2017**, *5*, No. 1.
- (14) Zuber, A.; Purdey, M.; Schartner, E.; Forbes, C.; van der Hoek, B.; Giles, D.; Abell, A.; Monro, T.; Ebendorff-Heidepriem, H. Detection of gold nanoparticles with different sizes using absorption and fluorescence based method. *Sens. Actuators, B* **2016**, *227*, 117–127.
- (15) Cottat, M.; D’Andrea, C.; Yasukuni, R.; Malashikhina, N.; Grinyte, R.; Lidgi-Guigui, N.; Fazio, B.; Sutton, A.; Oudar, O.; Charnaux, N.; Pavlov, V.; Toma, A.; Di Fabrizio, E.; Gucciardi, P. G.; Lamy de la Chapelle, M. High Sensitivity, High Selectivity SERS Detection of MnSOD Using Optical Nanoantennas Functionalized with Aptamers. *The. J. Phys. Chem. C* **2015**, *119*, 15532–15540.
- (16) Borrelli, A.; Schiattarella, A.; Bonelli, P.; Tuccillo, F. M.; Buonaguro, F. M.; Mancini, A. The Functional Role of MnSOD as a Biomarker of Human Diseases and Therapeutic Potential of a New Isoform of a Human Recombinant MnSOD. *BioMed Res. Int.* **2014**, *2014*, No. 476789.
- (17) Görlach, A.; Dimova, E. Y.; Petry, A.; Martínez-Ruiz, A.; Hernansanz-Agustín, P.; Rolo, A. P.; Palmeira, C. M.; Kietzmann, T. Reactive oxygen species, nutrition, hypoxia and diseases: Problems solved? *Redox Biol.* **2015**, *6*, 372–385.
- (18) Sheng, Y.; Abreu, I. A.; Cabelli, D. E.; Maroney, M. J.; Miller, A.-F.; Teixeira, M.; Valentine, J. S. Superoxide dismutases and superoxide reductases. *Chem. Rev.* **2014**, *114*, 3854–3918.
- (19) Robbins, D.; Zhao, Y. Manganese superoxide dismutase in cancer prevention. *Antioxid. Redox Signaling* **2014**, *20*, 1628–1645.
- (20) Jazayeri, M. H.; Amani, H.; Pourfathollah, A. A.; Pazoki-Toroudi, H.; Sedighimoghaddam, B. Various methods of gold nanoparticles (GNPs) conjugation to antibodies. *Sens. Biosens. Res.* **2016**, *9*, 17–22.
- (21) Moustauoui, H.; Movia, D.; Dupont, N.; Bouchemal, N.; Casale, S.; Djaker, N.; Savarin, P.; Prina-Mello, A.; de la Chapelle, M. L.; Spadavecchia, J. Tunable Design of Gold(III)–Doxorubicin Complex–PEGylated Nanocarrier. The Golden Doxorubicin for Oncological Applications. *ACS Appl. Mater. Interfaces* **2016**, *8*, 19946–19957.
- (22) Liu, Q.; Sacco, P.; Marsich, E.; Furlani, F.; Arib, C.; Djaker, N.; Lamy de la Chapelle, M.; Donati, I.; Spadavecchia, J. Lactose-Modified Chitosan Gold(III)-PEGylated Complex-Bioconjugates: From Synthesis to Interaction with Targeted Galectin-1 Protein. *Bioconjugate Chem.* **2018**, *29*, 3352–3361.
- (23) Arib, C.; Liu, Q.; Djaker, N.; Fu, W.; de la Chapelle, M. L.; Spadavecchia, J. Influence of the Aptamer Grafting on its Conformation and its Interaction with Targeted Protein. *Plasmonics* **2018**, *14*, 1029–1038.
- (24) Arib, C.; M, S.; Gerbino, A.; Spadavecchia, J. Aptamer Grafting onto (on) and into (in) Pegylated Gold Nanoparticles: Physicochemical Characterization and In vitro Cytotoxicity Investigation in Renal Cells. *J. Nanomed. Nanotechnol.* **2018**, *9*, 520–530.
- (25) Javier, D. J.; Nitin, N.; Levy, M.; Ellington, A.; Richards-Kortum, R. Aptamer-Targeted Gold Nanoparticles As Molecular-Specific

Contrast Agents for Reflectance Imaging. *Bioconjugate Chem.* **2008**, *19*, 1309–1312.

- (26) (a) Spadavecchia, J.; Apchain, E.; Alberic, M.; Fontan, E.; Reiche, I. One-step synthesis of collagen hybrid gold nanoparticles and formation on Egyptian-like gold-plated archaeological ivory. *Angew. Chem., Int. Ed.* **2014**, *53*, 8363–8366. (b) Spadavecchia, J.; Movia, D.; Moore, C.; Maguire, C. M.; Moustauoui, H.; Casale, S.; Volkov, Y.; Prina-Mello, A. Targeted polyethylene glycol gold nanoparticles for the treatment of pancreatic cancer: from synthesis to proof-of-concept in vitro studies. *Int. J. Nanomed.* **2016**, *11*, 791–822. (c) Politi, J.; De Stefano, L.; Giardina, P.; Casale, S.; Rea, I.; Spadavecchia, J. In *Hybrid Hydrophobin/Gold Nanoparticles: Synthesis and Characterization of New Synthetic Probes for Biological Applications*; Springer International Publishing: Cham, 2018; pp 169–176.
- (27) Liu, Q.; Sacco, P.; Marsich, E.; Furlani, F.; Arib, C.; Djaker, N.; Lamy de la Chapelle, M.; Donati, I.; Spadavecchia, J. Lactose-modified chitosan gold (III)-PEGylated complex-bioconjugates: from synthesis to interaction with targeted Galectin-1 protein. *Bioconjugate Chem.* **2018**, *29*, 3352–3361.
- (28) Moustauoui, H.; Movia, D.; Dupont, N.; Bouchemal, N.; Casale, S.; Djaker, N.; Savarin, P.; Prina-Mello, A.; de la Chapelle, M. L.; Spadavecchia, J. Tunable Design of Gold(III)-Doxorubicin Complex-PEGylated Nanocarrier. The Golden Doxorubicin for Oncological Applications. *ACS Appl. Mater. Interfaces* **2016**, *8*, 19946–19957.
- (29) Morel, A.-L.; Giraud, S.; Bialecki, A.; Moustauoui, H.; de La Chapelle, M. L.; Spadavecchia, J. Green extraction of endemic plants to synthesize gold nanoparticles for theranostic applications. *Front. Lab. Med.* **2017**, *1*, 158–171.
- (30) Monteil, M.; Moustauoui, H.; Picardi, G.; Aouidat, F.; Djaker, N.; de La Chapelle, M. L.; Lecouvey, M.; Spadavecchia, J. Polyphosphonate ligands: From synthesis to design of hybrid PEGylated nanoparticles toward phototherapy studies. *J. Colloid Interface Sci.* **2018**, *513*, 205–213.
- (31) Marguerit, G.; Moustauoui, H.; Haddada, M. B.; Djaker, N.; de la Chapelle, M. L.; Spadavecchia, J. Taxanes Hybrid Nanovectors: From Design to Physico-Chemical Evaluation of Docetaxel and Paclitaxel Gold (III)-PEGylated Complex Nanocarriers. *Part. Part. Syst. Charact.* **2018**, *35*, No. 1700299.
- (32) Melani, V.; Haddada, M. B.; Moustauoui, H.; Landoulsi, J.; Djaker, N.; de la Chapelle, M. L.; Spadavecchia, J. Pegylated doxorubicin gold complex: From nanovector to potential intercalant agent for biosensor applications. *Front. Lab. Med.* **2017**, *1*, 114–121.
- (33) Kammer, M. N.; Olmsted, I. R.; Kussrow, A. K.; Morris, M. J.; Jackson, G. W.; Bornhop, D. J. Characterizing aptamer small molecule interactions with backscattering interferometry. *Analyst* **2014**, *139*, 5879–5884.
- (34) Hsu, C.-L.; Lien, C.-W.; Wang, C.-W.; Harroun, S. G.; Huang, C.-C.; Chang, H.-T. Immobilization of aptamer-modified gold nanoparticles on BiOCl nanosheets: Tunable peroxidase-like activity by protein recognition. *Biosens. Bioelectron.* **2016**, *75*, 181–187.
- (35) Wang, W.; Ding, X.; He, M.; Wang, J.; Lou, X. Kinetic Adsorption Profile and Conformation Evolution at the DNA-Gold Nanoparticle Interface Probed by Dynamic Light Scattering. *Anal. Chem.* **2014**, *86*, 10186–10192.
- (36) Spadavecchia, J.; Perumal, R.; Barras, A.; Lyskawa, J.; Woisel, P.; Laure, W.; Pradier, C.-M.; Boukherroub, R.; Szunerits, S. Amplified plasmonic detection of DNA hybridization using doxorubicin-capped gold particles. *Analyst* **2014**, *139*, 157–164.
- (37) de Castro, M. A. G.; Höbartner, C.; Opazo, F. Aptamers provide superior stainings of cellular receptors studied under super-resolution microscopy. *PLoS One* **2017**, *12*, No. e0173050.
- (38) Monteil, M.; M, H.; Picardi, G.; Aouidat, F.; Djaker, N.; de La Chapelle, M. L.; Lecouvey, M.; Spadavecchia, J. Polyphosphonate ligands: from synthesis to design of Hybrid PEGylated Nanoparticles toward phototherapy studies. *J. Colloid Interface Sci.* **2018**, *513*, 205–213.
- (39) Spadavecchia, J.; Casale, S.; Landoulsi, J.; Pradier, C.-M. Tuning the shape and size of hybrid gold nanoparticles by porphyrins using seed-mediated synthesis. *Chem. Phys. Lett.* **2014**, *609*, 134–141.
- (40) Politi, J.; De Stefano, L.; Longobardi, S.; Giardina, P.; Rea, I.; Methivier, C.; Pradier, C. M.; Casale, S.; Spadavecchia, J. The amphiphilic hydrophobin Vmh2 plays a key role in one step synthesis of hybrid protein-gold nanoparticles. *Colloids Surf., B* **2015**, *136*, 214–221.
- (41) Pagba, C. V.; Lane, S. M.; Cho, H.; Wachsmann-Hogiu, S. *Direct Detection of Aptamer-Thrombin Binding via Surface-Enhanced Raman Spectroscopy*; SPIE, 2010; p 8.
- (42) He, H.; Dai, J.; Duan, Z.; Zheng, B.; Meng, Y.; Guo, Y.; Dan, X. Unusual sequence length-dependent gold nanoparticles aggregation of the ssDNA sticky end and its application for enzyme-free and signal amplified colorimetric DNA detection. *Sci. Rep.* **2016**, *6*, No. 30878.
- (43) Schreier, W. J.; Schrader, T. E.; Koller, F. O.; Gilch, P.; Crespo-Hernández, C. E.; Swaminathan, V. N.; Carell, T.; Zinth, W.; Kohler, B. Thymine Dimerization in DNA is an Ultrafast Photoreaction. *Science* **2007**, *315*, 625–629.
- (44) Catuogno, S.; Esposito, C. L.; de Franciscis, V. Aptamer-Mediated Targeted Delivery of Therapeutics: An Update. *Pharmaceuticals* **2016**, *9*, No. 69.
- (45) Dam, D. H. M.; Lee, H.; Lee, R. C.; Kim, K. H.; Kelleher, N. L.; Odom, T. W. Tunable Loading of Oligonucleotides with Secondary Structure on Gold Nanoparticles through a pH-Driven Method. *Bioconjugate Chem.* **2015**, *26*, 279–285.
- (46) Carlsson, L. M.; Marklund, S. L.; Edlund, T. The rat extracellular superoxide dismutase dimer is converted to a tetramer by the exchange of a single amino acid. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 5219–5222.
- (47) Sheng, Y.; Durazo, A.; Schumacher, M.; Gralla, E. B.; Cascio, D.; Cabelli, D. E.; Valentine, J. S. Tetramerization reinforces the dimer interface of MnSOD. *PLoS One* **2013**, *8*, No. e62446.
- (48) Chuang, C.-H.; Du, Y.-C.; Wu, T.-F.; Chen, C.-H.; Lee, D.-H.; Chen, S.-M.; Huang, T.-C.; Wu, H.-P.; Shaikh, M. O. Immunosensor for the ultrasensitive and quantitative detection of bladder cancer in point of care testing. *Biosens. Bioelectron.* **2016**, *84*, 126–132.
- (49) Ebrahim, A. H.; Alalawi, Z.; Mirandola, L.; Rakhshanda, R.; Dahlbeck, S.; Nguyen, D.; Jenkins, M.; Grizzi, F.; Cobos, E.; Figueroa, J. A.; Chiriva-Internati, M. Galectins in cancer: carcinogenesis, diagnosis and therapy. *Ann. Transl. Med.* **2014**, *2*, No. 88.
- (50) Besford, Q. A.; Wojnilowicz, M.; Suma, T.; Bertleff-Zieschang, N.; Caruso, F.; Cavalieri, F. Lactosylated Glycogen Nanoparticles for Targeting Prostate Cancer Cells. *ACS Appl. Mater. Interfaces* **2017**, *9*, 16869–16879.
- (51) Hong, S.; Choi, I.; Lee, S.; Yang, Y. I.; Kang, T.; Yi, J. Sensitive and Colorimetric Detection of the Structural Evolution of Superoxide Dismutase with Gold Nanoparticles. *Anal. Chem.* **2009**, *81*, 1378–1382.
- (52) Wang, D.; Zhao, X.; Vargak, M.; Spiro, T. G. Metal-Bound Histidine Modes in UV Resonance Raman Spectra of Cu, Zn Superoxide Dismutase. *J. Am. Chem. Soc.* **2000**, *122*, 2193–2199.
- (53) Movasaghi, Z.; Rehman, S.; Rehman, I. U. Raman Spectroscopy of Biological Tissues. *Appl. Spectrosc. Rev.* **2007**, *42*, 493–541.
- (54) Pieters, R. J. Inhibition and Detection of Galectins. *ChemBioChem* **2006**, *7*, 721–728.
- (55) Belitsky, J. M.; Nelson, A.; Hernandez, J. D.; Baum, L. G.; Stoddart, J. F. Multivalent interactions between lectins and supra-molecular complexes: Galectin-1 and self-assembled pseudopolyrotaxanes. *Chem. Biol.* **2007**, *14*, 1140–1151.
- (56) Mocak, J.; Bond, A. M.; Mitchell, S.; Scollary, G. A statistical overview of standard (IUPAC and ACS) and new procedures for determining the limits of detection and quantification: Application to voltammetric and stripping techniques (technical report). *Pure Appl. Chem.* **1997**, *69*, 297–328.
- (57) Zhao, X. J.; Wang, D. J.; Spiro, T. G. Detection of metal-bound histidine in ultraviolet resonance Raman spectra: Superoxide dismutase. *Inorg. Chem.* **1998**, *37*, 5414–5415.