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Aptamer-nanomaterial based biosensors for fluorescent detection of trace heavy metals

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ABSTRACT

Heavy metal pollution is a serious problem that affects our environment and health profoundly. Taking advantage of the nanotechnology and nanomaterials, researchers have been exploring new nano-biosensors for sensitive, selective, quantitative and rapid detection of heavy metals. This review article focuses on the recent progress in the research of fluorescence detection of heavy metals based on functionalized nanomaterials modified with nucleic acids including aptamers and DNAzymes.

KEYWORDS

Heavy metals;
Nanomaterials;
Aptamers;
Fluorescence;
Biosensors.

INTRODUCTION

Modern industries have brought tremendous benefits to human beings, but on the other hand, also caused severe pollutions including heavy-metal pollution which poses a constant threat to our environment and the public health[1]. As we know, many heavy-metal elements or their ions are highly toxic even at trace level. For example, exposure of mercury can lead fatal damages to brain, nervous system and other organs[2]. Uptake of lead may cause various neurotoxic effects and especially do harm to children brain development[3]. Chromium (VI) has been reported to be human toxic for causing lung, liver and kidney diseases[4]. Silver ions can do harm to human health by inactivating sulfhydryl enzymes and interacting with various metabolites[5]. Copper is an essential element in many biological processes. However, increased level of copper ions can induce gastrointestinal disturbance for a short period of time, while long time exposure causes damages of proteins, nucleic acids and organs[6]. In addition, many heavy metals cannot be degenerated naturally, so they will be accumulated through food chains and eventually do harm to human beings[7].

To protect the public health, it is therefore exigent to develop technology that can quickly monitor and analyze trace heavy-metals in our environment. Actually, there are already some widely utilized conventional tools, such as atomic absorption/emission spectroscopy[8], inductively coupled plasma mass spectrometry[9], mass spectrometry[10] and etc. However, most conventional approaches normally require expensive instruments and involve complicated and time-consuming operations. Therefore, researchers are still striving to search for new methods which can examine trace amount of heavy metals easily, rapidly and cost-effectively. For this purpose, research and development of miniature and elegent biosensors are currently of special interest. Generally, a

biosensor contains three components: a sensitive biological element that recognizes the target analyte, a transducer or detector element that transforms the signal upon interaction with the target analyte, and a sensor reader unit. For the biological element, DNA molecules are widely used because they can be readily synthesized according to the required sequence of bases which can interact with varied heavy metals. For example, the sequence containing thymines can be designed and synthesized to probe Hg^{2+} because the thymines can interact with Hg^{2+} to form T-T hairpin structure[11]. As for the sensor signal, fluorescence measurement has attracted special attention because of its high sensitivity, easy quantification and adaptability for infield detection applications[12]. In fact, many biosensors containing DNA recognition unit and fluorescence transducer mechanism have been proposed and developed. For example, Akira Ono et al. have devised an oligodeoxyribonucleotide (ODN)-based sensing system and made use of *fluorescence resonance energy transfer* (FRET) to detect Hg^{2+} and Ag^{+} ions[13,14]. For the choice of fluorophores, some fluorescent dyes such as TOTO-3 and Sybr Green 1 (SG) show dramatic fluorescence enhancement upon binding to double-stranded DNA (dsDNA) compared to relatively weak fluorescence upon binding to single-stranded DNA (ssDNA). Based on the folding of thymine-rich ssDNA into dsDNA in the presence of Hg^{2+}, Chang et al.[15] and Wang et al.[16] utilized TOTO-3 and Sybr Green 1 (SG) respectively for Hg^{2+} detection. Lin et al.[17] utilized SG to detect Ag^{+} based on the interaction between cytosine and silver ions. Dave et al.[18] achieved both detection and removal of Hg^{2+} through thymine-rich DNA functionalized polyacrylamide hydrogel.

To obtain high performance biosensors, now a new trend is to introduce nanomaterials and nanotechnology into the biosensor design. This can be attributed to invention and discovery of many new nanomaterials which possess excellent properties in optics, magnetics, electronics and etc. Application of proper nanomaterials can improve the detection sensitivity of biosensors significantly. For example, Liu et al.[19] used Rhodamine B isothiocyanate (RBITC)-poly(ethylene glycol) (PEG)-modified gold nanoparticles for Hg^{2+} detection. In their design, Hg^{2+} can remove the RBITC from the Au surfaces, resulting in the recovery of RBITC fluorescence. Li et al.[20] utilized poly(methacrylic acid) (PMAA)-templated Ag nanoclusters for Cu^{2+} detection. Guo et al.[21] detected Hg^{2+} with high sensitivity and selectivity through denatured bovine serum albumin (dBSA) stabilized Ag clusters. Besides Au/Ag nanoparticles and nanoclusters, some other functionalized nanomaterials can also be employed for heavy metal detection. For example, Jung et al.[22] designed aminonaphthalimide-functionalized $Fe_3O_4@SiO_2$ core/shell magnetic nanoparticles for detection of Hg^{2+} and CH_3Hg^{+} ions, and Wang et al.[23] achieved multiple and quantitative detection of heavy metal including Cu^{2+}, Cd^{2+}, Zn^{2+} and Hg^{2+} in aqueous solution based on 1,4-dihydroxyanthraquinone (1,4-DHAQ) derivative and 9-fluorenylmethyl chloroformate (Fmoc-Cl) co-modified Fe_3O_4 magnetic nanoparticals (MNPs). Water-soluble and stable quantum dots (QDs) are also used for heavy metal detection. Mohamed Ali et al.[24] capped glutathione (GSH) on CdTe and CdZnSe and applied them for selective detection of Pb^{2+}. Chan et al.[25] developed 16-mercaptohexadecanoic acid (16-MHA) modified CdSe QDs for detection of Cu^{2+}. Recently, some one-dimensional nanomaterials are also utilized for heavy metal detection such as nanorods[26] carbon nanotubes[27], nanofibers[28].

In view of the above mentioned advantages of nanomaterials and high sensitivity of fluorescence detection for biosensor design and application, another emerging trend for the heavy-metal sensor design is to modify the nanomateirals with certain special DNA sequences that can recognize and capture the taget heavy metal ions so that both detection sensitivity and specificity can be improved. For such a sensor design, however, it is crucial to find the right sequence of the DNA specific to the target analytes. Normally, DNA molecules are stablized by virtue of the forces such as electrostatic force, π-stacking and hydrogen forces[29]. The specific structural and functional nucleic acids that either have high binding affinity or excellent catalytic activity toward the target are called aptamers. Aptamers are obtained by means of *Systematic Evolution of Ligands by Exponential Enrichment* (SELEX), a protocol first proposed by Gold group and Szostak group independently[30,31]. The SELEX technique makes use of a nucleic acid library (10^{15}-10^{18} sequences of aptmers) and involves three processes including identification or selection of aptamers for target molecules, removal of non-binding ligands and amplification of

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bound aptamers[32]. The large aptamer pool ensures the structure, function and sequence variety of aptamers, whereas the separation step which requires sometimes special seperation protocols[33-36] plays a vital role in SELEX to guarantee the specific binding between the aptamer and the target. Due to accurate control in sequence synthesis, desired versatility and stability, many aptamers have been successfully found and isolated which can bind specifically toward a large number of targets ranging from organic molecules[37], proteins[38], bacteria[39], and cancer cells[40,41], and also to various metal ions such as K^{+}[42], Pb^{2+}[43], Cu^{2+}[44], Zn^{2+}[45], UO^{2+}[46] and etc.. For example, It is found that thymine-thymine (T-T) base pairs in a DNA sequence can interact with Hg^{2+}[47], cytosine in DNA can interact with Ag^{+}[14]. Actually, because of the high selectivity, the aptamers have become ideal recognition components for biosensor design in the combination of with varied detection techniques such as electrochemistry[48], resonance scattering spectroscopy[49], surface enhanced Raman spectroscopy (SERS)[50], colorimetry[51-53] and etc..

APTAMER-NANOMATERIAL BASED BIOSENSORS

As foregoing explicated, with the aid of nanotechnology, aptamer application and various fluorescence sensing mechanisms, many novel nanobiosensors for heavy metal detection are being exploited. To date a lot of of intelligent heavy-metal sensors with the combination of nanomaterials, aptamers and fluorescence detection mode been proposed and exploited. In the following, we would attempt to give some representative examples to demonstrate the research and development on this aspect.

Wang et al.[54] made use of FAM modified and rich-T bases contained DNA and achieved colorimetric and fluorescence detection of Hg^{2+} Figure 1. The detection mechanism is as follows: because ssDNA can be bound to citrate protected gold nanoparticles stronger than dsDNA, so the ssDNA can protect Au nanoparticles from aggregation[55]. In the presence of Hg^{2+}, ssDNA is folded into dsDNA, leading to the aggregation of Au nanoparticles and the corresponding colorimetric change. Correspondingly, the fluorescence of the dye is recovered, and so the change of fluorescence intenisty is proportional to the content of Hg^{2+} quantitatively. Such a biosensor exhibits a dynamic response range for Hg^{2+} detection from 9.6×10^{-8} to 6.4×10^{-6} M with the lower detection limit of 4.0×10^{-8} M.

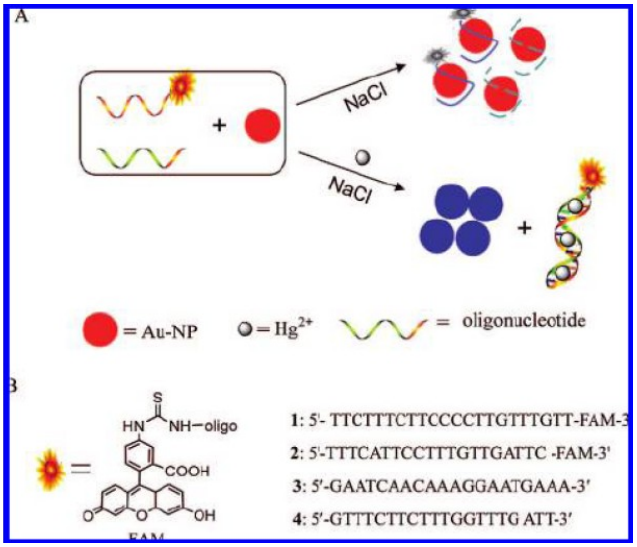

Figure 1 : The schematic plot shows the mechanism for the colorimetric and fluorescence detection of Hg^{2+}[54]

The organic dyes working as the donor in the FRET mechanism can be replaced by fluorescent quantum dots (QDs). Actually, QDs can offer some superior optical properties compared to organic dyes because QDs not only overcome the problem of photobleaching, but also possess high quantum yield, broad excitation spectrum and multiplexed detection ability[56]. Li et al[57] declared a *nanometal surface energy transfer* (NSET) strategy for ppb grade detection of Hg^{2+} Figure 2. They introduced CdS/ZnS core/shell QDs as the energy donor, Au nanoparticles as the energy accepter, and thymines contained mismatch DNA molecules as the linkers. In the presence of Hg^{2+}, the fluorescence of QDs is quenched because the distance between the QD and Au nanoparticles is shorten due to formation of T-Hg^{2+}-T pairs between the mismatch DNA. By recording the dramatic change of fluorescence intensity, Hg^{2+} can be probed with the lower detection limit 0.4 and 1.2 ppb in the buffer solution and in the river water, respectively.

Similarly, Huang and co-workers[58] adopted the "turn on" approach for Hg^{2+} detection. They used QDs as the donor and Au nanoparticles as the quencher. Due to long-lifetime fluorescence and unique photophysical properties of QDs, together with the excellent quenching performance of Au nanoparticles, high sensitivity with the lower detection limit 0.18 nM for Hg^{2+} detection

has been achieved. In the experiment, the 33-mer single-stranded DNA (stranded A) with a Mn:Cd/ZnS QDs attached at the 5' end was hybridized with a 10-mer single-stranded DNA (strand B) with a gold nanoparticle attached at the 5' end. This results in energy transfer from the Mn:CdS/ZnS QDs to the gold nanoparticles, leading to a decrease in the time-gated fluorescence intensity of the Mn:CdS/ZnS QDs. In the presence of Hg^{2+}, the folding of strand A leads to the release of strand B, so that the fluorescence of the Mn:CdS/ZnS QDs is increased.

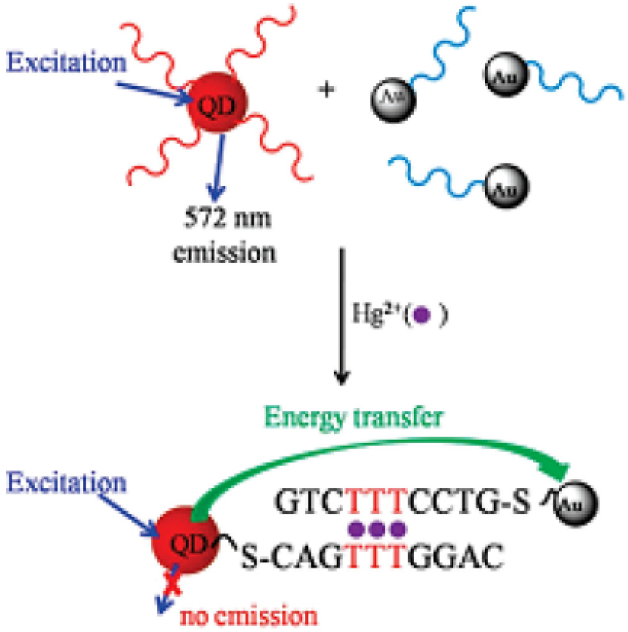

Figure 2 : The schematic plot explains the principle for the QD/DNA/Au nanoparticle-base sensor for Hg^{2+} detection[57].

Liu et al.[59] also employed "turn on" approach for selective and sensitive Hg^{2+} detection using thymine rich aptamer modified Au nanoparicles and OliGreen. In the presence of Hg^{2+}, the conformation of DNA changes from straight to folded conformation because of the formation of T-Hg^{2+}-T pairs, and some of DNA molecules are released from Au surface into solution and then conjugated with OliGreen. The enhanced fluorescence of OliGreen can thus be used for quantitative detection of Hg^{2+} ions. Besides the enhancement or the quenching of fluorescence, some other kinds of fluorescence detection methods have also been explored. Ye et al.[60] present a novel fluorescence polarization assay (FPA) based biosensor for Hg^{2+} detection Figure 3. Owing to the enhancement arising from gold nanoparticles and specifity due to the formation of T-Hg^{2+}-T complexes, this biosensor presents high sensitivity with 1.0 nM for the lower detection limit and high selectivity as well.

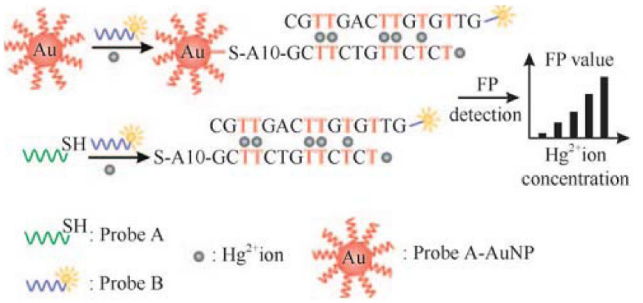

Figure 3 : The schematic plot illustrates the mechanism for Hg^{2+} detection based on fluorescence polarization enhancement by gold nanoparticles[60]

Graphene is a single-atom-thick and two-dimensional carbon material with remarkable electronic, thermal, mechanical properties[61,62]. It has been reported that graphene oxide (GO) can be bound to DNA nucleobases and nucleosides[63]. GO can be bound to ssDNA tightly based on the π-stacking interaction while dsDNA cannot be absorbed on graphene surface[64]. Therefore, GO can quench the fluorescence of the dye labeled on ssDNA, and the fluorescence can be recovered when the ssDNA molecules are released from the surface of GO. Based on this principle, Fan et al.[65] have successfully detected Ag^{+} with concentration as low as 20 nM Figure 4. This device obtains multiple detection ability and has the potential for other heavy metals detection. Similarly, carbon nanoparticles (CNPs) can also be used as a quencher for Ag^{+} detection[66].

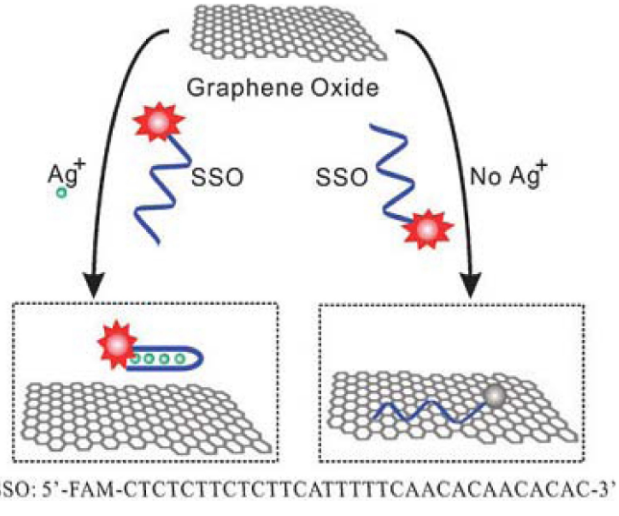

Figure 4 : The schematic illustration of the fluorescence sensor for Ag^{+} ions based on the target-induced conformational change of a silver-specific cytosine-rich oligonucleotide (SSO) and the interactions between the fluorogenic SSO probe and graphene oxide[65]

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Noble metal nanoclusters typically consist of no more than tens of atoms with dimension normally less than 2 nm. Because the dimension is comparable to the Fermi wavelength of electrons, they obtain molecule-like properties such as exhibiting strong and distinct fluorescence. Au[67] and Ag clusters[68] can be respectively synthesized by using BSA and ethoil as the stabilizing protector, and they have been adopted to detect Hg^{2+}[69,70] and monitor Cu^{2+}[71]. Compared with the BSA and ethoil modified nanoclusters, DNA-templateed Ag nanoclusters show higher quantum yield. Chang and co-worker[72] found that DNA can be used as the stabilizing agent to synthesize Ag nanoclusters due to the strong affinity between Ag and the cytosines in the single-stranded DNA, and they fabricated Ag nanoclusters by using 12 cytosine bases contained single-stranded DNA as the template. Taking advantage of DNA microarrays, Robert and co-worker[73] created new Ag nanoclusters with fluorescence tunable throughout the visible and near-IR region. And the as-synthesized nanoclusters have been used as the fluorescence donor for Hg^{2+} detection. The absorption and fluorescence characters of DNA-mediated Ag clusters can be adjusted through design of DNA sequence. Thymine-rich oligonucleotides can be used to synthesize blue/green-emitting Ag nanoclusters, while cytosine-rich oligonucleotides can be used as templates for synthesis of Ag nanoclusters which can emit either red- or bule/green fluorescence. Guo et al.[74] have used DNA protected Ag nanoclusters as fluorescent donor to detect Hg^{2+} in water. They synthesized the sequence of 5'CCCCCCCCCCCC 3'-stabilized Ag nanoclusters which have the excitaion and emission wavelengths at 580 and 650 nm respectively. This fluorescent probe shows the good selective recognition to Hg^{2+} and the detection limit reaches 5 nM. Su et al[75] developed a simple fluorescence sensor which combined with the properties of DNA-Cu/Ag nanoclusters and 3-mercaptopropionic acid (MPA). MPA can quench the fluorescence of DNA-Cu/Ag nanoclusters because MPA can be conjugated to the surface of Cu/Ag nanoclusters through the thiols and in the meantime, it weakens the interaction between the DNA and metal clusters. With addition of Cu^{2+} into solution, the thiols formed Cu-thiols complexes and they can be oxidized to form disulfide compounds. For this reason, thiols induced fluorescence quenching of Cu/Ag nanoclusters can be recovered in presence of Cu^{2+}. Such as a detection way may provide the detection of Cu^{2+} at the concentration as low as 2.7 nM, and it provides the potential for Cu^{2+} detection in pond water samples and Montana soil[75].

Besides aptamers, another functional DNA molecules called DNAzymes can also be used for biosensor desgin. DNAzymes can catalyze many of the reactions such as RNA/DNA cleavage and ligation worked as protein enzymes[76]. A series of heavy-metal specific DNAzymes have been obtained through the in vitro selection method including Pb^{2+}[77], Cu^{2+}[78], Zn^{2+}[79] and UO_2^{2+}[80]. For example, a Pb^{2+} dependent DNAzyme, named the 8-17 DNAzyme, show very high activity in the presence of Pb^{2+}. When the metal concentration is less than ppb grade, only Pb^{2+} can activate the reaction[81]. Due to their unique properties of high stability, low cost and ease of synthesis, they are applied for novel biotechnological applications, especially for heavy metal detection. Lu's group directed systematic work for utilization of DNAzymes with cleavage activity for detection of heavy metals such as Pb^{2+}[82], Cu^{2+}[83], Zn^{2+}[84] and UO_2^{2+}[80]. Wu et al.[85] introduced a kind of sensitive and specific fluorescence sensors for Pb^{2+} and Cu^{2+} detection with QDs and DNAzyme, respectively Figure 5. They made use of FRET between QDs and the the quencher labeled on the end of DNAzyme, and the fluorescence of QDs is enhanced when the DNA sequence is cleavaged by DNAzyme upon addition of heavy metals. This work also demonstrates the multiple

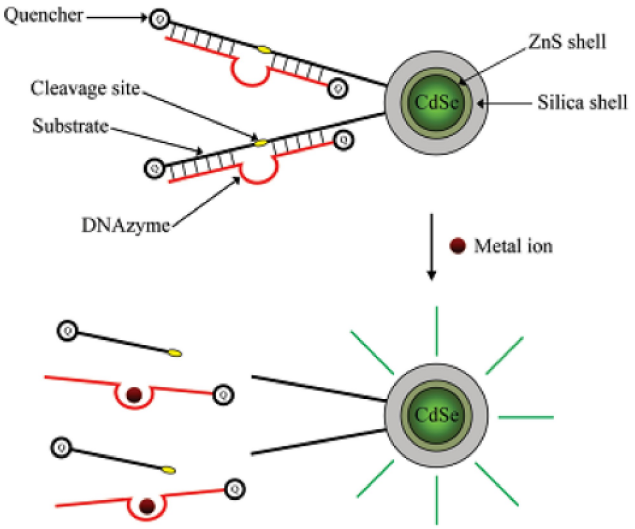

Figure 5 : The schematic illustrates the mechanism for the QD-based catalytical DNAzymes to metal ions the fluorescence from the QD is restored due to the cleavage of the DNAzyme substrate[85]

TABLE 1 : A list of DNA functionalized nanomaterials for fluorescence detection of heavy metals

Probe-design	Target	LOD	Ref.
Au/DNA-FAM	Hg^{2+}	4.0 nM	54
Quantumdot/DNA/Au	Hg^{2+}	2 nM	57
Au-DNA/ DNA-QD	Hg^{2+}	0.18 nM	58
Au-DNA/OliGreen	Hg^{2+}	25 nM	59
Au-DNA/DNA-FAM	Hg^{2+}	1.0 nM	60
DNA-Ag nanoclusters	Hg^{2+}	5 nM	74
Au-DNA/ DNA-QD	Hg^{2+}	0.49 nM	86
DNA-QDs	Hg^{2+}/ Ag^{+}	2/200 ppb	87
ssDNA-graphene	Ag^{+}	5 nM	65
ssDNA-carbon nanaoparticles	Ag^{+}	500 pM	66
DNA-Cu/Ag nanoclusters	Cu^{2+}	2.7 nM	75
DNA-Ag nanoclusters	Cu^{2+}	8 nM	88
DNA-Ag nanoclusters	Cu^{2+}	10 nM	89
DNAzyme-Au	Cu^{2+} /Pb^{2+}	1/1 nM	90
DNAzyme-QDs	Pb^{2+}/Cu^{2+}	0.2/0.5 nM	85
DNA-QDs/graphene	Pb^{2+}	90 pM	91

detection ability of QDs and high detection sensitivity for heavy metals.

CONCLUDING REMARKS AND OUTLOOK

With combination of the merits from both nanomaterials and biomaterials, the research on nano-biosensors now attracts increasing attention. For the application of nanotechnology, versatile nanomaterials can be employed, including Au/Ag nanoparticles and nanoclusters, quantum dots and nano-carbon materials such as carbon nanotubes and graphene, which are currently of special interest. For the use of biomaterials, because assorted aptamers can be readily synthesized and utilized to recognize and capture target analytes, they have now become a favorite candidate for the biosensor design. For the sensor signal recording, the measurement of fluorescence has been widely adopted due to its advantage of high sensitivity and convenience. In addition, the ways for the fluorescence measurement can be varied, such as recording of intensity change, color change, FRET signal switching-on and/or off and etc. As such, aptamer-based nano-biosensors provide high sensitivity, diversity, rapidity and convenience, and show a promising potential in the application for rapid detection of trace heavy metals in the environment. The development for the research of nano-biosensors is booming. This review article therefore only gives a glimpse of the rapid development in this research field, and definitely, there are many other good examples missed in this article. With the emphasis on the treatment of heavy-metal pollution, and with the development in nanoscience, bioscience and optical technology, we expect that more exciting results will come out in the near future.

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