

Deployment of functional DNA-based biosensors for environmental water analysis

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Abstract

Various functional DNA molecules have been developed for the detection of environmental contaminants in water, but their practical applications have been limited. To address this gap, this review highlights the efforts to develop field-deployable water quality biosensors. The biosensor devices include microfluidic, lateral flow and paper-based devices, and other more novel ideas such as the conversion of glucometers for the detection of environmental analytes. In addition, we also review DNA-functionalized hydrogels and their use in diffusive gradients in thin films (DGT) devices. We classify the sensors into one-step and two-step assays and discuss their practical implications. While the review is focused on works reported in the last five years, some classic early works are cited as well. Overall, most of the existing work only tested spiked water samples. Future work needs to shift to real environmental samples and the comparison of DNA-based sensors to standard analytical methods.

Keywords: aptamers; DNAzymes; gold nanoparticles; biosensors; fluorescence; lateral flow; microfluidics

Introduction

Environmental water quality monitoring is growing ever more important due to increasing human population and activities [1-3]. Heavy metal ions, metalloids, inorganic nutrients, pharmaceuticals, and pesticides are amongst the most common analytes, while tap, lake, river, groundwater and waste waters are the most common environmental matrices. Each analyte typically has one or a few instrumentation-based standard methods for detection. For example, metal detection typically relies on inductive-coupled plasma (ICP) atomic emission or mass spectrometry. Such methods require the shipment of samples to centralized labs for analysis, resulting in long turnaround times and high costs. On the other end, portable assays and kits based on simple chemical reactions such as the use of colorimetric reactions for the detection of nitrate, lead, mercury and arsenic are also available [4, 5]. However, such assays usually have low sensitivity and or specificity.

Biosensors provide a promising alternative solution for water quality monitoring due to their high selectivity [5]. Biomolecules such as enzymes and antibodies have been used to develop biosensors for a long time [6-9]. Some well-known examples include glucose meters (using glucose oxidase), pregnancy and Covid-19 test strips (using antibodies). Compared to proteins, DNA has much higher stability and programmable structures. In addition they are amenable to chemical synthesis and modification, making DNA well suited for biosensor development [10-13]. Moreover, in addition to simple hybridization DNA probes, functional-DNA-based biosensors have been extensively studied for environmental analyte detection [10]. Functional DNA refers mainly to two types of single-stranded DNA molecules: DNA aptamers and DNazymes. The former performs ligand binding while the latter functions as a catalyst [14, 15]. Since a DNzyme-based Pb^{2+} sensor was reported in 2000 by Lu and coworkers [16], the field has experienced a rapid growth in finding analyte-specific DNA sequences and designing new signal transduction methods [2, 15, 17].

Going from lab-based demonstrations to field applications is a challenging yet important step. Functional DNA biosensors have been reviewed multiple times, including their applications in environmental analysis [2]. The goal of this article is to address the practicality of deploying these sensors in the field. It needs to be pointed out that here that we rely mainly on the published literature. Practical designs that could lead to commercialization may have yet to be in the public domain. Nearly 400 papers have been published on functional-DNA-based portable biosensors and 64% of the papers were published in the last five years. In this article, we focus only on two types

of analytes: metal ions and small organic molecules. Other analytes such as proteins, environmental DNA, and bacterial cells are not included for the following reasons. Most protein detection methods are for biomedical applications. Environmental DNA detection typically requires some type of DNA amplification methods such as polymerase chain reaction (PCR) using DNA hybridization probes, where the use of functional DNA is limited. Lastly, the detection of bacterial cells is a more specialized topic and has been reviewed recently [18].

Functional DNA for metal ions and small molecules

DNA has a negatively charged phosphate backbone with nitrogen-rich nucleobases allowing it to bind various types of metal ions [19]. The majority of the available sensing DNA sequences have been obtained via in vitro selection using a large library of random DNA sequences, where the binding sequences are collected and amplified by PCR. While selection of aptamers for metal ions has been carried out [15], metal-binding aptamers have not been used widely, likely due to problems associated with non-specific metal binding to aptamers via electrostatic interactions.

DNAzymes provide an alternative solution for metal detection, where metal ions are recruited as specific cofactors to catalyze a chemical reaction [11]. This way, DNAzymes can overcome the nonspecific metal binding problem [20]. The most commonly used metal detecting DNAzymes perform the RNA cleavage reaction. Figure 1A, for example, illustrates the Pb^{2+} -specific 8-17 DNAzyme [21, 22]. The GR5 DNAzyme in Figure 1B demonstrates an even higher selectivity for Pb^{2+} [23]. The GR5 DNAzyme can react with Pb^{2+} at concentrations as low as 10 nM, yet remains inactive even in the presence of 10 mM Mg^{2+} [24]. Similarly, the 39E DNAzyme has over one-million-fold selectivity for UO_2^{2+} compared to other competing metal ions (Figure 1C) [25]. The Cd16 DNAzyme contains a phosphorothioate modification at the cleavage site, which provides selective binding for Cd^{2+} (Figure 1D) [26]. All these DNAzymes contain a substrate strand (upper strand) with a single RNA linkage, which can be cleaved by the enzyme strand (lower strand) in the presence of the target metal ion.

Metal ions can also be detected using aptamers. An interesting example is the recognition of Hg^{2+} by thymine-rich DNA forming T- Hg^{2+} -T base pairs (Figure 1E) [27]. Such simple DNA-based binding has also been used for the detection of Pb^{2+} by G-quadruplex DNA, and Ag^+ by cytosine-rich DNA [28]. In particular, for the detection of small molecules, aptamers have advantages over antibodies. In fact, riboswitches all rely on aptamers binding to small molecules

or ions [29]. For small molecule targets, competitive immunoassays have to be used because these molecules are too small to bind two antibodies at the same time. Figure 1F shows the aptamer for quinine. This aptamer was originally selected for the detection of cocaine [30], but exhibits a 20-fold higher affinity for quinine [31]. Figure 1G is a truncated aptamer for kanamycin [32] and Figure 1H is an aptamer for 17 β -estradiol [33]. These aptamers typically fold into a hairpin structure upon target binding, whereas their structure can be open before target binding, depending on the length of the stem region of the hairpin. It should be noted that not all reported aptamers actually bind to their intended targets with [34], and some environmentally relevant examples such as As(III) [35], ampicillin [36], chloramphenicol [37], and some pesticides [38]. Therefore, these non-binding aptamers are not included in this review.

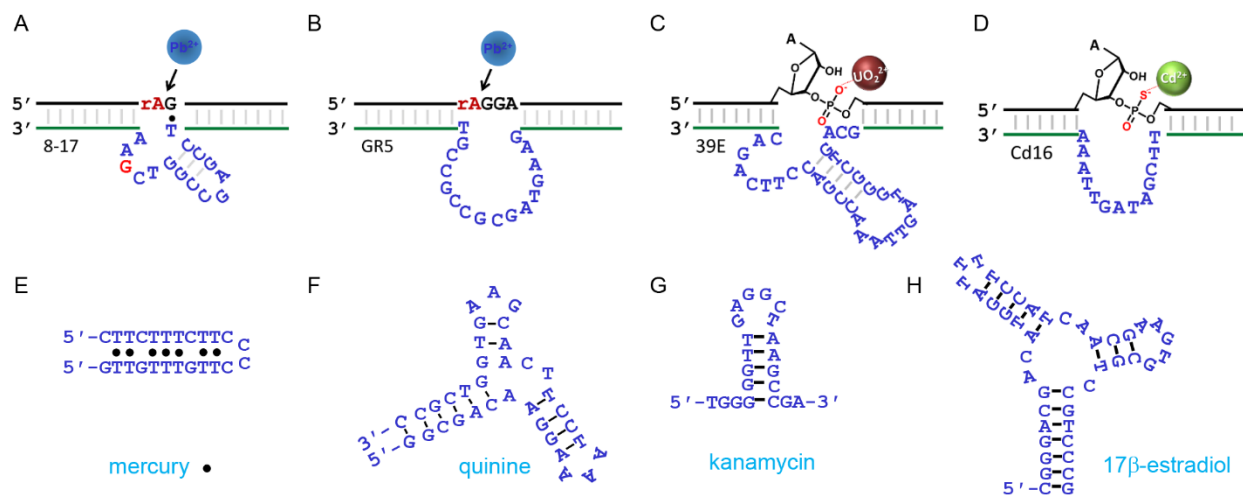


Figure 1. The structures of RNA-cleaving DNazymes for sensing (A) Pb^{2+} using the 8-17 DNzyme; (B) Pb^{2+} using the GR5 DNzyme; (C) UO_2^{2+} using the 39E DNzyme; and (D) Cd^{2+} using the Cd16 DNzyme. The lower row shows the secondary structures of the aptamers following binding of (E) Hg^{2+} ; (F) quinine; (G) kanamycin; and (H) 17 β -estradiol.

Signal transduction methods for robust detection

Compared to enzyme and antibody based biosensors, a key advantage of DNA-based biosensors is the versatility in signal transduction methods due to the ease of site-specific labeling and programmable DNA structures [10]. In addition to traditional fluorescence, colorimetric, and electrochemical detection, DNA binding has also been transduced to pressure [39] and temperature signals [40]. However, not all the signal transduction methods can be easily incorporated into a

robust sensing device and deployed for practical field applications. For effective field deployment, the signal transduction method needs to be robust, easy to calibrate, and resist to changes in buffer conditions. Here, we present three sets of detection methods for comparison.

Figure 2A shows a fluorescent sensor based on a DNAzyme, where a fluorophore is labeled on the substrate strand and a quencher is labeled on the enzyme strand [16]. Cleavage of the substrate strand results in the release of the fluorophore-bearing fragment causing enhanced fluorescence. Note that such catalytic-activity-based assays can also be calibrated using the reaction rate, which can yield a more robust calibration [11]. Figure 2B shows the structure-switching aptamer detection method [41], where a quencher-labeled complementary DNA is used to compete with aptamer binding. In both sensing approaches, the labels are covalently linked to the DNA. Such sensors usually have excellent sensitivity and stability. Figure 2C illustrates a label-free detection, where a DNA staining dye is added to probe target binding. Target binding usually displaces the dye leading to a fluorescence drop although, in some special cases, a large fluorescence enhancement can be achieved [42]. However, these sensors are more susceptible to changes in solution conditions.

By replacing fluorophores with gold nanoparticles (AuNPs), similar designs can be produced for colorimetric detection. The AuNPs are first densely functionalized with thiolated DNA to allow hybridization with DNAzymes [43] or aptamers [44]. In the presence of target analytes, DNAzyme cleavage or aptamer binding can both result in the disassembly of AuNPs and a color change from blue to red. The dense DNA layer also ensures high colloidal stability of the AuNPs. A label-free method without such a DNA layer has also been proposed [45], which relies on the adsorption of DNA aptamers to protect AuNPs against salt-induced aggregation. Our recent work has shown that this label-free approach is prone to interference by the adsorption of target analytes [46, 47]. In addition, salt and proteins in the sample matrix can also interfere with target detection by either destabilizing or stabilizing the AuNPs.

By using other types of labels, similar DNA designs can be used to generate various types of signals. For example, by labeling ferrocene or methylene blue, electrochemical sensors can be designed [48]. Using PtNPs to catalytically decompose H_2O_2 , pressure-based sensors can be produced [39]. By labeling an invertase (an enzyme that converts sucrose to glucose), the reactions can be detected using a commercial glucometer [8, 49].

One-step and two-step sensors

If all the reagents and reaction steps are integrated in a sensor device and the detection only requires the addition of the sample, we refer to these designs as one-step assays (Figure 2D). Ideally, field-deployable sensors should be one-step. However, the majority of the sensors described in the literature require more than one step. Either the DNAzyme or aptamer reactions happen in solution and the sensor device contains dried DNA staining dyes or enzyme substrates for signal generation (Figure 2E), or the enzymes or aptamers are immobilized on a platform to which dyes or substrates are added after reaction with the analyte of interest to generate a signal (Figure 2F). Such two-step sensors separate the reactions in time and the reagents in space. In contrast, one-step assays arrange all reagents in close proximity allowing for the reactions to happen without further intervention within a given time sequence.

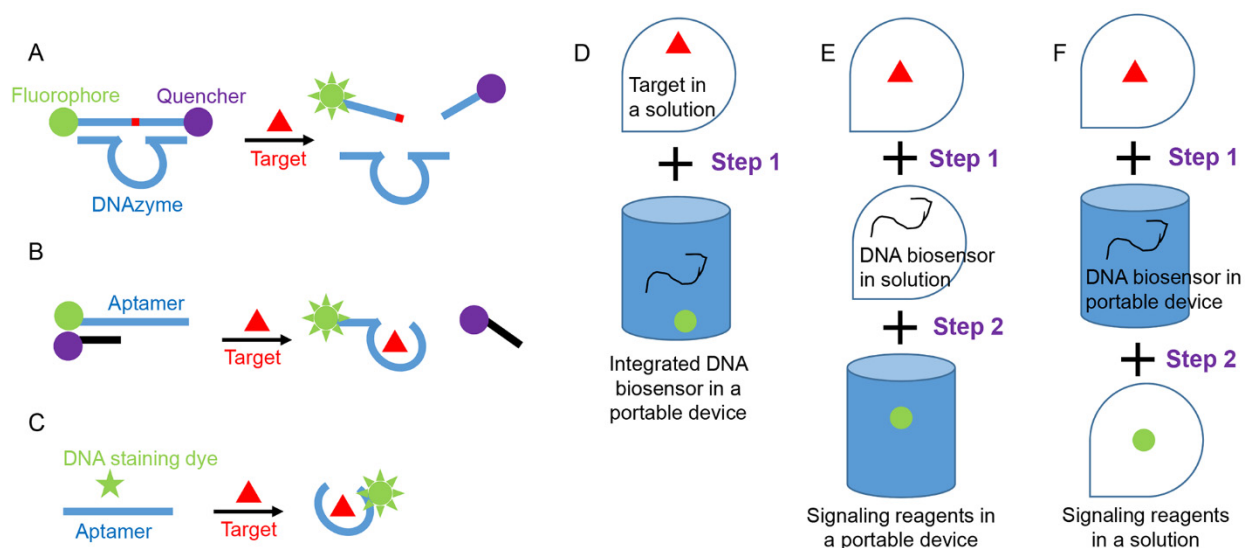


Figure 2. Schematic illustration of fluorescent sensor designs for (A) DNAzymes, (B) aptamers using covalently labeled fluorophores and quenchers, and (C) label-free detection of aptamer binding. Schematic illustration of field deployable biosensors using (D) all integrated 1-step detection; (E) 2-step detection with the water sample and functional DNA mixed in solution first and then the mixture is added to the device; and (F) 2-step detection with the water sample first incubated with immobilized functional DNA in the device followed by the addition of a solution containing signaling reagents.

In recent years, we are seeing growing efforts to integrate reagents within sensing instrumentation or platforms to produce easy-to-use assays that can potentially enable in-field deployment. Some of these are reviewed below.

Microfluidic devices

In microfluidic devices, microchannels guide the flow of samples and reagents to achieve programmed mixing and reaction steps. Microchannels are micrometer-sized and thus provide shorter light pathlengths compared to conventional assays (~10 mm), making colorimetric and fluorescent detection more challenging. Nevertheless, innovations in detection now make it possible to observe signals even at the shorter light pathlengths. Most proposed detection methods are based on microfluidic chips made from polydimethylsiloxane (PDMS) and plastics. One portable design was reported for the detection of Pb^{2+} [50]. This method used a DNAzyme to connect a polystyrene microparticle (PMP) and a magnetic microparticle (MMP). In the presence of Pb^{2+} , the DNAzyme was cleaved, separating the PMP from the MMP. The sample was then loaded into a microfluidic channel powered by capillary action. Using a small magnet, only free PMPs can flow and accumulate to form a thin particle dam strip visible by the naked eye (Figure 3A). The sensor has a LOD of 2.1 nM Pb^{2+} and was tested in a variety of water samples. By replacing the DNAzyme with a Cu^{2+} -specific one, Cu^{2+} was also detected with the same design [51].

Many of the recently proposed microfluidic solutions also use smartphones to take advantage of the optical units on them (namely the camera and ambient light modules), to quantify visual signals produced from the functional DNA biosensors. Li et al. reported a simple microfluidic chip made of PDMS that performs multiplexed detection of four cyanotoxins, including anatoxin-a, cylindrospermopsin, nodularin, and microcystin-LR [52]. The fluorescence-based detection was enabled by aptamers mixed with a DNA staining dye that was dried inside the microfluidic chip (Figure 3B). The dried dye had a 60-day shelf life. Using a smartphone, the detection process required just one step as only the sample was required to be added to the microfluidic chip. The fluorescence was excited with a portable battery-powered blue laser, which is housed in the smartphone containment device, and the emission was measured directly with the smartphone. All four cyanotoxins show a LOD between 1 and 3 nM when tested using spiked lake water samples. In another design, a smartphone camera was also used to read the color of aggregated AuNPs for quantification of the aptamer-based colorimetric sensing of Hg^{2+} [53].

Aside from color- and fluorescence- based detection, another strategy uses nanostructures for enhanced Raman signals. Fu et al. proposed such a detection method using a classic microfluidic chip design which includes two inlets through which the aptamer and sample were injected, followed by a joint mixing path in which the reaction mixture interacted with silver nanostructures to enhance the signal [54]. This design, however, was limited in portability since it required a spectroscope and injection pumps.

Overall, microfluidic chips coupled with functional DNA sensing show great promise for in-field applications. Innovative designs, such as the particle dam testing strip and the fluorescence-exciting smartphone discussed above, have demonstrated portability and deployment under real-world conditions. Table 1 summarizes some common designs for microfluidic devices, highlighting relevant metrics to allow for comparison of in-field performance. The majority of microfluidic designs require two-step detection, although examples of one-step detection systems have also been reported. Ongoing efforts need to focus on achieving a better balance between accuracy and portability. With further efforts into streamlining the testing steps and the development of real-time concentration detection software, we are hopeful that more microfluidic designs will soon be tested in the field.

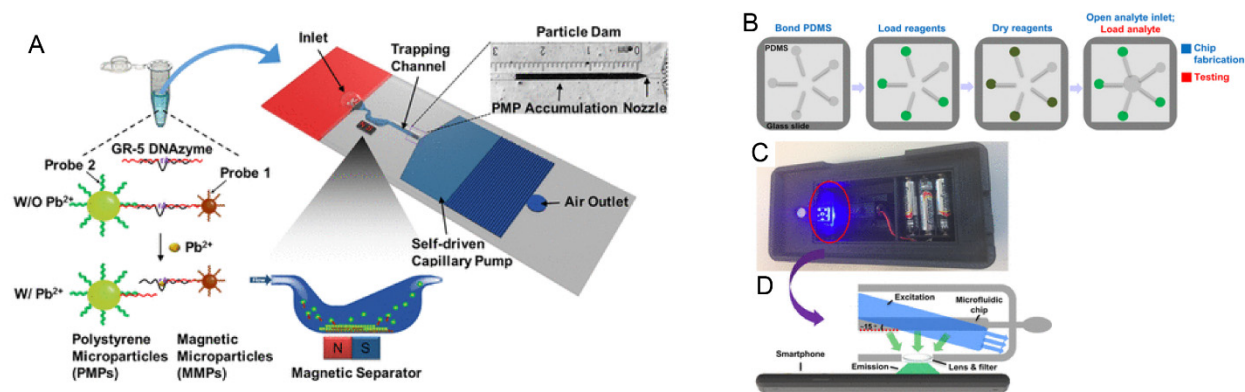


Figure 3 (A) Schematic particle dam detection using the Pb²⁺-specific GR5 DNAzyme. Figure adapted from ref. [50] with permission. Copyright 2020 American Chemical Society. (B) Procedure of reagent loading, drying and detection of four cyanotoxins. (C) A battery-powered blue laser for excitation, and (D) smartphone-based detection. (B-D) Adapted from ref. [52] Copyright 2020 American Chemical Society.

Table 1. Examples of microfluidic device and smartphone based detection. SERS: surface enhanced Raman scattering.

DNA	Target	LOD (nM)	Time (min)	Type	Real sample tested	Test steps	Year	Ref.
DNAzyme	Pb ²⁺	2.1		Particle dam – eye	Tap water	2	2020	[50]
DNAzyme	Cu ²⁺	33		Particle dam – eye	Tap and natural water	2	2021	[51]
DNAzyme	UO ₂ ²⁺	7.2×10 ⁻⁴	60	SERS	Tap and river water	2	2020	[55]
Aptamer	<u>Cyanotoxins</u>	< 3	5	Fluorescence	Spiked lake water	1	2019	[52]
Aptamer	Hg ²⁺	1.4	20	Colorimetry	Tap and river water	2	2016	[53]
Aptamer	PCB77	10		SERS	None	2	2015	[54]
Aptamer	Cd ²⁺	10	10	Colorimetry	Tap water	2	2020	[56]

Lateral Flow Devices

Lateral flow devices are popular because they are a mature technology, have quick processing times and are easy to use [57]. These sensors generally take the shape of a solid strip composed of nitrocellulose for protein immobilization and glass fiber for the drying of bio/nano-conjugates. Samples are placed on one end of the strip and allowed to flow laterally across the strip. The detection is commonly based on the accumulation of functionalized gold nanoparticles to induce a red-colored zone on the strip which can then be detected visually. While antibodies have been traditionally used for detection, aptamers and DNAzymes have also been used depending on the target analyte.

An example is based on the cleavage of the 8-17 DNAzyme to detect Pb²⁺ [58]. The DNAzyme was hybridized with a substrate strand bearing a thiol group on one end and a biotin tag on the other end. The substrate attached to the AuNPs through an Au-S bond (Figure 4A). This DNAzyme/AuNP conjugate was then dried on the conjugate pad. In the presence of Pb²⁺, the cleaved substrate strand released the enzyme strand, leaving only the thiolated substrate fragment on the AuNPs. The latter was captured by the biotinylated cDNA associated with the immobilized streptavidin producing a red line (Figure 4B). The uncleaved AuNPs continued to flow and were captured on a control marker through streptavidin-biotin interactions also producing a red color. Thus, a positive sample resulted in both markers appearing red. The lateral flow device was able to sense Pb²⁺ concentrations down to 0.05 nM in spiked drinking water. Because the device produced results in 10 min, it is ideal for the on-site testing of possible lead contamination.

In an earlier report by the Lu group on the detection of lead in paint, a similar DNAzyme was used but the test line and the control line switched position [59]. In that case, however, the LOD was found to be 5 μM Pb^{2+} , which was 100,000-fold higher than the device reviewed above. Given the activity of the 8-17 DNAzyme with a low (micromolar) apparent K_d [22], it is unlikely that the DNAzyme/AuNP conjugates were sensitive down to 0.05 nM Pb^{2+} with only a few minutes of flow time. Placing the test line before the control line therefore might confer a significant analytical advantage.

In both of the above Pb^{2+} sensors, the DNAzyme conjugates were dried on the lateral flow device. In another work, a Cu^{2+} -specific DNAzyme was used, but with the cleaving reaction first taking place in solution, followed by injection of the solution into a lateral flow device with dried DNA/AuNP conjugates for the detection of Cu^{2+} [60]. The Cu^{2+} sensor used a substrate that folded in a hairpin structure. The cleaved substrate attached to the AuNP conjugates of the test line, whereas the uncleaved hairpin substrate did not. An important advantage of the method is that the enzyme strand can be recycled and reused. This Cu^{2+} sensor had a visual LOD of 2 ng/mL (32 nM) and showed good recovery results with spiked local tap water and river water. Note that this two-step design may provide better sensitivity due to a longer DNAzyme reaction time, but less convenient as it required the transfer of small volumes of solutions into the lateral flow device.

Heavy metals can also be detected using aptamers. For example, a Hg^{2+} sensor based on the formation of T- Hg^{2+} -T base pairs used a thymine-rich DNA attached to AuNPs whereby the conjugate was dried on the conjugate pad. In the presence of Hg^{2+} , the DNA folded into a hairpin preventing hybridization to its complementary DNA that was attached to fluorescent microspheres (FMP) immobilized on the test line, causing fluorescence. Otherwise, hybridization took place and the fluorescence was quenched (Figure 4C) [61]. The authors reported a LOD of 0.13 ng/mL (0.65 nM Hg^{2+}). This assay has the advantage of being a one-step detection.

Two-step detection has also been used in aptamer-based lateral flow devices. In one such device, the kanamycin aptamer was attached to magnetic beads and hybridized with cDNA. When kanamycin was added, it displaced the cDNA and released it to solution [62]. The beads were then magnetically separated before the solution containing only cDNA flowed through the sensor (Figure 4E). The cDNA was sandwiched between the AuNP probe and the strand on the test marker to produce a signal. Without the cDNA, the probe only bound to the control marker further down

the strip. Kanamycin was detected with a LOD of 4.93 nM, although some pre-treatment of the sample was required.

Recently, a sensor for detecting erythromycin was developed combining recombinase polymerase amplification (RPA) and lateral flow [63]. RPA can amplify DNA in a way similar to PCR but takes less time and does not require a thermocycler. An erythromycin binding aptamer was immobilized by hybridization to a biotinylated DNA. The aptamer binding to its target disrupted this hybridization and released the aptamer to initiate RPA. The double-stranded RPA products including a biotin tag on one end and a FAM fluorophore on the other end then flew through the strip. The biotin can bind to AuNPs modified with streptavidin and the FAM was captured by the immobilized anti-FAM antibody producing a red test line. The authors claimed an LOD of 3 pM. The sensor was able to detect concentrations of erythromycin found in polluted water sources.

Lateral flow biosensors offer a quick and deployable way to measure common pollutants such as metal ions (lead, mercury, etc.) and antibiotics. Table 2 summarizes some of the examples of lateral flow sensors and other designs mentioned.

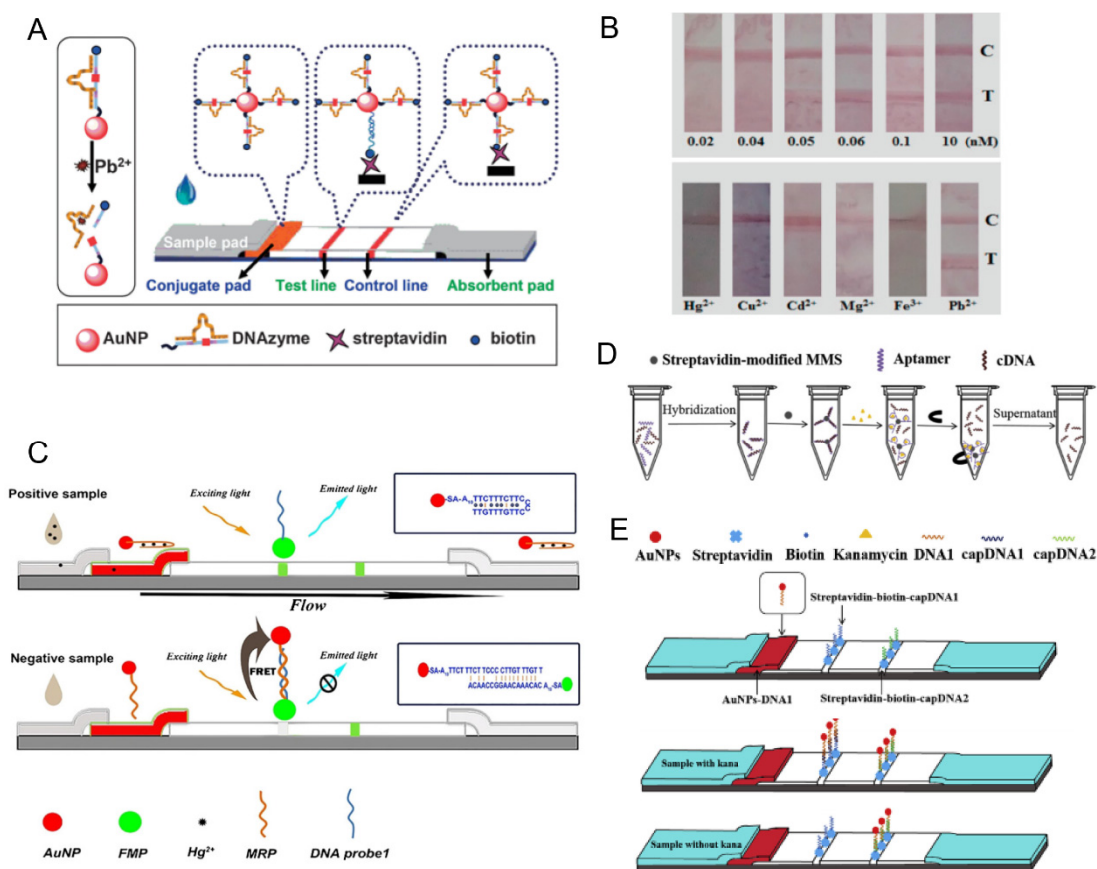


Figure 4. (A) Schematic showing the detection of aqueous Pb^{2+} using the 8-17 DNAzyme. (B) Results of the sensor in the presence of Pb^{2+} compared to other metal ions. (C) Aqueous Hg^{2+} detection using fluorescence quenching and the formation of T-T mismatches. Reprinted by permission from ref. [61] Copyright 2017 Springer Nature. (D) Example of a kanamycin sensor with pre-treatment using magnetic separation. (E) Lateral flow sensor design. Adapted with permission from ref. [62] Copyright 2019 Elsevier.

Paper based biosensors

Another type of field deployable sensors are based on paper [64, 65], most of which are two-step assays. Typically, a bioreceptor (aptamer or DNAzyme) solution is applied to a paper substrate with flow channels defined by hydrophobic barriers and allowed to dry. Next, the sample solution is blotted onto the paper causing reactions that produce a measurable signal. In an example involving the detection of Pb^{2+} [66], the GR5 DNAzyme was first immobilized on a magnetic bead and the substrate strand was then hybridized with DNA-functionalized AuNPs. In the presence of Pb^{2+} , the AuNPs were released due to the cleavage reaction and remained in the supernatant upon magnetic separation. The supernatant was blotted on paper, where it catalyzes the reduction of silver nitrate to silver nanoparticles. The paper thus changed color to grey, which was used for visual detection, with a reported LOD of 0.3 nM (Figure 5A). Synthetic urine and lake water samples were tested with recoveries ranging from 91% to 113%.

Another paper-based sensor for Pb^{2+} used distance and electrochemiluminescence. The visual distance marker was produced by 3,3-diaminobenzidine (DAB), a colorless molecule that turns brown upon polymerization into poly(DAB). In this sensor, AuNPs were functionalized with the 17E DNAzyme and a substrate that has a gold nanocube/horseradish peroxidase (HRP) complex attached. HRP catalyzed the formation of polyDAB from DAB (Figure 5B, 1). After addition of Pb^{2+} ions, the 17E DNAzyme cleaved the substrate leading to a decrease in the formation of the colored polyDAB, hence, providing a semiquantitative distance measurement. The fragment of cleaved substrate containing the gold nanocube/HRP then permeated the paper and enhanced the luminescence of luminol and CdSe quantum dots to produce a signal (Figure 5B, 2). This signal in conjunction with the DAB-based distance measurement in the first step yielded a 95-104% recovery of Pb^{2+} in spiked lake water samples with a LOD of 3 pM [67]. Lead was also detected using a paper electrode. Upon target addition, a modified cleaved substrate increased

conductivity when incubated with aniline by catalyzing the oxidative polymerization of aniline. This sensor detected lead in spiked lake samples with 94 -103% recovery and a LOD of 0.9 nM [68].

Zhang and coworkers reported a colorimetric sensor for mercury. The first step was to form Hg^{2+} -mediated T-T base pairing of an aptamer in solution, which enabled the formation of a catalytically active G-quadruplex DNA with hemin. This solution was then transferred to a paper test strip, where it reacted with dried 3,3,5,5-tetramethyl benzidine (TMB) to produce a blue-colored product [69]. A higher Hg^{2+} concentration resulted in a longer colored line (Figure 5C). This sensor had a limit of detection of 0.23 nM Hg^{2+} with recoveries ranging from 85-111% in spiked water samples.

Colorimetric detection of gentamicin was also achieved with paper-based sensors and aptamer functionalized AuNPs [70]. The gentamicin aptamer and AuNPs were first mixed in solution before being added to the reaction site of the paper sensor. The addition of gentamicin and NaCl to the inlet channels caused the aptamers to preferably bind to gentamicin leaving the bare AuNPs aggregated with a color change (Figure 5D). This sensor had a detection limit of 300 nM and a reaction time of 2 min with spiked milk samples.

Overall, the fabrication of paper-based devices can be very simple. For example, paper sensors can be inkjet printed on filter paper using aqueous ink composed of megadalton tandem repeating DNA aptamers. The aptamers were fixed on the paper yet retained sufficient mobility to undergo structure switching upon binding to target analytes (Figure 5E) [71]. Other ways of fabrication such as vacuum filtration techniques using carbon, metal nanomaterials, and aptamers can produce multiplex devices that can detect up to 16 different small molecules in a way that is cost-effective and generalizable [72].

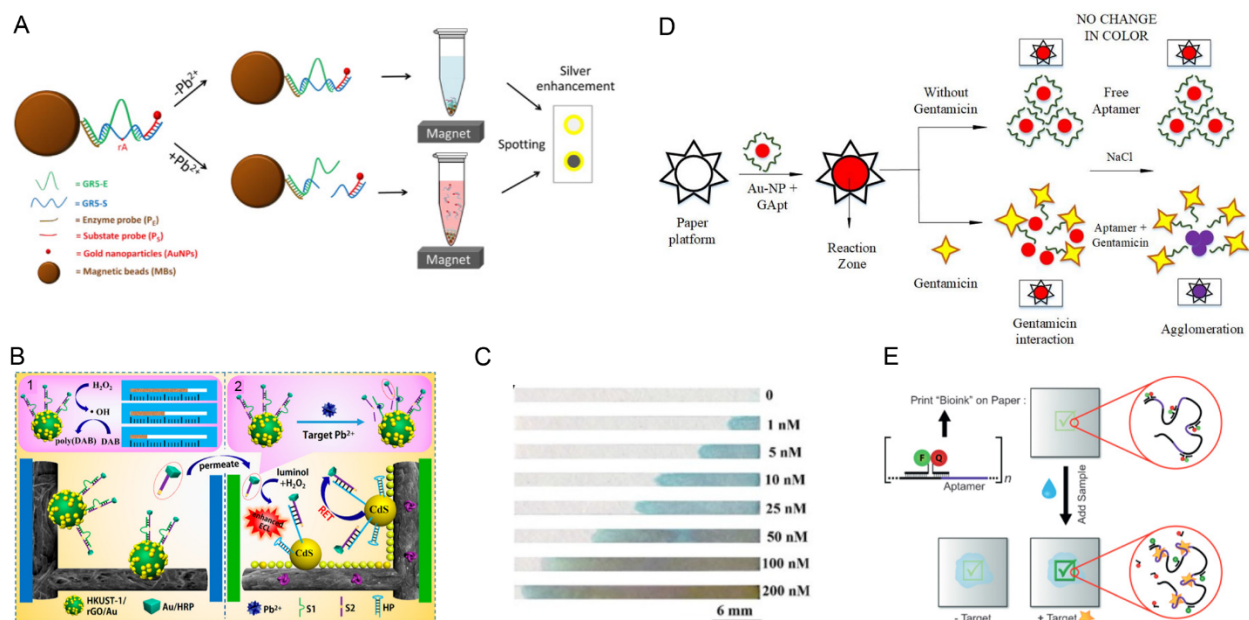


Figure 5. (A) Schematic of a colorimetric lead assay. Reprinted with permission from ref. [66] Copyright 2015 Elsevier. (B) Schematic illustration of semi-quantitative distance analysis and electrochemiluminescence output. Adapted with permission from ref. [67]. Copyright 2020 American Chemical Society. (C) Distance measurements based on the concentration of Hg²⁺ on a paper sensor. Reprinted from ref. [69] Copyright 2020 Elsevier. (D) Working principle of gentamicin-AuNP paper sensor. Adapted from ref. [70] (E) Inkjet printing of tandem DNA aptamers. Adapted from ref. [71] Copyright 2015 John Wiley and Sons.

Table 2. Summary of lateral flow and paper-based sensors. LF: lateral flow; PB: paper-based; KAN: kanamycin; GEN: gentamicin.

DNA	Target	LOD	Time (min)	Type	Real sample tested	Test Steps	Year	Ref.
DNAzyme	Cu ²⁺	4.2-5 nM	40	LF	Spiked municipal & river water	1	2017	[73]
DNAzyme	Cu ²⁺	2 ng/mL	20	LF	Spiked tap & river water	2	2019	[60]
DNAzyme	Pb ²⁺	25 nM	15	LF	Spiked tap & lake water	2	2018	[74]
DNAzyme	Pb ²⁺	0.05 nM	10	LF	Spiked drinking water	1	2014	[58]
DNAzyme	Pb ²⁺	5 μM	15	LF	Spiked paint	1	2010	[59]
Aptamer	Hg ²⁺	0.13 ng/mL	15	LF	Spiked river water	1	2017	[61]
Aptamer	KAN	4.96 nM	20	LF	Spiked food samples	2	2019	[62]
Aptamer	KAN	0.015 ng/L	10	LF	Wastewater	1	2021	[75]
DNAzyme	Pb ²⁺	0.3 nM	-	PB	Spiked river water	2	2015	[66]

DNAzyme	Pb ²⁺	0.5 nM	-	PB	Spiked tap & pond water	2	2020	[76]
DNAzyme	Ni ²⁺	3.1 nM	-	PB	Spiked lake water	2	2019	[77]
Aptamer	GEN	300 nM	2	PB	Spiked skimmed milk	2	2016	[70]
Aptamer	Hg ²⁺	0.23 nM	-	PB	Spiked lake, tap & river water	2	2020	[69]

Hydrogel-immobilized biosensors

Immobilization of DNA in hydrogels has a number of advantages, including the enrichment of target analytes for higher sensitivity, protection of DNA against nucleases, and sensor regeneration. We demonstrated this idea by co-polymerizing an acrydite-modified thymine-rich DNA in a polyacrylamide hydrogel for the detection of Hg²⁺, where adding SYBR Green I (SGI) dye produced a fluorescence signal (Figure 6A, B) [78]. By simply increasing the volume of the sample, the sensitivity increased by over ten-fold and even 10 nM Hg²⁺ could be visually detected. The same approach was also demonstrated for the detection of Pb²⁺ using a guanine-rich DNA [79]. Since SGI was added after the hydrogels reacting with Hg²⁺ and Pb²⁺, these were two-step sensors.

Recently, the performance of the Hg²⁺ hydrogel sensor was assessed under environmentally-relevant conditions [80]. When freely dispersed in solution (Figure 6A), the thymine-rich DNA sensor was very sensitive to variations in pH and the presence of metal ligands and natural dissolved organic matter (NDOM) such as fulvic acids. By incorporating the DNA in the hydrogel (Figure 6B), these solution effects were largely eliminated, except for NDOM. The competitive partitioning of Hg²⁺ between NDOM and the DNA, however, could be accounted for via equilibrium speciation calculations. The hydrogels thus allowed reproducible results over a broad range of experimental conditions, from simple buffer solutions to complex aqueous compositions mimicking natural lake waters, at pH 3-10 and 5-50 °C.

The DNA-functionalized hydrogel has been integrated with the diffusive gradients in thin films (DGT) devices (Figure 6C) [81]. Because in DGT devices the accumulation of the analyte(s) of interest in the binding layer is proportional to the exposure time, it becomes possible to detect even very low concentrations of trace analytes simply by increasing the deployment times. The performance of the Hg²⁺ DNA-DGT sensor with the DNA-functionalized hydrogel as the binding layer was evaluated over a broad range of pH and temperature conditions as well as hydrochemically diverse artificial and natural water samples. Equilibrium speciation calculations and temperature-corrected, multicomponent diffusion coefficients for aqueous Hg²⁺ systematically explained the Hg²⁺ quantification results and supported the use of the DNA-DGT sensor for

directly measuring aqueous Hg^{2+} concentrations down to the nanomolar level in freshwater environments. By extension, the same approach could be used to develop DNA-DGT sensors for other analytes.

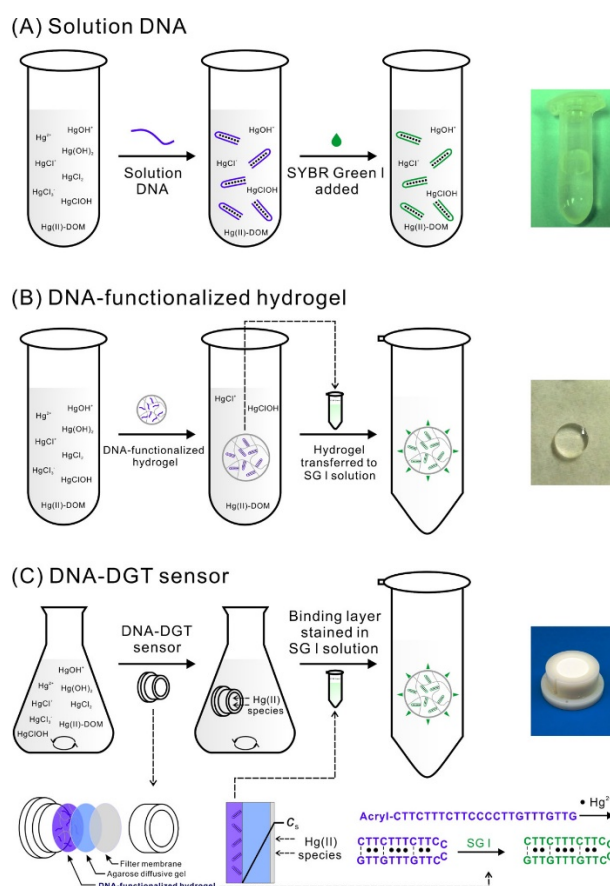


Figure 6. (A) A thymine-rich DNA freely dispersed in solution for detection of Hg^{2+} using SGI fluorescence. (B) DNA incorporated into a polyacrylamide hydrogel, and (C) DNA-functionalized hydrogel forming the binding layer of a DGT unit. Panel (C) also provides an expanded view of the binding and diffusion layers in the DNA-DGT sensor and illustrates the DNA's conformational change due to Hg^{2+} binding. Adapted from ref [81]. with permission. Copyright 2020 American Chemical Society.

Other types of biosensors

In this section, we describe a few other portable measurement devices. Personal glucose meters (PGM) have been engineered to be low cost and highly accurate. Lu and coworkers used PGM to detect other analytes by introducing an enzyme, invertase [49]. Using this idea, Zeng et al. recently

reported a PGM for quantitative Cd^{2+} detection [82]. The authors functionalized DNA1 on magnetic beads and hybridized it with DNA2, resulting in a duplex with overhangs at both ends as illustrated in Figure 7A. Through a recycling signal amplification process initiated by the binding of Cd^{2+} and its aptamers (DNA3), DNA4 was freed to hybridize with an overhang on the magnetic beads, causing the cleavage of DNA2 by exonuclease III (Exo III) to produce a large quantity of DNA1. An invertase-conjugated DNA complementary to DNA1 was then added and separated using a magnet. Invertase converted sucrose to glucose, producing a signal measured by a PGM for the quantification of Cd^{2+} concentration. The authors claimed a LOD of 5 pM Cd^{2+} . The PGM-based sensor yielded results within 7% of those measured by ICP-MS in lake water, river water, and pond water, demonstrating the accuracy and applicability of the PGM-based Cd^{2+} sensor. Although the design is highly portable due to the use of a PGM, it required multiple steps of mixing small volumes of solutions, incubation, magnetic separation and enzyme reactions. The numerous steps would require further engineering to become more streamlined for practical field applications. Moreover, replacing natural enzymes with nanozymes might be an interesting direction for developing robust portable biosensors as well [83, 84].

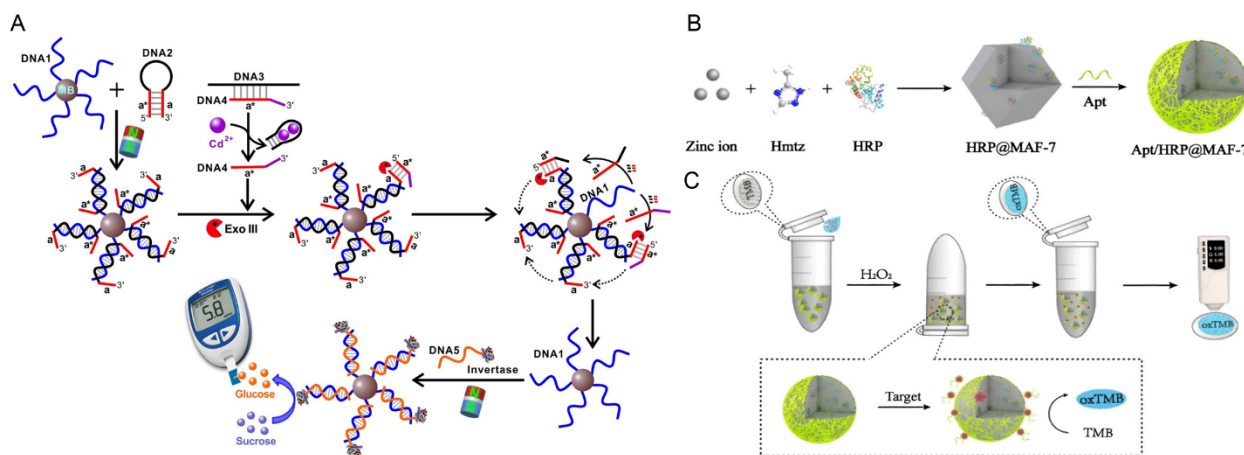


Figure 7. (A) Schematic representation of the Cd^{2+} detection mechanism based on a PGM. DNA is represented by different lines. DNA1-DNA5 are identified with different colors. Domain **a** is complementary to domain **a***. MB: magnetic beads; Exo III: exonuclease III. Adapted from ref. [82] Copyright 2019 Elsevier. (B) Schematic representation of the Apt/HRP@MAF-7 fabrication procedure and (C) the principle of the colorimetric sensor for on-site sensitive detection of targets. Adapted from ref. [85] Copyright 2020 American Chemical Society.

Colorimetric sensors are preferred for on-site detection, although using the naked eye can only achieve qualitative detection. Using portable colorimeters can solve this problem. Recently, Wang et al. entrapped HRP into a hydrophilic MAF-7 metal-organic framework material, and sealed the pores of HRP@MAF-7 with an aptamer shell, synthesizing Apt/HRP@MAF-7 (Figure 7B) for the on-site detection of streptomycin [85]. These authors speculated that hydrophilic MAF-7 not only prevented HRP aggregation but also ensured high enzymatic activity. In addition, the authors designed a microtube device with a TMB-based chip attached to the lid (Figure 7C). In the tube, HRP was released upon aptamer-target reaction to catalyze the conversion of colorless TMB to blue-green oxTMB in the presence of H₂O₂. A RGB signal can then be detected by a handheld colorimeter (Figure 7C). Under optimal conditions, this sensor for streptomycin had a LOD of 0.51 pg/mL. For the detection of streptomycin in raw milk and river water, this sensor exhibited recoveries ranging from 95 to 103% with a relative standard deviation (RSD) of 3.1% [85]. This method only required Apt/HRP@MAF-7 and the microtube device to be prepared in advance for use in on-site detection. The process did not require multiple sample additions and only needed 20 min incubation at ambient temperature, indicating promising potential for portable applications. Other recent works on this front are summarized in Table 3, from which we can see that PGM and colorimeters are by far the most popular detection meters.

Table 3. Summary of other types of biosensors. EB: electronic balance; PM: pressure meter; CM: colorimeter; GM: glucose meter

DNA	Target	LOD	Time (min)	Type	Real sample tested	Test Step	Year	Ref.
DNAzyme	Pb ²⁺	0.83 nM	30	EB	Tap and river water	2	2019	[86]
DNAzyme	Cu ²⁺	5.9 nM	40	CM	Tap and river water	2	2020	[87]
Aptamer	STR	0.51 pg/mL	1	CM	Raw milk and river water	2	2020	[85]
Aptamer	Quinine	0.32 uM	-	GM	Reclaimed wastewater	2	2018	[88]
Aptamer	Acetamiprid	0.42 nM	-	GM	Soil/tap/river/agricultural water	2	2022	[89]
Aptamer	Cd ²⁺	0.005 nM	40	GM	Lake/river/pond water	2	2019	[82]
Aptamer	Hg ²⁺	10 ⁻⁸ nM	100	GM	Tap/sewage/river water	2	2017	[90]

Summary and future perspectives

While DNazymes and aptamers are available for the detection of a growing number of aquatic contaminants, still DNA-based sensors for many other contaminants have yet to be discovered. For example, using DNA to detect very small anions remains very challenging. No DNA sensors exist for common pollutants such as nitrate, chloride and phosphate. Although a DNA aptamer for arsenic has been reported, its binding ability remains questionable [35]. Similar problems also exist for aptamers intended for other analytes of environmental concern [34], such as those targeting antibiotics [36, 37] and pesticides [38]. Thus, continued efforts to validate reported aptamers and obtain new and better ones will be needed.

The detection of metals (and metalloids) in freshwater environments using sensors based on DNazymes and aptamers shows great promise. However, it is worth re-emphasizing that the DNA sensors only detect free metal ions and do not directly measure total dissolved metal concentrations that are typically used to define water quality standards. Thus, the effects of complexation of target metals with inorganic and organic ligands must be considered to relate free and total dissolved concentrations. The example of the DNA-DGT sensor development shows how close collaboration with environmental scientists can help to overcome the gap between lab-based DNA sensor designs and real-world applications [80, 81].

Finally, the development of new sensors for environmental water quality applications require more attention at two critical aspects: calibration and detection limits. For instance, many colorimetric sensors use AuNPs, whose aggregation is strongly affected by the sample matrix. Thus, newly proposed sensors should be backed up with guidelines on the calibration requirements for specific environmental applications. From a practical perspective, methods that are easy to calibrate and are subjected to minimal matrix interferences should be prioritized. We also noticed that extremely low detection limits (in the pM range) are often reported, which are magnitudes lower than even the K_d of their respective DNA molecules. Control experiments should therefore be conducted to ensure that the reported LOD values are actually due to the intended DNA-target analyte interactions or reactions.

Achieving the lowest possible detection limit should not be the only goal of DNA sensor development. Sensor designs and associated LODs should be tailored to specific environmental analytical application. Semi-quantitative detection can be sufficient to monitor whether a threshold value is exceeded or not, while more detailed measurements may be needed for a scientific research

study. Lower LODs often come at a cost of more complex reaction designs (e.g., signal amplification by enzymes that take extra time and reagents). Simpler sensor systems may be more appropriate to support citizen science initiatives and to empower remote communities to access technologies for monitoring the quality of their drinking water sources.

Our review implies that the majority of the DNA-based sensors intended to be portable and field deployable are two-step (or more), meaning that they still require the transfer of small volumes of solutions along with other steps such as magnetic separation and incubation. These requirements hinder the general acceptance of these sensors. Ideally, all the reagents should be already inside the device in stable forms (e.g., in a dried state) so that the detection process can start automatically in the presence of the aqueous sample. Lateral flow devices are a representative example of such sensor devices that can be further developed for water quality applications. Future work also needs to include integrating DNA-analyte reactions in other field deployable devices and optimizing reagent drying conditions. Proposed sensors should be tested under environmentally relevant conditions so the design of prototypes and pilot studies will select the most promising lines of technology development. For metal ions, a key challenge is to obtain speciation information and correlation of biosensor data (related to bioavailability of metals) with ICP results (total metals). For small molecules, side-by-side comparison with standard analytical methods such as HPLC are needed to validate the assays. While such studies can be time consuming, they are critical for field deployment.

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