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DNA as Sensors and Imaging Agents for Metal Ions

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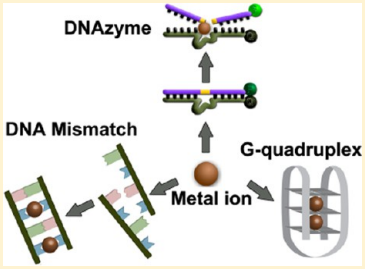
ABSTRACT: Increasing interest in detecting metal ions in many chemical and biomedical fields has created demands for developing sensors and imaging agents for metal ions with high sensitivity and selectivity. This review covers recent progress in DNA-based sensors and imaging agents for metal ions. Through both combinatorial selection and rational design, a number of metal-ion-dependent DNAzymes and metal-ion-binding DNA structures that can selectively recognize specific metal ions have been obtained. By attachment of these DNA molecules with signal reporters such as fluorophores, chromophores, electrochemical tags, and Raman tags, a number of DNA-based sensors for both diamagnetic and paramagnetic metal ions have been developed for fluorescent, colorimetric, electrochemical, and surface Raman detection. These sensors are highly sensitive (with a detection limit down to 11 ppt) and selective (with selectivity up to millions-fold) toward specific metal ions. In addition, through further development to simplify the operation, such as the use of "dipstick tests", portable fluorometers, computer-readable disks, and widely available glucose meters, these sensors have been applied for on-site and real-time environmental monitoring and point-of-care medical diagnostics. The use of these sensors for in situ cellular imaging has also been reported. The generality of the combinatorial selection to obtain DNAzymes for almost any metal ion in any oxidation state and the ease of modification of the DNA with different signal reporters make DNA an emerging and promising class of molecules for metal-ion sensing and imaging in many fields of applications.



1. INTRODUCTION

Sensing and imaging of metal ions have attracted much attention by scientists and engineers because of the important roles of metals in many fields such as environmental, biological, and medical sciences. Traditional analytical techniques for metal-ion detection, such as inductively coupled plasma mass spectrometry (ICP-MS) and atomic absorption spectroscopy (AAS), require expensive and bulky instrumentation and significant training to use properly, making it difficult for on-site and real-time detection. To overcome these limitations, significant progress has been made in developing sensors and imaging agents for the detection of metal ions, mostly based on organic molecules, peptides, proteins, or cells.[1−14]

DNA is a biopolymer that encodes the inheritable information of many organisms. At first glance, DNA does not appear to be a good candidate for sensing metal ions with high selectivity because the negatively charged phosphodiester backbones of DNA are known to be capable of binding cationic metal ions with poor selectivity for any particular metal ion. While the four DNA bases adenine (A), thymine (T), guanine (G), and cytosine (C) can also serve as ligands for metal ions,[15−19] many of these DNA−metal ion interactions are nonspecific and weak, making the use of DNA as sensors for metal ions very challenging because selectivity and sensitivity are required for the successful detection of a specific metal ion in the presence of other potentially interfering metals in complex samples.

To meet the challenge, two approaches have been established to identify metal-ion-selective DNA sequences. The first is through a combinatorial technique called in vitro selection, where random DNA libraries containing diverse DNA sequences are used to obtain desired sequences that can bind specific metal ions or use them as cofactors for catalysis.[20−25] The second approach utilizes DNA sequences discovered to be able to bind specific metal ions based on the study of the DNA structures or rational design.[26−33] By incorporation of signal reporters such as chromophores, fluorophores, electrochemical tags, and Raman tags, these metal-ion-specific DNA sequences found by these two approaches have been transformed into colorimetric, fluorescent, electrochemical, and Raman sensors and imaging agents for a broad range of metal ions with high sensitivity and selectivity.[23,25,34−63] This review covers the recent advances in this area (Table 1), with more focus on DNAzymes as sensors for metal ions.

Table 1. DNA Molecules Discussed in This Review as Sensors and Imaging Agents for Metal Ions

entry	DNA structures	mechanism of metal ion sensing	target metal ions	key refs
1	DNAzymes	catalytic cleavage of DNA substrates	Mg^{2+}, Zn^{2+}, Pb^{2+}, Cu^{2+}, UO_2^{2+}, Hg^{2+}, Co^{2+}, Mn^{2+}	20−25
2	DNA mismatches	formation of stable base pairs	Hg^{2+}, Ag^{+}, Cu^{2+}	31−33
3	DNA G-quadruplex	stabilization or destabilization of the G-quadruplex	K^{+}, Pb^{2+}, Cu^{2+}, Ag^{+}	26−30

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2. SENSORS BASED ON METAL-ION-DEPENDENT DNAZYMES

In the 1990s, DNA sequences with ligand binding (called DNA aptamers) or catalytic activities (called DNAzymes, deoxyribozymes, catalytic DNA, or DNA enzymes) were discovered through combinatorial techniques called in vitro selection or systematic evolution of ligands by exponential amplification (SELEX).[20,64−66] In these techniques, shown in Figure 1 as an example of the in vitro selection process for UO_2^{2+}-dependent DNAzymes, random DNA libraries containing up to 10^{15} different DNA sequences are applied under predefined selection pressures to isolate DNA sequences with desired properties out of the libraries; sequences thus selected are then amplified by polymerase chain reaction (PCR) to generate a new library for successive rounds of selection. After a few to a few dozen of such selection rounds, using negative selection to enhance metal-ion selectivity when necessary,[67,68] DNAzymes that are highly selective to use specific metal ions as cofactors to catalyze reactions can be obtained. In this way, DNAzymes that are dependent on Mg^{2+},[69,70] Zn^{2+},[71,72] Pb^{2+},[20,71] Cu^{2+},[21,73] UO_2^{2+},[74] Hg^{2+},[75] Co^{2+},[76] and Mn^{2+}[77] for various chemical and biological reactions have been successfully discovered. Among them, DNAzymes that can cleave or ligate nucleic acids have been widely applied in the development of selective and sensitive sensors for different metal ions, after they are conjugated with suitable signal reporters (Figure 2).[23,25,34−63]

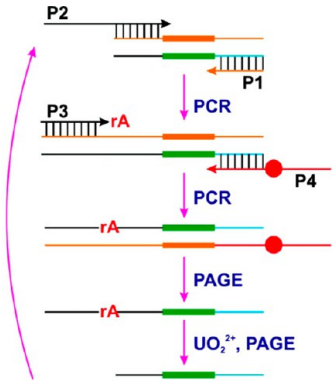

Figure 1. Scheme of the process for in vitro selection of UO_2^{2+}-dependent DNAzymes. The random DNA library (random regions shown in green) is amplified by PCR in the presence of primers P1−P4 and purified by polyacrylamide gel electrophoresis (PAGE) to generate an enriched pool. Then, those DNA sequences from the enriched pool that undergo UO_2^{2+}-induced cleavage are isolated by PAGE and used as the starting library for the next round of selection. After many such rounds of selection and negative selections, the DNA sequences in the final pool are cloned and sequenced. Adapted from ref 74.

2.1. Fluorescent Sensors Based on Metal-Ion-Dependent DNAzymes. *2.1.1. Fluorescent Sensors Labeled with Fluorophores and Quenchers.* Because of the ease in labeling DNAs with fluorophores and quenchers during or after the well-established automated solid-phase synthesis of DNA, the first report of a DNAzyme sensor was a fluorescent sensor for Pb^{2+} based on an 8−17 DNAzyme,[78] which showed much higher specificity to Pb^{2+} over other metal ions in catalyzing the cleavage of DNA substrates with a single RNA linkage (rA) at the cleavage site (Figure 3A). Such a high selectivity was attributed to the "lock-and-key" mode of catalysis for Pb^{2+}-dependent activity in comparison with other metal ions such as Zn^{2+} and Mg^{2+}.[79−84] The key to the sensing mechanism is to take advantage of the difference of DNA melting temperatures before and after metal-dependent cleavage. In the absence of a target metal ion, the enzyme strand (17E) can hybridize to its substrate strand (17DS) because the melting temperature can be designed to be above ambient temperature. Because 17DS and 17E are labeled with a fluorophore (FAM) and a quencher (Dabcyl), respectively, DNA hybridization resulted in placement of the quencher close to the fluorophore, resulting in a low fluorescent signal. Upon metal-catalyzed substrate cleavage, the melting temperatures of the two product strands become much less than those before cleavage, which can be designed to be lower than ambient temperature. As a result, the DNA duplex dehybridizes and the fluorophore-containing fragment is released, resulting in fluorescence enhancement due to separation of the fluorophore and quencher. Because the DNAzyme activity was dependent on the concentration of Pb^{2+} as the cofactor, the fluorescence enhancement rate was successfully used to determine the Pb^{2+} concentration in water.[78]

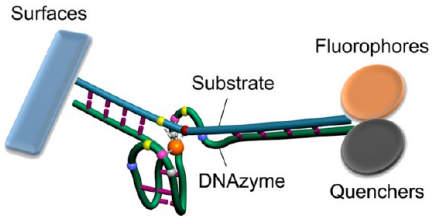

Figure 2. General sensor design based on nucleic acid cleavage of DNAzymes for metal-ion detection. The figure shows a typical fluorescent sensor. The fluorophore and quenchers may be replaced by other signal reporters, such as nanomaterials and electrochemical/Raman tags, to construct colorimetric, electrochemical, and Raman sensors. The DNAzyme may also be immobilized on a surface.

The above approach is named "catalytic beacon" because the increase of the fluorescent signal due to the catalytic activity is similar to a molecular beacon[85] but possesses several advantages. First, instead of detecting DNA/RNA only in the case of the molecular beacon, the catalytic beacon can detect a variety of targets such as metal ions and other molecules such as adenosine through a combination of DNAzyme and aptamer.[86] Second, the catalytic turnovers allow a single target to generate numerous products containing fluorophores or other labels, allowing amplification of the signals. Finally, instead of using the absolute intensity as the measure in the molecular beacon, which is vulnerable to background signal fluctuations due to autofluorescence by many species in cells or other sample matrixes, the catalytic beacon can rely on the rate of fluorescent increase, which is characteristic of target-induced cleavage.

While the above catalytic beacon approach allows the effective detection of metal ions, the background fluorescent signal is still relatively high because of potential dehybridization of the substrate strand from the enzyme strand in the absence of the target at ambient conditions. While an increase in the number of base pairs or GC contents can make hybridization stronger, a compromise has to be made to allow the cleaved product DNA strands to dehybridize in the presence of the target. To overcome this limitation, a dual-labeling approach was demonstrated by attaching fluorophore/quencher pairs to the two ends of the substrate DNA, and the background fluorescence of the sensor system was dramatically decreased for improved sensitivity in Pb^{2+} detection (Figure 3A).[87]

Because the melting temperature is the key to successful sensing, temperature can play a role in detection. To eliminate

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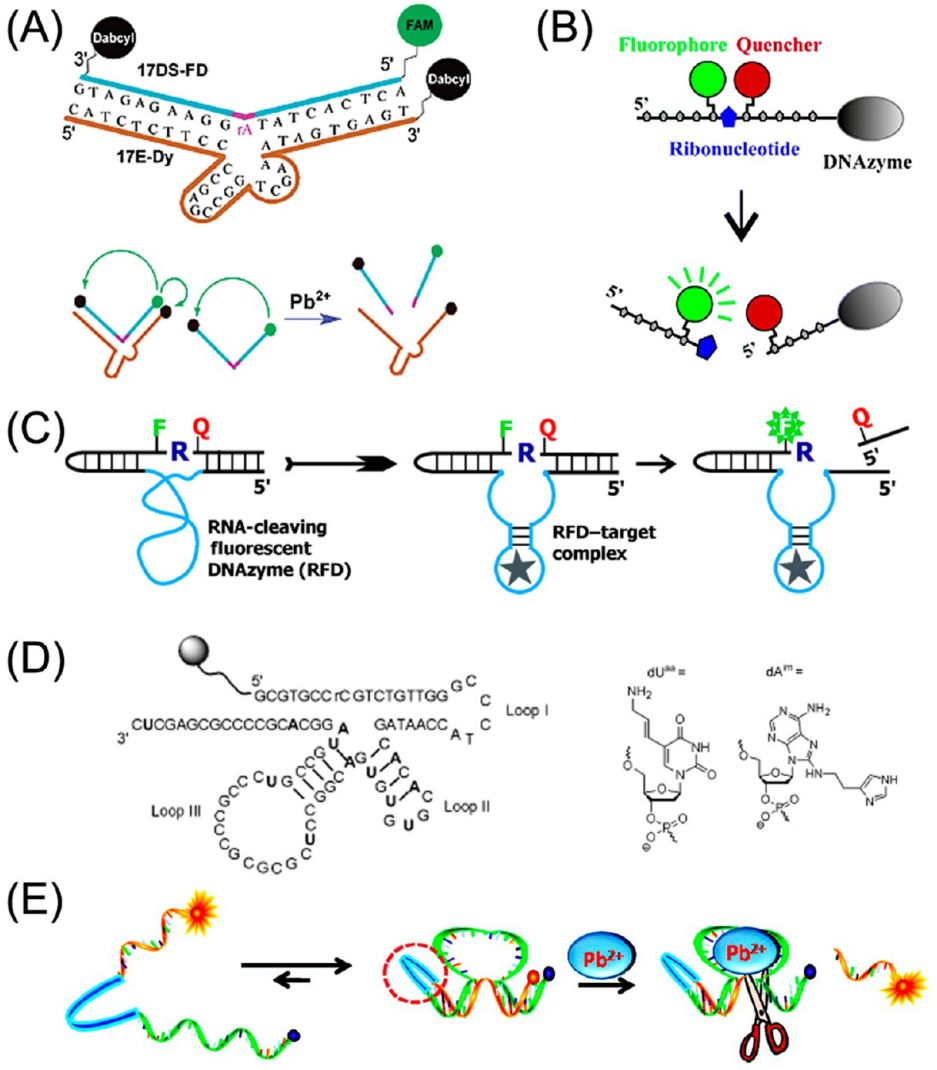

Figure 3. (A) Fluorescent sensor for Pb^{2+} based on a Pb^{2+}-dependent DNAzyme using a dual-labeled approach. (B) Attachment of the fluorophore and quencher pair close to the cleavage site of DNAzymes for DNAzyme selection and sensor design. (C) Bacterial detection using metal-ion-dependent DNAzymes with nearby fluorophore and quencher. (D) Hg^{2+}-dependent DNAzyme containing artificial nucleotides (bold U and A in the sequence, with corresponding chemical structures of modified dU^{aa} and dU^{im} shown on the right) for the development of Hg^{2+} sensors. (E) Single Pb^{2+} ion detection using a unimolecular DNA-catalytic probe. Adapted from refs 87, 94, 98, 75, and 103.

temperature as a confounding effect on the performance of the sensor, a temperature-resistant sensor for Pb^{2+} was developed by introducing site mutations to the binding arm of the DNAzyme.[88] When a classic Pb^{2+}-dependent DNAzyme was used in the sensor design, the sensitivity and selectivity of detection was improved because of the nature of the DNAzyme.[89] Later, taking advantage of the multiple turnover characteristics of catalytic and molecular beacons (CAMBs), the sensitivity of DNAzyme-based sensors for Pb^{2+} detection was further improved, and the system became easily compatible with aptazymes (a combination of an aptamer and DNAzyme or ribozyme, whose activity can be regulated by aptamer binding to its target) for the detection of other analytes.[90] In addition to the above Pb^{2+} sensors, using similar design concepts, UO_2^{2+}-,[74,91,92] and Cu^{2+}-dependent DNAzymes[21,73] were also successfully transformed into fluorescent sensors for the selective and sensitive detection of UO_2^{2+}[74] and Cu^{2+},[93] respectively. Notably, the detection limit of the UO_2^{2+} sensor was as low as 45 pM or 11 ppt,[74] lower than even the corresponding detection limit of ICP-MS. This work demonstrates the promise of DNAzyme-based sensors for high-performance metal-ion detection.

Instead of attaching fluorophores and quenchers to the ends of DNA strands, Li and co-workers inserted a fluorophore and a quencher close together at two nucleotides adjacent to a DNAzyme substrate's cleavage site, obtaining metal-ion-dependent DNAzymes through in vitro selection to cleave such substrate DNA for fluorogenic sensing (Figure 3B).[94−98] This approach enabled the synchronization of DNAzyme catalysis and fluorescence signaling[94] for many applications, including the discovery of fluorescent DNAzymes as sensors for wide pH ranges,[95] specific metal ions,[95] and bacteria (Figure 3C).[98] One of the most prominent advantages of such sensors is the low background fluorescence due to the closely localized pairs of fluorophores and quenchers, significantly improving the signal-to-noise ratio for more sensitivity detection.[94,97] However, one disadvantage of such design that the fluorophore and quencher can rarely be changed to other fluorophore/quencher pairs for multi-wavelength detection, because they are integral parts of the structure for the DNAzyme reactions.

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Perrin and co-workers introduced unnatural DNA bases into the random DNA library during in vitro selection and successfully selected a Hg^{2+}-dependent DNAzyme containing 8-histaminyl-dA and 5-aminoallyl-dU that hydrolyzes nucleic acid substrates (Figure 3D).[75] On the basis of a similarly modified DNAzyme, the Perrin group developed a sensor for Hg^{2+} with excellent selectivity and sensitivity via metal-ion-induced inhibition, although the signal readout is not through fluorescence.[99] Brennan and co-workers studied the quenching effect of heavy metal ions on fluorophore-labeled DNAs in sensor designs based on DNAzymes and provided general guidelines for the development of more efficient fluorescence-signaling DNAzymes.[100] Li and co-workers utilized a Mg^{2+}-dependent DNAzyme with a nonclassical allosteric design for the detection of metal ions and other molecules.[101] To enhance the performance of DNAzyme-based sensors, Willner and co-workers introduced ligation DNAzyme machinery coupled with peroxidase–mimic DNAzymes for the sensitive chemiluminescent detection of Cu^{2+}.[102]

In a typical sensor design, a nucleic acid cleaving DNAzyme and its substrate form a DNA duplex that brings close a fluorophore and quencher pair. Upon metal-ion-activated catalytic reaction, cleavage of the substrate causes fluorescence enhancement due to release of the fluorophore from the duplex.[74,75,78,87,93] In another design, Tan and co-workers connected the DNAzyme and substrate by a short oligonucleotide linker to construct a unimolecular form (Figure 3E).[103] In this case, the ratio between the DNAzyme and substrate was constant at 1:1, and the background signal originating from the unbound substrate was minimized. As a result, sensitive monitoring of a single Pb^{2+} ion was demonstrated, using a unimolecular DNA–catalytic probe containing both a Pb^{2+}-dependent DNAzyme fragment and a substrate motif (Figure 3E).[103]

In addition to using small organic molecules as fluorophores and quenchers, other nanomaterials can also be coupled with DNAzymes to develop metal-ion sensors. The Pb^{2+}-dependent DNAzyme was modified with biotin and then conjugated to multiwalled carbon nanotubes (MWNTs) coated with streptavidin (Figure 4A).[104] Catalytic cleavage of DNA substrates with multiple turnovers was maintained for the DNAzyme on the MWNTs compared to its unconjugated form in solution. The MWNTs quenched the fluorescence of nearby fluorophores so that a Pb^{2+} sensor could be designed based on cleavage of the fluorophore-labeled substrate by the Pb^{2+}-dependent DNAzyme. In addition to MWNTs, many other materials such as gold nanoparticles, graphene, and single-walled carbon nanotubes also exhibit strong quenching effects on fluorophores and can take the place of quenchers in the sensor design.[105−110] Pb^{2+}[105,107−110] and Cu^{2+}[106] sensors with improved performance were developed through covalent attachment or noncovalent adsorption of DNA on these nanomaterials. For example, graphene was used as efficient quenchers for the development of a Pb^{2+} sensor based on Pb^{2+}-dependent DNAzymes (Figure 4B),[110] where cleavage of fluorophore-labeled substrates significantly reduced the affinity of the fluorophore-labeled DNA fragment to graphene surfaces. In addition to methods based on the fluorescence intensity, gold nanoparticles[111] and graphene[112] were also conjugated to Cu^{2+}-dependent DNAzymes and substrates in order to induce changes in the fluorescence anisotropy upon Cu^{2+}-mediated cleavage of the DNA substrates. In another work, quantum dots were conjugated with DNA to serve as fluorophores for the multiplexed detection of Pb^{2+} and Cu^{2+} in one solution.[113]

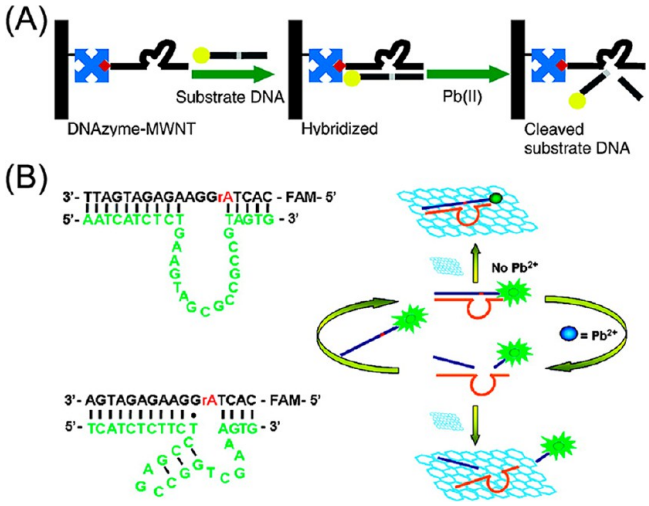

Figure 4. (A) MWNTs as quenchers for fluorophores for the development of Pb^{2+} sensors based on Pb^{2+}-dependent DNAzymes. (B) Graphene as an efficient quencher to bind a fluorophore-labeled DNAzyme–substrate duplex for the detection of Pb^{2+}. Adapted from refs 104 and 110.

2.1.2. Surface-Immobilized Fuorescent Sensors. To enable regeneration and long-term storage of the sensors for more practical applications, Pb^{2+}-dependent DNAzymes were immobilized on surfaces to develop solid sensor chips.[114−120] Gold surfaces were functionalized with thiol-modified and quencher-labeled DNAzymes, and then fluorophore-labeled substrates were hybridized with the immobilized DNAzymes. Upon coming in contact with samples containing Pb^{2+}, the substrates were cleaved by the DNAzymes and the fluorophores were released, resulting in Pb^{2+}-dependent fluorescence enhancement for Pb^{2+} detection (Figure 5A).[114] The immobilized sensors showed improved sensitivity over the original solution sensor while preserving the selectivity, and they could be regenerated after tests by the addition of a fresh fluorophore-labeled substrate, as well as stored in the solid state.[114] An internal standard was also introduced into the same sensors immobilized on nanocapillary array membranes to realize ratiometric fluorescence detection, which is more resistant to background fluctuation.[115] Later, microfluidic sensor devices for Pb^{2+} detection were developed by conjugating the same DNAzymes on poly(methyl methacrylate) microchannel walls.[116] By immobilizing DNAzymes and substrates on microarrays, Ye's[117] and Zhao's[118] groups constructed sensor arrays for the high-throughput detection of Pb^{2+} and Cu^{2+}, which combined the high selectivity and sensitivity of DNAzymes and the high-throughput analysis of microarrays. Sensitive flow-cytometric detection of Pb^{2+} was successfully achieved by Guo and co-workers using magnetic beads coated with labeled DNAzymes and substrates, where the ultrahigh performance was ascribed to the use of magnetic beads and flow cytometry to abstract a fluorescence signal from a complicated sample matrix and reduce the light scattering effects, respectively.[119] Brennan and co-workers trapped different DNAzymes and substrates in sol–gel-derived matrixes as sensors for a series of metal ions. This sol–gel sensor technology reduced the interference of metal-ion-induced fluorophore quenching and enabled the multiplexed detection of four metal-ion species using different DNAzymes in an array (Figure 5B).[120]

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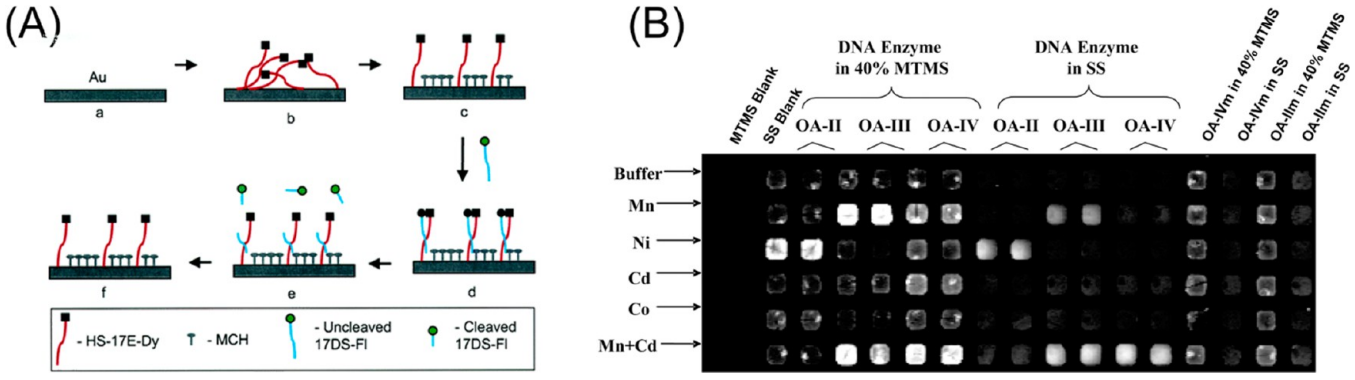

Figure 5. (A) Immobilizing Pb^{2+}-dependent DNAzymes and substrates on gold surfaces for fluorescent Pb^{2+} detection. (B) Sol–gel sensor array using different DNAzymes for the simultaneous detection of four metal-ion species. Adapted from refs 114 and 120.

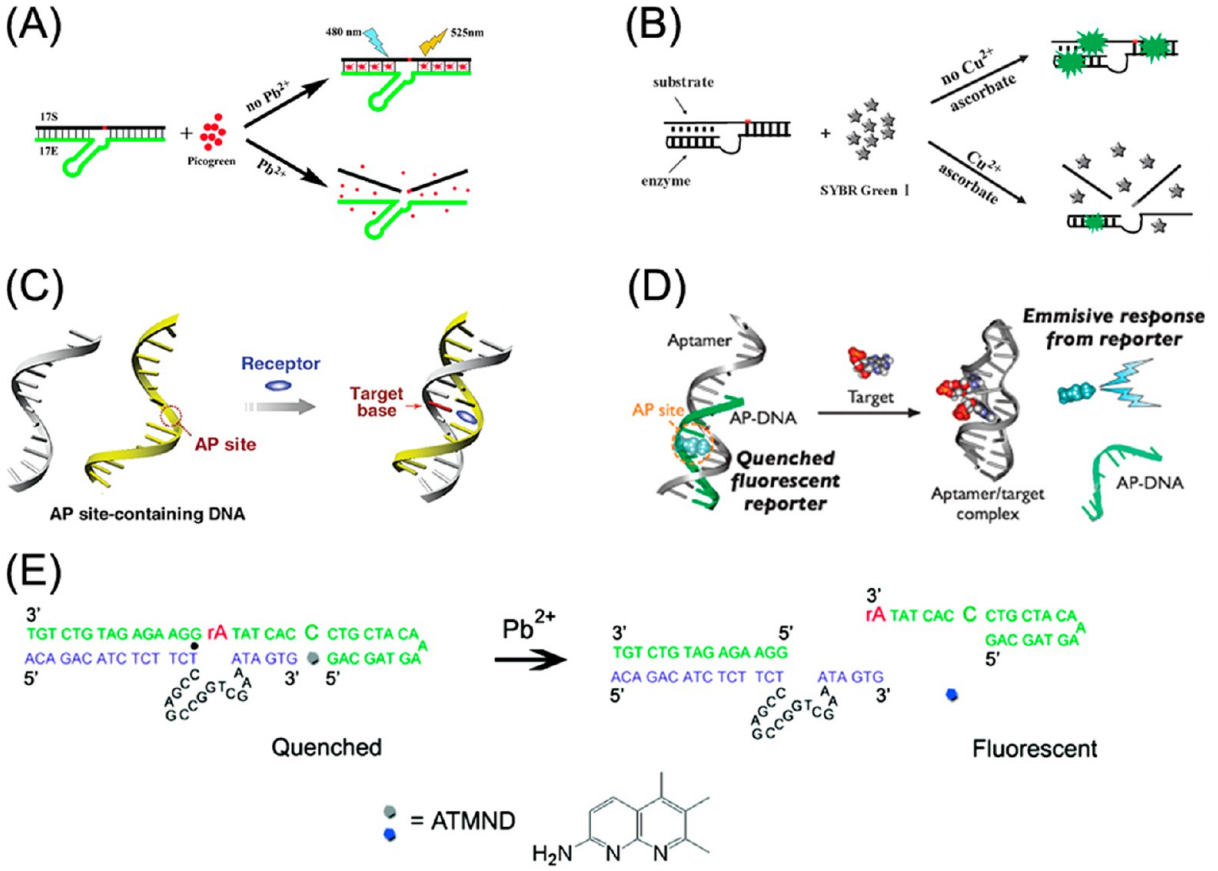

Figure 6. (A) Label-free fluorescent sensor for Pb^{2+} using Picogreen. (B) Label-free fluorescent sensor for Pb^{2+} using SYBR Green I. (C) Binding of ATMND (receptor) to a dSpacer (AP site) opposite to a cytosine (Target base) in a DNA duplex. (D) Label-free detection of small molecules using aptamers containing an AP site. (E) Label-free fluorescent sensors for Pb^{2+} using ATMND and a DNAzyme–substrate duplex containing a vacant site. Adapted from refs 125, 128, 129, 135, and 123.

2.1.3. Label-Free Fluorescent Sensors. Although covalent labeling of DNAzymes and substrates with fluorophores and quenchers has been widely applied as a general strategy for the design of various metal-ion sensors, such labeled DNAs are usually more complicated to synthesize and more expensive compared to DNAs without labels, and in some cases the labels may also interfere with the binding between DNAzymes and substrates or metal ions, reducing their activities. To overcome this challenge, label-free fluorescent sensors that do not require covalent labeling of DNAzymes and substrates have been developed.[121−128] A number of such studies have utilized DNA-intercalating dyes that exhibit distinct fluorescence characteristics when bound with double-stranded DNA (dsDNA) or single-stranded DNA (ssDNA) regions.[122,124−126,128] For example, Jiang and co-workers coupled cleavage of a DNA substrate by a Pb^{2+}-dependent DNAzyme in the presence of Pb^{2+} with quantitative PCR and subsequently measured the fluorescence of SYBR Green I upon its binding with the PCR products to detect the concentration of Pb^{2+} at a high sensitivity.[122] Using graphene as the quencher for the DNA-binding GelRed dye, a label-free Cu^{2+} sensor was developed based on a Cu^{2+}-dependent DNAzyme.[124] Picogreen (Figure 6A)[125] and SYBR Green I (Figure 6B)[128] were also applied as fluorescent dsDNA intercalators for the construction of label-free

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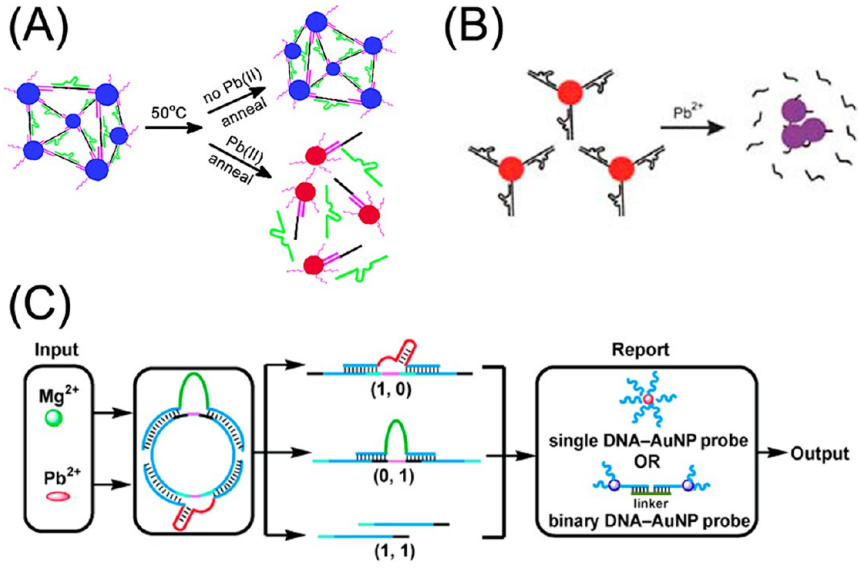

Figure 7. (A) Colorimetric Pb^{2+} sensor based on DNAzyme and functionalized gold nanoparticle assemblies that undergo disassembly in the presence of Pb^{2+}. (B) Colorimetric Pb^{2+} sensor based on a Pb^{2+}-induced assembly of DNAzyme-functionalized gold nanoparticles. (C) Logic response to Pb^{2+} and Mg^{2+} using gold nanoparticles as a signal output by a DNA duplex containing two DNAzymes as sensors. Adapted from refs 136, 146, and 147.

Pb^{2+} and Cu^{2+} sensors by Wang's and Yao's groups, respectively. In addition to small-molecule intercalating dyes, conjugated polymers that can distinguish dsDNA and ssDNA by changes in the fluorescence intensity were used to recognize the cleaved substrate by a Cu^{2+}-dependent DNAzyme for sensitive Cu^{2+} detection.[126]

Teramae and co-workers found that fluorescent compounds, such as derivatives of 2-amino-5,6,7-trimethyl-1,8-naphthyridine (ATMND) and riboflavin, could selectively bind to apurinic/apyrimidinic site (AP) sites (e.g., dSpacers and C3 spacers) in a DNA duplex and result in its fluorescence quenching via complementary hydrogen bonding with the opposite bases and $\pi-\pi$ stacking with the flanking bases (Figure 6C).[129−133] By designing a target-induced switch of DNA structures that caused ATMND binding or release, they also developed fluorescent sensors for DNA strands[129,131,133] and organic molecules (Figure 6D).[130,132,134,135] We took advantage of the specific binding between ATMND and AP sites (dSpacer or vacant sites) in a DNAzyme−substrate duplex to control the binding sites of fluorophores in the label-free metal-ion-sensor design (Figure 6E).[121,123,127] The more defined binding sites (AP sites) of ATMND in a DNA duplex compared to the nonspecific binding of intercalating dyes to DNA can help in the rational design of the sensors and minimize the risk of activity reduction due to the binding of dyes to the active cores of DNAzymes. In the presence of target metal ions such as Pb^{2+} and UO_2^{2+}, cleavage of the substrates by DNAzymes caused the deformation of duplex regions and released ATMND from the binding site because ATMND cannot bind to ssDNA. The metal ions were quantified by measuring the fluorescence enhancement of released ATMND.[121,123] The sensitivity and selectivity of this label-free method was found to be comparable with the previously reported labeled version, and it was further combined with the CAMB approach to develop more efficient label-free fluorescent sensors for a broader range of analytes.[127]

2.2. Colorimetric Sensors Based on Metal-Ion-Dependent DNAzymes. *2.2.1. Colorimetric Sensors Based on Gold Nanoparticles.* Besides fluorescence, colorimetry has also been used as the signal output for DNAzyme-based sensors, enabling the detection of metal ions by direct eye observation without any instrumentation or excitation light.[136−153] Taking advantage of the color changes of DNA-functionalized gold nanoparticles upon transformation between discrete and aggregated states as demonstrated by Mirkin et al.[154] and Alivisatos et al.,[155] our group has developed a series of colorimetric sensors based on different DNAzymes for the detection of a series of metal ions.[136−144] In 2003, the first colorimetric Pb^{2+} sensor based on gold nanoparticles and DNAzymes was developed (Figure 7A).[136] In this approach, the gold nanoparticles were cross-linked as aggregates by DNAzyme substrates through DNA hybridization, displaying a blue color with a broad absorption band around 700 nm. In the presence of Pb^{2+}, the cross-linker substrates were readily cleaved, so that no aggregates could be formed and the dispersed gold nanoparticles showed a red color with an absorption band at 522 nm. The light extinction ratio E_{522}/E_{700} could then be used as a measure for the quantification of Pb^{2+} concentrations in water. Interestingly, the dynamic range of Pb^{2+} detection was successfully tuned from 0.1−4 to 10−200 μM by changing the ratio of active and inactive DNAzymes.[136] A follow-up study further optimized the sensor design by testing different arm lengths of DNAzymes, gold nanoparticle alignments, ratios of DNAzymes and substrates, pH, and temperatures.[138] The detection of Pb^{2+} and formation of nanoparticle aggregates were accelerated at room temperature by the "tail-to-tail" alignment of DNA to 42 nm gold nanoparticles.[137] To make the sensor system less vulnerable to environmental fluctuations, a new approach of "light-up" (assembly) detection compared to the previous "light-down" (disassembly) response was developed with the assistance of invasive DNA.[139] When an improved design was applied utilizing asymmetric DNAzymes and substrates to form gold nanoparticle aggregates, the usage of invasive DNA was avoided to further simplify the detection.[140] In addition to the Pb^{2+}-dependent DNAzyme, Cu^{2+}- and UO_2^{2+}-dependent DNAzymes were also functionalized with gold nanoparticles for the development of colorimetric sensors for Cu^{2+}[141] and UO_2^{2+}[143] using a similar approach, respectively. Instead of cross-linking nanoparticles by DNA, Li and co-workers demonstrated that metal-ion-induced cleavage of

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substrates by DNAzymes on dispersed gold nanoparticles caused aggregation of the nanoparticles, and metal ions such as Pb^{2+} could be detected by a color change from red to purple (Figure 7B).[146] A colorimetric sensor with logic response to Pb^{2+} and Mg^{2+} was described by Zhang and co-workers based on two DNAzymes and cross-linked gold nanoparticles (Figure 7C).[147] When gold nanoparticles functionalized with DNAzymes were entrapped in hydrogels via DNA cross-linking, they could also serve as a colorimetric sensor for the detection of metal ions such as Cu^{2+}, as reported by Yang and co-workers.[149] Besides color change, DNAzyme-cross-linked gold nanoparticles were also used for ultrasensitive metal-ion detection via the light scattering signal change upon formation or dissolution of aggregates.[150]

In addition to thiol–gold interactions, DNA can bind to gold nanoparticles noncovalently via its nucleotide bases, with much higher binding affinities to gold for ssDNA than fully complementary dsDNA. Following this principle, label-free colorimetric sensors for metal ions have been developed using unmodified gold nanoparticles and DNAzymes.[143−145,148,151−153] Both Wang's[145] and our group[144] reported the use of a label-free Pb^{2+}-dependent DNAzyme and unmodified 13 nm gold nanoparticles as colorimetric sensors for Pb^{2+} detection in water. In the presence of Pb^{2+}, the DNAzyme–substrate duplex underwent cleavage and formed ssDNA fragments, which stabilized gold nanoparticles upon salt addition. Therefore, the concentration of Pb^{2+} in the samples was quantified by measuring the color change from blue to red. A similar approach was also applied in our group to a UO_2^{2+}-dependent DNAzyme, and the resulting label-free sensor successfully detected UO_2^{2+} in water without any modifications to the gold nanoparticles or DNAzymes.[143] For a label-free colorimetric Cu^{2+} sensor, Yang and co-workers utilized a unimolecular self-cleaving Cu^{2+}-dependent DNAzyme and unmodified gold nanoparticles.[148] Through nanogold-seeded nucleation amplification, a sensitive label-free UO_2^{2+} sensor was developed using a UO_2^{2+}-dependent DNAzyme and unmodified gold nanoparticles.[151] Similar to their labeled analogues, the label-free sensors based on DNAzymes and gold nanoparticles were also capable of serving as light-scattering sensors for the sensitive detection of Pb^{2+} and Cu^{2+}, with performances comparable to those of the labeled ones.[152,153]

2.2.2. Colorimetric "Dipstick" Tests Using Lateral-Flow Devices. Because the molar extinction coefficients of gold nanoparticles are much higher than those of most organic dyes, they are ideal materials for developing colorimetric test strips for metal-ion detection at low concentrations. Using lateral-flow devices similar to a previous approach for aptamers,[156] a Pb^{2+}-dependent DNAzyme was coupled with gold nanoparticles to construct an easy-to-use dipstick for Pb^{2+} in paints (Figure 8A).[157] In the presence of Pb^{2+}, cleavage of the substrates by the DNAzyme removed biotin from the surface of gold nanoparticles, enabling the capture of these red-colored nanoparticles on the test zone for the visible detection of Pb^{2+} concentrations. Zeng and co-workers utilized Cu^{2+}- and Pb^{2+}-dependent DNAzymes to fabricate a similar lateral-flow device for the detection of Cu^{2+} (Figure 8B) and Pb^{2+}, respectively.[158,159] The introduction of a catalytic DNA circuit in the Pb^{2+}-sensitive device dramatically enhanced the sensitivity of the sensor compared with previous solution-based approaches.[159]

2.2.3. Colorimetric Sensors Based on Hydrogen Peroxidase–Mimic DNAzymes. Another strategy to develop colorimetric DNAzyme-based sensors for metal-ion detection is the use of metal-ion-dependent DNAzymes to recognize target

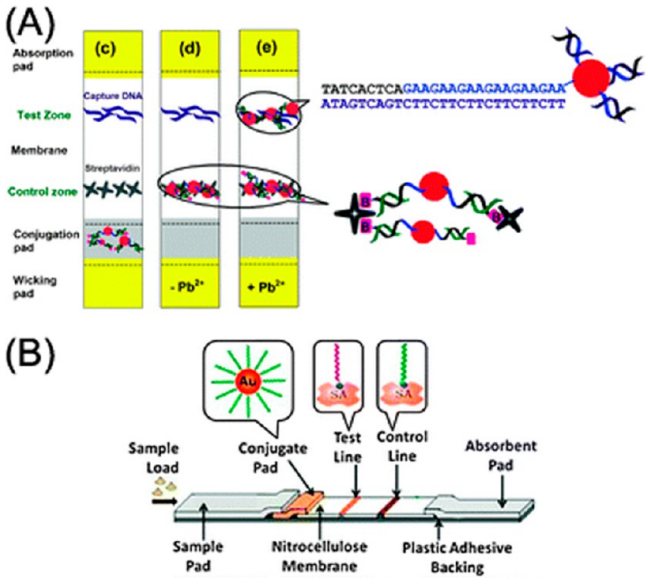

Figure 8. (A) Lateral-flow dipstick for the visible detection of Pb^{2+} in paint. (B) Lateral-flow dipstick for the visible detection of Cu^{2+}. Adapted from refs 157 and 158.

metal ions and hydrogen peroxidase–mimic DNAzymes to catalyze color-generating chemical reactions for signal output.[43,160−168] Willner and co-workers developed a DNAzyme cascade to transform Pb^{2+}-induced cleavage of the substrate by the Pb^{2+}-dependent DNAzyme into the production of colored oxidized 2,2′-azinobis(3-ethylbenzothiazoline)-6-sulfonate (ABTS), therefore achieving colorimetric detection of Pb^{2+} by monitoring of the increase of absorbance at 414 nm or direct observation of green color generation (Figure 9A).[164] The group also demonstrated a similar design for the colorimetric detection of UO_2^{2+} using a UO_2^{2+}-dependent DNAzyme and introduced one additional Mg^{2+}-dependent DNAzyme for the construction of a logic gate.[165] Tan and co-workers combined the Cu^{2+}-dependent DNAzyme, substrate, and a hydrogen peroxidase–mimic DNAzyme into a unimolecular sensor and achieved colorimetric detection of Cu^{2+} with high sensitivity (Figure 9B).[166] Similar dual-DNAzyme approaches were also applied by other groups for the detection of Pb^{2+} and Cu^{2+} using Pb^{2+}- and Cu^{2+}-dependent DNAzymes, respectively.[167,168]

2.3. Electrochemical and Raman Sensors Based on Metal-Ion-Dependent DNAzymes. Electrochemical and Raman signals have also been reported for developing metal-ion sensors based on metal-ion-dependent DNAzymes.[169−184] For example, Plaxco and co-workers achieved parts-per-billion-level electrochemical Pb^{2+} detection using an electrode-bound DNAzyme assembly, where cleavage of the substrates by Pb^{2+}-dependent DNAzymes brought the attached electrochemical tags much closer to the electrode to enhance the electrochemical signals (Figure 10A).[169] Shao and co-workers also developed an electrochemical Pb^{2+} sensor by immobilization of DNAzymes and DNA–gold biobar codes on electrodes for amplified detection (Figure 10B).[170] Similarly, other excellent works utilized different DNAzymes and nanomaterials to construct a series of sensors on electrodes and have successfully detected Pb^{2+},[173,177,179,182,184] Cu^{2+},[172,174,181] Mg^{2+},[178] and UO_2^{2+}[180] in various samples. In additional to the above sensors based on electrical signals, electrochemiluminescent sensors were also demonstrated for the sensitive detection of Pb^{2+}.[171,173,175,183] A study modifying DNAzyme-based sensors with Raman tags on

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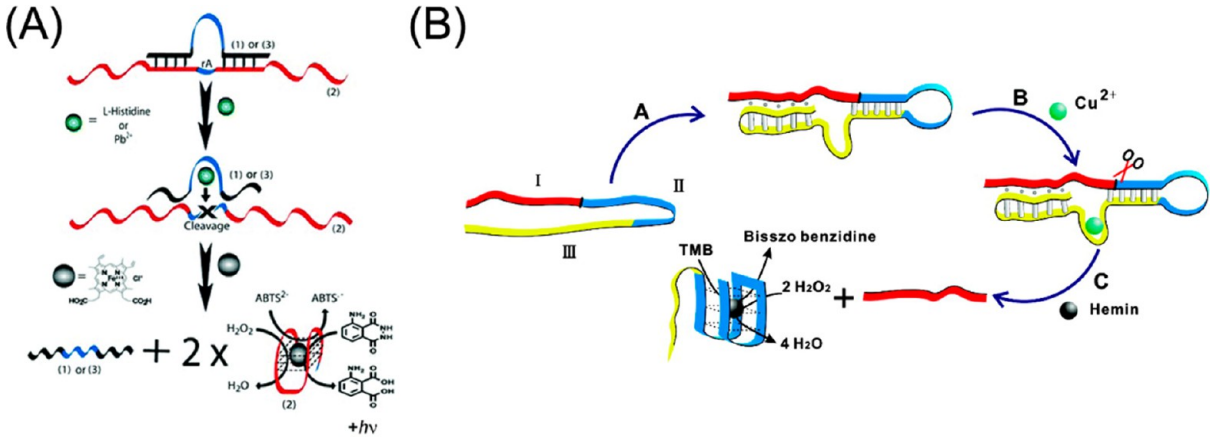

Figure 9. (A) Pb^{2+}-induced activation of a hydrogen peroxidase−mimic DNAzyme (red) by a Pb^{2+}-dependent DNAzyme (blue) for colorimetric Pb^{2+} detection. (B) Colorimetric detection of Cu^{2+} by a unimolecular sensor containing a hydrogen peroxidase−mimic DNAzyme (blue) by a Cu^{2+}-dependent DNAzyme (yellow). Adapted from refs 164 and 166.

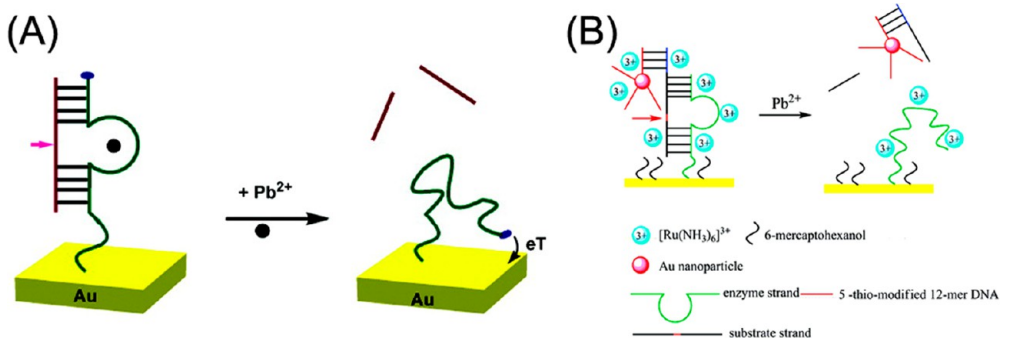

Figure 10. (A) Electrical detection of Pb^{2+} by Pb^{2+}-induced cleavage of the DNAzyme substrates that decreases the distance between the electrochemical tags and gold electrodes. (B) Electrochemical Pb^{2+} sensor based on Pb^{2+}-induced cleavage of the DNAzyme substrates that releases DNA−gold biobar codes containing ruthenium complexes. Adapted from refs 169 and 170.

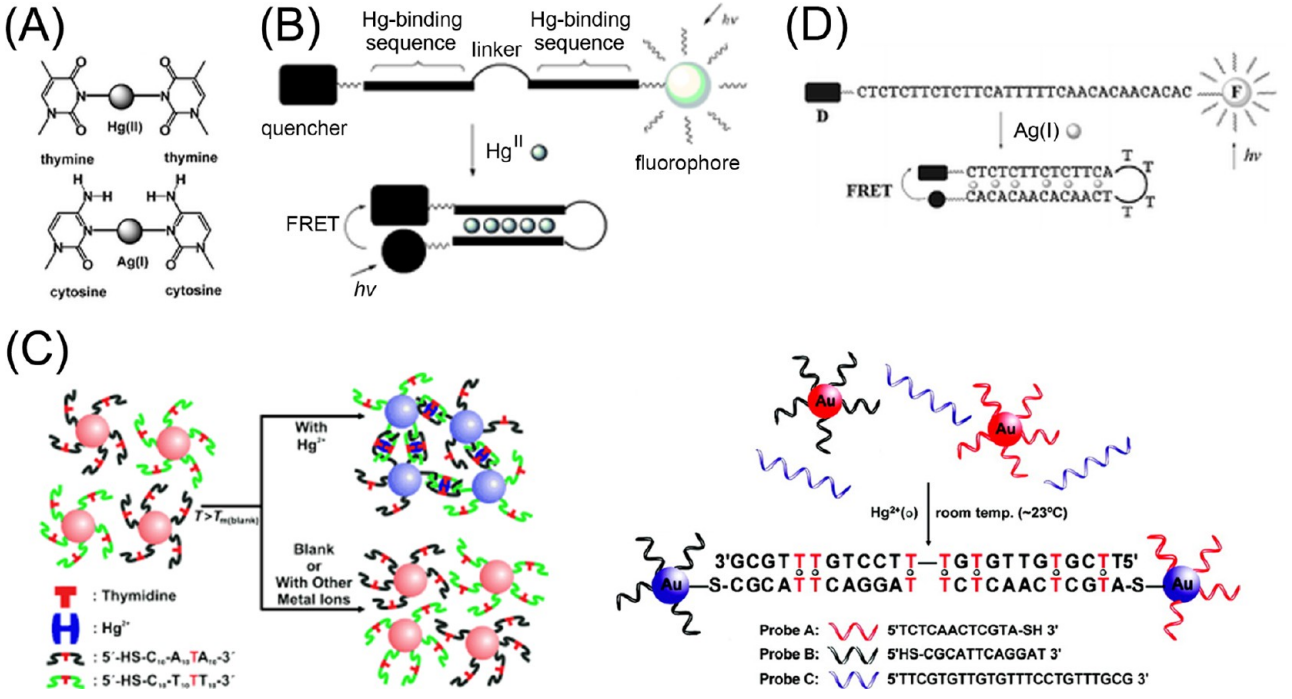

Figure 11. (A) Binding of Hg^{2+} and Ag^{+} by T−T and C−C mismatches in DNA. (B) Fluorescent Hg^{2+} sensor based on T−Hg^{2+}−T. (C) Fluorescent Ag^{+} sensor based on C−Ag^{+}−C. (D) Colorimetric sensors for Hg^{2+} based on nanomaterials. Adapted from refs 32, 185, 187, 198, 199.

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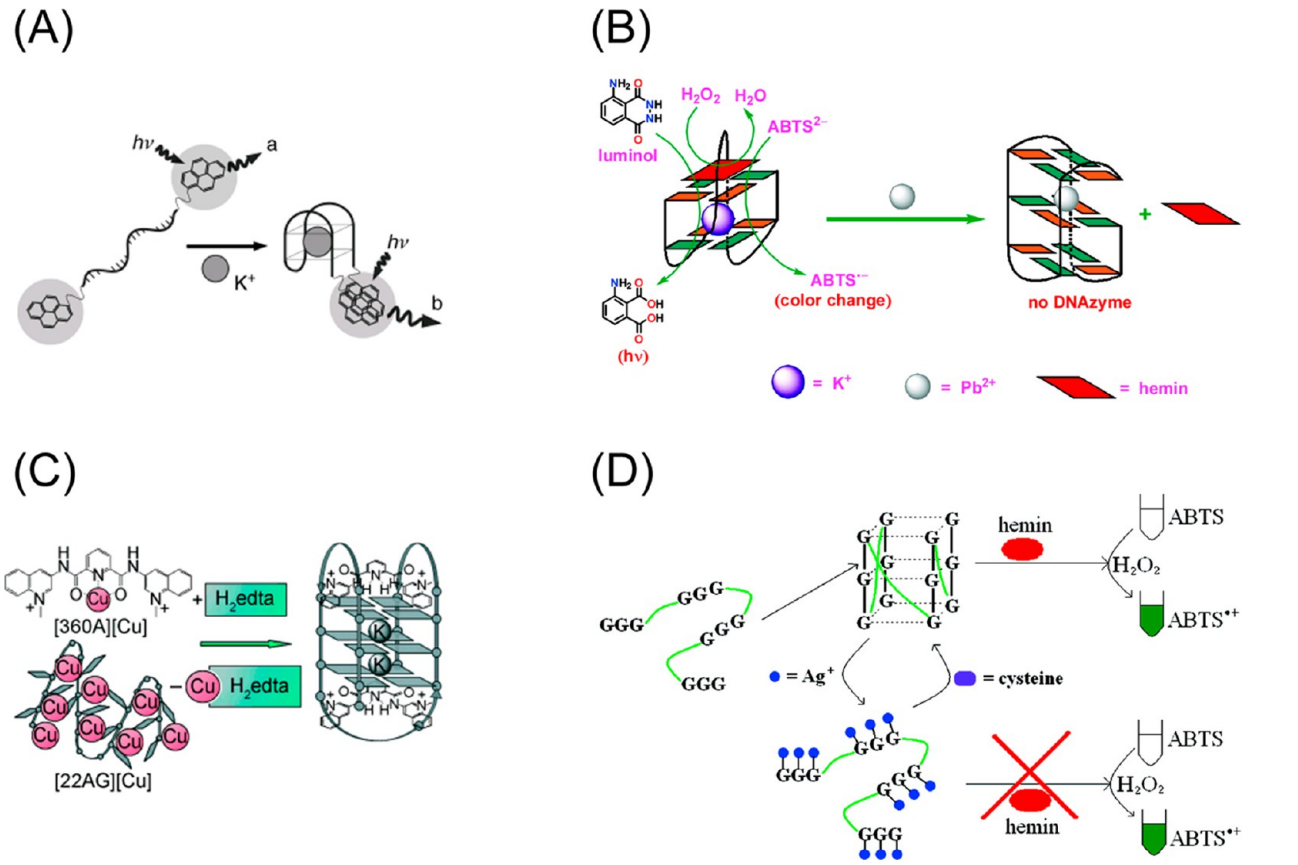

Figure 12. Sensors based on G-quadruplex DNA stabilized by K^+ (A), Pb^{2+} (B), and Cu^{2+} (C), while destabilized by Ag^+ (D). Adapted from refs 240, 197, 29, and 30.

gold nanoparticles instead of electrochemical tags enabled the detection of Pb^{2+} by surface-enhanced Raman spectra (SERS).[176]

3. SENSORS BASED ON METAL-BINDING STRUCTURES

In addition to metal-ion-dependent DNAzymes that were selected from random DNA libraries through combinatorial techniques, at least two types of DNA structures were also discovered to be efficient binding motifs for a series of metal ions. One of them is DNA mismatches that can bind specific metal ions to form stable "base pairs".[31−33] Examples include natural nucleobases such as T−T and C−C mismatches that can form stable T−Hg^{2+}−T[185,186] and C−Ag^+−C[187] structures in DNA duplexes with high specificity to Hg^{2+} and Ag^+, respectively (Figure 11), as well as artificial bases that form stabilized pairs with Ag^+ and Cu^{2+},[15,31,33,188−191] although the latter has not been widely applied in sensors because of the lack of commercial availability of the artificial bases required.[33] The other is the DNA G-quadruplex that is stabilized or destabilized by specific metal ions,[43,53,55,192,193] such as K^+,[26,194,195] Pb^{2+},[27,196,197] Ag^+,[30] and Cu^{2+}[28,29] (Figure 12). Taking advantage of the specific metal ion−DNA interactions, many metal-ion sensors based on such DNA structures have been developed in recent years.[32,43,53,55,192,193]

3.1. Hg^{2+} Sensors Based on T−Hg^{2+}−T-Containing DNA. Upon the first discovery of stable complex formation between T−T mismatches and Hg^{2+} in a DNA duplex with high specificity to Hg^{2+} by Ono and co-workers (Figure 11A),[32,185−187] Hg^{2+}-induced DNA duplex formation has been widely applied as a switch for Hg^{2+} sensor development.[58,102,142,198−230] Examples include, but are not limited, to DNA-based sensors for Hg^{2+} with colorimetry,[198,199,201,203,204,218] fluorescence,[142,200,208,209,211,219,220,224,225] electrochemistry,[210,215] SERS,[213] surface plasmon resonance,[217,227] and evanescent wave generation[216] as signal output. Ono and co-workers reported a fluorescence sensor for Hg^{2+} based on T−T mismatch (Figure 11B).[185−187] Upon Hg^{2+} binding to the DNA, it folded and brought the pair of quencher and fluorophore close enough to induce fluorescence resonance energy transfer (FRET) that caused a fluorescence quenching response to Hg^{2+}. Besides fluorescence signals, two independent groups of Mirkin's[198] and Liu's[199] developed colorimetric sensors for Hg^{2+} through the assembly of DNA-modified goldnanoparticles when T−T mismatches were stabilized by Hg^{2+} (Figure 11C).

3.2. Ag^+ Sensors Based on C−Ag^+−C-Containing DNA. Interestingly, in addition to the specific interaction between Hg^{2+} and T−T mismatches, Ono and co-workers also found that C−C mismatches in DNA could selectively bind Ag^+ (Figure 11).[187] Following this principle, a number of Ag^+ sensors have been developed (Figure 11D),[187,208,215,225,231−237] using colorimetry,[231,232,235,237] fluorescence,[208,225,233,236] light scattering,[234] electrochemistry,[215] and atomic force microscopy.[238]

3.3. Sensors for K^+, Pb^{2+}, Cu^{2+}, and Ag^+ Based on G-Quadruplex DNA. K^+ has been known to stabilize G-quadruplex motifs in DNA.[26] Fluorescent or fluorescence resonance energy transfer (FRET)-based K^+ sensors were developed by labeling G-quadruplex DNA sequences with fluorophores (Figure 12A)[195,239,240] or using label-free intercalating dyes.[241−243] Conjugated polymers that could distinguish K^+-bound and nonbound G-quadruplex DNA were also used for

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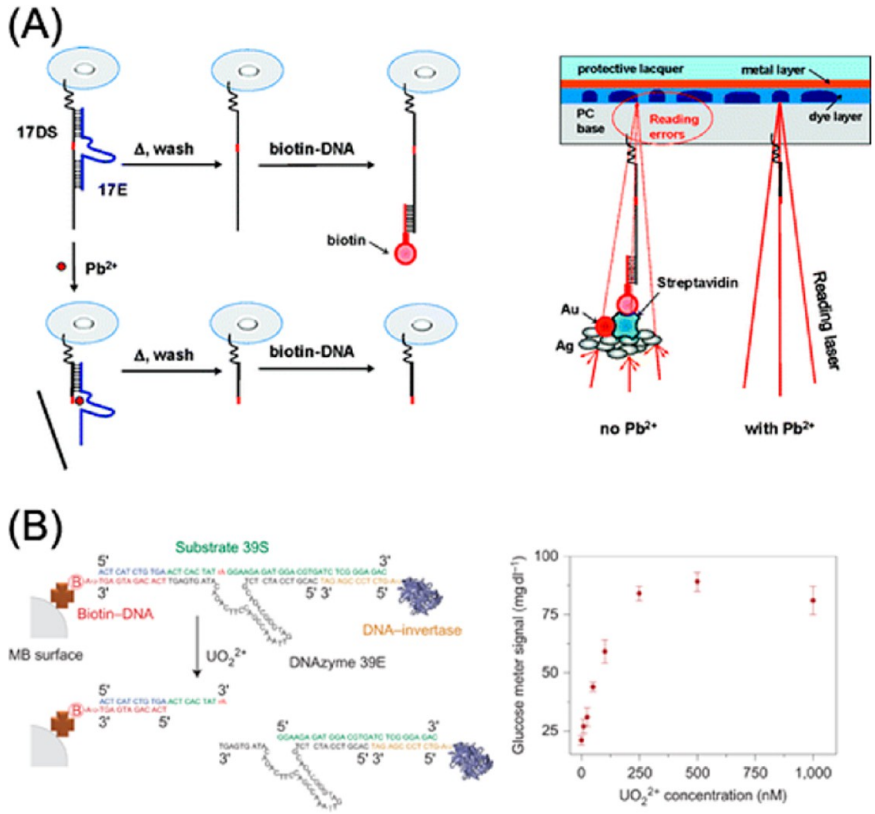

Figure 13. (A) Detection of Pb^{2+} using computer-readable disks based on a Pb^{2+}-dependent DNAzyme (17E). (B) UO_2^{2+} sensor based on a UO_2^{2+}-dependent DNAzyme (39E) conjugated to invertase and magnetic beads for the PGM detection of UO_2^{2+}. Adapted from refs 273 and 274.

the amplified fluorescence detection of K^+ in a homogeneous solution.[244,245] Colorimetric[246−249] and electrochemical[250,251] methods were also demonstrated based on G-quadruplex DNA for K^+ detection.

In addition to K^+, studies have also shown that Pb^{2+} is also capable of stabilizing the DNA G-quadruplex.[27,196] A colorimetric sensor (Figure 12B),[197] followed by a fluorescent version,[252] was developed by Wang and co-workers for selective Pb^{2+} detection, as an alternative of sensors based on Pb^{2+}-dependent DNAzymes. Since then, many other Pb^{2+} sensors have been reported using a similar DNA G-quadruplex, spanning between colorimetric,[253−255] fluorescent,[214,226,256−263] electrochemical,[215,264,265] and resonance scattering sensors.[266,267]

Compared to the roles of K^+ and Pb^{2+} in stabilizing DNA G-quadruplexes, the role of Cu^{2+} is less well understood but most likely provides stabilization in the form of a metal−ligand complex (Figure 12C).[28,29] Two studies by Wang and co-workers demonstrated success in developing fluorescent Cu^{2+} sensors based on this property.[29,268]

Unlike the above three metal ions, instead of stabilization, Ag^+ was found by Kong and co-workers to destabilize DNA G-quadruplexes (Figure 12D).[30] Through this approach, colorimetric[30,269] and fluorescent[270−272] sensors were also developed for selective Ag^+ detection.

4. SENSORS BASED ON COMBINATION OF DNAZYMES AND METAL-BINDING DNA STRUCTURES

By combining the selective metal recognition of the DNA structures and the sensitive signal amplification of the DNAzymes, a series of studies have taken the advantages of both metal-binding DNA structures and DNAzymes for sensitive and selective sensing of metal ions. The binding between specific metal ions and DNA structures can induce DNA structure changes and activate hydrogen peroxidase-mimic DNAzymes for colorimetric detection.[203,212,233,235] Besides peroxidase-mimic DNAzymes, nucleic acid cleaving DNAzymes have also been used for the signal amplification via their activation by the interaction between metal ions and metal-binding DNA structures for fluorescent sensor development.[142,222]

5. PORTABLE SENSORS USING WIDELY AVAILABLE DEVICES

In point-of-interest applications, such as in-field or at-home detection of metal ions, laboratory-based analytical instruments such as spectrometers and electrochemical workstations are not available, thus demanding new metal-ion sensors compatible with portable devices for quantitative detection. Although the lateral-flow devices mentioned above (section 2.2.2) can realize fast detection without the aid of instruments, this detection is only semiquantitative and based on observation by eye, which may suffer from human error. Quantitative detection, therefore, is difficult. To address this challenge, interesting technologies have been developed to design sensors based on publically commercialized devices, which would enable not only trained personnel but also the general public to monitor metal ions at almost any point of interest.

Yu and co-workers immobilized Pb^{2+}-dependent DNAzymes and substrates with nanomaterials on the surface of computer-readable disks (Figure 13A).[273] The nanomaterials cause error signals during laser reading of the disk. When samples containing Pb^{2+} were applied to such disks, Pb^{2+}-induced cleavage of the

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substrates caused the loss of nanomaterials, thus removing error signals. Using software that counted error numbers, the Pb^{2+} concentration could be successfully quantified. Any portable computer equipped with a CD drive should be able to use this method for Pb^{2+} detection.

In our group, we developed a new technology to take advantage of the most successfully commercialized public diagnosis device, personal glucose meters (PGMs), for metal-ion detection (Figure 13B).[274,275] In this approach, invertase, an enzyme that converts PGM-inert sucrose into PGM-detectable glucose, was conjugated with a UO_2^{2+}-dependent DNAzyme–substrate duplex and immobilized on the surface of magnetic beads. When samples containing UO_2^{2+} were applied to the sensor, cleavage of the substrates disrupted the duplex structure and released invertase from the surface into the solution. After removal of the magnetic beads by a magnet, the released invertase was allowed to catalyze the production of glucose, whose concentration was proportional to that of UO_2^{2+} in the sample. Finally, the concentration of UO_2^{2+} was successfully quantified via PGM measurement.[274] To minimize the interaction between metal ions and functionalized materials on the surface of magnetic beads, we further demonstrated an invasive DNA approach that separated the DNAzyme reaction and invertase release/catalysis and achieved sensitive detection of both Pb^{2+} and UO_2^{2+} at concentrations well below the regulated levels by the United States Environmental Protection Agency (U.S. EPA).[275] Xiang and co-workers also developed another approach using the PGM to detect Cu^{2+} in water via the ability of Cu^{2+} to catalyze azide–alkyne reactions,[276] which was previously applied by Mirkin and co-workers for the colorimetric detection of Cu^{2+} using gold nanoparticles.[277]

6. SENSING AND IMAGING OF METAL IONS IN LIVING CELLS

In contrast to the large amount of work on metal-ion detection in vitro using DNA-based sensors, the detection and imaging of metal ions in biological systems are of great significance for medical and biological studies. However, there are only very limited examples of DNA-based sensors for metal-ion detection in living cells. Difficulties include the delivery of sensor DNA into desired locations in cells, maintaining the stability of DNA strands against enzymatic degradation in living cells, and the controlled "activation" of sensors at specific locations inside cells. Our group has recently developed a fluorescent sensor for UO_2^{2+} utilizing DNAzymes for UO_2^{2+} recognition and gold nanoparticles for efficient cellular delivery (Figure 14).[278] The sensor was cell-compatible and efficiently delivered into living cells for UO_2^{2+} imaging, demonstrating the promise of DNAzyme-based sensors for cellular applications for the first time. By fluorescence microscopy analysis, the sensor was localized in lysosomes and indicated the accumulation of UO_2^{2+} in lysosomes when the living cells were in a UO_2^{2+}-polluted media. Further work is still needed to investigate the mechanism of uptake and the properties of the sensor in more detail and develop sensors for cellular imaging of other metal ions. Although this study just scratches the surface of metal-ion detection in vivo, it is apparent that DNA-based sensors will play larger and more important roles in in vivo applications in the future.

7. SUMMARY AND PERSPECTIVE

Through both combinatorial selection and rational design, a number of DNAzymes and DNA structures have been found to

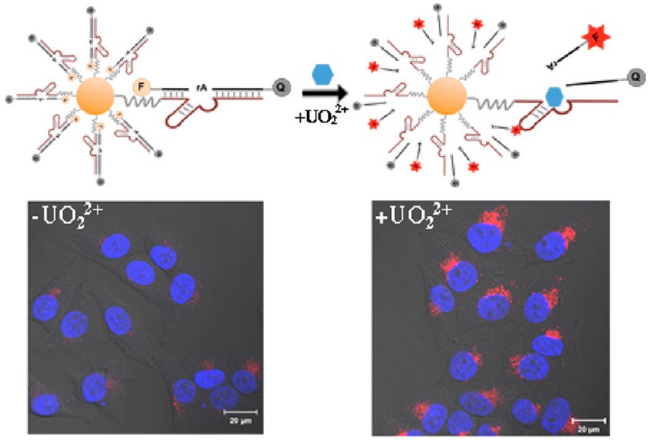

Figure 14. Live cell imaging of UO_2^{2+} by fluorophore-labeled uranyl-specific DNAzymes and substrates immobilized on gold nanoparticles. Adapted from ref 278.

display highly selective responses to specific metal ions. Using these DNA sequences as the basis, many metal-ion sensors have been developed utilizing various analytical techniques, including colorimetry, fluorescence, electrochemistry, SERS, and light scattering. For on-site and point-of-care applications, metal-ion sensors that can be used with commercially available portable devices, such as computer CD drives and PGMs, are already available for quantitative detection, whereas lateral-flow devices are ideal for instrument-free semiquantitative analysis of metal ions by direct color observation.

7.1. Advantages of DNA Molecules as Sensors and Imaging Agents for Metal Ions. Although DNA was not the first choice as sensors for metal ions because of perceived nonspecific electrostatic interactions between the phosphodiester backbone of the DNA and metal ions, reports from many laboratories around the world in the past decade have now firmly established that some DNA molecules can be effective sensors and imaging agents for metal ions. In the process, these studies have also demonstrated distinct advantages of DNA-based methods, particularly DNAzyme-based methods, over other methods.

The first advantage is that DNAzymes that are specific for almost any metal ion in specific oxidation states may be obtained using the same in vitro selection protocol, most notably even without prior knowledge of how the sensing molecules can bind selectively to that certain metal ion.[74,75,87,93,200] In contrast, it is usually difficult for most other techniques to apply successful strategies in designing sensors for one metal ion toward other metal ions, and thus extensive trial and error is required for sensor development for each additional metal ion. Until recently, antibodies were considered a general method to obtain sensing molecules for a broad range of targets. However, because of the need to elicit immune responses, it is often very difficult to generate antibodies selective for targets as small as metal ions. DNAzymes, because they are obtained in vitro, do not have the same issue as antibodies.

Another major challenge in designing sensors for metal ions is a lack of selectivity; the initially designed small molecule intended to bind one metal ion can often end up binding to other metal ions even more strongly. When this occurs, more work is required to redesign the molecules to better bind the intended target, which again is largely a process of trial and error. The in vitro selection method mentioned above is not immune to

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this problem when DNAzymes selected to be specific for one metal ion are more active in the presence of another metal ion. To meet this challenge, a "negative selection" strategy has been developed to remove the population of DNA sequences that bind competing metal ions, resulting in DNAzymes that are more selective for the target metal ion.[67,68] The reason why this strategy works for in vitro selection is that, instead of starting with one design and having to repeat the process of redesign, it starts with a large DNA sequence library (up to 10^{15} variations). Even though "negative selection" removes a large percentage of sequences, there are enough sequences left to perform the function in the presence of target metal ions.

Even though many molecules, especially biomolecules (e.g., proteins), are known to bind metal ions strongly and selectively, they are not metal sensors yet because another major component is signal transduction. It is often difficult to transform metal binding into signals in a general way without interfering with the binding. In contrast, because it is relatively easy to modify DNA with a reporter group, the reporter can be placed further away from the binding site and signal transduction can be realized based on the melting temperature differences before and after metal binding. Therefore, the third advantage of DNA-based sensors is the straightforward nature of transforming metal-ion recognition into different signal outputs and achieving efficient signal amplification for more sensitive detection without sacrificing metal-ion selectivity.[23,34−40,42−61,192,193,279−299] As shown in Figure 2, through the introduction of fluorophores, chromophores, nanomaterials, electrochemical tags, and Raman tags into the same design strategy, DNA-based sensors for metal ions based on fluorescence, colorimetry, electrochemistry, and surface Raman enhancement have been developed. In addition, many DNA-related enzymatic reactions and DNA-functionalized nanomaterials have been successfully incorporated into DNA-based sensors for signal amplification to achieve more sensitive detection of metal ions.

The fourth advantage of DNAzyme-based metal sensors is a general method to tune the dynamic range of detection.[108,121,144] This feature is especially important for the sensing and imaging of metal ions because every metal ion has a threshold level above which it is considered toxic. More importantly, this threshold level varies depending on the sample matrix or location where the metal ion resides. For example, the threshold for Pb^{2+} in soil is 400 ppm but is much lower in drinking water (15 ppb, as defined by the U.S. EPA). Even for the same matrix, the threshold can be different. For example, the defined Pb^{2+} level for paint on walls is 1.0 mg/dL but is lower for paint on toys (100 ppm) because Pb^{2+} in toys poses more danger to children. Therefore, it is not enough to have sensitive and selective sensors for metal ions; the sensors must also possess tunable dynamic ranges. DNAzymes can fulfill this requirement.

Unlike sensors for diamagnetic metal ions such as Ca^{2+},[300,301] Zn^{2+},[302−304] Cu^{+},[305,306] Hg^{2+},[307−309] and Pb^{2+},[310,311] designing and synthesizing sensitive and selective sensors for paramagnetic metal ions, such as copper and iron, remain a significant challenge. Even though fluorescent and chemiluminescent sensors based on various chelating agents have been reported for Co^{2+},[312−316] Cu^{2+},[317−335] Fe^{2+},[336−338] and Fe^{3+},[339−342] many of them are based on the quenching of fluorescence due to the paramagnetic metal ions' intrinsic fluorescence quenching properties, which is generally undesirable for analytical purposes because of the small dynamic range and potential false positives caused by nonspecific quenching in real samples. Other problems with current sensors include the requirement for oxidizing reagents such as hydrogen peroxide and poor selectivity. Therefore, the fifth advantage of DNA-based sensors is the ease of rational design to circumvent the quenching effect of paramagnetic ions by spatially separating the metal-recognition part from the fluorescent-signaling moiety, so that they are independent of each other. For example, our previously reported metal-ion-sensing platform based on DNAzyme catalytic beacons spatially separated the two elements (fluorophore/quencher and metal-ion binding site) by rigid dsDNA, resulting in fluorescent "turn-on" sensors, not only for diamagnetic metal ions such as Pb^{2+}[78,87,89] and UO_2^{2+}[74] but also for paramagnetic Cu^{2+},[93] with high sensitivity and selectivity.

Finally, DNA is biocompatible and biodegradable and is not recombinant. Thus, it is environmentally benign. Under physiological conditions, DNA is nearly 1000-fold more stable to hydrolysis than proteins/antibodies and nearly 100000-fold more stable than RNA.[343] The well-defined globular structures of catalytic DNA are also not easily recognized by endo- or exonucleases and thus are more resistant to nuclease attack than ssDNA or even dsDNA/RNA.[344] When folded, the compact globular catalytic DNAs are also less likely to bind other biomolecules in cells than ssDNA or dsDNA/RNA. In addition, unlike proteins or antibodies, most DNAzymes can be denatured and renatured many times without losing binding ability or activity. They can be stored under rather harsh, denaturing conditions and can be used when the correct conditions are restored. Therefore, DNA has a much longer shelf-life than many other biomolecules and is thus more suitable for field studies. Finally, DNA is adaptable to fiber-optic and microarray technology,[345−347] which is important for onsite or remote sensing of multiple metal ions simultaneously.

7.2. Future Directions. One important task in this field is the identification of new DNAzymes or DNA structures that can recognize more metal ions. Currently, DNA-based sensors are excellent in the detection of only a series of metal ions including K^{+}, Mg^{2+}, Zn^{2+}, Pb^{2+}, Cu^{2+}, Co^{2+}, Mn^{2+}, Hg^{2+}, Ag^{+}, and UO_2^{2+}. However, for other important metal ions such as Fe^{2+}, Fe^{3+}, Ni^{2+}, Cr^{3+}, and Cd^{2+}, there are very few DNA sequences found to show high specificity and affinity toward them. To address this challenge, more in-depth investigation is required to improve the classic DNA selection or discovery techniques. In addition, modified DNA bases and backbones with functional groups capable of metal binding are promising candidates for incorporation into DNA sequences to obtain DNAzymes that can recognize the metal ions that may be difficult for natural DNAs.

While a number of DNAzymes have been obtained that are specific for different metal ions, a fundamental understanding of the structural features responsible for the remarkable selectivity is still lacking. Biochemical studies have identified conserved sequences for metal binding and catalytic activities.[20,80,91,96,348−350] Biophysical studies, such as FRET and smFRET studies, have suggested that certain DNAzymes use the "lock-and-key" mode of metal binding and catalysis for the most active metal ions, similar to protein enzymes.[79,82,92,221,351,352] However, because of difficulty in obtaining three-dimensional (3D) structures of these DNAzymes, the exact 3D structural features remain to be elucidated.

Another challenge is the multiplexed detection of different metal ions simultaneously. Although a few studies using quantum dots and microarrays have demonstrated great promise, the number of metal-ion species that can be analyzed in one test is still limited. Not only are new DNA-based sensors needed for

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other metal ions, but a buffer condition compatible for the detection of many metal ions is also demanded. For the former, it can be anticipated that new DNA sequences for more metal ions will be identified through selection or discovery in the near future. For the latter, it is highly recommended that the selection or discovery of new DNA-based sensors for metal ions should be carried out under the same conditions (including buffer pH, ionic strength, and temperature) as those for known sensors to ensure that all of the sensors can be used in one solution for multiple metal ions without compromising the performance of any sensor.

As the public demand for monitoring hazardous metal ions quantitatively at the point of interest increases, a large market of portable sensors for metal ions is emerging and is expected to grow rapidly. Although achievements have been made in metal-ion detection using some commercial devices for the public, such as glucose meters and computer CD drives, there is still a need to make these methods of detection more user-friendly for public usage.

Finally, while DNA and DNAzyme sensors for the detection of metal ions in the environment have been relatively well developed, including commercially available products,[353] there are relatively fewer reports of using the DNA and DNAzymes as sensing or imaging agents for detection of metal ions in living cells and in vivo. Recent reports of DNAzyme-based imaging agents for the detection of uranyl in cells[278] and DNAzyme-based MRI contrast agents[354] are encouraging. Such sensors and imaging agents will provide more exciting opportunities for scientists to uncover the roles of metal ions in biological systems.

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Notes

The authors declare no competing financial interest.

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