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REVIEW

Analytical strategies based on quantum dots for heavy metal ions detection

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Abstract. Heavy metal contamination is one of the major concerns to human health because these substances are toxic and retained by the ecological system. Therefore, in recent years, there has been a pressing need for fast and reliable methods for the analysis of heavy metal ions in environmental and biological samples. Quantum dots (QDs) have facilitated the development of sensitive sensors over the past decade, due to their unique photophysical properties, versatile surface chemistry and ligand binding ability, and the possibility of the encapsulation in different materials or attachment to different functional materials, while retaining their native luminescence property. This paper comments on different sensing strategies with QD for the most toxic heavy metal ions (i.e., cadmium, Cd^{2+}; mercury, Hg^{2+}; and lead, Pb^{2+}). Finally, the challenges and outlook for the QD-based sensors for heavy metals ions are discussed. [DOI: 10.1117/1.JBO.19.10.101503]

Keywords: quantum dots; sensors; heavy metal ions; bio-inspired systems.

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1 Introduction

The detection and quantification of ions in different kinds of samples, i.e., environmental or biological samples, have long been of paramount importance for the scientific world. In fact, the development of novel methods based on nanoparticles (NPs) for sensing ions has experienced significant growth during the last decade.[1–8]

Among ions, heavy metal ions such as lead, cadmium, and mercury have attracted more attention due to their high toxicity and detrimental health effects.[7–11] Exposure to even very low levels of lead, cadmium, and mercury ions is known to cause cardiovascular diseases, cancer mortality, damage to liver, kidneys, and central nervous system, neurological, reproductive and developmental disorders, which are more serious problems for children particularly.[12,13] Accordingly, great efforts have been devoted to the development of the selective and sensitive detection methods.[14,15]

Traditional heavy metal analysis methods include atomic absorption spectrometry[16–18] and inductively coupled plasma mass spectrometry;[19–21] however, these instrumentally intensive methods only measure the total metal ion content, and often require extensive sample preparation. Electrochemical analyses have also been commonly used to detect metal ions present in biological or environmental specimens. Among the different electrochemical techniques, voltammetric and potentiometric techniques are the most reported for heavy metals detection.[22,23] However, selectivity, long-term stability, compatibility with aqueous environments, and ease of on-site sampling remain significant challenges for many of these techniques.

Optical detections (via fluorescence changes or colorimetric changes) are the most convenient and promising methods due to their simplicity and low detection limits.[24,25] One important advantage of a fluorescent probe would be the possibility of intracellular detection. In this area, the coupling of ion-sensitive fluorophores to NPs has been of special interest because they can be detected easily using fluorescence microscopy, therefore, allowing the monitoring of dissolved ion concentrations in living cells and organisms.[1–5]

Quantum dots (QDs) have been used in spectroscopic detection of metal ions since 2002,[26] and a wide range of ions have been investigated—for both transition metals[27] and heavy metals.[28,29] However, we focus here on the development of QD-based sensors for the most relevant toxic heavy metal ions, Cd^{2+}, Pb^{2+}, and Hg^{2+}. Although a number of original papers have been reported in the literature exploiting the particular properties of QDs for sensing heavy metal ions, so far there is not published a comprehensive review illustrating the different QD-based strategies for this particular range of target analytes.

We begin by describing some strategies to get ion selectivity when chemosensors for metal ions are designed. We follow with a brief summary on the use of a QD as a "passive" fluorescent label that replaces traditional organic fluorophores in many conventional assays, giving examples for the particular application of heavy metal ions detection. Then, we focus on "active" QD labels, such as charge transfer (CT) QD-based systems and energy transfer QD-based systems, which include fluorescence resonance energy transfer (FRET) or Dexter energy transfer (DET) mechanisms. In the last section, a special emphasis is made on the most innovative bioinspired QD-based sensors. These sensors use biological concepts, mechanisms, and functions as starting points on the design of novel sensing systems with improved analytical features. The functionalization of QDs with functional biomolecules (e.g., proteins, enzymes, nucleic acids, aptamers, and DNAzymes) has allowed the development

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of "bio-inspired" or "bio-mimetic" QD-based systems with great potential in the field of heavy metal ions analysis.

In summary, we provide a detailed evaluation of the different approaches developed so far for the analysis of the more toxic heavy metal ions (i.e., Cd^{2+}, Pb^{2+}, and Hg^{2+}) by using various types of QDs and in different configurations. A discussion of the analytical mechanisms behind each QD-based sensing system is also given.

2 Ion-Selectivity Strategies in Quantum Dot-Based Sensors

The critical requirement for the design of an ion-specific chemosensor is the selective binding of the target ion by the chemosensor. The selectivity of a QD-based sensor for specific metal ions can be achieved in different ways (Table 1). Here, we briefly point out the most useful ion-selectivity strategies which will be exemplified with articles commented throughout the following sections:

1. One way is using small molecules as chelating ligands (such as mercaptoacetic acid, MAA; L-cysteine, Cys; N-acetyl-L-cysteine, NAC; thioglycolic acid, TGA; glutathione, GSH; and dithizone, Dz), which present a higher affinity for some specific ions over others. For instance, MAA and Cys show a higher affinity for Hg^{2+} and Cu^{2+}, NAC for Hg^{2+}, whereas TGA and GSH present higher affinity for Pb^{2+}. The use of Dz is of particular interest because of its specific and strong Pb^{2+} binding capability. This strategy is not always as selective as others, but it is quite enough for many applications in which the potential interferences present in the medium are well known and controlled.
2. Approaches based on supramolecular chemistry, also called host-guest systems, open up new opportunities for developing ion-selective chemosensors. This strategy consists of functionalizing QDs with supramolecular ligands (i.e., crown ethers, calixarenes, cryptands, etc.) which act as host for the ions (guest). These ligands (host molecules) can be easily modified to modulate their selectivity toward different metal ions. For example, while the 18-crown-6 ether is able to selectively bind alkaline metal ions, mainly K^+, the 1, 10-diaza-18-crown-6 ether derivative exhibits a high Cd^{2+} selectivity over other transition metal ions, as well as alkali and alkaline earth metal ions. The design of a specific host group for target metal ions is the key point of this strategy.
3. Another way to get selectivity toward Hg^{2+} ions is based on the formation of HgS or HgTe particles on the surface of the QD after the addition of Hg^{2+}. The selectivity of this approach is attributed to the lower-solubility product constants (Ksp) of HgS and HgTe compared with those of other metal ions (CuS, CdS, ZnS, PbS, AgS, PbTe, etc.). The adsorption of the metal Hg^{2+} ions on the trap sites of the QD surface and concomitant formation of HgS or HgTe particles results in the surface passivation of the QDs.

Table 1 Different strategies to get ion selectivity in QD-based sensor.

Strategy	Selectivity	References
Based on small molecules as ligands		
Mercaptoacetic acid	Hg^{2+}	30, 31
L-cysteine	Hg^{2+}	32
N-acetyl-L-cysteine	Hg^{2+}	33
Thioglycolic acid	Pb^{2+}	34
Glutathione	Pb^{2+}	35–37
Dithizone	Pb^{2+}	38
Based on supramolecular chemistry		
Sulphur calix[4]arene	Hg^{2+}	39
Calix[6]arene	Hg^{2+}	40
1,10-diaza-18-crown-6	Cd^{2+}	41
Based on HgS or HgTe formation		
HgS particles	Hg^{2+}	42–45
HgTe particles	Hg^{2+}	46
Based on Hg^{2+}-sensitive organic dyes		
Spirolactam rhodamine derivative	Hg^{2+}	47
Based on metal-mediated DNA duplexes		
T—Hg^{2+}—T base pairs	Hg^{2+}	48–53
Pb^{2+}–stabilized G-quadruplex	Pb^{2+}	54, 55
Based on d^{10}—d^{10} metallophilic interactions		
Hg^{2+}—Hg^{2+} (HgS) QDs	Hg^{2+}	56
Based on designed metalloprotein		
Pb^{2+} ion-binding protein	Pb^{2+}	57
Based on DNAzymes		
Pb^{2+} ion-DNAzyme	Pb^{2+}	58–61
Cu^{2+} ion-DNAzyme	Cu^{2+}	58

4. The use of rhodamine-based Hg^{2+}-sensitive organic dyes is also a useful strategy to get selectivity in the development of sensing systems for Hg^{2+} detection. As example, a spirolactam rhodamine derivative can change its structure from the spirolactam state to the ring-opened state due to its complexation with Hg^{2+}. As a result, a new emission band appears which corresponded to the fluorescence emission of the dye in its open-ring state.
5. A more sophisticated strategy is to exploit the potential of oligonucleotides that selectively bind to some metal

ions to form stable metal-mediated DNA duplexes. This strategy has been widely used for Hg^{2+} sensing, since Hg^{2+} can selectively bind between two DNA thymine (T) bases and promote these T—T mismatches to form stable T—Hg^{2+}—T base pairs. Interestingly, these T—Hg^{2+}—T structures are more stable and have higher melting temperature than the Watson–Crick A—T pairs.

6. A less common strategy but not less interesting is based on the formation of specific and strong d^{10}—d^{10} interactions, also known as metallophilic interactions. Metallophilic bonds can occur between Hg^{2+} ions and Hg^{2+} present on the surface of the HgS QDs (i.e., $5d^{10}$—$5d^{10}$). Assuming the formation of a Hg^{2+}—Hg^{2+} metallophilic bond, the filled 5d and empty 6s orbital of each Hg^{2+} would overlap with each other, which then give rise to bonding and antibonding orbitals. These orbitals would reorganize leading to new energy-accepting levels on the Hg^{2+}, which facilitates the Dexter energy transfer to the Hg^{2+}. Based on this property, QD-based sensors for the selective detection of Hg^{2+} ions can be developed.

7. Metalloprotein can be designed to be selective towards specific ions; and therefore, they provide a useful way to achieve ion selectivity in the development of sensing systems. The advances in the protein design techniques have allowed adapting native ligand binding sites to selectively bind an alternative ligand of choice. Following this strategy, a Pb^{2+} ion-binding protein has been successfully generated and used for the construction of a selective QD-based sensor for Pb^{2+} ion detection.

8. The latest strategy consists in the use of metal ion-selective DNAzymes, which can be obtained with high specificity for any metal ion by using *in vitro* selection methods. The exceptional high metal ion specificity makes DNAzymes an excellent and general platform to sense metal ions.

3 Quantum Dot as Passive Fluorescent Labels

The simplest approaches are those in which the QD acts as a passive fluorescent label. Because the luminescence of QDs is very sensitive to their surface states, fluorescence transduction is based on the principle that chemical or physical interactions occurring at the surface of the QDs change the efficiency of the radiative recombination, leading to either photoluminescence activation or quenching. In general, the interaction of ions with QDs induces a fluorescence quenching that can be attributed to inner-filter effects, nonradiative recombination pathways, and electron transfer processes.[62] The observation of fluorescence enhancement is less frequent, and in these cases the mechanism ascribed for the observed increase in quantum yield is the passivation of trap states or defects on the surface of the QDs.[63,64] In any case, the changes induced by the direct interaction between the metal ions and the QD's surface, unmodified or functionalized with a given ligand, have allowed the sensitive detection of several toxic metal ions.

Many systems consisting of water-soluble QDs capped with different thiol ligands (e.g., mercaptoacetic acid, MAA; L-cysteine, Cys; and N-acetyl-L-cysteine, NAC) have been proposed for the development of QD-based sensors for heavy metal ions. In these systems, the ligand is, thus, simultaneously responsible for participating in the response to metal ions and for the solubility of QDs in the aqueous environment. Water-soluble mercaptoacetic acid-capped CdS and CdS/ZnS QDs (MAA-CdS and MAA-CdS/ZnS) were developed as fluorescent probes for Hg^{2+} ions. The method relies on the fluorescence quenching of the QDs observed in the presence of these ions. Under optimum conditions, the quenched fluorescence intensity of QDs was linearly proportional to the concentration of Hg^{2+} in the working range. The detection limits were in the nanomolar range, 4.2 nM for MAA-CdS[30] and 2.2 nM for MAA-CdS/ZnS.[31] In order to get a less toxic sensor, ZnS QDs modified by NAC (NAC-capped ZnS) were easily synthesized in aqueous medium via a one-step method.[33] The synthesized nanoparticles were applied to the trace determination of Hg^{2+} ions in water samples, achieving a detection limit of 5 nM.

In another work, monodisperse CdSe nanoclusters were prepared and functionalized with L-cysteine (Cys-capped CdSe).[32] These functionalized CdSe QDs exhibited strong specific affinity for Hg^{2+} through QDs interface functional groups. Based on the quenching of fluorescence signals of functionalized CdSe QDs, a simple, rapid, and specific array for Hg^{2+} was developed achieving a very low-detection limit (6 nM). The usefulness of this method was successfully demonstrated for Hg^{2+} detection in real samples (human urine and river water), as the results were in good agreement with those obtained by cold vapor atomic fluorescence spectrometry (CV-AFS). In a similar way, a method for the determination of Pb^{2+} was developed based on quenching of the fluorescence of TGA (thioglycolic acid)-capped CdTe QDs by Pb^{2+} in aqueous solutions.[34] To demonstrate their practical application, the proposed method was successfully applied to the analysis of Pb^{2+} in food samples (popcorn and instant noodles) with a Pb^{2+} concentration over $1.0\ \mathrm{mg} \cdot \mathrm{kg}^{-1}$ (ppm), and the results were satisfactory, i.e., consistent with those of flame atomic absorption spectrometry. According to EU legislation (EC/1881/2006), which sets maximum levels for chemical contaminants in foodstuffs, the Pb^{2+} content limits are $1.0\text{–}6.0\ \mathrm{mg} \cdot \mathrm{kg}^{-1}$ (ppm) depending on kind of food. Therefore, the proposed method presented enough sensitivity to fulfill the legal requirements. Among thiols, the use of glutathione (GSH), a linear tripeptide synthesized in the body, as QDs capping agent or ligand for the functionalization of QDs has stimulated recent research interests due to the biological significance of this molecule. GSH is not only an important water-phase antioxidant and essential cofactor for antioxidant enzymes, but it also plays roles in catalysis, metabolism, signal transduction, and gene expression. Thus, GSH-capped QDs as biological probe should be more biocompatible than other thiol-capping ligands. Moreover, GSH seems to be a very promising molecule, since GSH and its polymeric form, phytochelatin, are employed by nature to detoxify heavy metal ions in organisms. A sensor for Pb^{2+} was developed based on a selective fluorescence quenching of CdTe and CdZnSe QDs capped with GSH shells.[35] As a result of specific interaction, the fluorescence intensity of GSH-capped QDs was selectively reduced in the presence of Pb^{2+}, while no quenching response to alkaline and alkaline earth metal ions, Fe^{3+}, Al^{3+}, Ni^{2+}, and Zn^{2+}, was observed. A limitation of this sensor was the strong interference of Ag^+ and Cu^{2+} because they also showed a similar quenching effect as Pb^{2+} at similar concentration levels. In regard to the sensitivity, the detection limit was quite good (20 nM), although it was affected by the presence of a concentrated ionic mixture. In the presence of ionic mixtures, the system was still capable of Pb^{2+} detection with a detection limit as low as 40 nM, and only became less sensitive when the ionic mixture was as high as 50 μm. Similarly, GSH-capped ZnSe QDs were synthesized and used equally for the detection of Pb^{2+}.[36] This method presented improved sensitive and selective characteristics for the detection of trace Pb^{2+} in water, reaching a very low detection limit (0.71 nM). Since the U.S. Environmental Protection Agency (EPA) permits the maximum level of lead in drinking water to be 72 nM (15 ppb), this method amply complies with legal requirements. Gonçalves et al. also used GHS-capped CdTe QDs for detecting the presence of micromolar quantities of Pb^{2+}.[37] They carried out a parallel factor (PARAFAC) analysis of the fluorescence spectra to study the system. PARAFAC analysis of the excitation emission matrices of QDs acquired as a function of the Pb^{2+} ion showed that only one linearly independent component describes the quenching of the QDs by the Pb^{2+} ion, allowing robust estimation of the excitation and emission spectra and of the quenching profiles.

Even when the selectivity of these systems has been called into question several times, many articles have proved with experimental data that some ligands have a higher binding affinity for some ions than others. Therefore, this simple strategy can be useful for some practical applications. In order to increase the ion selectivity of the sensor, the use of supramolecular ligands, such as calixarenes, is an interesting and promising approach. Sulfur calixarene-capped QDs (Calix[4]-S-CdSe/ZnS) were prepared for the selective determination of Hg^{2+} ions in acetonitrile with high sensitivity (determination limit of 15 nM).[39] Similarly, but working in aqueous solution, Li et al. developed a simple method for the preparation of highly fluorescent and stable, water-soluble CdTe quantum dots in sol-gel-derived composite silica spheres coated with calix[6]arene.[40] These NPs allowed the ultrasensitive detection of Hg^{2+} ions, achieving a detection limit of 1.55 nM. The sensitivity of this method is quite good taking into account that the EPA sets the maximum level of mercury in drinking water at 10 nM (2 ppb). Both methods were based on the quenching of fluorescence in the presence of the target ions and exhibited superior selectivity than methods commented previously. By using dendrimers (i.e., highly branched macromolecules) as capping ligands, a sensor for Hg^{2+} was developed based on the quenching of the fluorescence of CdS QDs coated with polypropylenimine tetrahexacontaamine dendrimer generation 5 (CdS-DAB nanocomposites).[65] CdS-DAB nanocomposites allowed Hg^{2+} detection and quantification in aqueous solution, but they were also quite sensitive to the presence of Cu^{2+} and Pb^{2+} which could be considered as interfering species. These nanocomposites were shown to be less sensitive for Hg^{2+} ion than CdS QDs alone. This is due to the fact that the dendrimer confers to the QDs a high protection and stability so that the Hg^{2+} concentration has to be superior because the bonding between the dendrons (hydrogen bonding) is first disrupted by complexation and then the QDs by the quenching mechanism decreases the steady-state fluorescence properties. Thus, so far the use of dendrimers for the sensing of heavy metal ions has failed in terms of sensitivity and selectivity. Instead of working in solution as described in the above methods, Wang et al. proposed the use of fluorescent multilayer films fabricated with QDs for the detection of Hg^{2+}.[66] CdTe QDs capped with mercaptosuccinic acid were prepared in aqueous medium and deposited on the quartz slides to form multilayer films by electrostatic interactions with poly (dimethyldiallyl ammonium chloride) (PDDA). These PDDA/QDs multilayer films were easily fabricated and showed high photostability. More importantly, the fluorescence of these multilayer films could be quenched effectively by Hg^{2+} ions. However, the selectivity of this sensing configuration has not been comprehensively examined, and thus their practical application is not clear yet.

One example based on a fluorescence enhancement was reported by Zhao et al.[38] for the determination of Pb^{2+} ion by using Dz functionalized CdSe/CdS QDs. The binding of Dz to the QDs quenched the fluorescence of the QDs-Dz conjugates. Upon the addition of Pb^{2+}, the photoluminescence (PL) of QDs was recovered because the Dz ligands on the surface of the QDs were removed due to the high affinity of Dz to Pb^{2+} ions. The performance of this system was excellent, with a wide determination range (0.01 nM–20 μm), very high sensitivity (detection limit of 0.006 nM), and high selectivity. The selectivity of the system relies on the specific and strong Pb^{2+}-binding capability of Dz. The fluorescent probe was successfully applied to real samples (i.e., soil, tap water, and river water) with satisfactory results.

Based on a PL enhancement observation, two other sensors for Hg^+ ions were developed, but this time the strategy used to get the ion selectivity was different. PbS quantum dots capped with mercaptoethanol were synthesized in polyvinyl alcohol and used to investigate their photoluminescence response to various ions.[42] The enhancement in the PL intensity was observed with specific ions, namely Zn^{2+}, Cd^{2+}, Hg^{2+}, and Ag^+. Among these four ions, the PL response to Hg^{2+} even at submicromolar concentrations was quite high, compared to others. The high increase in the PL of PbS QDs after the addition of Hg^{2+} ions was attributed to the formation of HgS at the surface that has a bandgap higher than PbS. In this system, the excellent selectivity achieved toward Hg^{2+} over other ions is due to the much higher solubility product constant of HgS in comparison with CdS, ZnS, and AgS. The same approach but using MAA-capped InP QDs was used by Zhu et al.[43] to develop an Hg^{2+} sensor. Upon the addition of Hg^{2+} ions, a luminescence enhancement of the MAA-capped InP was observed due to the formation of HgS ultrasmall particles on the InP nanocrystals' surface. The HgS has a larger bandgap and efficiently confines the excitation to the InP QDs, eliminating nonradiative relaxation pathways, and thus increasing the PL intensity.

4 Quantum Dot as Active Fluorescent Labels

In many sensing configurations, the QDs are not used merely as passive labels but some kind of energy flow can happen between the components of the system. The principle is based on the fact that the charge/energy transfer at the nanoscale can be altered, set up, or disrupted by small perturbations at the surface of the QDs (Fig. 1). Here, we focus on the systems in which the binding or interaction of heavy metal ions with the nanoassembly is used to drive the association or dissociation of acceptors, or alter the donor-acceptor separation distance. Therefore, the modulation of the charge/energy transfer process efficiency provides an analytical signal.

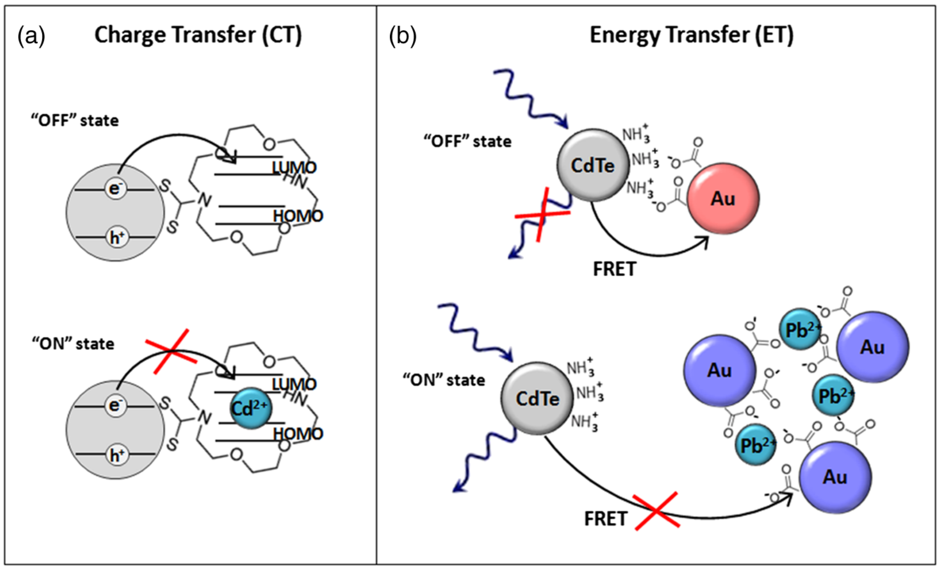

Fig. 1 Possible flow transfer processes between a QD (donor) and an acceptor: (a) charge transfer (CT) and (b) energy transfer (ET).

4.1 *Charge Transfer QD (Donor)–Acceptor Systems*

The optical excitation of QDs results in the formation of a bound electron-hole pair called an exciton. To generate efficient fluorescence, the rate of radiative recombination of the exciton must be competitive with the rates nonradiative recombination processes. Sometimes, this electron or hole can be transferred to other specie (acceptor) rather than lost as an emitted photon. Therefore, CT processes between QDs (donor) and other species (acceptor) provide a mechanism for the on/off switching of fluorescence, and thus a basis for the design of "turn on/off" sensors. It should be pointed out that CT mechanisms can only occur between species in intimate contact. Some "turn on/off" fluorescence approaches for the sensitive and selective detection of heavy metal ions have been developed. On the one hand, the use of crown ether molecules has provided an interesting approach based on supramolecular chemistry for the selective sensing of Cd^{2+} ions. Banerjee et al. functionalized QDs with 1,10-diaza-18-crown-6 rings to develop a novel Cd^{2+} selective chemosensor.[41] Core/shell CdS:Mn/ZnS QDs were synthesized using AOT/heptane/water water-in-oil microemulsion. Cd^{2+}-selective ligands (i.e., 1,10-diaza-18-crown-6) were directly attached to the QD surface via zero-length covalent coupling using CS_2. The 1,10-diaza-18-crown-6-capped QDs represented the "off" state because the QD fluorescence was quenched due to ligand assisted electron transfer process [Fig. 1(a)]. Such an electron transfer process could be prevented by complexation between Cd^{2+} and 1, 10-diaza-18-crown-6-capped QDs that led to fluorescence enhancement ("on" state). This system showed high selectivity for Cd^{2+} over other metal ions. The selectivity of this sensor relies on the higher binding constant of aza-crown ether for Cd^{2+} over other ions. On the other hand, the combination of QDs with organic dyes is another approach for the detection of metal ions based on a CT process. A turn-on fluorescence sensor based on xylenol orange (XO) functionalized CdSe/CdS QDs was developed for the determination of Pb^{2+} ion.[67] XO-functionalized CdSe/CdS QDs were formed via the layer-by-layer self-assembly reaction. QDs were first modified by mercaptoacetic acid, and then capped with the natural biopolymer chitosan and the negatively charged XO. In the XO-CdSe/CdS conjugate, the fluorescence of the QDs was quenched due to an electron transfer from XO to the QDs. Upon the addition of Pb^{2+}, a dramatic enhancement of the fluorescence intensity was observed, which resulted from the coordination between Pb^{2+} and XO on QDs surface and the disruption of the electron transfer mechanism. This system showed a limit of detection of 20 nM. Similarly, Gui et al. fabricated fluorescein isothiocyanate (FITC)-CdTe conjugates for developing a ratiometric fluorescence method for Cd^{2+} ion detection.[68] In this work, thioglycolic acid capped CdTe QDs were modified by cation polyelectrolyte polyethylenimine (PEI) to form PEI-stabilized QDs, and then combined with FITC through electrostatic interactions in aqueous solution. The intentional addition of S^{2-} quenched PL peak of QDs (at the "off" state), which was restored due to following addition of Cd^{2+} (at the "on" state). Interestingly, the introduction of S^{2-} and Cd^{2+} does not markedly affect the FITC PL peak which allowed the development of a ratiometric sensor. The detection limit of this on/off ratiometric PL method for the detection of Cd^{2+} was 12 nM. Although the sensitivity of this kind of systems is quite acceptable for their applicability to real samples, the discrimination between chemically closely related metal ions is not satisfactory enough.

4.2 *Energy Transfer Quantum Dot (Donor)-Acceptor Systems*

Energy transfer, which involves deactivation of an electronic excited state of the donor and concomitant formation of an excited state of the acceptor, can occur by two distinct mechanisms[69]: (1) FRET, which is a through-space mechanism; this requires spectral overlap between the emission of the donor and absorption(s) of the acceptor and arises from long-range dipole-dipole interactions between a donor molecule in the excited state and an acceptor molecule in the ground state.[70] (2) DET, which goes by a through-bond mechanism.[69] The Dexter mechanism[71] requires direct donor-acceptor orbital overlap and has an exponential-distance dependence with a range of approximately 1 nm. Based on one or the other of the described energy transfer mechanisms, several QD-based systems have been developed for the sensing of heavy metal ions.

4.2.1 *Fluorescence resonance energy transfer systems*

The development of ions sensors via analyte-induced changes in photoluminescence of QDs based on FRET is a very active research field. In these systems, the QD usually acts as donor, so the light energy absorbed by QDs (donor) is transferred to a nearby acceptor species, such as an organic fluorophore or another nanoparticle (acceptor). According to Forster's theory, the rate of energy transfer depends upon the following factors: the extent of spectral overlap between the donor emission and the acceptor absorption, the quantum yield of the donor, the relative orientation of the donor-acceptor transition dipoles, and the distance between the donor-acceptor molecules.[72,73] Several FRET systems using QDs as donors for the detection of heavy metal ions have been reported. In this kind of system, the detection strategy relies on the modulation of the effect of the target ions on the FRET efficiency between the QDs and the acceptors.

One example is the FRET-based system developed between the donor TGA (thioglycolic acid)-CdTe QDs and the acceptor butyl-rhodamine B in the presence of the surfactant of cetyltrimethylammonium bromide (CTMAB).[46] CTMAB micelles formed in water reduced the distance between the donor and the acceptor significantly and thus improved the FRET efficiency, which resulted in an obvious fluorescence enhancement of the acceptor. Upon addition of Hg^{2+}, the fluorescence of both donor and acceptor was quenched, but the quenching extent of the acceptor (butyl-rhodamine B) was much greater, and thus the detection relied on the spectral change of the acceptor. Since the fluorescence of free butyl-rhodamine B was not affected by the cation, the quenching was attributed to the binding of Hg^{2+} onto the surface of the TGA-CdTe QDs, which in result quenched the QD fluorescence by forming HgTe particles. This method was proved to be sensitive (detection limit of 20 nM) and repeatable in a wide range in aqueous solutions, where possible interferents had negligible effects on the determination of Hg^{2+}.

In another example, AuNPs were used as effective acceptors thanks to their high extinction coefficient (up to 10^9 M^{-1} cm^{-1}) and a broad absorption spectrum in visible light that is overlapped with the emission wavelength of usual energy donors. Wang and Guo reported a FRET system due to electrostatic interactions between the positively charged CdTe-QDs capped with cysteamine (CA-CdTe-QDs) and the negatively charged AuNPs capped with 11-mercaptoundecanoic acid (MUA-AuNPs).[74] This system allowed the determination of Pb^{2+} based on the modulation in FRET efficiency between QDs and AuNPs in the presence of Pb^{2+}, which congregates the AuNPs and thus inhibits the interaction of the QD-AuNP assembly. Under the optimum conditions, the detection limit was found to be 30 ppb of Pb^{2+} [Fig. 1(b)]. Concerning the selectivity, most metal ions showed little interference except for Ag^+ and Fe^{3+}. However, these interferences can be easily avoided by adding masking agents (Cl^- for Ag^+ and F^- for Fe^{3+}) when these interfering substances exist in the samples.

Recently, the potential of doped QDs has also started to be exploited in this direction. Two efficient FRET systems have been developed for the ultrasensitive detection of Hg^{2+} ions by using lanthanide-doped CdS QDs.[44,45] These composite nanoparticles, terbium(III)-doped CdS[44] and europium (III)-doped CdS,[45] were successfully synthesized through a straightforward one-pot process with GHS as capping ligand. In both cases, CdS nanoparticles act as energy donors whereas the lanthanide ions, Tb^{3+} or Eu^{3+}, are the energy acceptors. The emission intensity of the lanthanide(III) ions increased due to fluorescence energy transfer from the excited CdS particles to the emitting lanthanide(III). As a result of specific interaction, the fluorescence intensity of Ln(III)-doped CdS was obviously reduced in the presence of Hg^{2+}. This quenching can be attributed to the fact that the energy transfer system was destroyed by combining with Hg^{2+}, probably as a result of the formation of ultrasmall HgS particles on the surface of Ln(III)-QDs. Under the optimal conditions, the detection limits were 0.1 and 0.25 nM for Tb(III)-CdS and Eu(III)-CdS, respectively. It is important to note that the Ln(III)-doped CdS QDs provide better sensitivity toward Hg^{2+} than that of CdS QDs. Thereby, the Ln (III)-QDs composite takes advantage of the Ln complex, which has a very large Stokes shift and long fluorescence lifetime that permit more sensitive fluorescence detection. In addition to high sensitivity, both systems showed a unique selectivity toward Hg^{2+} ion with respect to common coexisting cations. An important drawback of the systems described above is the fluctuation of the fluorescence signal caused by external factors. Unlike those one-signal sensors, the ratiometric sensors compare fluorescence intensities at two different wavelengths before and after analyte recognition and use the ratio of the two fluorescence intensities to quantitatively detect the analyte.[2] Thus, they can significantly eliminate the external effects, such as instrumental drift and sensor concentration, by self-calibration of two different emission bands. These systems are therefore more precise and preferable as they avoid errors in the detection. Several QD-based ratiometric fluorescence probes for the detection of Hg^{2+} in different samples have been reported. Liu et al. developed a robust FRET-based ratiometric sensor for Hg^{2+} detection in water with multilayered QDs/silica composite nanoparticles.[47] By using the reverse microemulsion approach, CdTe QDs were first embedded into nanosized silica particles, forming the QDs/silica cores, and a positively charged ultrathin spacer layer was then deposited on each QDs/silica core so as to avoid contact between the QDs and the positively charged mercury ions (electrostatically repelled from the positively charged spacer layer). After a mercury ion probe (i.e., spirolactam rhodamine derivative) was covalently linked onto the particle surface. In the absence of Hg^{2+}, the probe is optically inert; whereas the presence of Hg^{2+} can induce the ring-opening reaction of the spirolactam rhodamine and turns the probe into the FRET acceptor. This method showed a detection limit of 260 nM (52 ppb), could be used in a wide pH range, and exhibited high selectivity toward Hg^{2+} ion due to the use of a specific mercury ion probe.

In another work, the QD-based ratiometric system consisted of a QDs nanocomposite with two differently sized CdTe/CdS QDs, allowing a visual detection of Hg^{2+}.[75] The red-emitting larger-sized CdTe/CdS QDs embedded in silica nanoparticles were insensitive to Hg^{2+}, while the green-emitting smaller sized ones were covalently conjugated onto the silica nanoparticles surface and sensitive to Hg^{2+}. The addition of Hg^{2+} can only quench green fluorescence in the dual-emission QDs nanocomposites, which trigger the change of fluorescence intensity ratio of two different emission wavelengths and hence induces the evolution of fluorescence color of the probe solution with variation of Hg^{2+} concentration. Concerning the mechanism, the adsorption of Hg^{2+} on green QDs surface changed the surface states of QDs and thus facilitated the electron transfer or the other nonradiative electron–hole recombination annihilation. As a result, the PL of QDs decreased remarkably. The performance of this method was successfully proved in the determination of Hg^{2+} content in fetal bovine serum and human urine with a high selectivity and sensitivity (detection limit of 3.1 nM). Another limitation of some FRET-based sensors which use QDs as the donor is that the background signals might interfere with the fluorescence of organic dyes or QDs, affecting the sensitivity of the sensors. To overcome this, Huang et al. described a time-gated fluorescence resonance energy transfer (TGFRET) sensing strategy employing water-soluble long lifetime fluorescence QDs and gold nanoparticles to detect trace Hg^{2+} ions in aqueous solution.[48] Mn-doped CdS/ZnS QDs were used as donors because of their high quantum yield and long lifetime fluorescence. The QDs (donor) and Au NPs (acceptor) were functionalized by two complementary single-stranded DNAs (ssDNAs), except for four deliberately designed T—T mismatches. When Hg^{2+} ions were present in the medium, deoxyribonucleic acid (DNA) hybridization occurred because of the formation of $T{-}Hg^{2+}{-}T$ complexes. As a result, the donor and acceptor were brought into close proximity allowing the energy transfer, and therefore, leading to a decrease of the QDs fluorescence. In comparison to other FRET systems, the proposed TGFRET sensor displayed the advantage of higher sensitivity, achieving a detection limit as low as 0.49 nM. This sensor was used to detect Hg^{2+} ions from samples of tap water, river water, and lake water spiked with Hg^{2+} ions, and the results showed good agreement with the found values determined by an atomic fluorescence spectrometer. The high sensitivity largely allows compliance with the legal requirements (EPA sets the maximum level of mercury in drinking water at 10 nM), demonstrating the practical use of this sensor.

4.2.2 *Dexter energy transfer systems*

Recent theoretical studies suggest that metal centers with a d^{10} electronic configuration have a strong affinity toward other closed-shell metal ions with similar electronic configuration.[76] In particular, this phenomenon associated with strong $d^{10}{-}d^{10}$ interactions is known as metallophilic interaction. This interaction originates due to dispersive forces which are further augmented by relativistic effects.[77] This kind of interaction can facilitate the DET process, and therefore, be used as an analytical strategy for the development of metal(d^{10})-sensitive sensors.

Goswami et al. developed a simple, two-step route for the synthesis of bovine serum albumin (BSA)-capped HgS QDs to be used for the detection of Hg^{2+} ions.[56] These as-prepared QDs demonstrated its potential as selective sensor materials for Hg^{2+}, based on selective luminescence quenching. In this system, i.e., $HgS{-}Hg^{2+}$, the probability of a FRET process was clearly ruled out due to the lack of absorption of the unsupported Hg ion. The sensing mechanism was demonstrated to be based on the formation of a metallophilic bond between Hg^{2+} ions and Hg^{2+} present on the surface of the HgS QDs (i.e., $5d^{10}{-}5d^{10}$). Assuming the formation of a $Hg^{2+}{-}Hg^{2+}$ metallophilic bond, the filled 5d and empty 6s orbital of each Hg^{2+} would overlap with each other, which then give rise to bonding and antibonding orbitals. Reorganization of these orbitals would therefore lead to new energy-accepting levels on the Hg^{2+}, which provides the basis of a double electron exchange process (Dexter). Therefore, in the excited state, the metallophilic bond facilitated the DET to the Hg^{2+}.

5 Bioinspired Quantum Dot-Based Sensors

The marriage of biomolecules with nanomaterials has produced a new technology named nanobiotechnology, which is used as a term to indicate the merger of biological research with various fields of nanotechnology. This discipline provides a broader perspective on bioinspired devices and applications related to nanomaterial-based sensing. Many of the currently investigated functionalized bionanosystems draw their inspiration from naturally occurring phenomena, prompting the integration of molecular signals and mimicking natural processes, at the cell, tissue, and organ levels. Many examples of "bio-inspired" QD-based systems for the analysis of heavy metal ions are emerging during the last decade. Figure 2 depicts some analytical strategies combining QDs with different functional biomolecules and different interaction mechanisms.

The fundamental and key feature of these systems is the use of a functional biomolecule as biorecognition element for the interaction with the target. Functional biomolecules with high

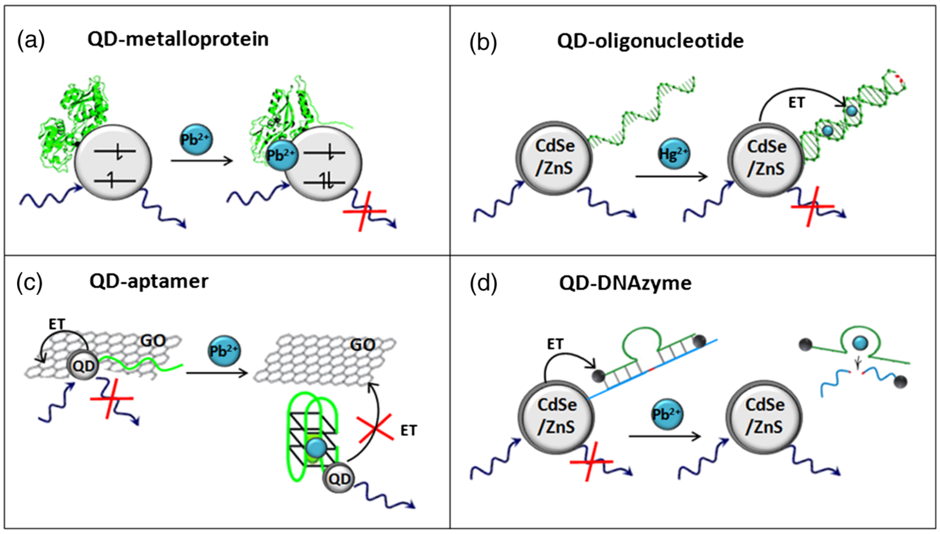

Fig. 2 Scheme of several analytical strategies using QD-biomolecule ensembles for the detection of heavy metal ions: (a) QD-metalloprotein, (b) QD-oligonucleotide, (c) QD-aptamer, and (d) QD-DNAzyme.

affinity and high specificity include proteins, enzymes, nucleic acids, aptamers, DNAzymes, and so on. The most promising examples are summarized as follows, classifying them according to the functional biomolecule used.

5.1 *Proteins and Enzymes*

While proteins and antibodies have been widely used as biorecognition elements in the design of sensors for almost any biomolecular target, they usually miss detection of any metal ion or a specific oxidation state of a given metal ion. Antibodies, which can be raised against large molecular targets such as proteins, often fail to recognize metal ions because they are too small to elicit a strong immunological response. Therefore, very few examples can be found for the particular application of heavy metal ions detection. By using metalloproteins, Shete and Benson[57] developed a selective QD-based strategy for the selective fluorescence imaging of Pb^{2+} ions around red blood cells. The metalloprotein was designed to be selective toward Pb^{2+} ion and attached to CdSe/ZnS QDs. This system showed a high sensitivity (detection limit of 100 pM Pb^{2+}) and high selectivity. Metal ion selectivity presumably comes from the coordination geometry selected to favor lone pair formation on Pb^{2+} ions and electrostatically disfavor tetrahedral coordination [Fig. 2(a)]. It should be pointed out that this sensor is reversible, which is a key feature for imaging Pb^{2+} ion fluxes. Replacement of CdSe/ZnS with InGaP/ZnS nanoparticles provided similar biosensors (100 pM limit of detection) but with excitation/emission wavelengths longer than the major absorbance of red blood cell hemoglobin (>620 nm). The metalloprotein-capped InGaP/ZnS QDs system provided a 5 nM Pb^{2+} detection limit in the presence of red blood cells. A promising application of these systems is to address inefficient Pb^{2+} ion chelation therapy for treating lead intoxication. Other interesting approach for the detection of Hg^{2+} was based on a signal amplification method by using the combination of QDs with a specific enzyme, i.e., a nicking endonuclease.[49] A nicking endonuclease (NEase) is a restriction endonuclease that recognizes specific nucleotide sequence in double-stranded DNA (dsDNA) but cleaves only one of the two strands. Streptavidin-coated QDs were conjugated to biotinylated hairpin-shaped probe A which was modified with a quencher BHQ-2. The fluorescence of the QDs was quenched by BHQ-2 through FRET quenching. In the presence of Hg^{2+}, probe B hybridized with the loop of probe A due to specific binding between thymine-thymine mismatches and Hg^{2+}, thus probe A was opened. Then, NEase recognized specific nucleotide sequences and cleaved probe A. After the dissociation of probe A fragments, the distance between QDs and BHQ-2 increased, which led to an increase of QDs fluorescence. The released Hg^{2+} and probe B could hybridize with another probe A to start a new cycle, and therefore, a remarkable signal amplification was achieved. Under optimal conditions, the detection limit of this assay was 0.8 nM.

5.2 *Nucleic Acids*

The ability of some metal ions that selectively bind to some nucleic bases to form stable metal-mediated DNA duplexes has been exploited for the design of numerous DNA-based sensors.[78] For example, mercury ions (Hg^{2+}) are capable of selectively coordinating thymine (T) bases to form stable $T—Hg^{2+}—T$ complexes,[79,80] and Ag^{+} interacts specifically with cytosine-cytosine (C—C) mismatches.[81] For Pb^{2+} detection, most sensors are based on the Pb^{2+}–stabilized G-quadruplex.[82] Based on this strategy and using QDs for the fluorescent read-out [Fig. 2(b)], several sensors for specific metal ions have been developed. However, different mechanisms are involved in the quenching of fluorescence. Both Freeman et al.[50] and Hao et al.[51] developed selective and sensitive sensing systems consisting of nucleic acid functionalized QDs, which enable the multiplexed analysis of Hg^{+2} and Ag^{+}. The first one used CdSe/ZnS QDs of different sizes, which were modified with T-rich or C-rich nucleic acids [Fig. 2(b)]. In the presence of Hg^{+2} or Ag^{+}, a rigid hairpin structure was formed, and a high decrease of the QD fluorescence was observed, which is attributed to electron transfer from the QDs to the metal ions bound to the thymine or cytosine bases. The detection limits were 10 nM for Hg^{+2} and 1 nM for Ag^{+}. Thus, this sensor presented a much higher sensitivity for the Hg^{+2} ions detection. In contrast, the system developed by Hao is based on a FRET mechanism between the donor (CdTe QDs functionalized with ss-DNA) and acceptor (TAMRA or Cy5-labeled ss-DNA). In the presence of Hg^{+2} or Ag^{+}, the donor and acceptor were brought into close proximity to another due to the formation of $T—Hg^{2+}—T$ and $C—Ag^{+}—C$ complexes allowing the energy transfer, which quenched the fluorescence of CdTe QDs and increased the acceptor fluorescence. This method showed better detection limits than the previous one, 1.8 nM for Hg^{+2} and 2.5 nM for Ag^{+}.

Using Au NPs as acceptors, an ultrasensitive fluorescent sensor based on the quantum dot/DNA/gold nanoparticle ensemble was developed for detection of Hg^{2+}.[52] Both CdSe/ZnS QDs and Au NPs were functionalized with ss-DNA, and DNA hybridization occurred when Hg^{2+} ions were present, leading to an energy transfer the QDs to the Au NPs, and therefore, quenching the fluorescence emission of the QDs. In this system, the fluorescence of QDs is quenched via nanometal surface energy transfer (NSET) mechanism rather than the conventional FRET process. This conclusion was reached by studying the dependence of the energy transfer efficiency with the separation distance between donor and acceptor. The key differences are that FRET is a dipole–dipole type energy transfer with $1/r^6$ distance dependence, while NSET is a dipole-surface type energy transfer following $1/r^4$ distance dependence.[83–85] This sensor was very sensitive with a detection limit of 0.4 and 1.2 ppb toward Hg^{2+} in the buffer solution and in the river water, respectively, values which satisfactorily meet the sensitive demands of U.S. Environmental Protection Agency (EPA) and World Health Organization (WHO).

5.3 *Aptamers*

Aptamers have demonstrated to be a useful functional biomolecule for the designing of QD-based sensors. Aptamers are single-stranded oligonucleotides (DNA or RNA sequence) that fold into distinct three-dimensional conformations capable of binding strongly and selectively to a target molecule. The huge potential of aptamers is that they can be selected against virtually any targets of choice using an *in vitro* process termed SELEX (systematic evolution of ligand by exponential enrichment).

Two "turn on" sensors based on the use of a G-quadruplex aptamer of DNA have been developed for the detection of Pb^{2+} ions. This aptamer consists of a four-stranded quadruplex helical structure formed by DNA sequences rich in guanine basic groups through hydrogen bond interactions. It can fold into a special and very stable secondary "G-quadruplex" structure with Pb^{2+} ions. Although both sensors use the same strategy to achieve the recognition of the Pb^{2+} ions, the detection method is different; one is a fluorescence method whereas the other is an electrochemiluminescence method. Li et. al developed a "turn-on" fluorescent biosensor using aptamer-functionalized CdSe/ZnS QDs and graphene oxide (GO).[54] The formation of GO/aptamer-QD ensemble led to the energy transfer from the QDs to the GO sheets, quenching the fluorescence of QDs. The fluorescence of QDs is quenched by GO via NSET. When Pb^{2+} ions were present, Pb^{2+} interacted with the aptamer inducing a conformational change in the aptamer, which led to the formation of a G-quadruplex/ Pb^{2+} complex. As a result, the QDs linked to the G-quadruplex/Pb^{2+} complex were detached from the GO sheet, which "turns on" the fluorescence of the QDs [Fig. 2(c)]. This sensor exhibited a limit of detection of 90 pM and excellent selectivity toward Pb^{2+} over a wide range of metal ions. The other system, which is a turn-on electrochemiluminescence (ECL) sensor using CdTe QDs, was developed by Hai et al.[55] In that work, Fe_3O_4—Au magnetic nanoparticles were modified by a layer of hairpin structure aptamer and CdTe QDs were used as the ECL markers. The presence of Pb^{2+} promoted the "stem-loop" structure of the aptamer to open, thus forming a stable G-quadruplex structure and exposing $3'$-NH_2, which bound to QDs. Thus, the magnetic nanoparticles were separated and enriched on the surface of the magnetic controlled gold electrode. This method presented an excellent sensitivity, achieving a limit of detection as low as 0.1 pM. In addition, the biosensor showed good selectivity, stability, and reproducibility. Although K^+, Ag^+, and Hg^{2+} can open the hairpin structure of the aptamer to form the G-quadruplex, its stability is lower than the G-quadruplex formed by Pb^{2+}. Therefore, the sensor showed a satisfactory selectivity toward Pb^{2+}. To verify the availability of the sensor in the analysis of real samples, river water and urine were tested and satisfactory results were obtained. In other work, a thymine-rich aptamer was used for the development of a room temperature phosphorescence (RTP) Hg^{2+} ions sensor. This system consisted of cetyltrimethylammonium bromide-capped Mn-doped ZnS quantum dots (CTAB/Mn-ZnS QDs) and label-free thymine (T)-rich aptamer.[53] The formed T—Hg^{2+}—T dsDNA could linearly quench the RTP of Mn—ZnS QDs through electron transfer and aggregation effect, and showed a detection limit of 1.5 nM.

5.4 *DNAzymes*

DNAzymes (catalytic DNAs or deoxyribozymes) are a new category of enzymes that are highly specific and sensitive for metal ions.[86–88] By using *in vitro* selection methods, DNAzymes with high specificity for any metal ion such as Pb^{2+}, Hg^{2+}, Cu^{2+}, and Zn^{2+} can be obtained, and by modifying fluorophore/quencher pairs attached to those DNAzymes, different types of heavy metal sensors can be developed. Combining the use of QDs as FRET donors and the potential of DNAzymes, a QD-DNAzyme sensor for the detection of Pb^{2+} and Cu^{2+} ions has been reported.[58] Quencher-labeled DNAzymes were conjugated to the surface of carboxyl silanized CdSe/ZnS QDs. The quencher on the DNAzyme efficiently quenched fluorescence of the QDs due to its close proximity. In the presence of Pb^{2+} ions, the quencher-labeled DNAzymes were cleaved off the QD and the QD emission was restored [Fig. 2(d)]. With this strategy, multiplexed detection was also demonstrated using two different colors of QDs, showing negligible cross talk between the Pb^{2+} detection and Cu^{2+} detection. The sensitivity of the system was really high, with experimental detection limits of 0.2 and 0.5 nM for Pb^{2+} and Cu^{2+}, respectively. Two electronic QD-DNAzyme sensors for highly sensitive detection of Pb^{2+} were developed by coupling the significant signal enhancement of PbS[59] or CdS[60] QDs with Pb^{2+} specific DNAzymes. In the former, the presence of Pb^{2+} cleaved the DNAzymes and released the biotin-modified fragments, which further hybridized with the complementary strands immobilized on the gold substrate. The streptavidin-coated, PbS-QD LBL (layer by layer) assembled nanocomposites were captured on the gold substrate through biotin-streptavidin interactions. Subsequent electrochemical signals of the captured QDs upon acid dissolution provided quantitative information on the concentrations of Pb^{2+} with a dynamic range from 1 to 1000 nM. Due to the dramatic signal amplification by the numerous QDs, subnanomolar level (0.6 nM) of Pb^{2+} was detected.[59] In the second approach, CdS QDs modified ssDNA were used as signaling probes. This QDs-labeled DNA was hybridized with the long ssDNA obtained after using the Pb^{2+}-specific DNAzyme as a recognition element and rolling circle amplification (RCA) as a signal amplification probe. The hybridized CdS QD-ssDNA was dissolved in nitric acid and the release Cd^{2+} atom could be conveniently quantified by square-wave stripping voltammetry. This system is even more sensitive than the previous one: ultra-low levels (7.8 pM) could be detected. Both systems showed good selectivity against other divalent metal ions and thus hold great potential for the construction of a general DNAzyme-based sensing platform for the monitoring of other heavy metal ions.[60]

Another strategy for Pb^{2+} monitoring based on CdS QD modified indium tin oxide electrode and photoelectrochemical analysis was developed by De-Man et al.[61] K^+-stabilized G-quadruplex DNAzyme accelerates oxidation of 4-chloro-1-naphthol by H_2O_2 to yield the insoluble and insulating product benzo-4-chlorohexadienone on the photoelectrode, reducing the photocurrent intensity. The sensing strategy was based on the Pb^{2+}-induced allosteric transition of G-quadruplex DNAzyme, which reduced the enzymatic biocatalytic precipitation on the CdS QDs photoelectrode. The photocurrent intensity ascended as the enhanced Pb^{2+} concentrations, indicating the Pb^{2+} controlled inhibition of DNAzyme catalytic capability. The percentage of the photocurrent increase was proportional to the Pb^{2+} concentration with a detection limit of 0.1 nM. However, further investigations are needed to eliminate some interferences caused by Cu^{2+} and Ag^+ ions.

6 Conclusions and Outlooks

Due to their unique features, the use of QDs as metal sensors has received great attention for years. These properties are not limited to their optical and electronic properties, but also include their versatile surface functionalization and high photostability, which make them far superior compared with organic dyes. Lately, remarkable progress has been made in this area, especially in regard to heavy metal ions detection.

In this article, we have reviewed the different analytical approaches developed so far for the analysis of the more toxic heavy metal ions (i.e., Cd^{2+}, Hg^{2+}, and Pb^{2+}). The classification of the QD-based systems according to the active or passive actuation of the QDs and also depending on the interaction mechanism that causes the analytical signal has allowed a global overview of the different alternatives in the design of novel configurations.

As ion selectivity is a critical point in the design of sensors for metal ions, new strategies to get higher affinity and specificity toward the target ion are progressively emerging. Besides, current efforts are focused on exploring the massive multiplexing capabilities of the QD-based systems for the simultaneous detection of several heavy metal ions with high sensitivity.

Focusing particularly on the functionalization of QDs with biomolecules, which act as a biorecognition element for the interaction with the target, and with special emphasis on the sensing configurations inspired by mimicking assemblies found in nature, the described bioinspired QD-systems show huge potential for improving the limitations of other systems, such as low sensitivity and selectivity. For example, CdS-ssDNA QDs in combination with a sensing system based on DNAzymes and RCA have allowed the detection of ultra-low levels (7.8 pM) of Pb^{2+}. However, most of the QD-based metal ion probes have only been demonstrated as proof-of-concept applications and tested mostly in buffer solution or in artificial matrices. For medical diagnosis and environmental monitoring applications, significant sample matrix effects need to be carefully evaluated. This will certainly be the challenge tackled in the near future with the continuous progress of the understanding of the exact toxicity and metabolic pathways.

In addition to the described approaches, there are some other strategies yet unexplored. The possibility of joining natural molecules with synthetic ones is a relatively unexplored realm of research. By combining biomolecules (i.e., enzymes, proteins, nucleic acids, etc.) with synthetic macromolecules, such as polymers, a novel class of organic-organic hybrid nanostructures can be produced aiming to matching designed properties not present in either one separately. These strategies are waiting to be explored.

We believe this research area will become more active due to the biological and environmental significance of Pb^{2+}, Cd^{2+}, and Hg^{2+}, and further investigations will continue to increase.

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