Chapter

Red or Blue? Gold Nanoparticles in Colorimetric Sensing

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Abstract

Gold nanoparticles (AuNPs) have been extensively used for the design of colorimetric sensors and probes due to their interesting photophysical properties. In particular, their surface plasmon resonance (SPR) is influenced not only by the size but also by the shape or the properties of the matrix surrounding the nanoparticles. This SPR band is sensitive to the proximity of other nanoparticles, and thus, analyte-triggered aggregation of AuNPs results in an important bathochromic shift of the SPR band and a change in the color of the solution from red to blue due to interparticle surface plasmon coupling. The selectivity of the AuNPs-based sensors toward the different analytes will depend on the recognition properties of the molecules attached to the surface of the nanoparticles. In this chapter, a selection of biologically active molecules has been considered as analytes: neurotransmitters, nerve agents, pesticides, and carboxylates of biological interest.

Keywords: gold nanoparticles, aggregation, neurotransmitter, nerve agents, pesticides, carboxylates

1. Introduction

A molecular sensor (or chemosensor) is a molecule or molecular ensemble designed to indicate the presence of a specific analyte through a detectable change in a macroscopic measurable signal. Molecular sensors are generally composed of two main elements: a recognition unit, which selectively interacts with the molecule or ion to be detected, and a signaling unit that is responsible of generating a macroscopically measurable signal (optical, electrochemical, mechanical...) upon the molecular recognition event. In general, in a chemosensor, the receptor binds the target analyte in a reversible manner. If the recognition event takes place through an irreversible chemical reaction, the chemosensor is better described as “probe.” The transduction mechanism, which is the mechanism by which the chemical interaction between the analyte and the recognition unit is converted into a change in the macroscopic signal of the signaling unit, will depend on the type of signaling unit and the structure of the receptor.

The use of chemosensors or probes for the colorimetric detection of small biologically active molecules offers some advantages over traditional instrumental analytical methods. Chromogenic probes are usually cheap, easy to use, and do not require of expensive instrumentation, and very often, the presence of the analyte can be detected by the naked eye, which allows for rapid in-situ detection.
Among the different approaches for colorimetric sensing, the use of gold nanoparticles (AuNPs) as scaffolds and signaling units for the construction of molecular sensors has attracted enormous interest for several reasons: They can be easily synthesized from Au(III) salts in various sizes and shapes. Their surface can be functionalized with a wide range of thiol- or disulfide-terminated organic ligands, by ligand exchange reactions, through the formation of strong Au–S bonds, leading to stable colloidal suspensions in water or organic solvents (depending on the ligand). Finally, gold nanoparticles have remarkable optoelectronic properties. In particular, the localized surface plasmon resonance (SPR) gives rise to a strong absorption band in the visible region. This SPR band is influenced not only by the size and shape of the nanoparticles but also by the dielectric properties of the environment and the proximity of other nanoparticles \[1, 2\]. This last property is the basis for colorimetric assays using AuNPs. Thus, analyte-triggered aggregation of AuNPs results in an important bathochromic shift of the SPR band (from ca. 520 to ca. 650 nm) and a change in the color of the colloidal solution from red (dispersed) to blue (aggregated) due to interparticle surface plasmon coupling \(\text{(Figure 1)}\). Moreover, AuNPs have very high molar extinction coefficients \(\varepsilon\) (ca. \(10^8–10^9\) \(\text{M}^{-1}\text{cm}^{-1}\) for AuNPs with diameters between 10 and 50 nm), which confers this sensing method a great sensitivity. In fact, the color change associated to the aggregation process can be observed by naked eye even at nanomolar concentration \[3, 4\].

The main challenge in the design of colorimetric sensors with AuNPs is the election of the recognition units to be attached onto the surface of the nanoparticles and the nature of the molecular interaction leading to the aggregation process.

A large number of target analytes (metal ions, anions, small organic molecules, or large biomolecules) have been detected using functionalized AuNPs as colorimetric probes. However, in this chapter, the discussion has been limited to the use of functionalized spherical gold nanoparticles for the detection of small molecules with biological activity, such as neurotransmitters, nerve agents, pesticides, and biologically relevant carboxylates.

### 2. Detection of Neurotransmitters

Among the neurotransmitters, biogenic amines (BA) are of particular interest due to their impact in areas ranging from biomarkers of specific diseases \[5–7\] to quality control of foodstuffs \[8, 9\]. Nitric oxide (NO) is also very important neurotransmitter in the central, peripheral, and enteric nervous systems (ENS) \[10, 11\].

Dopamine, the simplest biogenic catecholamine (CA), is an important neurotransmitter of the central and peripheral nervous systems \[12\]. An approach for the colorimetric detection of dopamine has been developed using 4-amino-3-hydrazino-5-mercaptop-1,2,4-triazol functionalized AuNPs \(\text{(Figure 2)}\).
Dopamine induced the aggregation of the AuNPs through hydrogen bonding interactions \([13]\). Each dopamine molecule has three H-donor groups, which are able to form hydrogen bonds (the amino and both hydroxyl groups). On the other hand, the 4-amino-3-hydrazino-5-mercaptop-1,2,4-triazol presents two hydrogen bond acceptors that can interact with the target molecule, inducing aggregation with the concomitant color change. Epinephrine, norepinephrine, and isoprenaline were tested as possible interferants. The three compounds showed lower responses than dopamine.

Following the same approach, several functionalized AuNPs have been reported \([14–16]\) for dopamine detection in biological media. This neurotransmitter has also been detected using unmodified citrate-capped gold nanoparticles \([17]\). A net of hydrogen bonds among dopamine molecules and dopamine with citrate is responsible for the aggregation of the nanoparticles (Figure 3). Selectivity toward dopamine is achieved by modifying nanoparticle size.

Serotonin (5-hydroxytryptamine, 5-HT) is a neurotransmitter that plays a key role in the regulation of various biological functions. 5-HT has been used as a biomarker to detect the presence of carcinoid tumors. A selective and sensitive probe based on AuNPs for detecting serotonin has been reported \([18]\). In this case, AuNPs were bi-functionalized with dithiobis(succinimidyl propionate) (DSP) and with N-acetyl-L-cysteine (NALC). DSP reacts with the amino group of 5-HT, and NALC is able to interact with the hydroxyl group of serotonin through electrostatic interactions and hydrogen bonds formation, and additionally, it also acts as a stabilizer of the colloidal solutions of AuNPs due to its negative charge at neutral pH (Figure 4). In the presence of the analyte, aggregation takes place and the solution color changes from red to blue, being this change observable by naked eye.

Nitric oxide (NO) is one of the gaseous neurotransmitters. NO is generated in the nitric oxide synthase catalyzed oxidation of L-arginine to L-citrulline and is involved in a variety of important biological processes. For example, it stimulates

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**Figure 2.**
Dopamine detection using triazol functionalized AuNPs.

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**Figure 3.**
Dopamine detection using unmodified citrate-capped AuNPs.
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the production of cyclic guanosine monophosphate (cGMP) which acts as a second messenger. In addition, nitric oxide is released by some neurons that innervate the gastrointestinal tract, penis, respiratory passages, and cerebral blood vessels. Nitric oxide is also released as a neurotransmitter in the brain and has been implicated in the processes of learning and memory. Detection of this gas has been carried out using functionalized AuNPs that aggregates through NO-induced “click” reaction [19]. To detect the analyte, both azide- and terminal alkyne-functionalized gold nanoparticles were synthesized.
The sensing mechanism is shown in Figure 5. The initial aqueous solution containing a mixture of azide- and terminal-alkyne functionalized AuNPs remains dispersed in the presence of Cu(II), with its characteristic red-wine color. When NO is added to the solution, Cu(II) is reduced to Cu(I), and then, the “click” reaction between azide and alkyne-terminated nanoparticles takes place, giving rise to the nanoparticles aggregation with the subsequent change in the color of the solution.

3. Detection of nerve agents and pesticides

The current rise in international concern over the use of chemical warfare (CW) agents in different conflictive sceneries has resulted in an increasing interest in the detection of these lethal chemicals. Among CW species, nerve agents are greatly dangerous and their high toxicity and easy production underscore the need to detect these deadly chemicals via quick and reliable procedures. AuNPs have been used as sensors for some nerve agent simulants with good results. Different sensing mechanisms have been used for detecting these compounds, for example, compensation of charges has been applied for this process [20]. The designed sensing protocol takes advantage of the nucleophilic reactivity of pyridine moieties toward nerve gases [21]. This reactivity generates positive charges on the surface of the gold nanoparticles, diminishing their colloidal stability and inducing aggregation (Figure 6).

Several pyridine derivatives were studied, and their ability to act as probes for DCNP detection was evaluated by UV-vis spectroscopy. After addition of increasing amounts of DCNP, the intensity of the surface plasmon peak of the monodispersed AuNPs at 526 nm decreased and a new peak at c.a. 660 nm appeared as the AuNP clusters were formed. The best results were obtained with compound 1 (Figure 6c) that showed a limit of detection of 76 ppm under the experimental conditions used.

Following the same approach, compound 2 (Figure 7) was bound to gold nanoparticles. In this case, the positive charge appears as a consequence of the reaction described in Figure 7. The limit of detection determined in this case was 81 ppm.

Also, triarylcarbinols have been used as recognition motifs. These compounds can be converted into their corresponding carbocations in the presence of nerve agent simulants through phosphorylation of the OH group followed by S_N1 elimination. Consequently, AuNPs functionalized with this type of compounds have been used in detecting simulants of these dangerous compounds (Figure 8, X = F, CN) [22].

An approach based on enzymatic reactions has also been described for detecting nerve agents GB, GD, and VX and the highly toxic pesticide paraoxon (Figure 9). The prepared sensor showed high sensitivity [23].
In this method, thiocholine (TCh), which is generated from S-acetylthiocholine (ATCh) through acetylcholinesterase (AChE) enzymatic hydrolysis, induces aggregation of AuNPs stabilized with lipoic acid. When the analytes are present in the solution, the production of TCh is suppressed, and a disaggregation of the particles is observed with a change of color from blue to red (Figure 10).
Neither AChE nor ATCh can induce aggregation of the AuNPs stabilized with lipoic acid, whereas TCh is capable of producing this process. The analytes inactivate AChE via nonreversible phosphorylation even with very low concentrations. In consequence, ATCh cannot be hydrolyzed which inhibits the aggregation. Limits of detection are in the pM range and the system can be used in complex matrices such as in apple juice.

Other dangerous pesticides have been detected using AuNP-based colorimetric aptasensors [24]. The sensing mechanism is summarized in Figure 11. Aptamers (organophosphorous pesticide aptamers in this case) are able to be adsorbed on the surface of gold nanoparticles through coordination between Au and N atoms in DNA bases. When salt is added, this adsorption gives rise to stable dispersions of gold nanoparticles with the characteristic red color. However, in the presence of the analytes, the aptamers desorb from the surface of the AuNPs, giving rise to the aggregation process with the concomitant change of color. Because double-stranded DNA is not adsorbed by gold, single-stranded DNA was used in these experiments. Although the sensitivity of these assays is not high enough, this design shows a new approach for preparing sensors based on gold nanoparticles.

4. Detection of biologically important carboxylic acids

Detection of dicarboxylates is very important, since some of them have critical roles in the most important metabolic cycles of living organisms. Specifically, succinate plays a fundamental role in processes, such as the Krebs cycle and oxidative phosphorylation. It is an inhibitor of mitochondrial lipid peroxidation, preventing or delaying most of the damage caused by the peroxidation on different mitochondrial structures and functions. For all these reasons, it is of interest to detect this compound. Among the different strategies used in designing sensors
for these compounds, functionalized gold nanoparticles have shown interesting applications [25].

The sensing protocol, in this case, is related to the well-known interaction between the carboxylate and thioureas groups. For this reason, AuNPs containing thiourea groups were prepared, and their behavior in front of different dicarboxylates was evaluated. Among the different dicarboxylates studied (oxalate (C2), malonate (C3), succinate (C4), glutarate (C5), and adipate (C6); all of them as TBA salts) only with succinate, a color change of the solution from red to blue was observed (Figure 12, right, bottom). The limit of detection determined for this dianion was 0.5 μM.

Following a similar approach, maleate and fumarate were distinguished [26, 27]. Compound 3 was used to cap gold nanoparticles (Figure 13). The prepared AuNPs were able to recognize trans-dicarboxylates such as fumarate, one of the key components generated in the Krebs cycle, over its cis-isomer, maleate. The trans-isomer, fumarate, seems to have the appropriate geometry to induce the nanoparticle aggregation, whereas the cis compound presents a similar behavior to that of flexible dicarboxylates like oxalate, malonate, succinate, glutarate, propionate, and 4-pentenoic acid.

Pyruric acid (2-oxopropanoic acid, PA) is the simplest alpha-keto acid. It plays important roles in several biochemical pathways. For example, it supplies energy to cells through the citric acid cycle when oxygen is present and under hypoxic conditions produces lactate. It also appears as an intermediate in several metabolic processes such as the glycolysis of glucose or the synthesis of carbohydrates or fatty acids. Gold nanoparticles have proved to be useful for detecting this acid [28]. The approach, in this case, is also based on the use of unmodified AuNPs and Cytidine-rich oligonucleotides (C-rich DNA). C-rich DNA can fold into one closely packed four-stranded structure called i-motif [29] through protonated cytosine-cytosine (C-C+) base-pair formation under slightly acidic conditions. The principle of the designed PA sensor is similar to that previously indicated in Figure 11. The rigid i-motif structure
is unable to stabilize gold nanoparticles, but when a change of pH is induced in the medium from acid to neutral, the C-rich DNA changes its structure and prevents gold nanoparticles aggregation. The change in pH is induced by the addition of PA and pyruvate decarboxylase (PDC). The enzyme transforms PA into acetaldehyde and CO with the corresponding change of pH from acid to neutral. At neutral pH, the C-rich DNA presents its extended single-stranded structure and effectively binds to AuNPs, stabilizing them against NaCl-induced aggregation. Based on this principle, PA can be selectively detected by the color change of the AuNPs (Figure 14).

UV-Vis spectra were recorded to demonstrate the proposed sensing mechanism. Solutions of AuNPs (3 nM) containing C-rich DNA showed a maximum absorption peak centered at 520 nm. After addition of PA, the changes induced in the absorption band depend on the acid concentration. At 5.6 mM, a hypochromic effect was observed, but at 16.8 mM, a bathochromic shift can be observed from 520 to over 600 nm with the corresponding change of color. After addition of PCD, the disaggregation is produced and the solution recovers its red color. Selectivity against lactic acid, ascorbic acid, and glucose was established, and the limit of detection determined was 3.0 mM.

Ascorbic acid (AA, also known as vitamin C), an antioxidant compound, is present not only in biological fluids but also in foodstuffs and pharmaceuticals. Taking into account the red-ox properties of ascorbic acid, an approach using gold nanoparticles and Cr(VI) has been described for its detection [30]. Gold nanoparticles were stabilized with sodium tripolyphosphate (Na$_5$P$_3$O$_10$), and the sensor was prepared using this AuNPs and Cr (VI) salts. As Cr(VI) exists in the form of HCrO$_4^-$, CrO$_4^{2-}$, or Cr$_2$O$_7^{2-}$, there is an electrical repulsion with tripolyphosphate that precludes the nanoparticles aggregation. By contrast, Cr(III), a hard lewis acid strongly coordinates to the polyphosphate ligand, giving rise to the charges compensation and in consequence the nanoparticles aggregation (Figure 15). Ascorbic acid is able to trigger the process by reducing Cr(VI) to Cr(III). Selectivity studies were carried out with PO$_4^{3-}$, Zn$^{2+}$, Cu$^{2+}$, SO$_4^{2-}$, Ni$^{2+}$, Li$^+$, Na$^+$, Mg$^{2+}$, Br$^-$, NO$_3^-$, glucose (Glu), citric acid (CA), and oxalic acid (OA) as interferences, and no appreciable changes were observed with any of the studied compounds. The limit of detection determined for this method was 0.15 μM.

Finally, also amino acids have been detected using functionalized gold nanoparticles. Thus, tyrosine (Tyr) was detected using N-acetyl-L-cysteine modified gold nanoparticles [31]. In this case, the chiral ligand N-acetyl-L-cysteine (NALC) was chosen to include chirality in the sensors and study its use in selective recognition and separation of enantiomers.

The chiral selectivity is attributed to a chemical interaction between chiral NALC–Au NPs and L-Tyr at the molecular level as is shown in Figure 16.

Figure 14. (Left) Mechanism for pyruvic acid detection. (Right) UV-Vis spectra of the AuNP suspensions (3 nM) containing C-rich DNA after incubation with (b) 5.6 mM PA, (c) 16.8 mM PA, (d) 5.6 mM PA + PDC, (e) 16.8 mM PA + PDC, and (f) PDC only; curve (a) is the background signal (reproduced with license number Reprinted with permission from Li et al. [28]. Copyright 2014 Royal Society of Chemistry).
Tyr interacts with the ligand NALC through hydrogen bonds that involve carboxylic, amino, and hydroxyl groups. The selectivity response may be attributed to the conformation of L-Tyr that seems to be more appropriate for forming the complex with NALC.

5. Conclusions

The use of gold nanoparticles for the preparation of colorimetric sensors is a very active field. The changes in the color of colloidal gold nanoparticles in solution because of the change in the surface plasmon absorption band upon aggregation or disaggregation processes can be easily used to transform the molecular recognition event into a macroscopic measurable signal. This change from red to blue can be observed by the naked eye, allowing in this way cheap and easy detection of the target analytes. In this chapter, different mechanisms for the direct or indirect analyte-triggered aggregation of the AuNPs have been considered, including chemical reactions, supramolecular interactions, or changes in the pH of the medium. The selectivity observed in the sensing response in some cases depends on the conformation or configuration of the analyte, but also it can be achieved by using enzymes that catalyze specific reactions or aptamers able to interact with an analyte. Reactions induced by the analyte have also been
explored, for example based on redox transformations. Taking into account the
interesting photophysical properties of the gold nanoparticles, their easy func-
tionalization, the use of aqueous solutions, and the detection by naked eye, we
can conclude that the red or blue question will continue to be very present in the
molecular sensing field.

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