CHEMICAL REVIEWS

REVIEW

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Recent Trends in Macro-, Micro-, and Nanomaterial-Based Tools and Strategies for Heavy-Metal Detection

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1. INTRODUCTION

In small quantities, certain heavy metals (e.g., iron, copper, manganese, and zinc) are nutritionally essential for a healthy life. However, heavy metals show a great trend to form complexes, especially with ligands of biological matter containing nitrogen, sulfur, and oxygen. As a result, changes in the molecular structure of proteins, breaking of hydrogen bonds, or inhibition of enzymes can occur. These interactions, among others, may explain the toxicological and carcinogenic effects of heavy metals such as those affecting the central nervous system (Hg^{2+}, Pb^{2+}, As^{3+}); the kidneys or liver (Cu^{2+}, Cd^{2+}, Hg^{2+}, Pb^{2+}); or skin, bones, or teeth (Ni^{2+}, Cu^{2+}, Cd^{2+}, Cr^{3+}).[1−3]

Heavy metals are not biodegradable and therefore they remain in ecological systems and in the food chain indefinitely, exposing top-level predators to very high levels of pollution. For this reason, safe limits or maximum contaminant levels have been defined for drinking water by different organizations from all over the world. Table 1 summarizes the standards and guidelines for heavy metals in drinking water recommended by the World Health Organization (WHO) and Environmental Protection Agency (EPA) based on toxicity data and scientific studies.[4]

There is an ongoing search to develop sensing strategies for trace heavy metal for the early detection of pollution in various chemical systems, including living systems and the whole environment. Hence, the purpose of the present review is to provide a general overview on the latest trends in the development of heavy-metal-detection devices and systems.

As for other sensing systems, there are two important parts to be considered: the receptor and the immobilization/transducing platform. While the first part, the receptor (i.e., heavy-metal ionophores, biological receptors, etc.), is the responsible for the device selectivity, the second one is the responsible for the device stability as well as the sensitivity, which will depend on the technique used (i.e., optical, electrochemical).

The chemical recognition of heavy-metal ions is the base for the mentioned techniques to enable a proper sensing through specific substances/species called receptors of the detection system.

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The specific interaction of heavy-metal ions with the selected receptor usually involves noncovalent bonding, such as hydrogen bonding, metal coordination, hydrophobic forces, van der Waals forces, $\pi-\pi$ interactions, and electrostatic and/or electromagnetic effects. The specific recognition defined as the different affinity of a specific ligand for different metal ions is of special interest in this review.

As is well-known, standard techniques for trace heavy-metal analysis (even in the ppt and ppq range) require sophisticated analytical techniques such as atomic absorption spectroscopy (AAS),[5,6] inductively coupled plasma-mass spectrometry (ICP-MS),[7,8] mass spectroscopy (MS),[9] X-ray fluorescence spectroscopy (R-FS),[10] and potentiometric methods[11,12] and specialized personnel to carry out the operational procedures. Efforts are ongoing to prepare rapid and inexpensive techniques for heavy-metal detection. For this reason, techniques such as colorimetry,[13−15] fluorimetry,[16] and voltammetry[17,18] among others have been adapted to allow the miniaturization and in-field applications.

The introduction in the past few years of nanostructured materials, such as noble metal nanoparticles (MNPs), quantum dots (QDs), and magnetic nanoparticles or nanotubes, which enhance selectivity, sensibility, and reproducibility, has been of great importance to improve detection limits and to allow an adequate miniaturization of the sensing devices. Additionally, nanomaterials can be fashioned with a wide range of small organic ligands and large biomacromolecules by using tools and techniques of surface modification, giving rise to highly selective heavy-metal ion sensing systems.[19] The synergy of the properties of these materials with those of the mentioned receptors gives rise to highly sensitive and cost-efficient heavy-metal-detection systems.[20,21] In this review, we devote our focus mostly into that borderline to show the benefits of a merger that involves modified macro and nanostructurated materials for heavy-metal detection. (Scheme 1)

Table 1. Standards and Guidelines for Heavy Metals in Drinking Water Recommended by the WHO and EPA

metal	WHO (mg/L)	EPA (mg/L)
Ni	0.07	0.04
Cu	2	1.3
Zn	3	5
Cd	0.003	0.005
Hg	0.001	0.002
Pb	0.010	0.015
As	0.010	0.010

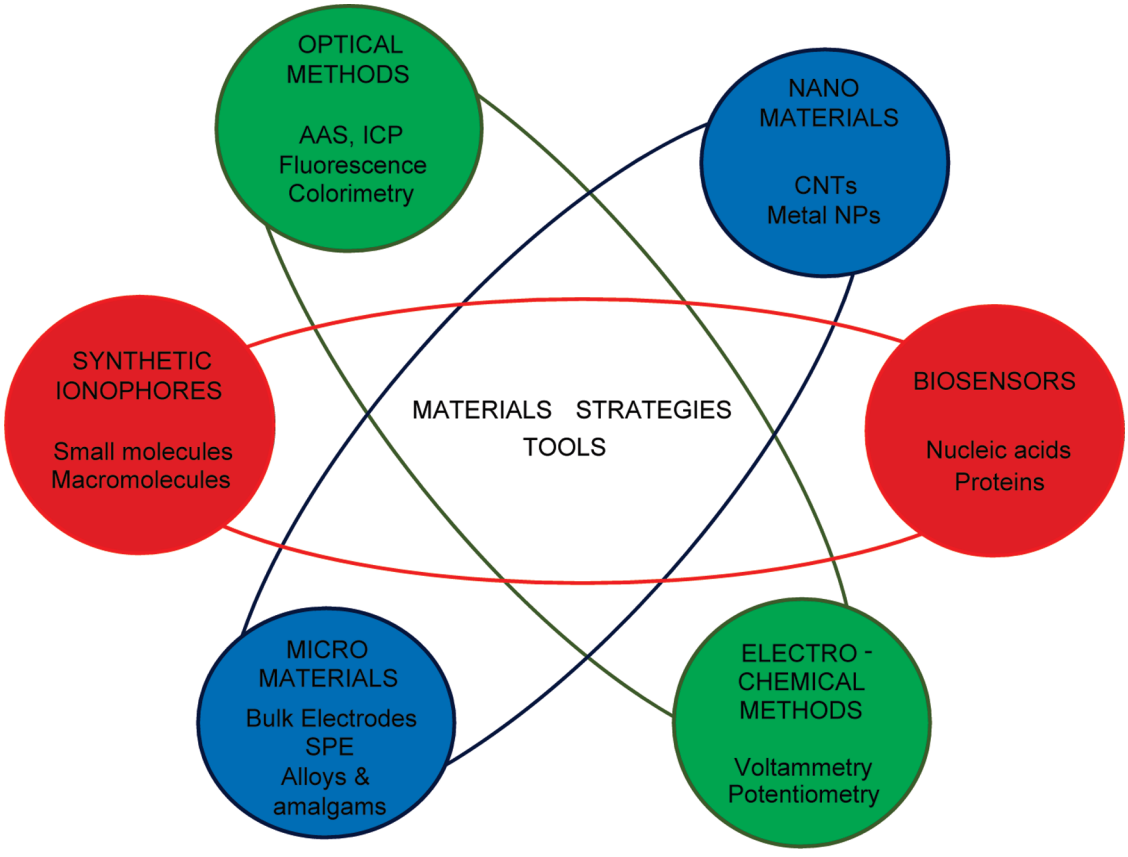
2. HEAVY METAL RECOGNITION MATERIALS

Different types of materials (inorganic, organic, biological, and combined materials) for specific/selective recognition of heavy metals have been reported in the literature. In the next section we will focus on the most used recognition materials in terms of developing real sensing systems overall with interest in the integration of robust, simple, and low cost devices.

2.1. Alloys and Amalgams

Several metal−metal alloy systems play a critical role in the dramatic improvement of the heavy-metal sensors. For several

Scheme 1. General Schematic of the Tools, Strategies and Materials Used for Heavy-Metal Detection and Reviewed in This Paper



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decades, mercury amalgam formation has been the most used for electrochemical detection of heavy metals. The use of mercury electrodes for metal detection has been reported since the middle of the 20th century.[22] Mercury-modified electrodes coupled with stripping techniques have been recognized as the most-sensitive devices for determination of heavy metals, especially for lead detection. The use of a hanging mercury drop electrode (HMDE, the most common mercury electrode) and mercury-film electrode has allowed a sub-ppb lead determination.[23] However, these techniques require tedious experimental precautions regarding the stability and recovery of the mercury drop after each experiment or careful manipulation of mercury solutions during film deposition.

The combination of screen-printed electrodes (SPEs) modified with mercury films reduces the amount of mercury used for each analysis compared to the ones using a mercury drop. SPEs related instruments can be made portable by including a compact battery, achieving a user-friendly field-deployable device that allows the direct monitoring of heavy metal traces for situ control of pollution.[24−27]

Nowadays, the development of alternative materials less toxic and more environmental friendly than mercury are the subject of interest. Nontoxic metal solid electrodes (M SE, where M is Ag, Au, Cu, Ir, etc.),[28] for example, can in many cases successfully substitute mercury electrodes and, in some special cases, offer possibilities not available to mercury electrodes, as described by Barek et al. in a recent review.[29] Bismuth and antimony film electrodes have similar electroanalytical performance as those of mercury, with the advantage of being more environmental friendly. While amalgam formation is responsible for the stripping performance of mercury electrodes, the attractive and unique behavior of bismuth and antimony film electrodes is attributed to the formation of multicomponents alloys. Bismuth electrodes offer a well-defined, undistorted, and highly reproducible stripping response, excellent resolution of neighboring peaks, high hydrogen evolution, wide linear dynamic range, with signal-to-background characteristics comparable to those of common mercury electrodes.[30] Elemental antimony forms very hard alloys with copper, lead, and tin. In 2007, Hocevar et al. reported for the first time an antimony film electrode (SbFE) as a possible alternative for electrochemical stripping analysis of trace heavy metals.[31]

Although the electrochemical techniques using alloys and amalgams have been highly important during the past decades, they will not be further discussed in this review, as these have been extensively discussed in previous ones.[23,29,32−35]

2.2. Synthetic Receptors

The specific recognition of heavy metals by using synthetic or natural receptors called ionophores or carriers is one of the most reported. Different kinds of ionophores have been described so far, including simple molecules, macromolecules, or caged molecules, which can operate by two different ways of recognition: chemical affinity, cavity entrapment, or both ways simultaneously. Not only the binding affinity is important for the chemical sensing but also the chemical response of the ionophore after the analyte binding. Some typical commercial and noncommercial ionophores are depicted in Table 2.

2.2.1. Small Molecules. Small molecules can offer a simple way to develop heavy-metal probes with low nonspecific interactions and low backgrounds compared to macromolecules and biological receptors. Some highly selective ionophores for different analytes are commercially available. For example, the dioxadithioamide 3 (molecule **1** in Table 2) is described to behave as a highly selective ionophore for Cd^{2+} in solvent polymeric membranes.[36] The ionophore contains S-donor groups that are considered soft Lewis bases and have a certain affinity for Cd^{2+}, which is a soft Lewis acid. The soft acid−soft base affinity along with the formation of a certain chelating rings size around the Cd^{2+} and the adequate coordination geometry environment results in the high selectivity of the ionophore for metal cation Cd^{2+}.

Basu et al. developed a small molecule, 4,4-dimethyl-4*H*-5-oxa-1,3-dithia-6,11-diazacyclopenta[*a*]anthracen-2-one (molecule **2** in Table 2), for lead detection by turn-on ratiometric fluorescence in the presence of lead. It has a thiol-based binding site and differs from other fluorophores with harder donors such as oxygen or nitrogen.[37] Lead is also a soft metal and therefore favors sulfur-rich binding sites.

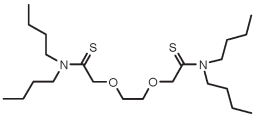
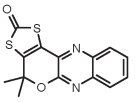
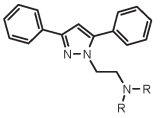
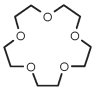
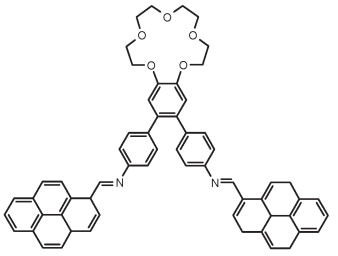
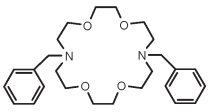
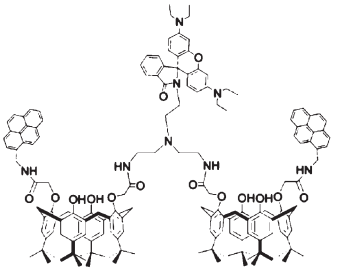
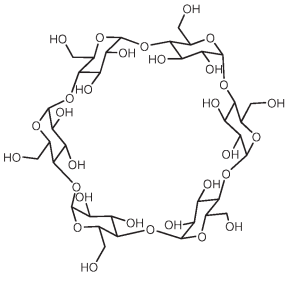
Amino-based ligands are an attractive class of ligands due to their binding abilities through heavy-metal ions. Our group has recently synthesized *N*-alkylaminopyrazole-derived ligands[38,39] (1-[2-(octylamino)ethyl]-3,5-diphenylpyrazole, molecule **3** from Table 2) and studied their reactivity toward different heavy metals, such as Zn^{2+}, Cd^{2+}, and Hg^{2+}.[40,41] Between various ligands, pyrazoles have attracted considerable attention mainly because their conjugate bases, pyrazolates, have been found to bind heavy metals in a variety of coordination modes and, in particular, are robust bridging ligands. These ligands can act as mono- or bidentante ligands in their coordination to the metal center. In addition, some of these ligands have recently been used for the formation and stabilization of AuNPs, and have been applied in a AuNP-based sensing system for Hg^{2+} ions.[42]

2.2.2. Macromolecules and Caged Molecules. Macromolecules and caged molecules are ideal targeting species, as their specificity for an individual metal ion can be tuned as a function of their structure. As a result, controlled binding of the desired analyte in a heterogeneous mixture can be achieved.

Crown ethers, first synthesized by Pedersen in 1967,[43] are commonly used in the design of selective receptors based on their fairly high selectivity and their unique ability to bind metal cations. The selectivity of the complexes of crown ethers is based on the size of the substrate and the ring size and donor atoms distribution in the ether crown. It is obvious that crown ethers of larger inner cores tend to bind larger ions. Crown ethers are commonly used as sensors for alkali metals, such as Na^{+}, K^{+}, and Ca^{2+}. However, many examples about the use of crown ethers for heavy-metal detection have been reported.[44] The most basic crown ether commercially used as lead ionophore is 15-crown-5 (molecule **4**, Table 2). Srivastava et al. presented a poly(vinyl chloride) (PVC) based membrane of **4** which exhibited a good response for Pb^{2+} ions over a wide concentration range.[45] The ionic radii of the Pb^{2+} ion is known to be of 1.33 Å, while the cavity size of this simple crown ether is ca. 1.2−2.2 Å. These data confirm the good fit of the ions inside the cavity of the receptor.

Crown ethers can not only act as receptor cavities but also can introduce some properties to the ionophore which can be useful for the further detection technique. A good example is presented in the paper recently published by Bhalla et al.[46] They reported a terphenyl-based receptor with a 15-crown-5 modified with a pyrene as fluorophore with a highly aromatic structure (molecule **5**, Table 2) which has proved to have high selectivity and sensitivity "off−on" fluorescence signaling behavior for Hg^{2+} ions. This efficiency may be attributed to the electrons on the

Table 2. Synthetic Receptors Used for Heavy-Metal Detection[a]

Molecule	Synthetic receptor	Metal	Ref
1		Cd^{2+}	[36]
2		Pb^{2+}	[37]
3		Hg^{2+}	[38],[39] [42]
4		Pb^{2+}	[45]
5		Hg^{2+}	[46]
6		Hg^{2+}	[47]
7		Hg^{2+}	[48]
8		Pb^{2+} Cd^{2+}	[49]

[a] **1**, dioxadithioamide; **2**, 4,4-dimethyl-4*H*-5-oxa-1,3-dithia-6,11-diazacyclopenta[*a*]anthracen-2-one; **3**, 1-[2-(octylamino)ethyl]-3,5-diphenylpyrazole; **4**, 15-crown-5; **5**, terphenyl-derived 15-crown-5; **6**, *N*,*N*′-dibenzyl-4,13-diaza-18-crown-6; **7**, calix[4]arene; **8**, cyclodextrin.

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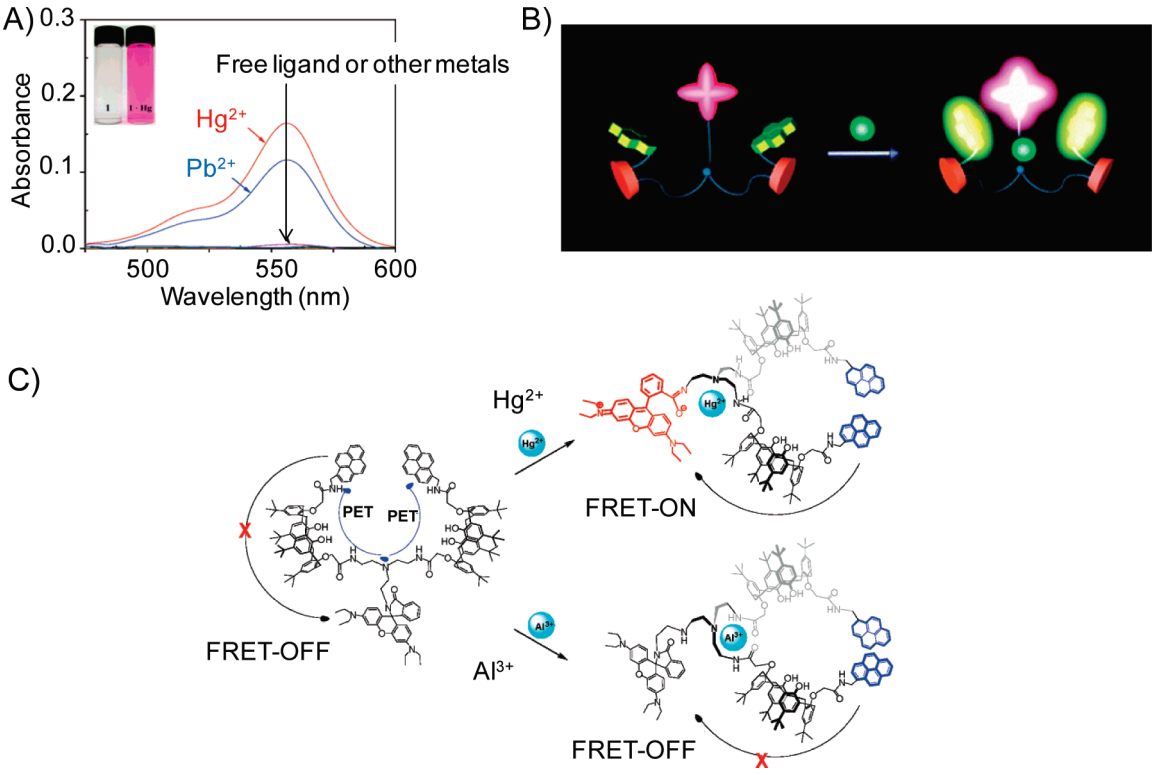

Figure 1. (A) UV−Vis spectra of molecule **7** (see Table 2) (0.015 mM) in CH_3CN in the presence of various metal cations, including Na^+, K^+, Rb^+, Cs^+, Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Pb^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+}, Fe^{2+}, Co^{2+}, and Ag^+ (100 equivalents, as perchlorates). Inset: Color change of molecule **7** upon addition of Hg^{2+} ion. (B) Scheme of the fluorescence enhancement provoked by Hg^{2+} ions. (C) Proposed different complexation behaviors of **5** with Hg^{2+} and Al^{3+}. Reprinted with permission from ref 48. Copyright 2007 American Chemical Society.

oxygen atoms of the ether crown ring moiety, which enhance the photoinduced electron transfer (PET) to the fluorophore moiety and make the receptor a very weak fluorescent, enhancing in this way the fluorescence percentage while coordinating the specific metal ion.

An efficient way to change the metal ion affinity of crown ethers is to insert nitrogen atoms into the crown ether's structure instead of oxygen atoms, making it more sensitive to Ag^+, Hg^{2+}, and Cd^{2+} ions. One example is *N,N′*-dibenzyl-4,13-diaza-18-crown-6 (molecule **6**, Table 2), where two oxygens of an 18-crown-6 have been replaced by nitrogens to enhance the affinity for Hg^{2+}.[47]

A mercury ionophore based on calixarene units (molecule **7**, Table 2) has also been reported.[48] Calixarenes are macrocyclic compounds obtained by the reaction between *p*-substituted phenol and formaldehyde in basic medium, which has a π-electron-enriching hydrophobic cavity formed by benzene rings. The size of the cavity can be adjusted and modified with specific functional groups with the purpose of enhancement of the selective recognition ability to specific metal ions. In addition, calixarene-based compounds have been widely exploited as the framework of many fluorescent chemosensors toward heavy metals due to their flexible structural property. Vicens et al.[48] presented a modified calixarene which belongs to the class of energy transfer sensors and undergoes a complex process upon mercury complexation. The structures of tripodal $N(CH_2CH_2NH_2)_3$ (tren) and rhodamine spirolactam give a suitable binding structure for some specific cations. Although it is not highly mercury selective binding, only the presence of the Hg^{2+} and Pb^{2+} ions leads to the appearance of a new absorption band centered at 555 nm. The Hg^{2+} ions induce a relatively larger change than Pb^{2+} ions. Other metal cations do not induce any distinct UV−vis spectra/color changes, implying that the molecule shows special binding ability toward Hg^{2+} and Pb^{2+} ions. Mercury is known to interact with the spirolactam unit and induce a ring-opening of the rhodamine. Other metal ions like Al^{3+} prefer to coordinate with the tren-diamine unit (Figure 1), provoking a clear different fluorescence change compared to the one observed for the Hg^{2+} ions detection (for more detail, see section 5.2.3).

Cyclodextrins (CDs), first described by Villiers in 1891,[49] can be applied in many different areas (molecule **8**, Table 2).[50] The principal factors involving the host−guest complex formation with metals are covalent interactions between the metal and the deprotonated secondary hydroxyl groups located inside the molecular cavity.

Although the data about the interaction of unmodified cyclodextrins with heavy-metal ions are not very numerous, some examples can be found in the literature.[51,52] Lead− and cadmium−cyclodextrins complexes are the most reported. Both metal complexations involve the deprotonation of cyclodextrins in alkali solutions in order to strengthen the complexing ability of β-cyclodextrin to form the metal complexes.[50] Usually the sensing mechanism for heavy metals using cyclodextrins makes use of the modification of membrane gels or carbon paste electrodes with these compounds.

To briefly resume this section of synthetic receptors, it can be concluded that the typical specific recognition mechanism of heavy-metal ions consists of a noncovalent binding of metals guests by an organic host molecule. This selective binding can be

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explained in different ways. On the one hand, chelating ligand systems are compounds with more than one donor atom, which can form chelate rings when they form coordination complexes with metal ions. The stability of the coordination complexes of chelating ligands is higher than those formed with unidentate analogs due to the chelate effect. However, both make use of the high affinity of oxygen, nitrogen, and sulfur donor atoms toward metal ions to bind those selectively. The planarity of the ligand is also important in its complexation properties. Some factors, related to the cation and influencing the selectivity behavior, include the geometric shape of the ion, its charge density, and its size (the ionic radius). The last factor seems to have a large influence on the selectivity.

An important issue to be considered for heavy-metal interaction with ligands is the stability of the formed complex. One of the most applied methods to predict the stability of a metal−ligand complex formation is the Pearson acid base concept (HSAB),[53,54] which essentially describes that soft acids react faster and form stronger bonds with soft bases, whereas hard acids react faster and form stronger bonds with hard bases, all other factors being equal. Hence, Cu^{+}, Ag^{+}, Cd^{2+}, and Hg^{2+}, classified as soft acids, bind favorably mainly to ligands containing sulfur, while the borderline acids Co^{2+}, Ni^{2+}, Cu^{2+}, Pb^{2+}, and Zn^{2+} prefer binding to receptors with nitrogen donor atoms. Cr^{3+} and Al^{3+}, classified as hard acids, prefer the coordination with ligands containing oxygen as donor atom. However, Pearson's classification is in many ways arbitrary and in many cases should be used with a certain amount of caution.[55]

On the other hand, the use of supramolecular chemistry with macrocyclic compounds and caged molecules such as podands, crown ethers, cryptands, spherands, or calixarenes takes advantage of their molecular cavities, basket, or similar hosts to accept metal ions inside these cavities and bind them selectively and reversibly.[56−58] According to this concept, the size of the cavity determines the selectivity for the different heavy metals.

2.3. Biological Receptors

Perhaps the biggest challenge in heavy-metal-sensing research is the design and synthesis of specific receptors with strong metal-binding capabilities. The interaction of heavy-metal ions with biological molecules such as proteins, antibodies, or nucleic acids offers in this field remarkable advantages in terms of selectivity and limits of detection.[59] Some examples of biological receptors are depicted in Table 3.

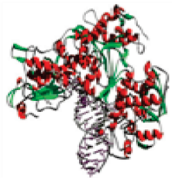
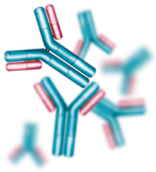
2.3.1. Nucleic Acids. The use of DNA probes for heavy-metal detection is mainly based on two well-established scientific foundations: the use of DNA hybridization and the fact that thymine−thymine (T−T) mismatches of complementary sequences can bind heavy-metal cations. Since Ono et al. reported that the chelating ability of the thymines that form mismatches in the oligonucleotide duplex is extremely selective for Hg^{2+} to promote these T−T mismatches to form stable base pairs,[60] an increasing interest in the development of this type of biosensors using this tight interaction has been noted in the last few years.

The thymine interaction with Hg^{2+} ions has a special interest for sensing applications.[61] Figure 2 shows the ^{1}H NMR spectra of the Hg^{2+} duplex formation. In the absence of Hg^{2+}, four imino proton resonances of the T−T mispairs (10.4−11.1 ppm) are observed, in conjunction with those of the Watson−Crick base pairing (12.5−13.5 ppm; Figure 2A). With the Hg^{2+} ions addition, the imino proton resonances of the T−T mispairs almost disappear (Figure 2C), suggesting that the imino protons of the T−T mispairs in the mercury-free duplex are substituted with Hg^{2+} ions. All these data confirm that Hg^{2+} ions bind directly to the thymine nitrogen in place of the imino proton and bridge two thymine residues to form the T−Hg−T pairs. All the methods based on this kind of interaction show excellent selectivity against other heavy-metal ions due to the high specificity and strength of the chelate complex formed with Hg^{2+} ions, as shown in Figure 2D.

Other nucleic acid based strategies are being used for other heavy-metal sensing. DNAzymes are DNA sequences that catalyze chemical reactions, such as cleavage of ribonucleic acid targets. DNAzymes may provide a general solution for metal detection because of their relatively high stability, low cost, and easy synthesis. DNAzymes attracting most attention are those that are divalent metal ion cofactor specific. As shown in Figure 3A, in these catalytic reactions, in the presence of specific metal ions, the substrate is irreversibly cleaved into two fragments at the cleavage site.[62] The cleavage is catalyzed by a specific metal, which means DNAzymes can be used as heavy-metal sensors. The cleaved pieces can be further detected selectively by immobilizing fluorophores, gold nanoparticles, or other detection labels at the end of the DNAzyme strands.

Different sensing strategies using DNAzymes, such as the one described by Wang et al. for mercury ion detection using G-quadruplex-based DNAzymes, are also being applied for metal detection.[63]

Table 3. Biological Receptors Used for Heavy-Metal Sensing

Receptor	Metal	Reference
Nucleic acids 	Pb^{2+} Hg^{2+}	[62],[63],[139]-[142], [158]-[165]
Proteins 	Pb^{2+} Cd^{2+} Hg^{2+}	[65]-[71]
Antibodies 	Pb^{2+} Co^{2+} Cu^{2+} Ag^{+} Cd^{2+} Hg^{2+}	[72]-[79],[182]

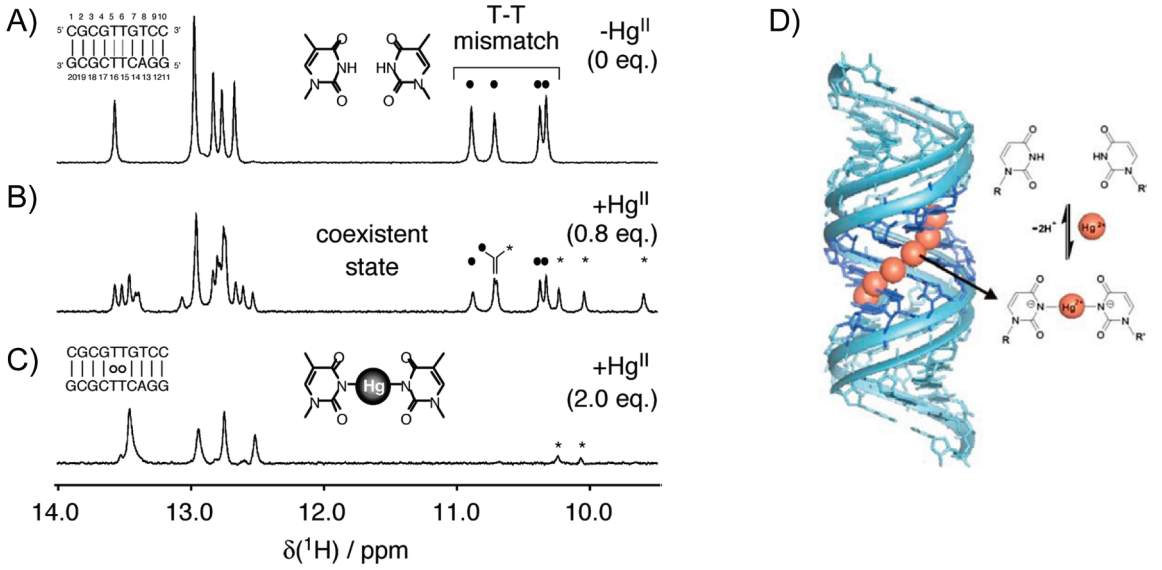


Figure 2. The imino proton region of the 1D ^{1}H NMR spectra of DNA−Hg^{2+} complexes: (A) 1D ^{1}H NMR spectrum in the absence of Hg^{2+}, (B) in the presence of Hg^{2+} (0.8 equivalents to the duplex), and (C) in the presence of Hg^{2+} (2.0 equiv to the duplex). (D) Scheme of the Hg^{2+} interaction with DNA. Reprinted with permission from ref 61. Copyright 2006 American Chemical Society.

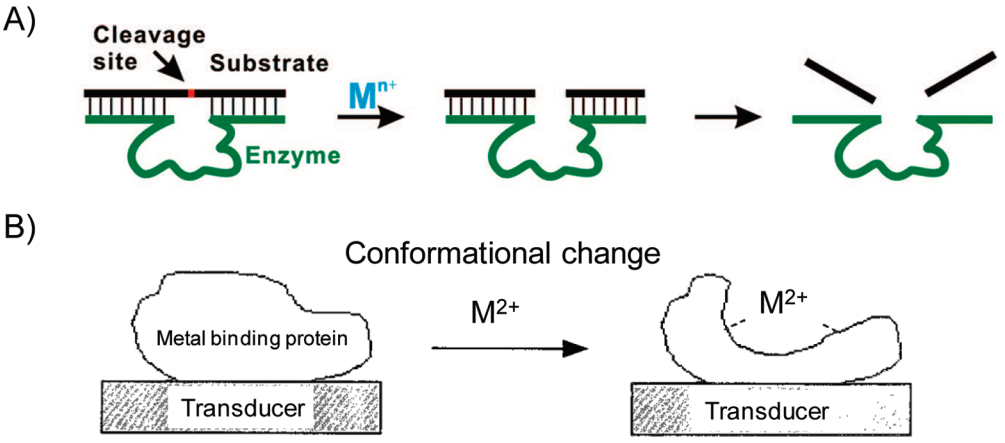


Figure 3. (A) The general reaction scheme of a substrate cleaving DNAzyme. Reprinted with permission from ref 62. Copyright 1994 American Chemical Society. (B) Protein-based biosensor concept for measuring conformational change upon binding of heavy-metal ions. Reprinted with permission from ref 65. Copyright 1998 American Chemical Society.

2.3.2. Proteins. Metal cations assist in protein folding or bind to folded proteins to make them functional and/or enhance, diversify, or tune their properties. Understanding the molecular basis of metal binding affinity and selectivity in metalloproteins is of high importance to shed light on the mechanism of some catalytic reactions and metal-induced protein folding and aggregation and also to develop novel metalloproteins and biosensors with preprogrammed properties with the aim of heavy-metal sensing.

Although a lot of information has been reported on protein−metal interactions,[64] the exact mechanism and physicochemical principles governing protein−metal recognition remain elusive. It is known that many factors play critical roles in the metal−protein recognition process, such as the metal and its coordination sphere, the protein matrix, the bulk solvent, and the cellular environment. Briefly, the affinity of a protein ligand for a given metal cation mainly depends on the ligand's charge, dipole moment, polarizability, charge-donating ability, and denticity.

Protein-based heavy-metal biosensors are not as common as the ones based on nucleic acids previously described. However, different detection principles, such as the measurement of the conformational changes of the protein when a heavy-metal ion is bound to it[65] (Figure 3B) or fluorescence,[66−70] among others,[71] have been reported.

In addition to that, immunoassays have emerged as an alternative approach for metal ion detection, since they offer significant advantages over traditional detection methods, such as high sensitivity, selectivity, and species-specificity.[72] Although most environmental immunoassays are directed toward low molecular weight organic compounds, including industrial pollutants, pesticides, and herbicides, the technique is theoretically applicable to any pollutant for which a specific antibody can be generated. Metal ions have an affinity for amino acid side chains[73] containing sulfur, nitrogen, and oxygen atoms, and antibodies contain these amino acids in their complimentarily determining regions. Thus, it might be expected that antibodies have the

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ability to bind tightly to metal ions or metal−chelate complexes. An antibody-based immunoassay for ionic mercury described in 1991 is the basis for the metal ion immunoassay presently commercially available.[74] This assay captures soluble ionic mercury on a reactive sulfhydryl surface and uses a mercury-specific monoclonal antibody to bind the mercury−sulfhydryl complex.

Moreover, different monoclonal antibodies have been generated to recognize metal−EDTA complexes of Cd^{2+}, Hg^{2+}, Cu^{2+}, Pb^{2+}, Co^{2+}, and Ag^{+}, besides many other metal ions.[75−79]

3. IMMOBILIZATION PLATFORMS

A crucial point in the design of heavy-metal-sensing systems is the immobilization of the specific receptors onto a proper platform. This immobilization can be carried out by different methods, depending on the nature of the receptor and the material that constitutes the sensing platform. In this section different macro-, micro-, and nanomaterial sensing platforms, including the respective receptor immobilization strategies that apply to heavy-metal detection, are reviewed.

3.1. Conventional Platforms

Different methods for the immobilization of synthetic heavy-metal receptors onto conventional platforms (i.e., micro- or macrometallic electrodes, synthetic/polymeric platforms) are used.[80] Sol−gel encapsulation,[81,82] polymer entrapment,[83] polymerization of the receptor itself onto the electrode surface, physical adsorption (electrostatic and/or hydrophobic adhesion), or covalent bonding leading to the formation of irreversible covalent bonds[84,85] are the most reported.

Physical adsorption of receptors onto polymer supports is rarely used because weak bonding leads to a loss of the receptor. In contrast, some works with the use of a polymer entrapment technique or covalent bonding are described.[83−85]

A well-known non-cross-linked perfluorosulfonate cation-exchange polymer, Nafion, which consists of hydrophobic, negatively charged ionic clusters, is widely chosen as a soft template to immobilize different heavy-metal receptors such as the one for cadmium reported by Jiang et al.[86] or for lead described by Pan et al.,[87] the thiol self-assembled monolayers described by Yantasee et al.,[88] or even for nanosized material such as graphene nanocomposite films recently described by Li et al.[89]

Biological receptors can also be linked to an electrode surface, forming biosensing platforms. The immobilization of the bioreceptors in a thin layer at the transducer surface can be performed using different techniques, such as sol−gel encapsulation,[90] entrapment within self-assembled monolayers,[91] or covalent bonding on membranes or activated surfaces or the bulk modification of entire electrode material.[92]

3.2. Nanoparticles as Immobilization Platforms

The interest in metal nanoparticles (MNPs) for sensing applications is growing nowadays due to their strong emission and stability to photobleaching and also thanks to their possibility to be easily functionalized by specific receptors to afford water compatibility and increase target specificity.

As is well-known, MNPs can be synthesized using a wide variety of techniques, such as chemical reduction,[93,94] UV photoactivation synthesis,[95] laser pulse methods,[96] or sonochemical methods,[97] among others. However, the most important method followed for the synthesis of MNPs is the chemical reduction of metal salts. The physical features of the MNPs can be controlled during the reaction step by different parameters, such as the precursor concentration, the nature of the solvent, the acting of the reducing agent, and the capping agent employed.

Although obtaining homogeneous MNPs is of high importance for the sensibility of the sensing techniques, the most important step to achieve easy-to-functionalize MNPs is the stabilization mechanism. Two methods are the most used to enable the stabilization of MNPs: electrostatic stabilization and steric stabilization. The electrostatic interaction consists of the formation of an electric double layer (EDL) around the MNPs with a Coulombic interaction between oppositely charged ions. The EDL stabilization mode (typical of citrate-reduced AuNPs) not only imposes a significant limitation on the stability of the corresponding colloids due to its dynamic nature and its sensitivity to the environment but also opens up exceptional possibilities for further chemical steps toward the modification of the MNPs' surface. Actually, the deposition of a layer of another inorganic substance (inorganic core−inorganic shell composites), the attachment of certain organic capping agents, and the covalent binding of biomolecules (i.e., DNA, antibodies) are the most typical surface derivatizations of MNPs.

On the other hand, the steric stabilization method is based on the use of organic ligands to form a protective layer. This is accomplished by the use of polymers, surfactants, or ligands. Concretely, surfactants such as sodium bis(2-ethylhexyl)sulfosuccinate, cetyltrimethylammonium bromide, or tetraoctylammonium bromide (AOT, CTAB, or TOAB, respectively) offer great colloid stabilization, avoiding the Van der Waals interparticle attractions that lead to agglomeration. The stabilization is achieved when the polar group of the surfactant adsorbs on the surface of MNPs and the organic apolar "tail" faces the peripherals of the resulting assembly, thereby providing steric repulsion. Generally, colloids stabilized with surfactants (or polymers) have limited stability due to the lack of strength on the binding. Organic ligands that contain a donor group with substituents of varying steric bulk are bound stronger to MNPs surface than the mentioned surfactants due to their pronounced covalent character. To reach the same stability, a smaller mass capping ligand per unit mass of MNPs is required than if surfactant stabilizer is used. This ability allows the displacement of the surfactant by organic molecules in a moderate certain time. In gold nanoparticles (AuNPs), the thiol exchange method is the most common way to replace the original capping molecules, since the metal−sulfur bond is known to be the strongest bond compared to other general functional groups (i.e., amines, carboxylic acids, alcohols, and phosphors).[98−102] Biomolecules, such as DNA and small molecule ligands, are first functionalized with an alkylthiolated linker and then bound to gold nanoparticles through Au−S bonds after a few hours of reaction.[103] For complete exchange, sonication and heating might be required to take the other capping agent off while preventing the nanoparticles' aggregation.

The option of using a reducing agent for formation of AuNPs that can act as capping agent and remain available for sensing is of great interest to avoid the different exchange reactions. The use of a pyrazole-derived amino ligand, which has a triple functionality, has been recently described in our group: it induces gold nanoparticle formation, stabilizes the formed AuNPs, and at the same time remains available for heavy-metal detections.[42]

4. ELECTROCHEMICAL DETECTION

Electrochemical measurements have shown numerous advantages for trace heavy-metal detection, including rapid analysis,

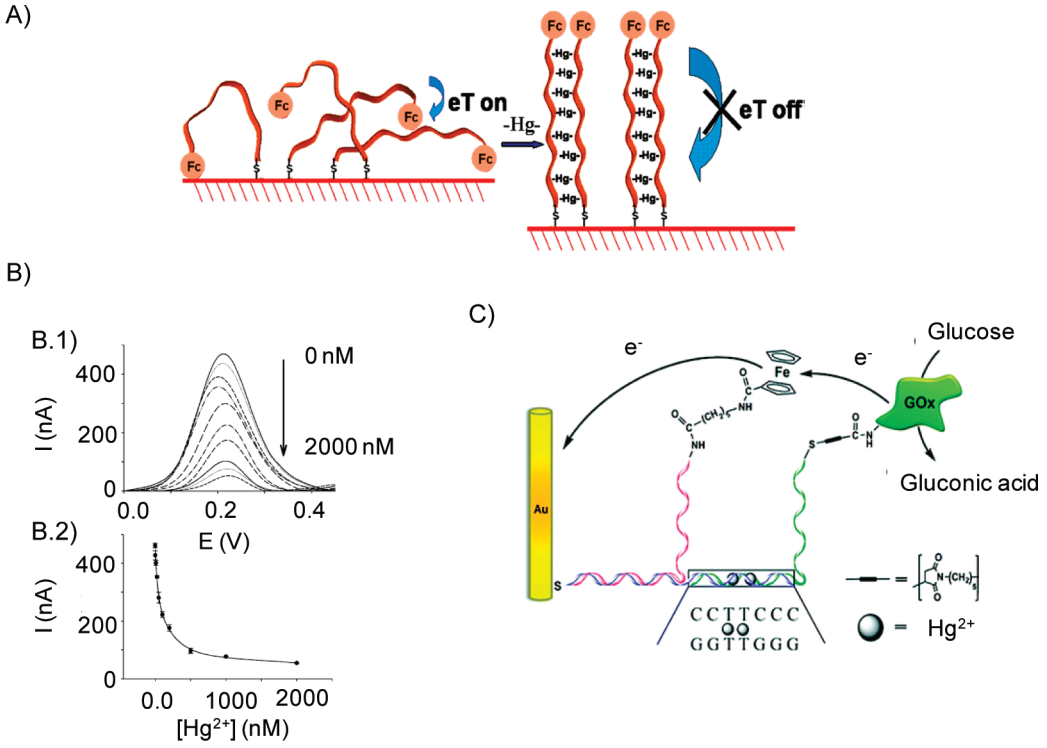

Figure 4. (A) Electrochemical sensing strategy for Hg^{2+} based on conformational switch mediated by T−Hg^{2+}−T coordination. (B.1) Typical DPV curves of the sensor in response to Hg^{2+} ions of varying concentrations. (B.2) Plot of DPV peak currents versus Hg^{2+} concentrations. Reprinted with permission from ref 92. Copyright 2009 American Chemical Society. (C) Enzyme glucose oxidase based biosensor used for Hg^{2+} detection. Reprinted with permission from ref 108. Copyright 2010 American Chemical Society.

good selectivity, and sensitivity. Therefore, some works related to the electrochemical analysis of heavy metals are discussed in the next section.

4.1. Chemically and Biochemically Modified Electrodes

Modified electrodes for electroanalytical determination of heavy metals using stripping voltammetry are commonly employed and present many advantages over the nonmodified electrodes, such as the enhancement of the sensibility and selectivity of the technique. A wide range of modifications have been reported in the literature with the aim of electrochemical detection of heavy metals, ranging from synthetic metal ionophores to biological receptors such as DNA or proteins.

α-Cyclodextrin- or β-cyclodextrin-modified carbon paste electrodes have been used to determine Pb^{2+} by means of anodic stripping voltammetry.[51,52] Both modified electrodes display good resolution of the lead oxidation peak. The analysis of the results indicates that the carbon paste electrodes modified with β-cyclodextrin exhibit a better analytical response than the ones modified with α-cyclodextrin. Detection limits of 6.30×10^{-7} M and 7.14×10^{-7} M of Pb^{2+}, respectively, are obtained. The principal factors involved in guest−host complex formation are covalent bonds formed between metal ions and deprotonated $^{-}$O-groups of cyclodextrins.

Although the modified carbon paste electrodes are still being used for sensing purposes, due to their mode of preparation, these devices usually lack reproducibility between batches and their use is time-consuming. While this is not a big problem for in-lab research, it would be a considerable burden in mass-production systems.

Because of their exceptional selectivity and sensibility, biomodified electrodes involving DNA, proteins, and antibodies have emerged as a new type of electrochemical sensing strategy for heavy-metal ions. Sensors based on proteins with distinct binding sites for heavy-metal ions are being developed and characterized. A capacitive signal transducer is used to measure the conformational change following heavy-metal binding (Figure 3B). The proteins can be immobilized in different ways on a self-assembled thiol layer on a gold electrode placed as the working electrode in a potentiostatic arrangement in a flow analysis system. Metal ions can be detected down to femtomolar concentration using the biosensor described by Bontidean et al.[65] Figure 4A shows a developed electrochemical sensor based on cooperative coordination with Hg^{2+} by a pair of short poly-T oligonucleotides that induce conformational switching of the ferrocene-tagged probes from a single-strand to a duplex-like structure, modulating the electron transfer efficiency.[92] This strategy exploits the cooperation of proximate poly-T oligonucleotides in coordination with Hg^{2+}. Ferrocene (Fc)-tagged poly-T oligonucleotides are immobilized on the electrode surface via self-assembly of the terminal thiol moiety. In the presence of Hg^{2+}, a pair of poly-T oligonucleotides can cooperatively coordinate with Hg^{2+}, which triggers a conformational reorganization of the poly-T oligonucleotides from flexible single strands to relatively rigid duplex-like complexes, thus drawing the Fc tags away from the electrode with a substantial decrease of the redox current. Figure 4B.1 shows typical differential pulse voltammetry (DPV) curves of the sensor in response to Hg^{2+} ions of varying concentrations, while Figure 4B.2 is a plot of DPV peak currents versus Hg^{2+} concentrations. A similar design was proposed for Pb^{2+} detection using methylene blue as electrochemical label.[104]

Other approaches related with biochemically modified electrodes involve enzyme-based biosensors, such as oxidase,

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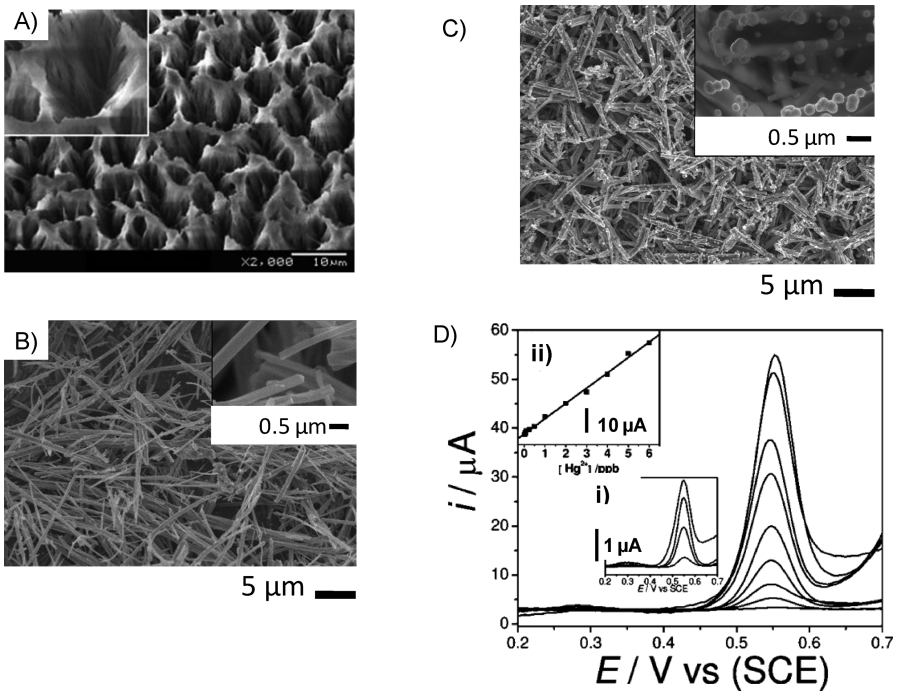

Figure 5. Typical SEM images: (A) the bunch-like Bi composed of Bi nanowires (inset: the nest formed by the nanowires), (B) the as-synthesized 3,3′,5,5′-tetramethylbenzidine (TMB) based nanofibers (NFs), (C) Au−PtNPs/NF inorganic−organic hybrid nanocomposite on a glassy carbon electrode (GCE) (inset: corresponding magnified SEM image), (D) stripping voltammograms of increasing mercury concentrations (from bottom to top, 0, 0.1, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, and 6.0 ppb, respectively) [inset i: the stripping voltammograms of Au−PtNPs/NFs/GCE toward the lower concentrations of Hg^{2+} (from bottom to top, 0.02, 0.08, 0.10, and 0.25 ppb, respectively); inset ii: the calibration curve]. Reprinted with permission from ref 120. Copyright 2010 Springer. Reprinted with permission from ref 125. Copyright 2010 American Chemical Society.

dehydrogenase, and urease, which detect metal ions by relying on their inhibition of enzymatic reactions.[105−107] The enzyme glucose oxidase has been recently used by Willner et al. (Figure 4C). The system is based on the use of a DNA scaffold for the amplified detection of Hg^{2+} using an electrically contacted relay/enzyme structure as a transducing element.[108] The detection limit for Hg^{2+} corresponds to 100 ± 10 pM. This great sensitivity originates from the amplification path provided by the biocatalytic system. Although the system has high sensitivity, working with enzymes requires narrow operation parameters (e.g., of pH, temperature, ionic strength) and long incubation times. In this context, the application of the enzyme-based system in real samples might be difficult or even impossible if the sample is under extreme conditions.

Point-of-care devices are being developed nowadays to provide low-cost analytical systems useful for environmental monitoring of heavy metals. In this context, paper-based systems are highly novel and important.[109,110]

4.2. Nanostructurated Electrodes

In the recent years, the use of nanomaterials [i.e., carbon nanotubes (CNTs), metal nanoparticles (MNPs), or metal nanotubes (MNTs)] for the construction of electrochemical sensors and biosensors has become one of the most active areas in analytical chemistry.[111−114] These materials often exhibit unique chemical, physical, and electronic properties that cannot be achieved by the bulk material.[115] Such nanomaterial-modified electrodes for stripping analysis of heavy metals show dramatically increased sensitivity due to their large specific surface area and high surface free energy.

4.2.1. Metal Nanoparticle Modified Electrodes. MNP-modified electrodes may serve as random arrays of microelectrodes. They show distinct advantages over the conventional macroelectrodes, such as increased mass transport, decreased influence of the solution resistance, low detection limit, and better signal-to-noise ratio.

Bismuth and antimony nanoparticles have been proven to be highly sensitive and reliable for trace analysis of heavy metals in conjunction with anodic stripping voltammetry. The attractive and unique behavior of bismuth and antimony nanomodified electrodes is attributed to the formation of multicomponents alloys, as well as the enhanced sensibility coming from the combination of the great properties of the nanostructured material.[116−119] A new self-organized morphology of Bi (called bunch-like bismuth, see Figure 5A) grown in a bare electrode has been recently described by Zhang et al. and exhibits a good performance for detection of heavy-metal ions.[120]

In a parallel way, AuNPs-based electrodes have been proven to be a promising approach for heavy-metal detection.[121−124] Recently, bimetallic NPs have been extensively investigated due to their extraordinary properties, such as good conductivity, and better catalytic activities than their monometallic counterparts. Gong et al. have just reported the use of a bimetallic Au−PtNPs inorganic−organic hybrid nanocomposite modified glassy carbon electrode for Hg^{2+} ion determination.[125] Bimetallic Au−PtNPs are homogenously distributed in the interlaced nanofibers (NF) matrix (see Figure 5B,C), building a 3D porous network. Such a 3D porous nanostructured composite film greatly facilitates electron-transfer processes and the sensing behavior for Hg^{2+} detection, leading to a remarkably improved sensitivity and selectivity. The calibration

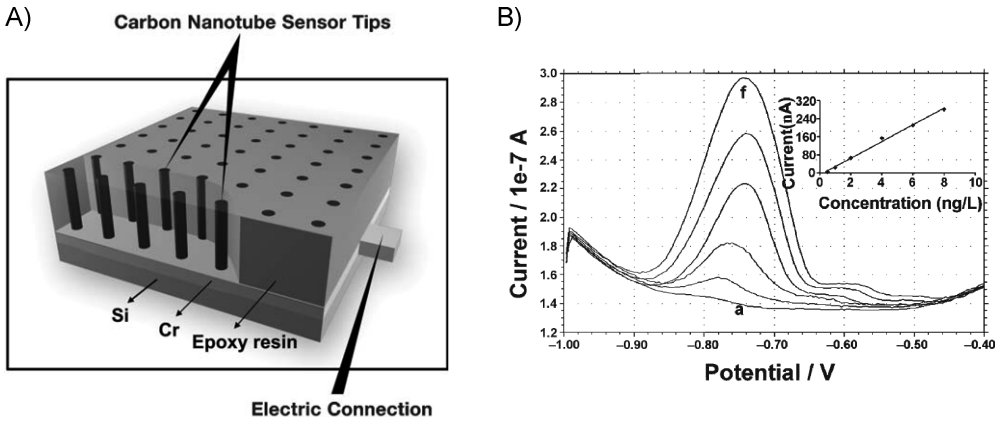

Figure 6. (A) Structure of carbon nanotubes nanoelectrode array. (B) Square wave voltammetric response for an increasing Cd^{2+} the concentration of 0.5, 1, 2, 4, 6, 8 mg L^{-1}. Also shown (inset) is the resulting calibration plot. Reprinted with permission from ref 130. Copyright 2005 Royal Society of Chemistry.

plot is linear up to 10 ppb, and the detection limit of 0.008 ppb (8 ppt) is obtained with the calculation based on a signal-to-noise ratio equal to 3 (see Figure 5D).

In the same field, Domínguez et al. reported a novel method for the anodic stripping voltammetry determination of Sb^{3+} using AgNPs-modified screen-printed electrodes.[126] The detection limit for Sb^{3+} using the silver and gold modified electrode was 6.79×10^{-10} M for an accumulation time of 200 s.

4.2.2. Carbon Nanotube Modified Electrodes. The unique electronic, chemical, and mechanical properties of carbonanotubes (CNTs) make them extremely attractive for heavy-metal electrochemical sensors.[127] Sensing using CNTs takes advantage of their bulk properties, such as the increased electrode surface area and fast electron transfer rate compared to bulk carbon electrodes. Compared with the traditional macroelectrodes, the nanomodified electrodes have big mass sensitivity, an increased mass-transport rate, a decreased influence of the solution resistance, and a higher signal-to-noise ratio.[128,129] Liu et al. reported a CNT nanoelectrode array in connection with a bismuth film that shows potential to detect very low concentrations of heavy-metal ions compared with the traditional bulk and macroelectrode sensing platforms.[130] Figure 6A shows a scheme of the structure of the CNT nanoelectrode array. A linear relationship between the stripping current and the cadmium concentration is obtained covering the concentration range from 0.5 to 8 mg L^{-1} (Figure 6B). A detection limit of 0.04 ng L^{-1} (40 ppt) in connection with a 240 s accumulation time is reported.

Other works have also been reported in this field, such as the one presented by Wang et al.,[131] Hwang et al.,[132] and by Xu et al.[133] using multiwall CNTs combined with bismuth films for heavy-metal detection.

The combination of multiwall CNTs with screen-printed electrodes (SPE) and mercury nanodroplets has also been investigated for simultaneous detection of Cd^{2+}, Pb^{2+}, and Cu^{2+}.[134] Although the mercury concentration used in the analysis is low, the use of free-mercury electrodes to avoid additional environmental problems should be of greater interest. On the other hand, metal nanotubes are a good alternative to metal NPs and CNTs. The oxidative detection of As^{3+} on highly ordered platinum nanotube array electrodes by linear scan voltammetry has been reported by Xu et al.[135] Considering the detection limit and the reported reproducibility, this method has practical utility for environmental monitoring of arsenic.

In addition, the combination of CNTs with metal nanoparticles for electrodes modification has been also considered for trace detection of As^{3+} ions.[136] The higher electroactive area resulting from smaller size Pt nanoparticles combined with the increase of the large local rates of mass transport to the electrode coming from the CNTs leads to detection limits for As^{3+} ions of ppb to ppt level.

4.2.3. Nanomaterials Combined with Synthetic Receptors Modified Electrodes. The use of nanomaterials combined with the specific complexing ability of the receptors results in an improved electrochemical sensing platform toward heavy metals with high sensitivity and excellent selectivity for stripping analysis. Pan et al. reported a nanosized hydroxyapatite/ionophore-modified glassy carbon electrode for the determination of lead.[87] The nanostructurated material provides a unique three-dimensional network structure and particular multiadsorbing sites, while the specific complexing ability of the ionophore for lead enhances the sensibility and selectivity of the electrochemical platform for this metal.[137] The electrode has a linear range of response from 5.0 nM to 0.8 μM with a 10 min accumulation time at open-circuit potential. The sensitivity and detection limit of the proposed sensor are 13 μA/μM and 1.0 nM, respectively. Interferences from other heavy-metal ions such as Cd^{2+}, Cu^{2+}, and Hg^{2+} ions associated with lead analysis can be effectively diminished.

A similar work presented by Huang et al. for trace detection of mercury ions combining the use of 2-mercaptobenzothiazole adapters in a SiO_2 3D gold micro/nanopore array has been recently reported showing an excellent linear range (0.05−10 nM) and a good repeatability (relative standard deviation of 2.10%).[138]

4.2.4. Nanobiomodified Electrodes. Electrochemical biosensors based on modifications with DNA and nanomaterials have received increasing attention, mainly for mercury detection.[139,140] During the past decade, oligonucleotide-functionalized gold nanoparticles have been employed as amplifying tags for novel biosensing protocols due to their unique properties. Recently, Kong et al. have reported a highly selective electrochemical biosensor based on the strong and specific interaction of Hg^{2+} ions by two DNA thymine bases and the use of AuNP-functionalized reporter DNA to achieve signal amplification (see Figure 7).[141] The electrochemical mercury biosensor is composed of three elements: a 5′-thiol-modified

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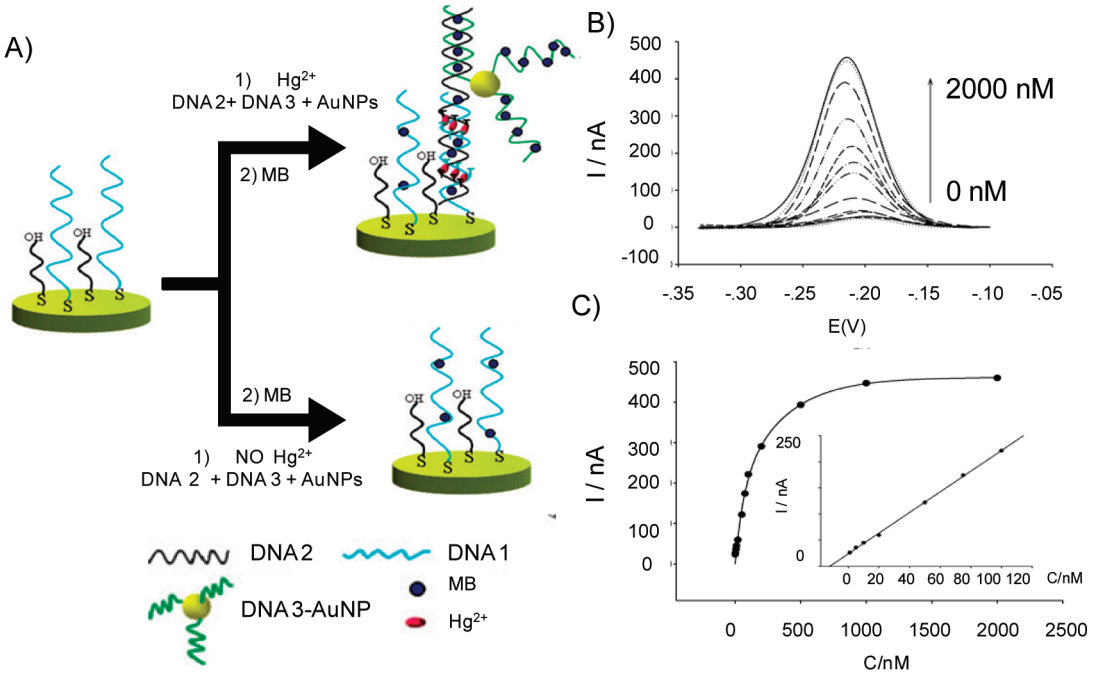

Figure 7. (A) A description of the electrochemical Hg^{2+} sensing strategy based on the signal amplification of AuNP-functionalized reporter DNA. (B) DPV responses of the sensor after the addition of different concentrations of Hg^{2+} ions (0 , 0.5, 1, 5, 10, 20, 50, 75, 100, 200, 500, 1000, and 2000 nM). (C) Calibration curves of peak current as a function of Hg^{2+} concentration. Inset: the linearity of the relative peak current (in %) with respect to the Hg^{2+} concentration over the concentration range 1−100 nM. Reprinted with permission from ref 141. Copyright 2009 Royal Society of Chemistry.

oligonucleotide containing six T-bases for Hg^{2+} binding as a capture probe (DNA **1**), an appropriate oligonucleotide linker (DNA **2**) with the 3′-terminal partially complementary with the capture probe sequence except six T−T mismatches, and finally a DNA **3** functionalized with AuNPs, which could specifically hybridize with the partial linker sequence close to the 5′-terminal. When the sensing platform is incubated with solutions containing mercury and the DNA linker, the capture probe can hybridize with the linker via specific T−Hg−T interaction, and the AuNP-functionalized reporter DNA subsequently hybridizes with the linker. In the absence of mercury, the linker does not hybridize with the capture probe, because the melting temperature is lower than the incubating temperature due to the T−T mismatches. Figure 7B shows DPV responses of the sensor after the addition of different concentrations of Hg^{2+} ions (0, 0.5, 1, 5, 10, 20, 50, 75, 100, 200, 500, 1000, and 2000 nM). Moreover, Figure 7C shows the calibration curves of peak current as a function of Hg^{2+} concentration and the linearity of the relative peak current with respect to the Hg^{2+} concentration over the concentration range 1−100 nM. The described electrochemical sensor can achieve a detection limit of 0.5 nM, which makes the detector favorable for Hg^{2+} ions assays in practical samples, such as tap or river water with very low Hg^{2+} concentrations. Even though the system presents low detection limits, issues related with the reusability (by immersing the electrode into 1 M NaOH at 50 °C) would represent a drawback for long-term and in-field applications.

Shen et al. have developed a DNA−Au biobar code assay for electrochemical detection of lead.[142] The biobar code assay is based on AuNPs functionalized with a large number of oligonucleotide strands (the bar codes) and a corresponding recognition agent. The bar code strands are used as a surrogate target and as a means of amplification. The electrochemical DNAzyme biosensor based on DNA−Au biobar code amplification provides a platform for analysis of many small molecules, especially for metal ions. A redox mediator has to be selected as an electrical signal readout. As depicted in Figure 8A, exposure of the modified electrode to an aqueous solution of a redox mediator cation $Ru(NH_3)_6^{3+}$, coming from the electroactive hexaaminеruthenium(III) chloride (RuHex) complex, at a low ionic strength leads to an ion exchange equilibrium between RuHex and the native charge compensating ions associated with the anionic DNA backbone. The electrochemical behavior of the interaction of RuHex with DNA can be investigated by DPV. Figure 8B shows DPV curves for DNAzyme-modified electrodes (in the presence of DNA−AuNPs biobar codes) upon analyzing different concentrations of Pb^{2+} (from 1 to 1000 nM). In addition, in Figure 8C, DPV signal changes for DNAzyme-modified electrodes (in the presence of DNA−AuNPs biobar codes) after reaction with various divalent metal ions (all at 0.5 μM) are shown. When the modified electrode is incubated with solutions containing Pb^{2+}, the catalytic strand carries out catalytic reactions and the substrate is broken into two pieces and dissociated from the catalytic strand. As a result, the amount of the RuHex electrostatically bonded to DNA modified on the surface decreases, resulting in lower DPV signals.

Despite being a highly sensitive system, the in-field application of such a rather complicated strategy should be carefully studied in terms of reproducibility and robustness, including the avoidance of time-consuming procedures.

5. OPTICAL DETECTION

Optical detection systems are other alternatives to the electrochemical detection methods. These represent attractive analytical tools whenever continuous monitoring and real-time information is desired.[143−145]

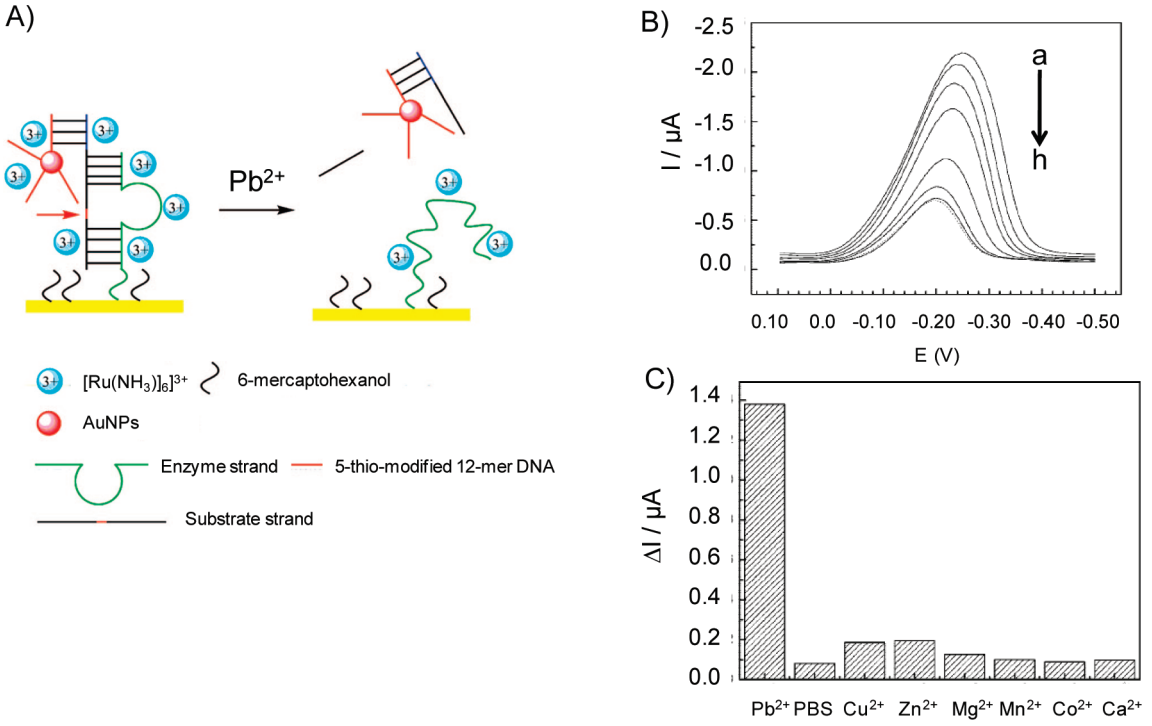

Figure 8. (A) The principle of the electrochemical DNAzyme sensor for Pb^{2+}. (B) DPV curves for DNAzyme-modified electrodes (in the presence of DNA−AuNPs biobar codes) upon analyzing different concentrations of Pb^{2+}: (a) 0 nM, (b) 1 nM, (c) 5 nM, (d) 10 nM, (e) 50 nM, (f) 100 nM, (g) 500 nM, and (h) 1000 nM. The solution is 5 mM Tris buffer (pH 8.2) containing 10 μM hexaminethenium(III) chloride (RuHex). (C) DPV signal changes for DNAzyme-modified electrodes (in the presence of DNA−AuNPs biobar codes) after reaction with various divalent metal ions (all at 0.5 μM). Reprinted with permission from ref 142. Copyright 2008 American Chemical Society.

5.1. Light Absorption Techniques

5.1.1. Nonmodified Metal Nanoparticles. There is great interest in the development of the next generation of heavy-metal sensors, being those of cost-effective production and user-friendly format with an in-field readable result. To achieve these reachable next-generational goals, colorimetric detection is a good alternative method. In this kind of sensor setup, the detection signal is through a visual color change in the reaction medium. Typical agents in this analytical method are AuNPs, due to their robust properties that are a direct effect of their small size.[146]

It is known that the optical response of spherical AuNPs exhibits a single absorption band attributed to the collective dipole oscillation (surface plasma resonance). The optical properties of noble metal particles originate from these localized surface plasmons. These phenomena occur when an electromagnetic field interacts with conduction band electrons and induces the coherent oscillation of electrons. As a result, a strong absorption band appears in some region of the electromagnetic spectrum, depending on the particle size and shape and the dielectric constant of the medium. Hence, by exploitation of this property, AuNPs can be used as effective tools to design sensing systems.[147] The sensing mechanism is based on the measurement of the change of surface plasmon property either due to small changes in refractive index of the surrounding medium that occur during the binding of external analyte at or near the surface of a AuNP or due to the changes in internanoparticle interactions in a nanoparticles assembly.

A label-free AuNPs (citrate-capped) based assay for Hg^{2+}, Pb^{2+}, and Ag^{+} ions has been recently reported by Hung et al.[148] The sensing system is based on AuNPs aggregation induced by alkanethiols, which act as strong electron-releasing ligands of high polarizability and preferably form complexes with soft Lewis acids such as gold. Depending on the chain length of such thiol ligands, when they are present on the AuNPs suspension, these readily access the surface of the AuNPs, displacing the citrate ions through the formation of stronger Au−S linkages inducing the nanoparticles aggregation. The competition between the heavy-metal ions and AuNPs for their binding toward alkanethiols is the key for the label-free assay developed by Hung et al., who achieved detection limits up to nanomolar metal concentration.

A lot of effort has been applied toward identifying the ideal plasmon sensor with a large spectral shift for a given change in refractive index. In this field, rod-shaped nanoparticles remain popular for plasmonic sensing applications.[149] It is known that the absorption spectra of gold nanorods are characterized by the dominant longitudinal surface plasmon band (SP_{long}, at a longer wavelength) and the much weaker transverse surface plasmon band (SP_{trans}, at a shorter wavelength, ca. 520 nm), with the position of the SP_{long} band being strongly dependent on the aspect ratio of the gold nanorods. The λ_{max} of the SP_{long} absorption band responds nicely to the binding event in terms of sensitivity and linearity of the target analyte concentration. This sensing response mode of the gold nanorods exhibits much higher sensitivity compared with that of the gold nanospheres.[150]

Interesting approaches using nonmodified gold nanorods to monitor Hg^{2+} ions upon changes in the surface plasmon resonance have been reported.[151] The outstanding selectivity and sensitivity results from the well-known amalgamation process that occurs between Hg^{0} (Hg^{2+} previously reduced using $NaBH_4$) and Au nanorods, provoking a blue shift of the maximum absorption wavelength of the longitudinal mode band of

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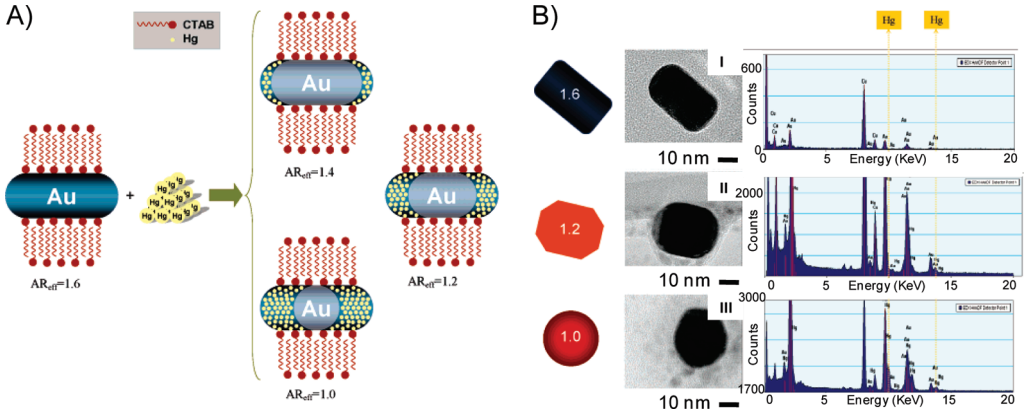

Figure 9. (A) Schematic diagram showing the amalgamation of Hg^0 with Au nanorods. (B) TEM and EDX analysis of Au nanorods in the absence and the presence of Hg^{2+}: (I) no Hg^{2+}; (II) 1.25×10^{-5} M Hg^{2+}, and (III) 1.57×10^{-4} M Hg^{2+}. Reprinted with permission from ref 151. Copyright 2006 American Chemical Society.

Au nanorods in the presence of ultralow traces of Hg^{2+} (detection limit of 6.6×10^{-13} g·L^{-1}). Figure 9A depicts a schematic diagram showing the amalgamation of Hg^0 with the gold nanorods. Figure 9B shows TEM and EDX analysis results of Au nanorods in the absence and the presence of Hg^{2+}.

5.1.2. Synthetic Receptor Modified Metal Nanoparticles. Appropriately functionalized AuNPs with adequate heavy-metal ion receptors can be used as specific sensing platforms via an ion-chelation-induced aggregation process. The aggregation also enhances the hyper-Rayleigh scattering (HRS) response from nanoparticle solutions, providing an inherently more sensitive method of detection. Hupp et al. were pioneers in the development of colorimetric detection systems using AuNPs functionalized with mercaptoundecanoic acid (MUA) as a proof-of-concept sensor system that causes aggregation of AuNPs upon the presence of Pb^{2+}.[152] The developed system lacks of selectivity and sensibility and can just be considered a proof-of-concept for the colorimetric heavy-metal sensing using AuNPs. After this initial idea, many other works have been reported using similar strategies but altering the receptor ligand in order to improve the selectivity.

In subsequent works, a highly selective sensor for Pb^{2+} detection was accomplished using more specific receptor-modified AuNPs and modulating parameters such as pH. Guan et al. demonstrated that the citrate-capped AuNPs show selective response to Pb^{2+} ions at pH 11.2 due to the formation of $Pb(OH)_3^-$ and as a result of suppressed coordination of other interfering heavy-metal ions with the carboxyl groups.[153] Gallic acid has been also used by Yoosaf et al.[154] as a lead-specific receptor in connection with gold nanoparticles. The detection limit was improved using the same receptor by Tseng et al.,[155] minimizing the nanoparticle size distribution and interparticle repulsion with the addition of $NaClO_4$.

N-Alkylaminopyrazole ligands used for AuNPs formation (reducing and capping agent) which remain available for nanomolar concentration of heavy-metal ions have been reported recently by our group.[42] The ligand seems to be selective to Hg^{2+} ions at a low range of heavy-metal ion concentrations (up to 2 nM Hg^{2+}) while at high concentration range (10 to 100 ppm) all heavy metals induce AuNPs aggregation (see Figure 10A). The schematics of the mechanism of heavy-metal sensing with *N*-alkylaminopyrazole-modified gold nanoparticles is depicted in Figure 10B. These new gold nanoparticles are expected to be of special importance for applications like lab-on-a-chip, where the sensing reagents can be prepared in situ and used immediately afterward.

Quite a similar mechanism of detection has been described by Kim et al. in a recent work based on the use of AuNPs modified with thiolated ligands.[156] However, in this work the detection limit is 2 orders higher (100 nM) than the system based on AuNPs modified with *N*-alkylaminopyrazole ligands.[42]

Gold nanorods, as described in the previous sections, are a good and more sensitive alternative to gold nanoparticle sensing systems. Modified gold nanorods have been recently described by Ni et al. as pH and metal sensing devices using resonance-coupling-based systems and plasmon-enhanced spectroscopy.[157]

5.1.3. Biomodified Metal Nanoparticles. AuNPs colorimetric biobased sensors have also been widely used for heavy-metal sensing in aqueous solutions. DNA-functionalized AuNPs have been employed for colorimetric Hg^{2+} ion detection by Mirkin et al.[158] Metal concentration can be determined by the change of the solution color at a given temperature or the melting temperature of the DNA−AuNPs aggregates. The colorimetric detection method is based on the reversible association of two cDNA−AuNPs forming DNA-linked aggregates followed by a concomitant purple-to-red color change (see Figure 11A). When mercury is present in the sample, it coordinates selectively to the bases that make up a T−T mismatch, raising in this way the melting temperature of the resulting aggregates structures, allowing one to quantify the Hg^{2+} concentration in the sample. Parts B and C of Figure 11 show normalized melting curves of aggregates with different concentrations of Hg^{2+} ions and the graph of the melting temperature (T_m) for the aggregates as a function of Hg^{2+} ions concentration, respectively.

Although almost all the research focus on this DNA-based detection methodology has been concerned with using AuNPs, systems involving other noble metallic nanoparticles such as silver nanoparticles (AgNPs) have also been reported.[159] The principle based on AgNPs colorimetric biosensing is similar to that of AuNPs as described by Yang et al. for the detection of Hg^{2+} ions using DNA target-responsive structural variation, such as the change from unfolded ssDNA conformation to rigid dsDNA or hairpin structure.

The major part of the described setups seems to be for "disposable" uses. The incorporation of these sensing systems in fully portable, power-free, and cost-effective microfluidic devices is of high importance for in-field applications. He et al.

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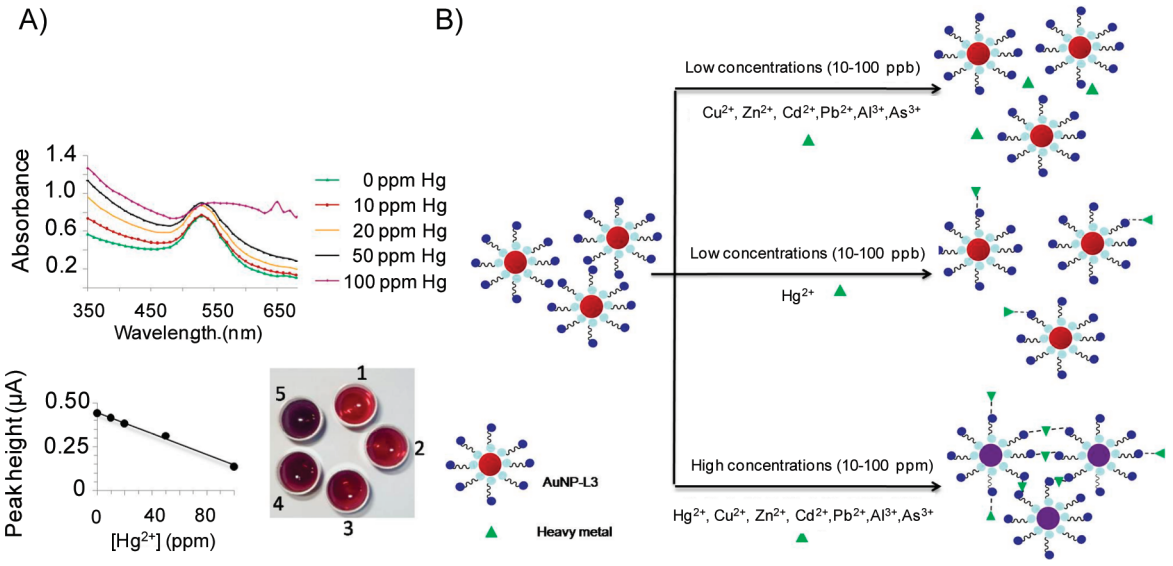

Figure 10. (A) Absorption spectra (higher figure) corresponding to the analysis of high concentrations of Hg^{2+} ions (10−100 ppm) in the presence of AuNPs-L3 solution and its linear evolution (lower figure). (B) Scheme of the mechanism of heavy-metal sensing with *N*-alkylaminopyrazole-modified AuNPs. Reprinted with permission from ref 42. Copyright 2010 American Chemical Society.

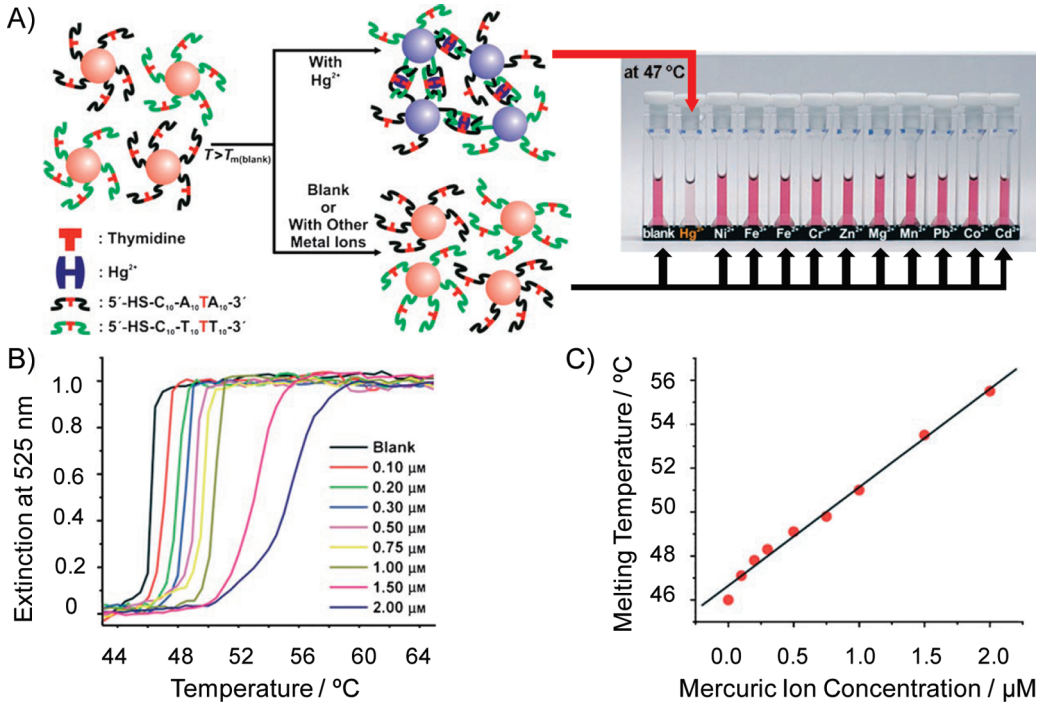

Figure 11. (A) Colorimetric detection of Hg^{2+} ions using AuNPs−DNA. The color change of the aggregates (probes A and B, each at 1.5 nm) in the presence of various representative metal ions (each at 1 mm) upon heating from room temperature to 78 °C. (B) Normalized melting curves of aggregates (probes A and B) with different concentrations of Hg^{2+}. (C) Graph of the melting temperature (T_m) for the aggregates as a function of Hg^{2+} concentration. Reprinted with permission from ref 158. Copyright 2007 Wiley-VCH.

successfully directed their research on the adaptation of this sensing methodology (DNA-based AuNPs for Hg^{2+} detection) in a simple microfluidic device at room temperature using PDMS.[160] Since the detection does not rely on an expensive instrument, the developed device might by suitable for in-field application in monitoring systems. However, the benefits of these simple continuous monitoring systems should be carefully considered in terms of robustness and stability.

Biobased sensing methods for Hg^{2+} ions are the most studied and developed due to the well-known high affinity of mercury toward DNA thymine bases, as described in previous sections. However, some methods for Pb^{2+} cations have also been reported using DNAzymes. DNAzymes (also called catalytic DNA or desoxyribozymes), as described in other previous sections, are DNA molecules that can catalyze many chemical and biological reactions, with most of them requiring specific

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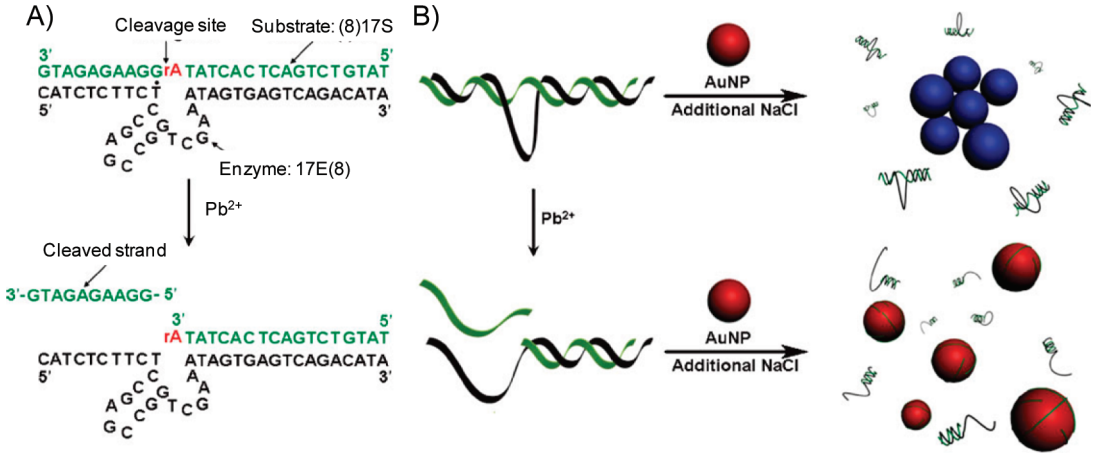

Figure 12. (A) Secondary structure of the DNAzyme complex, which consists of an enzyme strand [17E(8)] and a substrate strand [(8)17S]. After lead-induced cleavage, 10-mer ssDNA is released, which can absorb onto the AuNPs surface. (B) Schematic of the label-free colorimetric sensor. The lead-treated/untreated complex and NaCl are mixed with AuNPs. The AuNPs aggregate in the absence of lead but remain dispersed in the presence of lead. Reprinted with permission from ref 162. Copyright 2008 Wiley-VCH.

metal ions as cofactors. This property of the DNAzymes for the Pb^{2+} catalytic DNA cleavage has been exploited for heavy-metal sensing. In 2003, Lu et al. reported for the first time the use of a selected DNAzyme-directed assembly of AuNPs for lead determination.[161] It consists of a 5′-thio-modified 12-mer DNA attached to 13 nm AuNPs (DNA_{Au}), a DNAzyme [17E(8)], and its substrate (Sub_{Au}). The sequence of Sub_{Au} can hybridize specifically to DNA_{Au} on each end, while the 17E(8) recognition portion is maintained. This hybridization causes aggregation of the AuNPs system. In the presence of Pb^{2+}, the 17E(8) catalyzes hydrolytic cleavage of Sub_{Au} and prevents the formation of aggregates. In a more recent work, Lu et al. improved their system, reporting a label-free colorimetric detection of lead ions with nanomolar detection limit using gold nanoparticles and DNAzyme separately.[162] The design of the colorimetric sensor is shown in Figure 12. It is based on the 8-17 DNAzyme selected, which is again highly specific for Pb^{2+} cleavage. When AuNPs are added to DNAzyme complex without Pb^{2+}, a large extent of AuNPs aggregation (purple-blue AuNPs aggregates) in the presence of high salt conditions is observed. In the presence of Pb^{2+}, the enzymatic strand is able to cleave and release ssDNA products that bind to AuNPs and prevent aggregation, resulting in a red colored solution. The detection limit of the sensor has been improved to a concentration of 3 nM of Pb^{2+} ions in a fast and simple label-free colorimetric sensor.

To extend the applicability of this methology, Lu et al. reported the application of the same sensing mechanism in an easy-to-use dipstick test, applied to the detection of lead in paints, that used immobilized nanoparticle−DNAzyme conjugates on a lateral flow device.[163] In Figure 13 a scheme of the lateral flow test is depicted showing the assembled lateral flow device (13A), a negative control (13B), and a positive control (13C) of lead-containing paints. A real picture of the lateral flow strips after the sensing assay is shown in Figure 13D. The high detection limit achieved with this sensing device (5 μM of Pb^{2+}) is a limitation for its applicability in water samples, where lower levels need to be reached.

DNAzyme in combination with AuNPs have also been used for Cu^{2+} detection.[164] However, a ligation-based DNAzyme system was used due to the intrinsically more sensitive system for the lack of background in comparison to the ones described for Pb^{2+}. In addition, the optical Cu^{2+} detection using DNAzyme has been described without the use of nanostructurated material.[165] This probe simultaneously employs two catalytic functions: (1) a Cu^{2+}-dependent self-cleavage catalytic activity to detect Cu^{2+} ions and (2) an HRP-mimicking function to give the colorimetric readout signal. The method exhibits a sensitivity of 1 μM (65 ppb) of Cu^{2+} in drinking water.

Although to date the majority of the colorimetric AuNPs sensing strategies have used nucleic acids as sensing elements, Naik et al. demonstrated the potential of peptide-functionalized AuNPs as colorimetric sensors for heavy metals.[166] Peptides are versatile ligands, and complexation with metal ions is driven by the interaction of the peptide backbone and amino acid side chain, and hence, modification in the peptide sequence results in a change in metal speciation and coordination chemistry.

The above-described plasmonic coupling of self-assembled nanoparticles systems involving synthetic or biological receptors has some limitations. It cannot provide nanoscopic resolution due to the aggregation of many particles nor does it provide molecular specific information about the metal ions that are being detected. In order to overcome the limitations of these techniques, Lee et al. reported a nanospectroscopic metal ion detection technique based on metal−ligand coordination chemistry as well as on plasmonic resonance energy transfer (PRET).[167] This technique improves the ones described above in terms of sensitivity and selectivity due to two different aspects: reducing the dimensions of the detection site to a single nanoscale probe and achieving molecular specificity due to the selective formation of a resonant metal−ligand complex on the surface of the gold nanoplasmonic probe and ion-specific resonant quenching in the Rayleigh scattering (see Figure 14). The detection system is applied for Cu^{2+} ions sensing in aqueous solution using ethylenediamine ligands. Coordination of Cu^{2+} ions with an ethylenediamine-functionalized probe leads to striking changes in the Rayleigh scattering profile, showing a drastic decrease in intensity with a concomitant decrease in dark-field transmittance, making possible the detection of Cu^{2+} ions at concentrations down to 1 nM.

5.2. Light Emission Fluorescence Techniques

Among light emission techniques, fluorescence has been widely used for the determination of heavy metals in environmental and

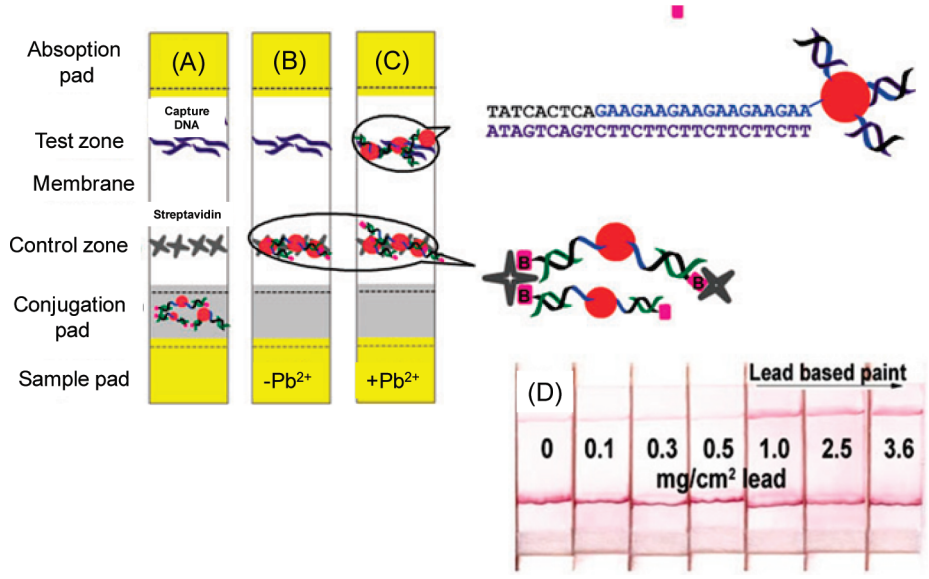

Figure 13. (A) Assembled lateral flow device and (B) negative control. In the absence of Pb^{2+}, AuNPs-uncleaved substrate is captured at the control zone via streptavidin−biotin interaction, producing a single red line. (C) Positive test. Substrate is cleaved in the presence of Pb^{2+} and the AuNPs-cleaved product migrates beyond the control zone to be captured at the test zone by hybridization to cDNA. Two red lines are produced. (D) Real picture of the detection of lead in paint around the US federal threshold for leaded paints (1 mg cm^{-2}) using the dipstick designed. Reprinted with permission from ref 163. Copyright 2010 Royal Society of Chemistry.

clinical samples. Fluorescence is a member of the ubiquitous luminescence family of processes in which susceptible molecules excited to high energy levels decay to lower levels, emitting transition-allowed radiation. An important feature of the sensing systems based on fluorescence is their highly sensitive detection compared to absorbance-based techniques.[168] While light absorbance is measured as the intensity difference between light passing through the reference and the sample, in fluorescence the intensity is directly measured. Fluorescence sensing requires a change in a spectral property as a response to the change of the analyte. Changes can occur in the intensity, excitation or emission spectra, anisotropy, or lifetime of the sensing probe. However, the fluorescence intensity is the most used parameter for heavy-metal detections due to the direct sensing response.

Sensors based on metal ion induced changes in fluorescence appear to be particularly attractive, and they are one of the first choices because of the simplicity and the low detection limits that may be achieved by using this technique.[169] Thus, the synthesis of new molecules that specifically bind metal ions with fluorescent signal transduction has drawn worldwide attention.[170,171] Fluorescence typically occurs from aromatic molecules due to the rigid conjugated structure and the high ring density of π electrons.

The complexation of a metal ions results in either enhanced fluorescence, also termed chelation-enhanced fluorescence (CHEF), or in a decreased fluorescence−chelation-enhanced quenching (CHEQ). Mechanisms commonly involved to explain the response of fluorescent sensors to heavy metals are electron transfer (ET) and charge transfer (CT). These two general categories include photoinduced electron transfer (PET), intramolecular charge transfer (ICT), and photoinduced charge transfer (PCT).[170] Some details about the different strategies that take advantage of turn-off or turn-on of the fluorescence used for heavy-metal detections will be given in the following sections.

In addition to fluorescence techniques, chemiluminescence and electrochemiluminescence are also being applied for heavy-metal ion detection.[172,173]

5.2.1. Turn-off Detection. As happens with the light absorption based techniques, methods for Hg^{2+} ions sensing are the most reported ones due to the well-known high toxicity of this metal.[174] Because Hg^{2+} ions have a propensity to quench fluorophore emission, turn-off detection is a facile approach for monitoring this metal. Fluorescence quenching by heavy-metal ions is a well-documented phenomenon and occurs by a number of pathways that include spin−orbit coupling, ET, and CT.[175−177]

A strategy that has recently been employed for turn-off detection of Hg^{2+} is metal ion replacement.[178] This approach takes advantage of the ability of various metal ions to differentially modulate the emission of a single ionophore. Wu et al. demonstrated the fluorescent quenching of a Zn^{2+} complex with a phenylene-bridged bis(pyrrol-2-ylmethyleneamine) ligand when Zn^{2+} cation is varied by Hg^{2+} cation (Figure 15A). As shown in Figure 15B, the addition of Hg^{2+} ions causes the decomposition of the complex and the formation of a nonemissive dinuclear complex. Figure 15C shows the fluorescence change in CH_3CN with the concentration of Hg^{2+} ions (from 2.67×10^{-6} to 1.60×10^{-5} M). Although the sensing strategy is interesting, the detection limits achieved are rather high, which would affect the real application possibilities.

Other strategies have been described for turn-off sensing, such as the ones described by Métivier et al.[179] In this work, based on calixarene fluoroionophores with dansyl reporting groups, the fluorescence quenching upon the increase of the Hg^{2+} ions concentration is attributed to a nonradiative energy transfer process consisting of the reduction of Hg^{2+} ions by the excited dansyl chromophore and the subsequent back electron transfer, which returns Hg^{2+} ions to their ground state (see Figure 16A). Figure 16B shows the corrected emission spectra of the described fluoroionophore in the presence of increasing concentration of

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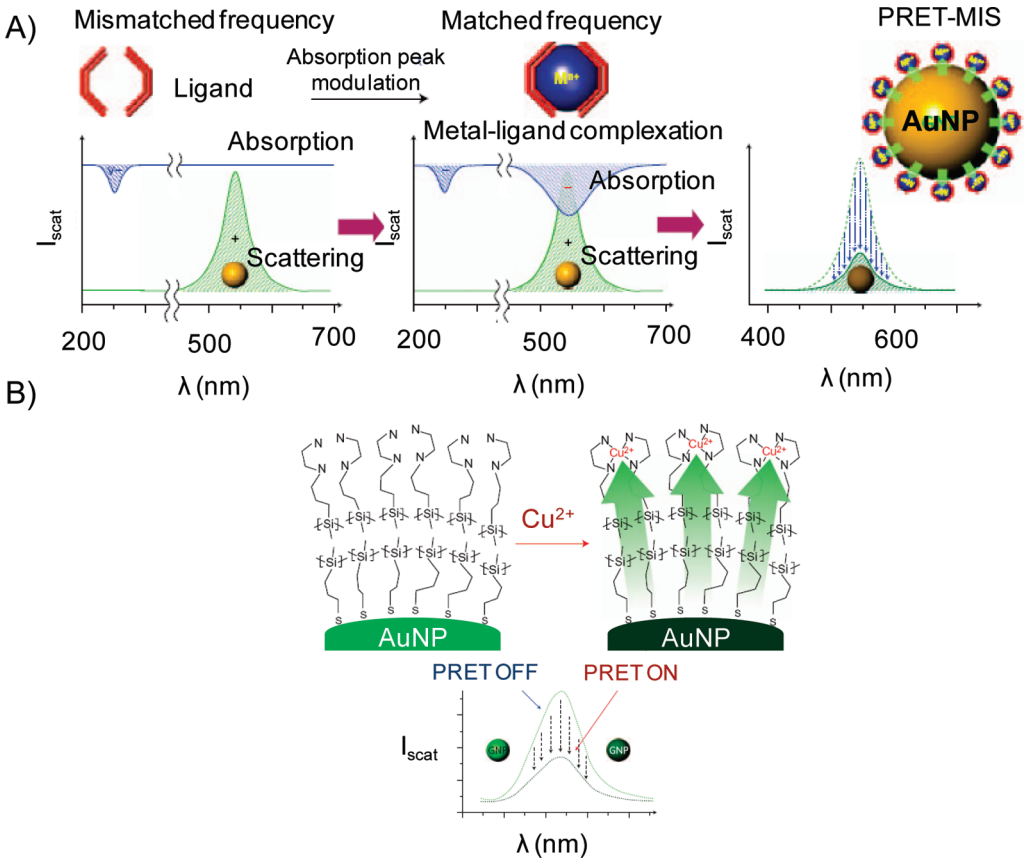

Figure 14. (A) There is no spectral overlap between ligands without the metal ion and the AuNPs (left). When the electronic absorption frequency of the metal−ligand complex matches with the Rayleigh scattering frequency, the selective energy transfer is induced by this spectral overlap (middle) and the distinguishable resonant quenching on the resonant Rayleigh scattering spectrum is observed (right). (B) A schematic illustrating the detection of Cu^{2+} via plasmonic resonance energy transfer (PRET) between a single AuNP and conjugated resonant complexes $[Cu(TMSen)_2]^{2+}$ (the hydrogen atoms are not shown), and the expected Rayleigh scattering profile (I_{scat}, scattering intensity). Reprinted with permission from ref 167. Copyright 2009 Nature Publishing Group.

Hg^{2+} ions. The detection limit of the sensing system for the Hg^{2+} ions is 0.3 μM.

5.2.2. Turn-on Detection. The sensors that show fluorescence enhancement on binding to the cation of interest [turn-on fluorescent sensors or CHEF (chelation-enhanced fluorescence) sensors] are preferred because they allow a lower detection limit and a high-speed spatial resolution via microscope imaging. This is the case of a terphenyl-based receptor (molecule **5**, Table 2) recently described by Bhalla et al. together with two other fluorescent molecules based on terphenyls which have pyrene and quinoline moieties.[46] Upon addition of increasing amounts of Hg^{2+}, a remarkable enhancement of fluorescence intensity is shown. This enhancement is attributed to the formation of the metal complexes as a result of the suppression of the photoinduced electron transfer (PET) from imino nitrogen to the photoexcited quinoline or pyrene moiety (the energy of the nπ^* state of the emission is increased and that of the $\pi\pi^*$ state of emission of the quinoline or pyrene group becomes the lowest excited state). In the PET mechanism, electrons are induced in the free ligand by exciting radiation to transfer from lone pairs on donor atoms such as *N*-donors to the π-system of the fluorophores and in so doing quench their fluorescence. Involvement of these same lone pairs in forming bonds to metal ions lessens the PET quenching effect, leading to the CHEF effect, where metal ions can be sensed by the increased fluorescence intensity.

Moreover, some of the molecules described by Bhalla et al. present crown rings moieties that further increase the fluorescence enhancement by the involvement of the electrons of the oxygen atoms of the crown in the PET phenomenon, which makes the receptor very weakly fluorescent and the percentage of fluorescent enhancement is greater on coordinating with the specific metal ion. Among these receptors, the lowest detection limit achieved has been 4×10^{-8} M of Hg^{2+} ions in THF/H_2O.

A more simply structured molecule has been applied for Hg^{2+} ion selective turn-on fluorescent sensor by Li et al.[180] The receptor is an amine-based molecule with both imine and aniline groups. Hg^{2+} ions are shown to interact with the aniline site, provoking a strong metal-induced intramolecular electron transfer (MICT) followed by a push−pull system that cause a distinct change in the optical properties (color change and fluorescence enhancement) of the receptor.

Vicens et al.[48] designed a *N*-tripodal molecule consisting of two branched calixarene units (each bearing one amido pyrenylmethyl moiety), a Hg^{2+}-induced fluorescence resonance energy transfer (FRET) chemosensor (molecule **7**, Table 2). Addition of Hg^{2+} ions to a solution of **7** results in highly enhanced fluorescence due to the energy transfer from the pyrenyl excimer to a ring-opened rhodamine moiety. A possible structure according to this mechanism is shown in Figure 1B,C. Figure 1A shows UV−vis spectra of **7** (0.015 mM) in CH_3CN in

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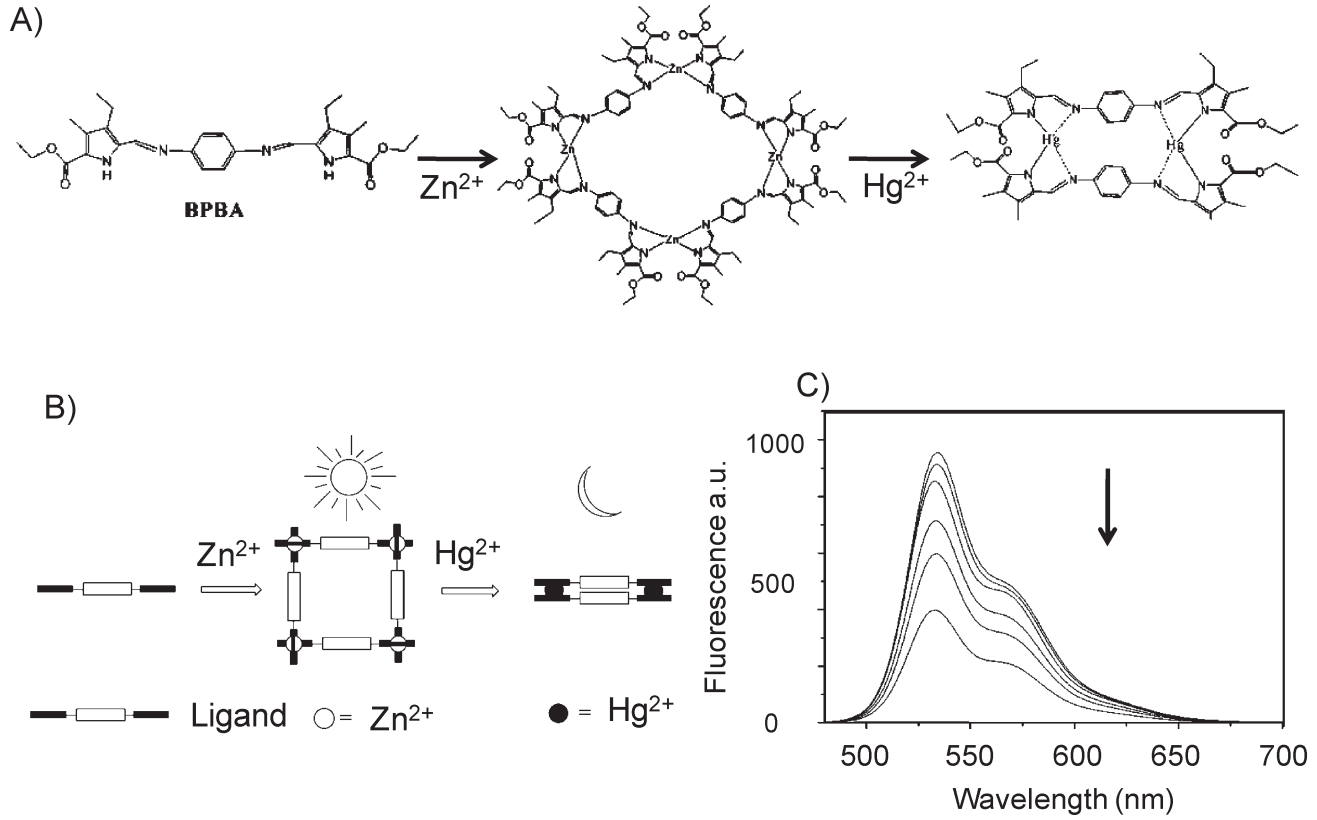

Figure 15. (A) Process of the complex (BPBA, phenylene-bridged bis(pyrrol-2-ylmethyleneamine)) transition. (B) Ratiometric Zn^{2+} sensor and strategy for Hg^{2+} selective recognition by central metal ion replacement. (C) Fluorescence change in CH_3CN with the concentration of Hg^{2+} (2.67×10^{-6}, 4.0×10^{-6}, 5.33×10^{-6}, 6.67×10^{-6}, 8.0×10^{-6}, and 1.6×10^{-5} M, respectively). Reprinted with permission from ref 178. Copyright 2006 American Chemical Society.

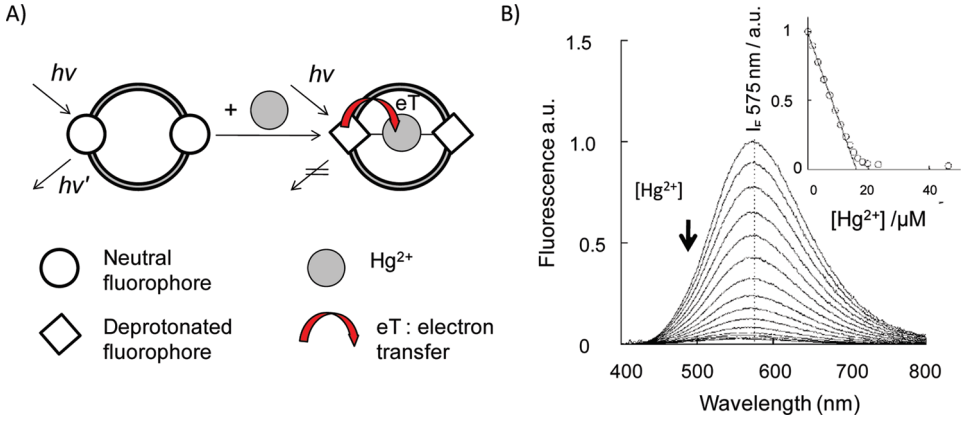

Figure 16. (A) Schematic representation of the Hg^{2+}−calixarene fluoroionophore complex and involved photoinduced electron transfer. (B) Corrected emission spectra of calixarene fluoroionophore in the presence of an increasing concentration of Hg^{2+} in CH_3CN/H_2O (60:40 v/v) at pH 4.0. λ_{exc} = 350 nm. Inset: Calibration curve as a function of mercury concentration. Reprinted with permission from ref 179. Copyright 2004 Wiley-VCH.

the presence of various metal cations, including the color change of 7 upon addition of Hg^{2+} ions.

Although most of the reported works on fluorescent sensing are based on Hg^{2+} ion detection, other heavy-metal cations detection systems have also been described.[181] Actually, Qian et al. recently described a cadmium-selective turn-on fluorescent sensor based on the PET mechanism that can operate in aqueous solution and also in living cells. The living cell image experiments presented in their work demonstrate its value in the practical application of biological systems.[171]

5.2.3. Biobased Turn-off Detection. Fluorescence methods for heavy-metal sensing based on biomolecules such as proteins,[66] antibodies[75,182] or nucleic acids[183−186] are being exploited due to their specificity, water-solubility, and operability under physiological conditions. In 2007 Varriale et al. reported a protein-based fluorescence cadmium biosensor using metallothioneins (MTs).[66] MTs are small cysteine-rich proteins that bind heavy metals such as zinc, cadmium, copper, or mercury. Mouse MTs bind Cd^{2+} ions with higher affinity than other metals. With these basics, Varriale et al. reported the use of a

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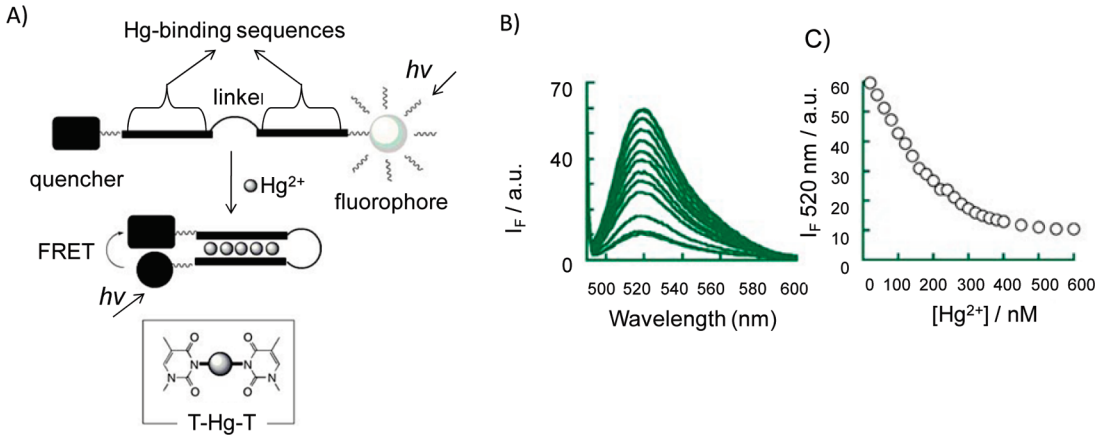

Figure 17. (A) A schematic representation of the hairpin structure induced by Hg^{2+} ion mediated T−Hg−T pair formation, which results in the quenching of fluorescence from the fluorophore. (B) Fluorescence response (10 Nm) upon addition of Hg^{2+} ion (0, 20, 40, 60, 80, 100, 120, 140, 160, 200, 300, 400, 500, and 600 Nm). The intensity of fluorescence emission decreased as the Hg^{2+} ion concentration increased. (C) Fluorescence emission intensity (520 nm) versus Hg^{2+} concentration. A buffer solution of 3-(*N*-morpholino)propanesulfonic acid (10 Mm, pH 7.0), NaCl (25 Mm), $NaNO_3$ (500 Mm), and ethylenediamine (0.1 Mm) was used. Reprinted with permission from ref 184. Copyright 2004 Wiley-VCH.

mouse MT to selectively detect cadmium ions in water solution labeled with an extrinsic fluorescence probe, rodhamine.[66] The followed strategy consists of binding the rodhamine-labeled MT to a previously Zn^{2+} ion saturated Chelex resin. Since the affinity for cadmium is higher than for zinc, cadmium causes the displacement of the rodhamine-labeled MT. Increasing concentrations of cadmium provoke a fluorescence intensity decrease. The limits of detection achieved are lower than 0.5 μM in water samples.

Regarding fluorescent antibody sensing systems, Matsushita et al. presented a cofactor approach employing stibenylboronic acid in combination with blue fluorescent antibody 19G2 as a fluorescent signal tuner and amplifier to detect Hg^{2+} ions.[182] The key of the mercury specificity in this work is not the antibody but the stilbene derivate. Arylboronic acid moiety undergoes transmetalation with Hg^{2+} ions to form aryl mercuric chlorides, further dramatically decreasing the fluorescence of the antibody.

As described in previous sections, the use of nucleic acid based systems provides an attractive methodology for mercury sensing. Fluorescence methods are not an exception. Exceptional detection limits for mercury have been achieved using nucleic acid derived sensing probes. Most of these systems are based on the fluorescence resonance energy transfer (FRET) process between a fluorophore (F) and a quencher (Q) moiety located on the 3′ and the 5′ oligonucleotide chain (ON) ends.[184−186] The mechanism of FRET involves a donor fluorophore in an excited electronic state, which may transfer its excitation energy to a nearby acceptor chromophore in a nonradiative fashion through long-range dipole−dipole interactions.

In 2004, Ono et al. described a biosensor based on a fluorophore−ON−quencher (F−ON−Q) system divided into two parts: the thymine-rich mercury-binding sequence and the linker sequence.[184] The mechanism for the detection of Hg^{2+} ions by F−ON−Q is shown in Figure 17A. In the presence of Hg^{2+} ions, mercury-mediated base pairs (T−Hg−T) are formed between thymine residues from two Hg-binding sequences in the oligonucleotide to give rise to a hairpin structure. Both termini of the oligonucleotide chain are brought close to each other upon the formation of the hairpin structure, which leads to the FRET process between the F and the Q moieties (Figure 17A). Consequently, this provokes a significant quenching of the fluorescent emission relative to a random coil. Figure 17B shows the fluorescence response of the sensor upon addition of Hg^{2+} ions. The intensity of fluorescence emission decreases as the Hg^{2+} ion concentration increases. Fluorescence emission intensity (520 nm) versus Hg^{2+} concentration is shown in Figure 17C.

Using the same sensing strategy, Chang et al. recently described a thrombin-binding aptamer probe labeled with the donor carboxyfluorescein and the quencher 4-([4-(dimethylamino)phenyl]azo) benzoic acid for sensitive detection of Pb^{2+} and Hg^{2+} ions.[186]

5.2.4. Biobased Turn-on Detection. Being sensitive and selective, turn-off-based systems may give a "false positive" result, caused by external quenchers or other environmental factors that can induce fluorescence decrease. Turn-on fluorescence based systems involving the use of biomolecules are being developed in order to improve this methodology. Lu et al. have demonstrated that the combinatorial selection of DNAzymes can be employed for heavy-metal detection, developing a catalytic beacon for mercury based on a uranium-specific DNAzyme.[187] The optimal DNAzyme was labeled with fluorophores and quenchers to construct a catalytic beacon, and a mismatch was introduced in the substrate binding arm and it was mixed with UO_2^{2+} ions to become a mercury sensor. In the absence of mercury, the DNAzyme is incapable of binding UO_2^{2+} ions, because its active secondary structure cannot be formed due to the mismatched introduced. The addition of mercury ions quickly restores the stem-loop structure with the aid of UO_2^{2+} ions and activates the DNAzyme to cleave the substrate, releasing the fluorophore-labeled piece and resulting in an increased fluorescence.

Other variations of the work of Ono et al., with the aim of developing turn-on systems instead of turn-off ones, have been recently reported by Lu et al.[185] In this work, the folding induction of the fluorophore-labeled DNA strand by thymine−Hg−thymine formation releases the hybridized DNA strand with the quencher, which is previously hybridized to the strand labeled with the fluorophore, and consequently increases the fluorescence of the probe. The sensor has a detection limit of 3.2 nM Hg^{2+} ions in water.

Conjugated polymers have been used for their optical amplification properties combined with oligonucleotides and DNA intercalators.[182] The electrostatic interactions between the

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positively charged polymers and the negatively charged oligonucleotides bring the conjugated polymers and intercalators into close proximity to one another, and efficient energy transfer from conjugated polymers to intercalators occurs when Hg^{2+} ions are present. The energy transfer process results in the amplification of fluorescence signals as a result of the large overall absorption cross sections and collective optical response of the conjugated polymers.

5.2.5. Nanostructured Light-Emitting Sensors. It is described that metal nanoparticles (MNPs) can quench or enhance the fluorescence intensity of a fluorophore.[188,189] Fluorescence quenching and enhancement are determined by the interactions between fluorophores and metal surfaces, depending on the location of the fluorophore around the particle, the orientation of its transition dipole moment relative to the particle surface, and the particle−fluorophore distance. When the separation distance between fluorophores and metallic surfaces is smaller than 5 nm, strong quenching of fluorescence emission and a dramatic reduction of lifetimes of the excited states are observed.[190−194] In contrast, when the distance is in the range of 10−20 nm, enhanced fluorescence emission can result because of the local concentration of the incident excitation field by metallic nanoparticles.[195−197]

AuNPs have highly size-dependent fluorescence properties, which make them very sensitive to the environment. For this reason, some works involving fluorescence based in AuNPs have been reported for heavy-metal detection.

AuNPs coated with different alkanethiol ligands have been demonstrated to have fluorescent properties.[198] Moreover, because the fluorescent AuNPs possess long fluorescent lifetimes (more than 20 ns), they present great potential for their use in heavy-metal sensing. In this way, Chang et al. presented one type of alkanethiol-modified AuNPs (11-mercaptoundecanoic acid coated AuNPs, AuNPs−MUA) for Hg^{2+} ions sensing.[198] In the presence of Hg^{2+} ions AuNPs−MUA solution undergoes to a fluorescence quenching phenomenon, due mainly to the aggregation caused by the interaction of Hg^{2+} ions through an ion-templated chelation process with the carboxylate groups present on the AuNPs' surface. Comparing this technique to those based on absorption wavelength previously described, fluorescence is more sensitive to changes in the AuNPs' size.

Tian et al. reported another turn-off fluorescent AuNP-based system to detect the presence of Hg^{2+} ions.[199] The quenching of the fluorescence is attributed to the enhanced PET process from aniline subunit to naphthalimide chromophore of the AuNPs' coating ligands as a result of their approaching to each other. The sensing ability of their sensor can be tuned to the maximum efficiency level by the use of a suitable surfactant in its critical micelle concentration.

The quenching effects of AuNPs have been used in many different works.[200,201] The sensor system described by Zhu et al.[202] for sensing Cu^{2+} ions is based on the modulation of the photoluminiscent quenching efficiency between the chromophore (perylenebisimide) and AuNPs in the presence of Cu^{2+} ion. In the absence of Cu^{2+} ion, the fluorescent chromophore containing pyridyl moieties coordinates to AuNPs through weak N and Au interactions, which causes quenching of the fluorescence of the chromophore. In the presence of Cu^{2+} ions, AuNPs are displaced from the chromophore because of the stronger coordination ability of Cu^{2+} ions with pyridyl moiety in comparison to that of AuNPs giving rise to a turn-on of the fluorescence of the chromophore.

AuNPs enhancement functions have been employed to substantially improve the performance and sensitivity of various biosensing systems. Ye et al. recently developed a Hg^{2+} sensing system using thymine−Hg^{2+}−thymine coordination chemistry and fluorescence polarization enhanced by AuNPs.[203] The fluorescence polarization value P is sensitive to changes in the rotational motion of fluorescently labeled molecules. The P value of a fluorophore is proportional to its rotational relaxation time, which is turn depends upon its molecular volume. If a molecule is small, it will rotate faster and hence will have a smaller P value. The work of Ye et al. consists of two complementary probes that contain six strategically placed T−T mismatches. Probe A is modified with AuNPs and probe B with a fluorescent dye. When Hg^{2+} ions are present, the hybridization of probes A and B takes place with the consequent increase of the P value. The increase is greater than when AuNPs are not present. The enlargement of the molecular volume of the AuNPs functionalized with thymine−Hg^{2+}−thymine complexes results in a substantial increase in the P value upon the rotational correlation time. The substantial sensitivity improvement is mainly attributed to the slower rotation of the fluorescent unit when dye-labeled oligonucleotides are hybridized to the AuNPs surface.

Semiconductor quantum dots (QDs) represent a revolution in the application of fluorophores in fluorescent labeling compared to traditional organic dyes due to the high photochemical and chemical stability of their quantum yield. Therefore, the use of QDs as alternative for organic dyes in sensing, biosensing, and imaging is increasing. In a recent work reported by Wu et al., the combination of the benefits of DNAzymes and QDs to develop a highly sensitive and selective sensor for Pb^{2+} and Cu^{2+} ions is studied.[204] One of the advantages of using QDs instead of conventional organic dyes is the possibility of multiple detection using a single laser excitation. In this way, Pb^{2+} and Cu^{2+} ions can be monitored and quantified at the same time.

Other kinds of semiconductor nanocrystals such as CdTe nanocrystals have also been applied in the heavy-metal-sensing field.[205]

Despite the reported advantages of semiconductor nanocrystals in the sensing field, there is the need to understand the potentially harmful side effects of these materials, due to the fact that QDs may also pose risks to human health and the environment under certain conditions. A trade-off between their contribution to the sensing field and their drawbacks related to the environment and human health should be assessed.

6. OTHER MATERIALS AND STRATEGIES

Finally, it is important to note that other materials beside nanoparticles and nanotubes, such as mesoporous materials or optical fibers, are being used for heavy-metal detections.

Mesoporous materials offer an alternative means of immobilizing small molecules.[206] One of the strategies to modify these materials is to include triethoxysilane groups in the receptors, which will chemically bind to the substrate.[207,208]

Organic−inorganic hybrid silica nanomaterials with large specific surface areas have been recently reviewed by Jung et al.[209] Jung et al. reported a selective chromogenic sensing receptor for Cu^{2+} ions which was immobilized onto a silica nanotube, giving a red-colored solid.[210] Upon the addition of Cu^{2+} ions, the red-colored solution undergoes a color change from red to slightly yellow. The change in the color is attributed to the formation of the complex between the carbonyl groups of

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the receptor attached to the silica nanotubes. As an extension of these works, the same research group reported on the immobilization of a fluorescence receptor onto the surface of silica nanotubes but this time for Pb^{2+} ions sensing.[211] In this case, the sensor exhibits a selective change in fluorescence monomer and excimer emission due to the fact that the two oxygen atoms of the two amides that the ligand contains bind to Pb^{2+} and cause conformational changes of the two amides, giving a decreased excimer emission.

Optical fiber chemical sensors for heavy metals have also been described. Optical fibers are useful as sensing platforms because they accommodate multiplexing and can be delivered to remote locations, such as deep waters, which could be of interest for heavy-metal sensing.[212] The modification of this type of material is usually by polymerizing the carrier into a quartz glass plate surface treated with a silanizing agent to prevent the leakage of the receptor. Yu et al. reported on the use of an optical fiber consisting of a liophilic porphyrin dimer for Hg^{2+} ions detection.[212] The use of twin porphyrin rings enhances the selectivity of the sensor toward Hg^{2+} ions with respect to other transition metals. The basis of the Hg^{2+} ions determination lies in the quenching effect provoked by the metal while coordinating to the ligand which exhibits high fluorescence due to the conjugated double bond system and the high mobility of its π-electrons.

Atomic force microscopy (AFM) can also be applied as a quantification tool for sensing purposes. A recent example shown by Fang et al. consists of the use of graphene surfaces modified with 1-octadecanethiol for Hg^{2+} ions detection.[213] Although it is a sophisticated sensing setup which even lacks sensibility and sensitivity (detection limit of 10 ppm Hg^{2+} ions), it opens up new opportunities for graphene-based electronics as heavy-metal sensors.

Chemical sensing using nanomotors has also been described by Wang et al.[214] This sensing strategy is based on the change of the acceleration of bimetal nanomotors when heavy metals (e.g., Ag^{+} ions) are present. This work seems to be very interesting for microdevice applications and probably toxicity-related studies in in vitro systems rather than for robust and in-field applications.

7. CONCLUSIONS AND FUTURE PERSPECTIVES

Research in the area of design and fabrication of sensors and biosensors for heavy-metal detection has become of great interest to a variety of scientific communities ranging from biological and chemical sciences to engineering communities. Several tools and techniques for heavy-metal detection based on the latest trends in (nano)materials and micro- and nanotechnologies are being developed. These are offering new opportunities for new heavy-metal-sensing technologies with advantages such as high sensitivity and selectivity, rapidness, and cost efficiency due also to the possible integration with existing simple platforms and technologies beside the development of new ones.

Optical techniques based on absorption of light by nanoparticles make use of the colorimetric and size-dependent properties of nanoparticles to develop simple, chemistry-based, fast, user-friendly, and reliable in-field devices. Among optical techniques, fluorescence is found to be of special interest for the determination of heavy metals in environmental and clinical samples. An important feature of the sensing systems based on fluorescence is their highly sensitive detection compared to absorbance-based techniques. In addition fluorescence techniques offer a variety of detection formats. Strategies such as fluorescence turn-off or turn-on in connection to the use of either biologically or chemically modified nanoparticles are offering novel, interesting tools.

Electrochemical techniques coupled to nanomaterials are also showing to be applicable to fast-responding electrochemical sensors for heavy-metal detection in online monitoring or flow-injection systems. The combination of nanomaterials such as MNPs or CNTs among others with synthetic or biological receptors possesses a high degree of specificity, which makes developed sensing formats attractive for the design and fabrication of integrated detection systems for real sample applications. Moreover, the incorporation of nanostructured materials in the development of such sensors has led to an increase in the sensitivity, sensibility, and reproducibility of these sensors, achieving very low detection limits (in the range of ppt).

The mentioned optical and electrochemical based systems, thanks to the use of nanomaterials and nanotechnologies, are offering opportunities for the development of novel sensing platforms, such as simple colorimetric kits, lateral flow paper based devices, or electrical/electrochemical disposable electrodes that can be of special interest for in-field detections of heavy metals in a simple and cost-effective way. In addition, due to the synergy of nanomaterials' properties with nanotechnologies, the development of highly integrated detection systems that can even ensure *online* or even implanted heavy-metal detectors applicable not only to environmental studies but also to other fields like clinical analysis or safety and security can be previewed.

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BIOGRAPHIES

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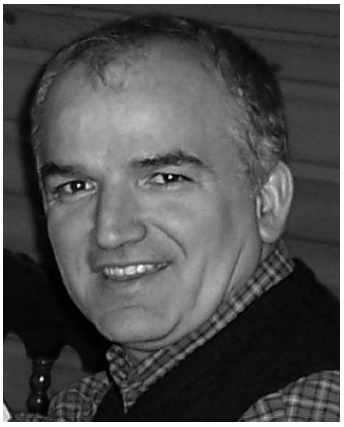
Josefina Pons studied chemistry at Universitat Autònoma de Barcelona (UAB) and received her doctoral degree at the same university in 1986. She became an assistant professor two years later and finally a Chemistry Professor at Universitat Autònoma de Barcelona in 1988. She has been a member of the group "Coordination Chemistry with Functionalized Ligands" at the Chemistry Department of UAB since 1994. She is an expert in the design and synthesis of novel ligands for various applications, including heavy-metal detection.



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