HHS Public Access

Author manuscript

Curr Opin Biotechnol. Author manuscript; available in PMC 2018 June 01.

Published in final edited form as:

Curr Opin Biotechnol. 2017 June ; 45: 191–201. doi:10.1016/j.copbio.2017.03.002.

DNAzyme sensors for detection of metal ions in the environment and imaging them in living cells

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Abstract

The on-site and real-time detection of metal ions is important for environmental monitoring and for understanding the impact of metal ions on human health. However, developing sensors selective for a wide range of metal ions that can work in the complex matrices of untreated samples and cells presents significant challenges. To meet these challenges, DNAzymes, an emerging class of metal ion-dependent enzymes selective for almost any metal ion, have been functionalized with fluorophores, nanoparticles and other imaging agents and incorporated into sensors for the detection of metal ions in environmental samples and for imaging the metal ions in living cells. Herein, we highlight the recent developments of DNAzyme-based fluorescent, colorimetric, SERS, electrochemical and electrochemiluminscent sensors for metal ions for these applications.

Introduction

Metal ions are major targets for environmental sensing, because the presence, distribution and speciation of metal ions in the environment – such as in ground water, lakes, rivers and oceans – as well as in living cells can have a significant impact on human health. A primary example is the elevated levels of lead (Pb) in the drinking water in cities such as Flint, Michigan, due to aged water pipes and improper water treatment. Fast, accurate detection and quantification of metal ion concentrations in different locations in the environment is important in preventing heavy metal contamination. At the same time, it is also imperative to investigate the effects of distribution and speciation of these metal ions in living cells to determine their potential effects on biological species such as plants, animals and humans.

While metal analysis has been established using techniques such as atomic absorption spectroscopy, atomic emission spectroscopy (AES), inductively coupled plasma (ICP)-AES and ICP-mass spectroscopy, these methods of instrumental analysis are limited to laboratory settings, are expensive to operate and cannot provide real-time information. To overcome these limitations, the field of portable detection has been growing over the past decade, which has been facilitated in part by the application of advances in biotechnology. In

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particular, a new class of sensors based on DNAzymes have been used to impart metal ion selectivity into otherwise non-selective sensing modalities [1-5].

DNAzymes are single-stranded (ss) DNA sequences which fold into complex tertiary structures and are able to catalyze a number of reactions, including cleavage of the phosphodiester backbone at a ribonucleotide or deoxyribonucleotide site [6]. Metal ions have been shown to play a critical role in the catalytic process and are constitutively required for the catalytic activity of most known DNAzymes. DNAzymes are discovered by an iterative screening and amplification process known as *in vitro* selection (Fig. 1A) [6,7]. *In vitro* selection starts with approximately 10^{15} different DNA sequences and, based on the selection conditions, can produce DNAzymes which are highly specific for metal ion cofactors in a range of concentrations. Thus far, DNAzymes have been selected that are specific for Pb^{2+} [6]-[8], Zn^{2+} [9,10], Mg^{2+} [8,11], Cu^{2+} [12], Ca^{2+} [13], $UO2^{2+}$ [14], Na^{+} [15], Hg^{2+} [16], Cd^{2+} [17], Cr^{3+} [18], Ln^{3+} [19-21], Ce^{3+} [22], and Ag^{+} [23].

DNAzymes typically have three main components in their secondary structures: two binding arms, an active site, and an enzymatic region (Fig. 1B). The ability to select a DNAzyme with metal ion specific activity without prior chemical knowledge of the DNAzyme structure, and then to subsequently modify DNAzyme binding arms and other non-essential nucleotides with minimal to no effect on selectivity and sensitivity has made DNAzymes ideal metal-selective components for new metal ion sensing technologies. These attributes in conjunction with the relatively low-cost synthesis, chemical stability, and ease of DNA functionalization with a plethora of chemical modifications available, has made DNAzymes easy to attach to nanomaterials and other molecular signaling groups such as fluorophores and electrochemical agents. Due to this ease of conjugating different sensing agents with different DNAzyme sensors in a modular fashion, these sensors can readily be adapted for multiplexed sensing, allowing for simultaneous imaging of multiple metal ions within the same system. Additionally, the modular structure of DNAzymes presents a unique advantage because it allows them to be used interchangeably to detect different metal ions using the same sensing strategy. If a new sensing platform is developed for detection of a certain metal ion using DNAzymes, it can often be easily adapted to sense various other metal ions simply by exchanging the DNAzyme with another. Because of this advantage, while this review highlights DNAzyme-based sensors developed for specific metal ions, the same sensing technique can theoretically be applied to detect any of the previously listed metal ions by simply exchanging the DNAzyme. By adapting DNAzymes for different sensing platforms, metal ions have been selectively detected using colorimetric [24], fluorescent [25], electrochemical [26,27], electrochemiluminescent, chemiluminescent [28], luminescent [29], SERS, glucose-meter [30-32], PCR [33], nanopore [34,35] and cantilever-based methods [36,37]. This article seeks to review recent progress, focusing largely on new developments within the past year, of DNAzyme-based metal ion sensing, with an emphasis on fluorescent, colorimetric, SERS, electrochemical, and electrochemiluminescent detection methods.

Fluorescent Metal Ion Sensors for Environmental Monitoring and Cellular Imaging

One of the earliest and most established DNAzyme-based sensors is the fluorescent sensor based on the novel design of the catalytic beacon, which typically places a fluorophore-quencher pair on adjacent ends of the enzyme and substrate strands, respectively (Fig 1C) [14,38]. Upon metal ion-dependent cleavage of the substrate strand at a specific ribonucleotide (e.g. rA) or deoxyribonucleotide site, the fluorophore will dissociate to generate a turn-on fluorescent signal, due to a change in the melting temperature after the cleavage reaction. The metal ion concentration can then be determined based on the kinetic rate of fluorescence increase. Since the invention of the catalytic beacon, it has been modified to decrease the background and has utilized various different fluorophores. The ability to change fluorophores and the separation between the metal ion binding site and the fluorophore give a distinct advantage to DNAzyme-based fluorescent metal ion sensors. These fluorescence sensors typically have a limit of detection (LOD) in the low nanomolar to picomolar range and have been successfully applied to the detection of metal ion contaminants, including $UO2^{2+}$, Pb^{2+}, Hg^{2+}, Cu^{2+}, Ag^{+}, Cr^{3+}, and Ce^{3+}, in tap [39,40], ground [18], pond [41,42], waste, river and lake water [23,43-45], as shown in Table 1. As a result, fluorescent sensors based on DNAzymes are commercially available and have been used to monitor drinking water systems in public schools [46]. Recent work has focused on signal amplification by protein enzymes like exonuclease [42] and alkaline phosphatase [47], or by enzyme-free amplification methods which are solely DNA-based, such as hybridization chain reaction (HCR) [39].

While the high selectivity and sensitivity of the catalytic beacon has made it widely applicable to environmental sensing, a key advantage for its application in cells is the ability to easily choose the desired fluorophore, which allows it to be used with other cellular dyes and sensors without worry of overlapping fluorescence and even allows for detection of multiple metal ions simultaneously. Therefore, in 2013, the Lu Group reported the first application of metal ion sensing in cells using a catalytic beacon [48]. In this study the catalytic beacon was delivered into cells via functionalization to gold nanoparticles, and used phosphorothioate modifications on the 3′- and 5′-ends to prevent degradation by exonucleases. Subsequent studies have used cationic liposomes [49], MnO2 nanosheets [50], DNA nanostructures [51] and cell penetrating peptides [49,52] to deliver DNAzymes to cells. To increase biostability, other groups have used non-natural L-DNA, which has similar reactivity to the D-DNA enantiomer but cannot be degraded by native nucleases [53,54]. Perhaps the most significant advancement in cellular imaging was the introduction of a photocaging group, to allow for light-controlled activation of DNAzymes; in this way, nonspecific cleavage of DNAzymes during their delivery into cells can be prevented and metal ion imaging can be controlled both temporally and spatially using light. Photoactivation of DNAzymes by modifying the active site or backbone, and subsequent light activation was demonstrated as early as 2004, and earlier yet for ribozymes in 1998 [55-57]; however, not until 2014 was a photocaged DNAzyme used to detect the presence of metal ions in cells [49]. The photocaging process requires synthesis of a photolabile nitrobenzyl group on the 2′ -OH of the phosphoroamidite which will be then become the

RNA active site. Though this provides for reliable photocaging, the synthesis of the photocaged phosphoroamidite is complex, thus in 2016 Yu Xiang's lab simplified the photocaging process to a simple post-synthetic modification of a non-bridging phosphorothioate with bromo-4′ -hydroxyacetophenone on three nucleobases essential for DNAzyme activity [58]. Furthermore, Xiang *et al.* was able to detect both $UO2^{2+}$ and Pb^{2+} simultaneously in cells using DNAzyme-DNA nanostructure complexes, using another photocaging method [51]. All of these studies have enhanced our ability to probe labile metal ion distributions in cells; however, further work needs to be performed in order to use DNAzymes to accurately quantify metal ion concentrations in the cellular environment.

Colorimetric sensors

While fluorescent sensors provide quantitative information about metal ions, the need for a fluorimeter and costs associated with it may deter the application of fluorescent sensors for on-site and real-time environmental monitoring. The visible spectrum (300 nm – 700 nm) offers a myriad of opportunities for the development of sensors where the presence of an analyte can be observed by the naked-eye, allowing for fast and simple detection of analytes without the need of sophisticated instrumentation [79]. Over the past decade, there have been significant advancements in the field of colorimetric-based biosensors. One interesting emerging trend in the past decade was the extensive use of colorimetric gold and silver nanomaterials for the detection of metal ions [80,81].

In the past several years, several systems utilizing DNAzymes for colorimetric metal ion sensing have been reported. Most of these sensor designs take advantage of recent progress in the field of nanotechnology to couple activity of the DNAzyme with a colorimetric output. Yun *et al.* [75] designed a sensor which uses a DNAzyme to cleave a DNA hairpin with low stability (Fig. 2A). In the presence of Pb^{2+}, the cleaved hairpin dissociates, generating short DNA stands that adsorb on the surface of gold nanoparticles, thus stabilizing the nanoparticles and yielding a bright red solution. In the absence of lead, the hairpin remains uncleaved and so is unable to stabilize the nanoparticles. The gold nanoparticles aggregate without the stabilizing single-stranded DNA ligands, which shifts the surface plasmon resonance, resulting in a purple solution. This detection scheme has a reported LOD of 20 pM [75]. Similar nanoparticle-based colorimetric sensing strategies have been demonstrated in hydrogel systems. In these systems, DNAzymes were embedded with gold nanoparticles within a hydrogel which is cross-linked with the substrate strand. In the presence of the target metal ion, the DNAzymes cleaves the substrate strands which destabilize the hydrogel. The subsequent release of the embedded gold nanoparticles resulting in a similar change in color, based on detection of the desired metal ion. This hydrogel-DNAzyme-based colorimetric biosensor was demonstrated effectively for sensing lead [82], lanthanide [76] and uranyl ions [73] (Fig. 2B) with 20 and 37 nM LOD values, respectively.

Other approaches to colorimetric sensing involving DNAzymes not covered by this review use redox chemistry to induce a color change in certain redox active compounds, using the redox-active horseradish peroxidase (HRP) mimicking-DNAzyme [79].

SERS sensors

Surface-enhanced Raman scattering (SERS) is the enhanced Raman signal of a molecule in close proximity to or direct contact with a roughened metal surface or nanoparticle. This analytical technique is capable of producing a Raman enhancement factor of 10^{11} as compared to that of single molecule fluorescence detection methods [83,84]. Therefore it is not surprising that SERS-based DNAzyme metal ion sensors have been utilized to develop sensitive metal ion sensors for lead and uranyl ions [85-89]. Inspired by a previously reported highly reproducible SERS system [90], Shi et al. [78] reported using the 8-17 DNAzyme to detect Pb^{2+} with a SERS gold-silver nanoparticle satellite core structure system on a silicon chip (Fig. 2C). This system yielded a LOD of 8.9 pM with high selectivity and was able to be recycled over multiple rounds. In another study, the 8-17 DNAzyme was functionalized on the surface of gold nanoparticles in the presence of Raman-active Victoria blue B dye to detect Pb^{2+} with a LOD of 10 fM [91]. In a different system, the 39E DNAzyme was used to detect $UO2^{2+}$ using a plasmonic nanowire interstice sensor to reach a LOD of 1 pM with high selectivity [77]. The use of DNAzymes in SERS applications has seen its translation into field of environmental monitoring for Pb^{2+}[78,85], $UO2^{2+}$ [77,87] and Hg^{2+} metal ions [90].

Electrochemical Sensors

While fluorescent, colorimetric and SERS sensors are powerful methods to detect metal ions, they are often vulnerable to high background noise from environmental samples. Therefore, DNAzyme-based electrochemical detection of metal ions has been a popular field since its conception in 2007, because of its relative imperviousness to signal interference [92]. The field has expanded significantly since the original concept of a DNAzyme-based electrochemical sensor, which used the cleavage and release of a substrate of the 8-17 DNAzyme in the presence of Pb^{2+} to increase the flexibility of a thiol-DNA-methylene blue (MB) tethered enzyme and thus decrease the distance between the electrode and MB [26,69]. Much of the progress in this field has dealt with amplifying the signal either via the introduction of enzymes, changing the redox active group or through use of hybridization chain reaction (HCR), for which this review will focus.

In order to overcome the inherent limitation of one signal output per single DNAzyme reaction, several differing methods of HCR have been applied. One of the most sensitive of these methods employed the highly selective $UO2^{2+}$-dependent DNAzyme, 39E [14]. Yun *et al.* was able to achieve an LOD of 2 pM (Fig. 3A) by using a non-functionalized 39E DNAzyme which, when cleaved, would release an initiator sequence to open hairpin 2 (H2). Hairpin 1 (H1) would subsequently bind to H2, which is functionalized on the electrode through a thiol modification, resulting in release of the initiator sequence. Methylene blue (MB), a minor groove binder, would then bind the dsDNA attached to the electrode to generate an electrochemical signal [63]. A similar method was employed again using the 39E DNAzyme, which allows the H1/H2 complex to form upon cleavage by the tethered DNAzyme, revealing the substrate HCR initiator on the electrode. The initiator opens H2, which subsequently opens H1 to form a nicked dsDNA polymer chain, which is again filled with minor groove binder MB to give an electrochemical signal, with an LOD of 20 pM

(Fig. 3B) [64]. It should be noted that a further study uses the formation of a hairpin polymer for the electrochemical detection of Pb^{2+} by tethering the enzyme sequence of a DNAzyme to a magnetic bead, and using the cleavage of the substrate and exposure of the enzyme sequence to initiate a hairpin chain assembly on the magnetic beads with a ferrocene-tagged hairpin, generating an electrochemical signal, with an LOD of 37 pM [67].

Electrochemiluminescence

Even though electrochemical sensors are quite useful in detecting metal ions in complex environmental samples with minimal interference, these sensors, often do not have enough sensitivity for environmental detection of metal ions without resorting to the signal amplification methods described in the previous section. A further method of enhancing the sensitivity of these metal ion sensors is with electrochemiluminescence (ECL), which excites a luminophore via a high-energy electron transfer reaction. This technique has recently gained popularity in analytical detection because of its relatively simple detection equipment, coupled with the reactivity-based luminescence signal output, leading to low background and high sensitivity ranging from low picomolar to low nanomolar analyte concentrations (Table 1). In 2009, Zhu *et* al. developed a lead sensor by functionalizing an Au-electrode with a thiol-modified 8-17 DNAzymes, which can cleave the substrate sequence in the presence of Pb^{2+}. The substrate is labeled with the luminophore Ru(bpy)3-NHS, so the subsequent release of the cleaved substrate generates a turn-off signal (Fig. 4A) [93]. The sensitivity imparted by this technique meant that the LOD was 11 pM. Using a different approach, Guangpeng Liu *et* al. was able to detect Ag^{+} with an LOD of 2 pM, by using DNAzyme nanowires composed of horseradish peroxidase-mimicking DNAzyme, also called the hemin/G-quadruplex DNAzyme, to accelerate the luminescence of luminol [62]. In the presence of Ag^{+}, the C-Ag^{+}-C interaction promoted the release of the DNAzyme nanowires, which inhibits peroxidase activity and decreases overall luminescence.

To further improve the sensitivity, decrease background and mitigate the risk of false positives, Zhang and co-workers were able to report a turn-on DNAzyme-based ECL sensor for Pb^{2+} with an LOD of 1.4 pM (Fig. 4B) [94]. They were able to accomplish this feat by tagging the DNAzyme enzyme sequence with Ru(bpy)3-NHS and annealing the enzyme and substrate sequence to form the rigid hybridized structure which keeps the luminophore further from the electrode. Upon cleavage and release of the substrate, the ssDNA of the enzyme is able to bend and move the luminophore closer to the electrode, increasing electron transfer to ruthenium and thereby increasing the luminescence signal. Wu and co-workers built upon this design to create another turn-on sensor with an LOD of 6.4 pM Pb^{2+} by attaching the enzyme sequence to the electrode via a 4-amino benzoic acid linker, and using the substrate strand to block hybridization of free Ru(bpy)3-NHS-labeled hairpin sequences, which are complementary to the enzyme [59]. In the presence of Pb^{2+}, the substrate will cleave and be replaced by the Ru(bpy)3-NHS-labeled hairpin, placing the luminophore next to the electrode. However, even with the significantly improved sensitivity and decreased background signal of the turn-on ECL sensors, further improvements to sensitivity and selectivity have been shown with the “on-off-on” signaling method. A DNAzyme-based “on-off-on” ECL sensor for Cu^{2+} with picomolar sensitivity, and with selectivity for 1 nM copper over 1 μM levels of competing metal ions has thus been

developed (Fig. 4C) [61]. This sensor used a 3,4,9,10-perylenetetracarboxylic acid (PTCA)/aniline mixture to generate a turn-on signal. The presence of ferrocene, tagged to the substrate sequence would quench the PTCA luminescence signal, generating a “turn-off” signal proportional to the amount of fully formed enzyme-substrate complex. The “on” signal is then regenerated by cleavage and dissociation of the ferrocence-tagged substrate in the presence of the analyte. Overall, the highly sensitive ECL sensor platform has shown great promise developing turn-on, turn-on and “on-off-on” sensors.

Conclusions and Perspectives

In this mini-review, we have summarized recent advances in DNAzyme-based metal ion sensing, focusing on fluorescent, colorimetric, SERS, electrochemical and electrochemiluminescent sensors. Of these techniques, fluorescent, colorimetric, electrochemical and ECL sensors have been able to successfully detect metal ions in environmental samples and fluorescent sensors have been developed for the detection of metal ions within living cells. Improvements on DNAzyme-based metal ion sensors have successfully lowered the limit of detection for many of these techniques by changing the method of signal output, whether by converting systems with turn-off signals is to turn-on outputs, or by incorporating methods of signal amplification such as HCR. Additionally, DNAzyme fluorescent sensors have been modified to allow temporal control through the introduction of a photo-caged switch, which can enable time-dependent detection of native metal ion concentrations. Furthermore, initial studies have demonstrated the simultaneous monitoring of two different metal ions in cells. The generality of many of these techniques means that they can typically be used for multiple detection methods and across different sample conditions, including both environmental and cellular samples. There can be room for significant growth in detection of metal ions in cells by applying techniques like HCR to amplify fluorescent signals, and further work must be done to accurately quantify metal ions in a cellular environment using catalytic beacon sensors. The field of DNAzyme-based environmental sensors can also be improved by generating robust portable sensors for all sensing modalities, and for multiplex detection of many metal ions simultaneously. Overall, the field of DNAzyme-based metal ion sensing is continuing to develop with promising prospects for future cellular and portable detection technologies.

Acknowledgments

We wish to thank Lu group members who contributed to the work described in this review, Mr. Ryan Lake for proof-reading, and the U.S. National Institutes of Health (R21MH110975 and R41MH111337) for financial support.

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Highlights

- DNAzymes are an emerging class of metal ions sensors with high selectivity
- Recent progress has been made in using DNAzymes for environmental detection of metal ions with high sensitivity
- Cellular imaging of metal ions in living cells using DNAzymes with high temporal control has also bfigeen reported

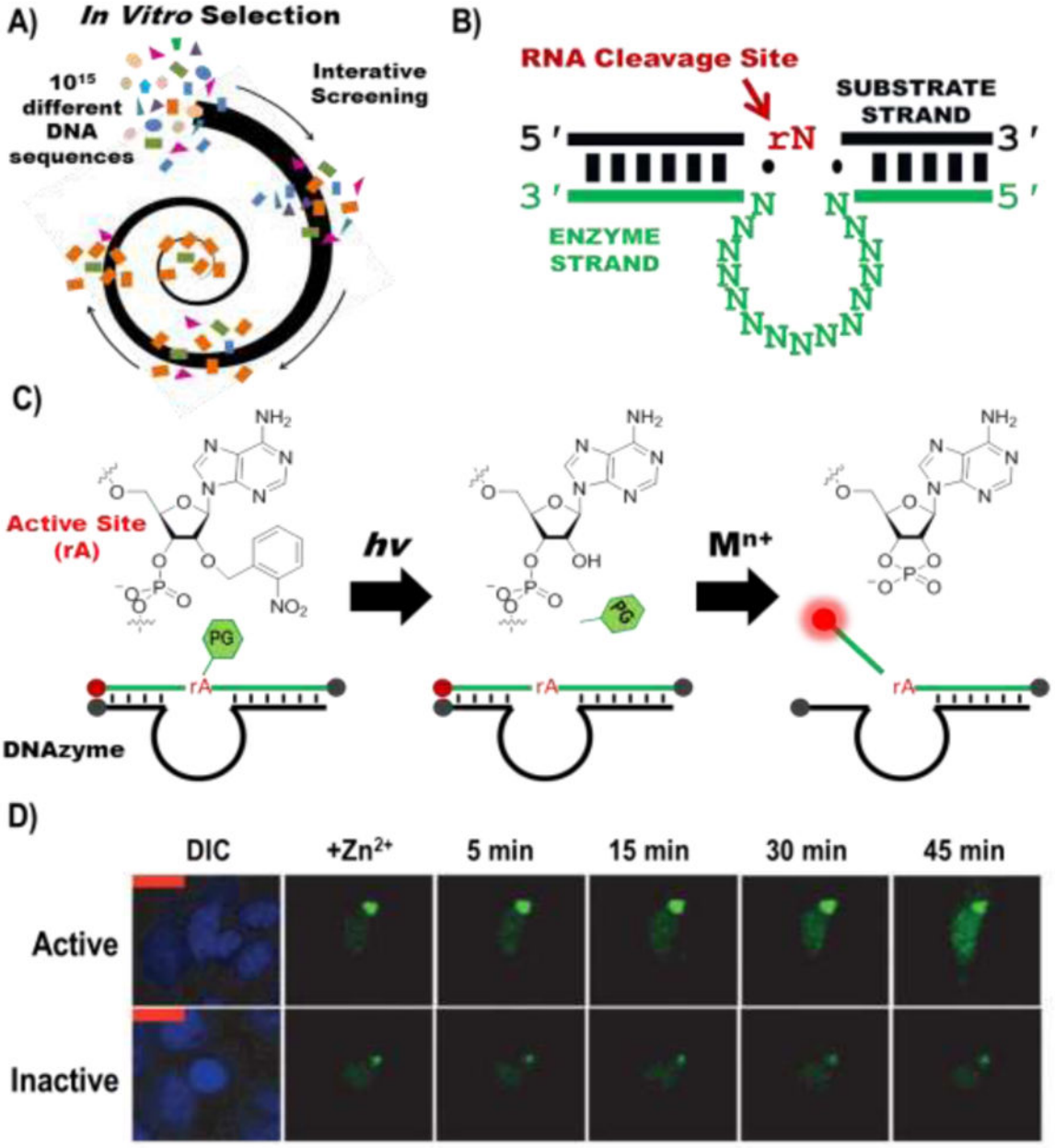

Figure 1.

DNAzymes are selected via an iterative combinatorial selection strategy called *in vitro* selection (A). The resulting DNAzyme (B) has binding arms which hybridize according to Watson-Crick base pairing, indicated by the black and green bars, which are highly programmable, an enzymatic region, shown as repeated N, where N is A, C, G or T, and an RNA cleavage site indicated in red at rN, where N can be A, C, G, T or U. (C) By functionalizing the DNAzyme with fluorophores (red) and quenchers (grey), it can be turned into a catalytic beacon, which has a turn-on fluorescent signal in the presence of a selective monovalent, divalent, or trivalent metal-ion cofactor. The catalytic beacon can be caged using the photolabile nitrobenzyl group on the 2′-OH. An example of the efficacy of the photocaged catalytic beacon is shown in D (figure modified from ref. 49)

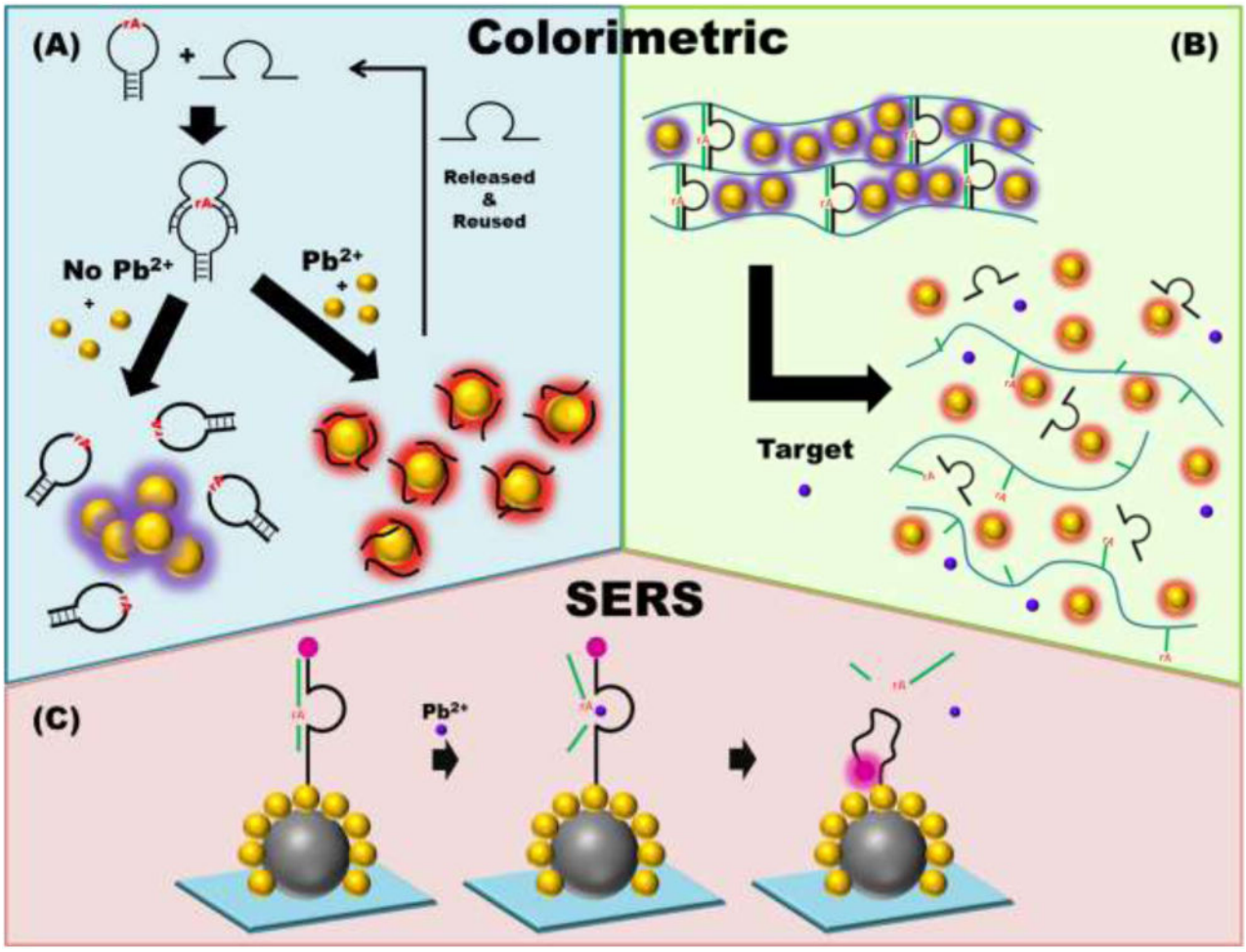

Figure 2.

A schematic representation of DNAzyme-based colorimetric and SERS detection methods using nanoparticles. In (A), the presence of the target analyte (Pb^{2+}) cleaves the DNA hairpin, and as a result the released ssDNA is able to adsorb on the gold nanoparticle surface, stabilizing the nanoparticles and preventing aggregation, resulting in a red solution. Without analyte-induced cleavage by the DNAzyme, no free singled-stranded fragments are available to stabilize the nanoparticles, leading to aggregation of the nanoparticles and a purple colored solution. In section (B), nanoparticles are enclosed within a hydrogel polymer with DNAzyme cross linkers. The presence of target analyte cleaves the DNAzyme, destabilizing the hydrogel system and releasing the gold nanoparticles to allow a recovery of absorbance unique to gold nanoparticles. In the bottom section, gold satellite coated silver core nanoparticles were synthesized. Using gold-thiol chemistry, DNAzymes were functionalized on the surface of the nanoparticles where the Cy5 fluorescence dye is far from the surface of the probe. The presence of target would cleave the substrate strand, resulting in the deformation of the enzyme strand to increase the proximity of the Cy5 dye and the nanoparticle.

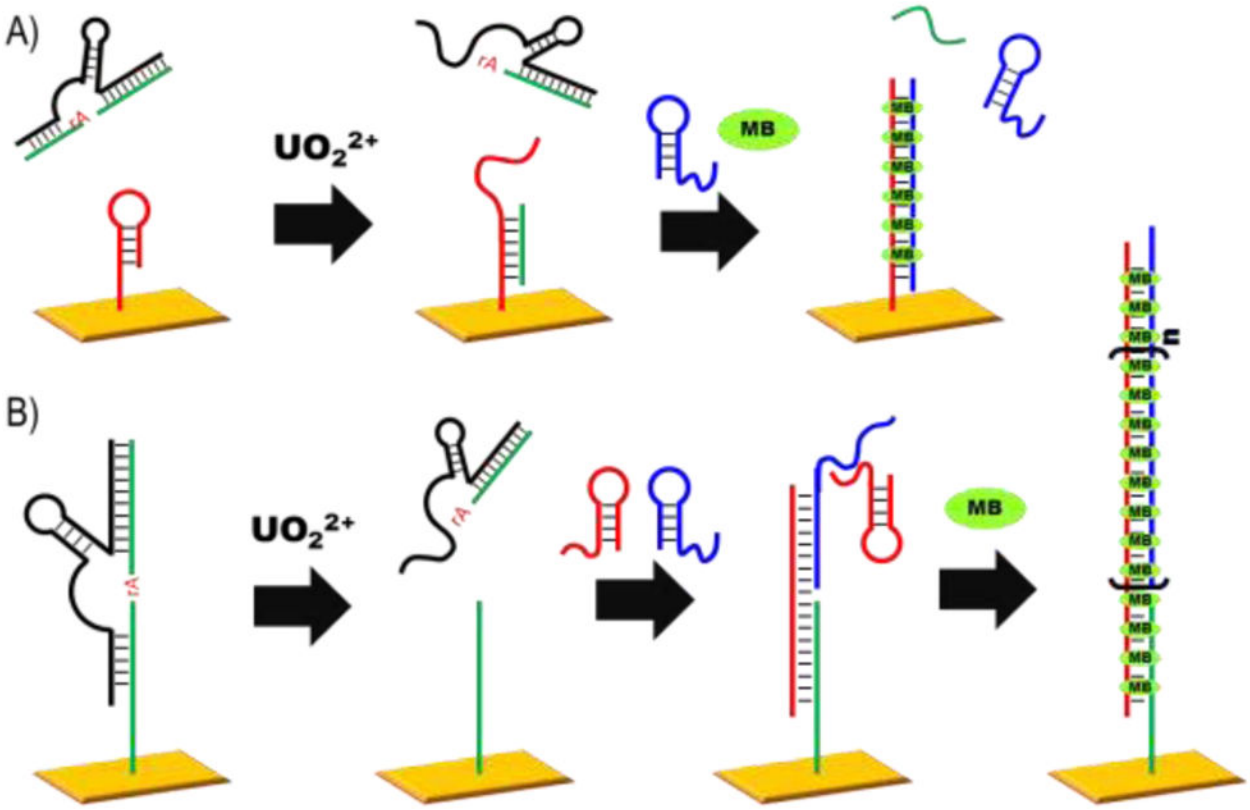

Figure 3.

Electrochemical signals enhanced by HCR, with the substrate acting as an initiator for H2 (shown in red) to open and hybridize with H1 (in blue), which kicks-off the substrate initiator so it can open another H2 (A). The formation of a nicked dsDNA polymer chain initiated by cleavage of the DNAzyme tethered to the electrode (B).

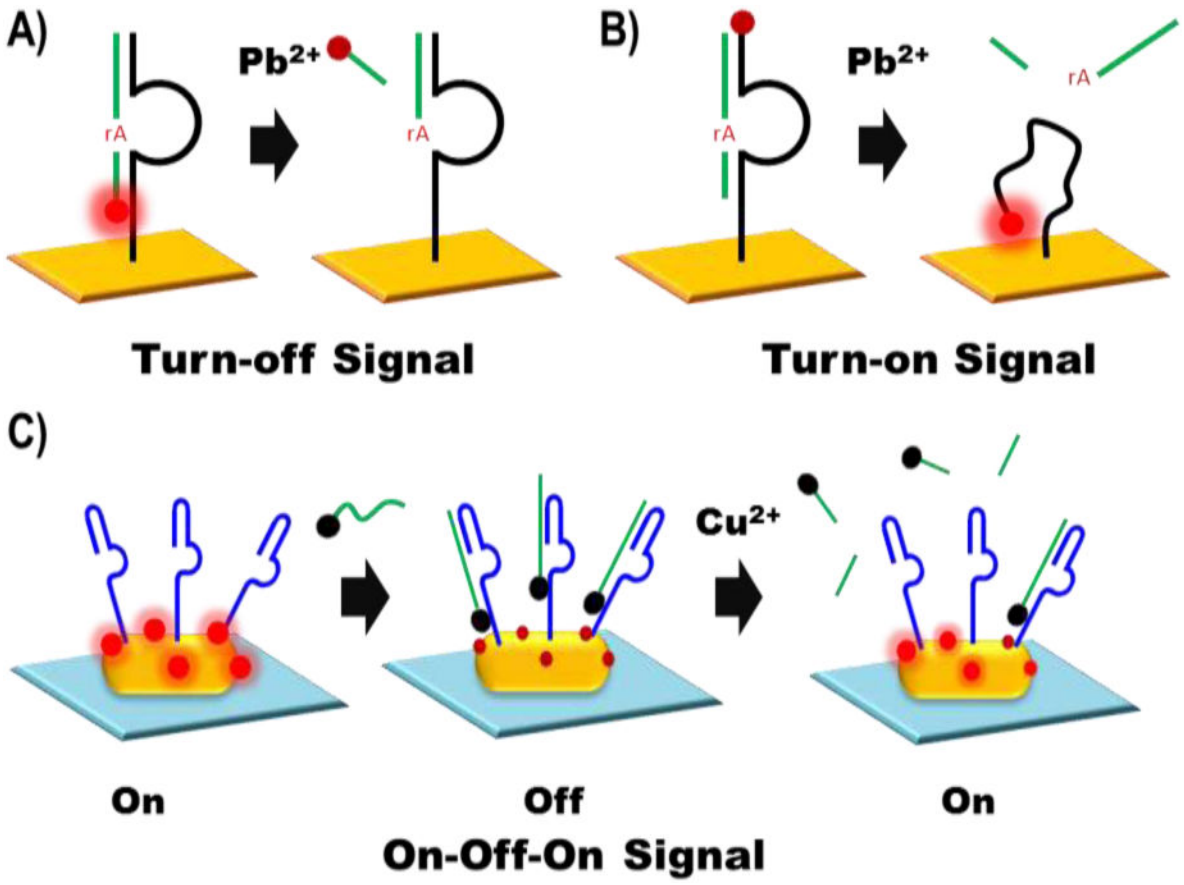

Figure 4.

Schematics of the turn-off (A) turn-on (B) and "On-Off-On" signal (C) DNAzyme sensor constructs. The turn-off sensor (A) uses a DNAzyme enzyme sequence (black) tethered to an electrode (gold surface) to cleave a DNAzyme substrate sequence (green) at the RNA active site (indicated by rA in red). The cleavage reaction will release the Ru(bpy)3-NHS tag (shown in bright red when "on" and dark red when "off") into solution turning "off" the luminescence signal. The turn-on sensor uses release of the DNAzyme substrate to allow the Ru(bpy)3-NHS labeled enzyme sequence to bend towards the electrode and generate a turn-on signal. The "On-Off-On" sensor (C) initially has a gold nanorod (yellow oval) coated in 3,4,9,10-perylenetetracarboxylic acid (indicated in bright red when excited and dark red when quenched) and the enzyme sequence of the Cu^{2+}-dependent DNAzyme (dark blue). The introduction of a substrate strand (green) attached to a ferrocene quencher (black circle) turns off the signal and the subsequent introduction of Cu^{2+} cause the cleavage and subsequent release of the quencher to regenerate a turn-on signal.

Table 1

Recent metal ion sensors developed using DNAzymes, the type of DNAzyme and the samples in which sensors were tested

Detection Mode	Analyte	LOD	DNAzyme Name	Sample	Ref.
Fluorescence-B Detectionased					
	UO_2^{2+}	2.4 pM	39E DNAzyme	Mine and Pond water	[42]
	UO_2^{2+}, Pb^{2+}	0.6 nM, 3.9 nM	39E and 8-17 DNAzymes	Cellular	[51]
	Pb^{2+}	1.7 nM	8-17 DNAzyme	River, Lake, and Waste water	[45]
	Pb^{2+}	2 nM	GR-5 L-DNAzyme	River water + salt and buffer	[53]
	Hg^{2+}	None Given	UV1C DNAzyme	Tap Water	[40]
	Cu^{2+}	500 pM	CA3 variant DNAzyme	Tap	[39]
	Cu^{2+}	1.6 nM	PsCu10 DNAzyme	Lake water	[44]
	Cd^{2+}	1.6 nM	BN-Cd16 DNAzyme	Rice extract	[17]
	Ag^{+}	24.9 nM	Ag10c DNAzyme	Lake water	[23]
	Cr^{3+}	70 nM	Ce13d DNAzymes	Groundwater	[18]
	Zn^{2+}	None Given	8-17 DNAzymes	Cellular	[58]
Electrochemiluminescent Detection					
	Pb^{2+}	6.4 pM	8-17 DNAzyme	Buffer	[59]
	Pb^{2+}	~50 pM	8-17 DNAzyme	Mineral, Drinking, and Snow water	[60]
	Cu^{2+}	1 nM	CA3 variant DNAzyme	Human hair extract buffer	[61]
	Ag^{+}	2 pM	HRP-mimicking DNAzyme	River water	[62]
Electrochemical Detection					
	UO_2^{2+}	2 pM	39E DNAzyme	River water	[63]
	UO_2^{2+}	20 pM	39E DNAzyme	River water	[64]
	Pb^{2+}	0.034 pM	8-17 DNAzyme	Tap and lake water	[65]
	Pb^{2+}	0.005 nM	8-17 DNAzyme	Lake water	[66]
	Pb^{2+}	37 pM	8-17 DNAzyme	Buffer	[67]
	Pb^{2+}	43 pM	8-17 DNAzyme	Tap water (5%)	[68]
	Pb^{2+}	0.25 nM	8-17cis DNAzyme	Lake water sample	[69]

Detection Mode	Analyte	LOD	DNAzyme Name	Sample	Ref.
	Pb^{2+}	500 pM	HRP-mimicking DNAzyme	Buffer	[70]
	Hg^{2+}	5.8 nM	HRP-mimicking DNAzyme	Rap and Waste water	[71]
	Cu^{2+}	0.1 aM	CA3 variant DNAzyme	River water	[72]
Colorimetric Detection					
	UO_2^{2+}	37 nM	39E DNAzyme	Filtered (0.25 μM) Lake water	[73]
	Pb^{2+}	12 pM	8-17 DNAzyme	Tap and River water, and Landfill leachate	[74]
	Pb^{2+}	20 pM	8-17 DNAzyme	River water	[75]
	Ce^{3+}	20 nM	Multiple DNAzymes	Lake water	[76]
Surface Enhanced Raman Scattering Detection					
	UO_2^{2+}	1 pM	39E DNAzyme	Sea, River, and Pond water	[77]
	Pb^{2+}	8.9 pM	8-17 DNAzyme	Lake, Tap, and Industrial waste water	[78]