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DNAzyme-Functionalized Gold Nanoparticles for Biosensing

Yu Xiang, Peiwen Wu, Li Huey Tan and Yi Lu

Abstract Recent progress in using DNAzyme-functionalized gold nanoparticles (AuNPs) for biosensing is summarized in this chapter. A variety of methods, including those for attaching DNA on AuNPs, detecting metal ions and small molecules by DNAzyme-functionalized AuNPs, and intracellular applications of DNAzyme-functionalized AuNPs are discussed. DNAzyme-functionalized AuNPs will increasingly play more important roles in biosensing and many other multi-disciplinary applications.

Keywords Biosensing · DNAzyme · Gold nanoparticle · Intracellular imaging

Abbreviations

AuNP	Gold Nanoparticle
DNA	Deoxyribonucleic Acid
dsDNA	Double-stranded DNA
PEG	Poly-(Ethylene Glycol)
SERS	Surface Enhanced Raman Spectroscopy
SPR	Surface Plasmon Resonance
ssDNA	Single-stranded DNA

Contents

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1 Introduction: DNAzymes and Gold Nanoparticles

Deoxyribonucleic acid (DNA) is a biopolymer made of four types of deoxynucleotides: adenosine (A), thymidine (T), guanosine (G), and cytidine (C). Complementary DNA strands assemble into a double helix (DNA duplex) via the formation of A–T and G–C base pairs [1, 2]. Such DNA was long considered solely a genetic material to encode the inheritable information of many organisms through its sequence. However, this understanding was challenged in the 1990s when DNAs with catalytic activities [3–5] and ligand-binding abilities [6–9] were discovered from libraries containing random DNA sequences through combinatorial techniques such as in vitro selection (Fig. 1) or systematic evolution of ligands by exponential enrichment (SELEX) [3–9]. The DNAs with catalytic activities are DNAzymes (also called deoxyribozymes or catalytic DNAs). Since their discovery, many different DNAzymes have been isolated by different research groups to catalyze the cleavage [3, 10–18], ligation [4, 19, 20], phosphorylation [21], adenylation [22], depurination [23], and thymine dimer repair [24, 25] of nucleic acids, as well as formation of nucleopeptide linkage [26, 27], porphyrin metallation [28], and other chemical reactions [29–31]. DNAzymes are generally more stable than protein and ribozymes due to the prominent stability of DNA against hydrolysis and denaturation, and many artificial modifications can be further introduced into DNAzymes to enhance their resistance to nuclease degradation [32, 33]. As a result of these properties, DNAzymes have been widely applied in many research fields, including biosensing [34–45], logical DNA computing and machines [39, 46–48], gene therapy [49–54], and others [55–60].

Among many nanomaterials, gold nanoparticle (AuNP) is one of the most studied [61]. The growing research interest on AuNPs is mainly because of their excellent properties such as high stability, strong plasmonic effects, good catalytic activities, and low cytotoxicity [62]. Many techniques have been developed for the synthesis of AuNPs of different sizes and shapes [63–68], including Au spheres [69–72], rods [73–80], prisms [81–84], cages [85–87], and wires [88, 89]. AuNPs are widely applied in nanoassembly [90–99], chemical catalysis [90, 100–106], sensing [96, 107–122], and biological applications [90, 123–131].

In this chapter, we focus on DNAzyme-functionalized AuNPs in biosensing and dynamic assembly [43, 96, 98, 121, 132–138]. The related works that have been conducted in our group are also introduced. Readers are directed to other outstanding reviews about more general perspectives of DNAzymes [34–60, 139–172], AuNPs [61, 62, 90], and biomolecule-functionalized nanomaterials [92, 95, 97–99, 122, 173–178].

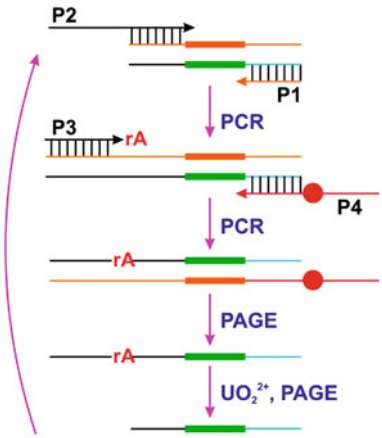

Fig. 1 A scheme of in vitro selection technique for selecting DNAzymes specifically using uranyl ions (UO_2^{2+}) as a cofactor to catalyze nucleic acid cleavage. The random DNA library is amplified by PCR using primers P1 and P2, and repeated using P3 and P4 containing rA as a cleavage site and an overhang/spacer as a tag, respectively. After PCR and PAGE purification, the DNA library is incubated with UO_2^{2+}. DNA sequences (containing both DNAzyme (*bold* and *green*) and substrate (*black* and *rA*) motifs) that undergo cleavage in the presence of UO_2^{2+} are isolated and used for the next round of selection. After a few rounds of selection, UO_2^{2+}-specific DNAzymes are identified by cloning and sequencing (Adapted from Ref. [16])

2 DNAzyme-Functionalized Gold Nanoparticles for Biosensing

2.1 Fabrication of DNA-Functionalized AuNPs

Before AuNPs can be used with DNAzymes, they have to be functionalized with DNA through strong ligand–Au bindings or in some other cases by weaker DNA–Au interactions. AuNPs synthesized by various methods are generally coated by ligands to stabilize the colloid aqueous solutions [62, 63, 71]. These ligands on the surface of AuNPs can be efficiently replaced by thiol-containing molecules because of the thiophilic nature of Au [179–181]. Based on this principle, thiol-modified DNAs (thiol-DNAs) were successfully functionalized onto the surface of AuNPs to direct the assembly of AuNPs via DNA hybridization (Fig. 2a) [182, 183]. After DNA functionalization, the AuNPs were stabilized by the strong electrostatic repulsion between negatively charged DNA strands. A fluorescence-based method could be utilized to quantify the surface coverage and hybridization efficiency of thiol-DNAs on AuNPs, using mercaptoethanol to displace the surface-bound fluorophore-labeled thiol-DNAs [184]. DNA loading on AuNPs of different sizes can be significantly enhanced by aging in concentrated salt solution or introducing a PEG spacer to the DNA [185]. Gel electrophoresis was used to study the conformation of thiol-DNA attached on AuNPs [186] and separate DNA-functionalized AuNPs containing different DNA coverage or in different

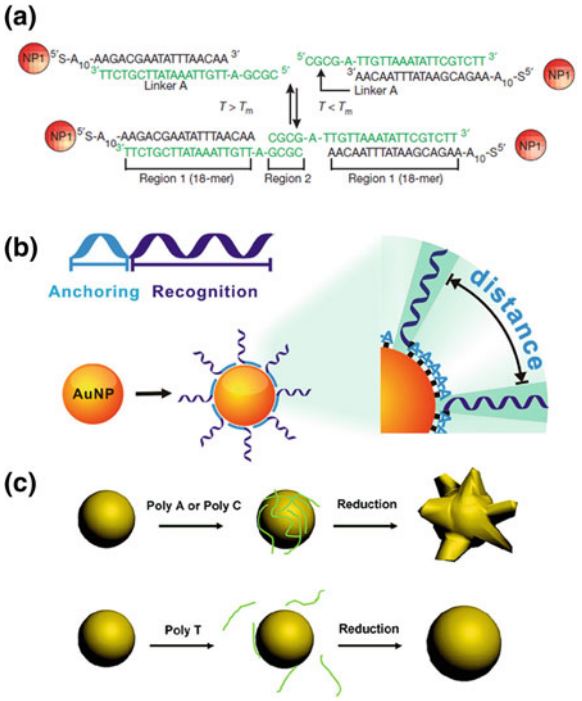

Fig. 2 Functionalization of DNA on AuNPs through **a** thiol–Au interaction, **b** poly A tags, and **c** in situ embedding during AuNP growth (Adapted from Ref. [182, 193, 196])

assemblies [183, 187–190]. In addition to the DNA-functionalized AuNPs with multiple DNA strands on each AuNP, mono-DNA-functionalized AuNPs (one DNA strand per AuNP) could also be prepared and purified [183, 187, 191, 192]. In addition to thiol-DNA, DNA with a poly A block was found to bind AuNPs with sufficient affinity to form DNA-functionalized AuNPs, and the lateral spacing and surface density of DNA strands on AuNPs was controlled by adjusting the length of the poly A block (Fig. 2b) [193].

In our lab, DNAzymes and their nucleic acid substrates were used as cross-linkers to form assemblies of DNA-functionalized AuNPs via DNA hybridizations on both binding arms, and the assemblies underwent disassembly upon cleavage of the substrates by the DNAzymes in the presence of metal ion cofactors [194, 195]. In addition to the postsynthetic methods that attach DNA on the surface of pre-formed AuNPs, our lab developed a new in situ method to prepare DNA-functionalized AuNPs simultaneously during the growth of AuNPs in the presence of DNA (Fig. 2c) [196, 197]. In this approach, DNA noncovalently bound to the surface of spherical Au nanoseeds through its poly A or poly C blocks, and then Au salts and reductants were added to initiate the growth of the nanoseeds into larger flower-shaped AuNPs. DNA was partially embedded in the newly formed Au layers during the growth of these Au "nanoflowers", and the DNA fragment on the surface of the AuNPs was still active to hybridize with AuNPs functionalized with a complementary DNA strand to form nanoassemblies [196]. One advantage

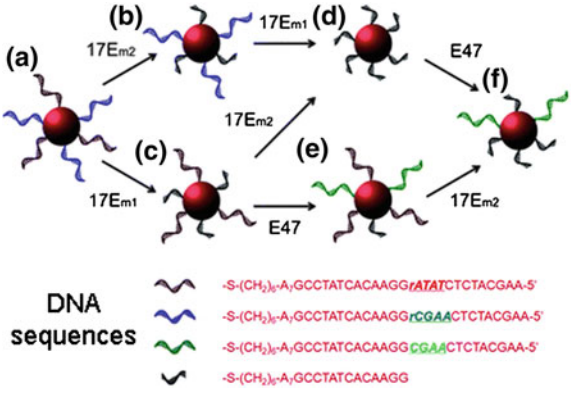

Fig. 3 Selective and stepwise modification of DNA sequences immobilized on AuNPs using DNAzymes (Adapted from Ref. [204])

of this in situ DNA-functionalization method over postsynthetic methods is that the attachment of DNA on the AuNPs by embedding is so stable that the treatment of high concentrations of coadsorbed diluent molecules such as mercaptoethanol could not displace the DNA from the AuNPs [196], whereas under this condition thiol-DNA on the surface of AuNPs was efficiently removed from the AuNPs [184]. The mechanism on how DNA is embedded and which sequence is preferred for the embedding during the growth of Au nanoseeds is currently not clear. Further studies are under way in our lab to answer these questions to enable more efficient DNA functionalization on AuNPs and better preservation of DNA activities.

On the other hand, despite the above progress made to attach DNA on AuNPs, few methods are capable of modifying the DNA sequences already attached [189, 198–203]. The "post-attachment" modification can enable more flexible transformations of DNA sequences to control AuNP nanoassemblies and tune their functions [189, 198–203]. We have recently developed a DNAzyme-based method to modify the sequences of DNA on AuNPs (Fig. 3). The DNA is processed by DNAzymes to cut off (cleave) unwanted fragments and then add (ligate) desired new fragments, enabling DNA sequence modifications to provide new DNA functions to the DNA-functionalized AuNPs [204]. More importantly, the DNA sequence modifications catalyzed by the DNAzymes are sequence-specific [3, 4], so that multiple DNA sequences on one AuNP can be modified selectively and in a stepwise manner [204]. Another advantage of this method is that the size of DNAzymes is comparable to that of DNA attached on AuNPs, thus little steric effect is present [203, 204]; whereas protein enzymes that catalyze sequence-specific DNA cleavage and ligation are much larger and may encounter low efficiency or incomplete cleavage of DNA on AuNPs [198–201]. In our current method, a ribonucleotide (rA) serves as the designed cleavage site in the DNA when DNAzymes are present. This rA is not necessary if a recently discovered new DNAzyme that can catalyze the cleavage of unmodified DNA is utilized [18].

2.2 DNAzyme-Functionalized AuNPs for Biosensing

The major application of DNAzyme-functionalized AuNPs is biosensing [43, 98, 121, 132, 135, 137]. DNA-functionalized AuNPs were first found to assemble via DNA hybridization between complementary DNA strands in 1996 [182, 183], where a color change from red to blue was observed. A red shift in absorption spectra was observed when discrete AuNPs assembled into macroscopic aggregates [182]. Due to the extremely large extinction coefficient of AuNPs compared to organic dyes and their other prominent properties such as light scattering, surface enhanced Raman spectroscopy (SERS), and surface plasmon resonance (SPR), AuNPs have been widely applied for highly sensitive enzyme-free detection of nucleic acids [205–240].

By introducing DNAzymes into such DNA–AuNP systems and taking advantage of AuNPs, a new series of DNAzyme-based biosensors has been developed for a broad range of targets [194, 221, 241–278]. In most of these studies, DNAzymes have played two distinct roles in target recognition and in signal enhancement. In the former case, nucleic acid cleaving or ligating DNAzymes [3, 4, 165] are used to recognize specific cofactors such as metal ions and small organic molecules; then the DNAzyme-catalyzed cleavage or ligation of the nucleic acid substrates alters AuNP assemblies, producing physically detectable signal changes for sensing the cofactors as targets [194, 241–252, 255–261, 263, 265–267, 269, 271–275, 277, 278]. In the latter case, peroxidase-mimicking DNAzymes [28, 29, 40, 169], usually containing G-quadruplex motifs, serve as signal generators or enhancers on AuNPs to transform target recognition by other molecules into physically detectable signals [221, 253, 254, 262, 264, 268, 270, 275, 276].

In addition to the above examples of in vitro detection, cellular or in vivo biosensing is also an active field of research and can provide useful information of the analytes in live cells and organisms. Although DNA-functionalized AuNPs have been successfully applied to biosensing of nucleic acids and small molecules in cells based on nucleic acid hybridization and aptamers [230, 279–282], such application based on DNAzymes and AuNPs for metal ion detection in live cells is still very challenging and has been reported only recently [283].

2.2.1 Nucleic Acid Cleaving/Ligating DNAzymes and AuNPs for Biosensing

In 2003, a colorimetric biosensor for lead ion (Pb^{2+}) was developed based on the DNA-directed assembly of 13 nm AuNPs in our lab (Fig. 4a) [194]. DNA-functionalized AuNPs were mixed with 8–17 DNAzymes and nucleic acid substrates. The nucleic acid substrates cross-linked the AuNPs into aggregates via DNA hybridization after mild heating at 50 °C and annealing, resulting in a blue solution and an absorption band around 700 nm. However, in the presence of the target (Pb^{2+}, the cofactor of the DNAzyme), the nucleic acid substrates that crosslinked

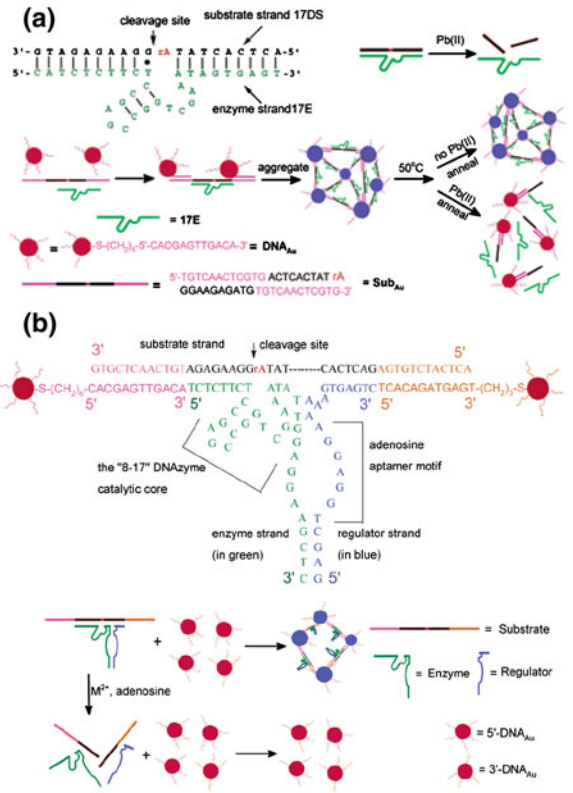

Fig. 4 Colorimetric detection of (**a**) Pb^{2+} and (**b**) adenosine using DNAzyme-functionalized AuNPs by an 8–17 DNAzyme and an adenosine-specific aptazyme, respectively (Adapted from Ref. [194, 244])

the AuNP together were cleaved by the DNAzymes so that no AuNPs aggregates could form. A red color and an absorption band at 522 nm were observed in this case. When an inactive DNAzyme was used, the DNA-directed assembly of AuNPs still occurred, but no color change was observed regardless of whether the samples contained Pb^{2+}. Interestingly, by changing the ratio of active and inactive DNAzymes, the dynamic range of the biosensor could be tuned from 0.1 $\sim$ 4 to 10 $\sim$ 200 μM [194]. This method was further optimized by testing the biosensor using different DNAzyme lengths, AuNP alignments, stoichiometries of DNAzyme to its substrate, buffer pH, and temperatures [242].

To enable fast Pb^{2+} detection at ambient temperature, the "tail-to-tail" alignment of 42-nm AuNPs was used for the biosensor design. The alignment and size of AuNPs were the major determining factors to achieve fast color changes and assembly of AuNP aggregates [241]. In another work, DNA functionalized AuNPs first assembled into aggregates cross-linked by 8–17 DNAzymes and substrates. Then, the aggregates were used as a biosensor system to detect Pb^{2+} by disassembling them in the presence of Pb^{2+} with the assistance of invasive DNA, resulting in color changes from blue to red and a blue shift in absorption spectra [245]. Compared with the previous methods [194, 241, 242], this new design

exhibited a "light-up" response to Pb^{2+} rather than a "light-down," and thus was less vulnerable to interference from other species that could inhibit the DNAzyme activity [245]. To eliminate the requirement of invasive DNA usage, an improved design using asymmetric DNAzymes to form the AuNP aggregates as the biosensor system was also developed [246].

In addition to Pb^{2+}, the biosensor system was further modified to detect adenosine by replacing the 8–17 DNAzyme with a DNA aptazyme (Fig. 4b). The aptazyme was a combination of an 8–17 DNAzyme and an adenosine aptamer, with the latter sequence inserted to one binding arm of the 8–17 DNAzyme. In this case, only in the presence of both adenosine and Pb^{2+} could the DNAzyme motif be activated to cleave its substrate and disassemble the AuNP aggregates [244].

In addition to the 8–17 DNAzyme that is specific to Pb^{2+}, other DNAzymes such as a nucleic acid ligating E47 DNAzyme for cupric ion (Cu^{2+}) and a nucleic acid cleaving 39E DNAzyme for uranyl ion (UO_2^{2+}) were also functionalized with AuNPs for the detection of Cu^{2+} and UO_2^{2+} through the color change of AuNP assemblies [247, 248]. In the former case, the E47 DNAzyme catalyzed the ligation of two short DNA strands into a long strand in the presence of Cu^{2+}. The long DNA strand could then bind two types of DNA-functionalized AuNPs by linking two short DNA fragments via DNA hybridization, respectively, to form blue colored aggregates as a response to Cu^{2+} in the solution [247]. In the latter case, the 39E DNAzyme induced the cleavage of a nucleic acid substrate with UO_2^{2+} as the cofactor. Upon the addition of UO_2^{2+} to AuNP aggregates cross-linked by 39E DNAzymes and their substrates, the cross-linker substrates were cleaved and caused the disassembly of the AuNP aggregates, resulting in color changes from blue to red [248].

In contrast to the above examples of AuNP aggregate formation by DNA cross-linkers [182], non-crosslinking DNA hybridization could also induce rapid aggregation of AuNPs [284]. For example, Li and coworkers utilized the 8–17 DNAzymes and substrates to functionalize discrete AuNPs without cross-linking, where Pb^{2+}-induced cleavage of the substrates reduced the stability of the AuNP colloid solution and caused aggregation. The detection of Pb^{2+} in this simple method was successfully achieved by monitoring the color change from red to purple or the red shift in absorption spectra due to the Pb^{2+}-induced aggregation of AuNPs [285].

To make the metal ion detection more user-friendly, lateral-flow devices were also developed based on DNAzymes and AuNPs for dipstick tests. In one design, the 8–17 DNAzymes and substrates were modified with biotin and functionalized on AuNPs. In the absence of Pb^{2+}, the AuNPs in the lateral-flow device were captured by the streptavidins immobilized on the device in the control zone, thus showing a colored band in this zone as a sign of Pb^{2+} free samples (Fig. 5a). However, when Pb^{2+} was present, the cleavage of the substrates removed the biotin labels from AuNPs and allowed them to go through the control zone on the device to reach the test zone containing immobilized complementary DNA, where biotin-free AuNPs were captured and displayed a colored band, indicating the presence of Pb^{2+} in the samples [256]. In another design by Zeng and coworkers, a

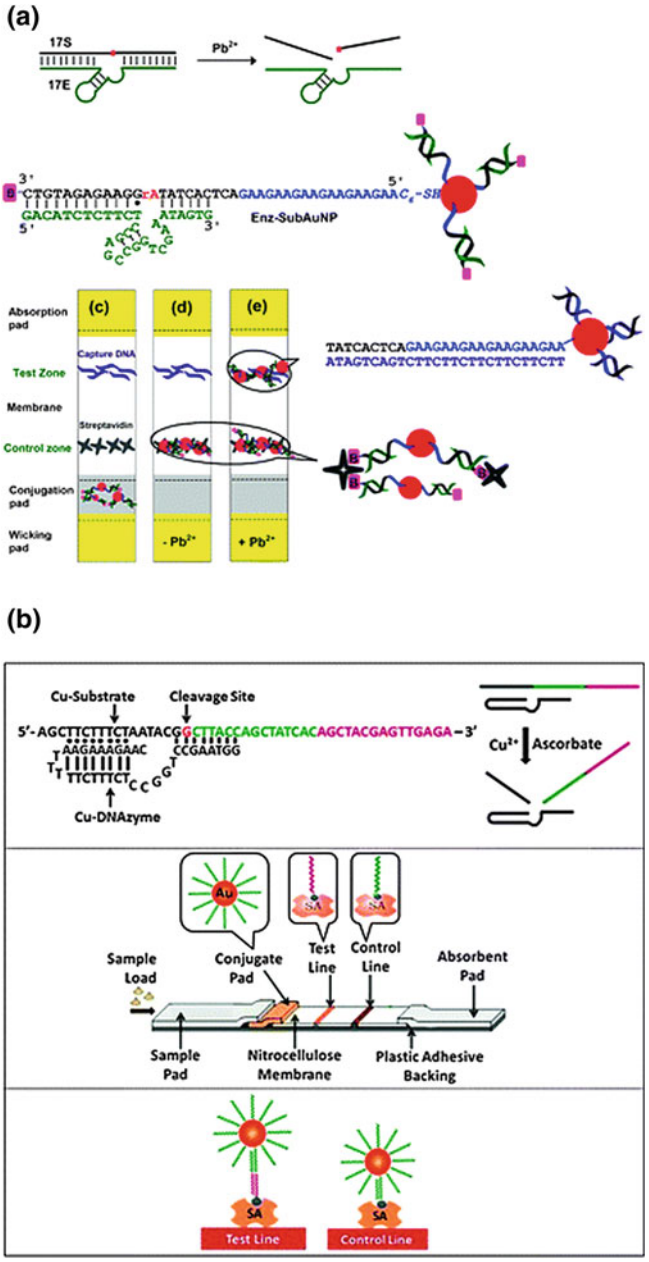

Fig. 5 Lateral-flow devices using DNAzyme-functionalized AuNPs for the detection of (**a**) Pb^{2+} and (**b**) Cu^{2+} (Adapted from Ref. [255, 256])

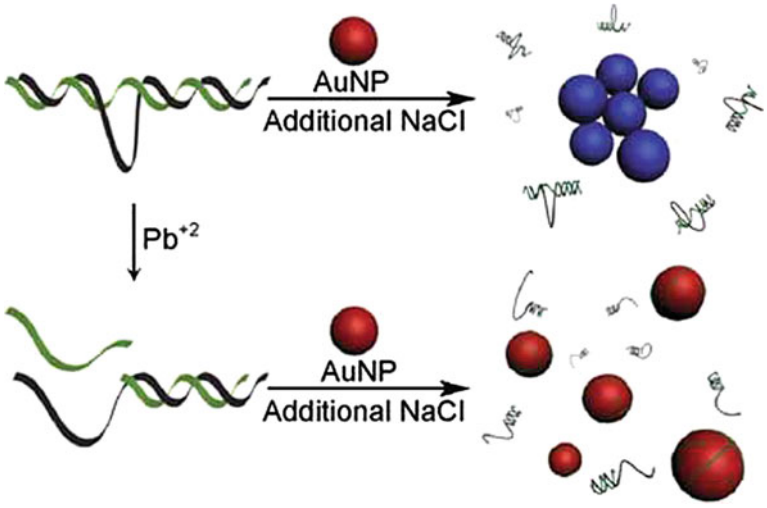

Fig. 6 Label-free detection of Pb^{2+} using DNAzymes and unmodified AuNPs (Adapted from Ref. [250])

nucleic acid cleaving DNAzyme specific for Cu^{2+} and its substrate were used without being prefunctionalized on AuNPs. The target Cu^{2+} interacted with the DNAzyme and substrate first, releasing a single-stranded DNA (ssDNA) piece that could link DNA-functionalized AuNPs to the test line by DNA hybridization (Fig. 5b). In the absence of Cu^{2+}, however, no release of the ssDNA occurred, thus the AuNPs moved across the test line and were captured on the control line where complementary DNA was immobilized. Therefore, a red line appeared on the test line only when Cu^{2+} was present in the samples [255]. Later, the same research group further incorporated a catalytic DNA circuit that could amplify the release of a ssDNA by the 8–17 DNAzyme and its substrate, achieving the detection of Pb^{2+} on a lateral-flow device with high sensitivity [277].

In addition to the lateral-flow device, an interesting study by Yu and coworkers utilized a conventional compact disc as the platform for Pb^{2+} detection based on DNAzymes and AuNPs. In their method, Pb^{2+}-induced cleavage of the substrate strand prevented the attachment of DNA-functionalized AuNPs onto the disc coated with complementary DNA. When Pb^{2+} was present in samples, fewer AuNPs were decorated on the disc to induce error in disc reading. Any optical drive from a computer could be used as a reader for this Pb^{2+} detection with the disc [265]. We also developed a method to use a low-cost commercial device, a glucose meter, to detect Pb^{2+} using DNAzymes, although no AuNPs were involved in the method [286].

In 2008, unmodified AuNPs were found to have the ability to couple with label-free DNAzymes and substrates for Pb^{2+} detection (Fig. 6) [250, 251]. The approach was based on a previous finding by Rothberg and coworkers that ssDNA

and double-stranded DNA (dsDNA) had distinct binding affinities and stabilization effects on unmodified AuNPs [218–220, 287]. Upon cleavage of the nucleic acid substrate by the 8–17 DNAzyme in the presence of Pb^{2+}, a ssDNA fragment was released from the DNA duplex to bind unmodified AuNPs. Because this ssDNA fragment had a stronger binding affinity to AuNPs to stabilize the colloid solution, the red color of the solution was only preserved when Pb^{2+} was present. By measuring the absorbance ratio at two wavelengths, the Pb^{2+} concentration was quantified [250, 251]. Through a very similar design but using another nucleic acid cleaving DNAzyme for Cu^{2+} with unmodified AuNPs, Yang and coworkers also developed a label-free method for detection of Cu^{2+} [257].

In addition to the above colorimetric methods based on DNAzyme-functionalized AuNPs, other analytical techniques have also been utilized to detect the target-induced change of DNAzyme-substrate duplexes and AuNPs. For example, AuNP-induced light scattering was used to develop biosensor systems for the detection of Pb^{2+} (Fig. 7a) and Cu^{2+} [263, 271, 273, 274]. The approach was based on the cleavage of substrates in the presence of metal ions by DNAzymes to either disassemble the DNA cross-linked AuNP aggregates [263] or prevent the aggregation of unmodified AuNPs [271, 273, 274]. In all these studies, the quantification of metal ions was achieved by measuring the difference in light-scattering properties between discrete AuNPs and aggregates [263, 271, 273, 274]. SERS [267] and SPR [275] (Fig. 7b) were also used to detect Pb^{2+} by measuring the release of AuNPs [267] and the activation of catalytic motifs [275] by the DNAzyme-catalyzed cleavage of substrates on surfaces, respectively.

Instead of generating signals directly, AuNPs can also serve as efficient fluorescence quenchers for the design of fluorescent biosensors [224, 288]. Following this principle, a Pb^{2+} sensor was developed by attaching fluorophore-labeled 8–17 DNAzymes and substrates to AuNPs, which underwent fluorescence enhancement in the presence of Pb^{2+} as a result of the increase in fluorophore-AuNP distance upon DNAzyme-catalyzed cleavage of the substrate [260]. Another study used a similar design for a Cu^{2+}-specific DNAzyme but was applied for ascorbic acid detection rather than Cu^{2+}, taking advantage of the requirement of ascorbic acid to reduce Cu^{2+} for the DNAzyme's activity [272]. Similarly, rod-shaped AuNPs coated with positively charged surfactants acted as binders and quenchers for flurophore-labeled 8–17 DNAzymes and substrates, allowing the detection of Pb^{2+} by fluorescence enhancement [266]. In addition to fluorescence quenching, AuNPs could serve as fluorescence anisotropy generators for the coated fluorophores due to the large size compared to free fluorophores and DNA. Based on this principle, a fluorescence anisotropy sensor was constructed using DNAzyme-functionalized AuNPs for the detection of Cu^{2+} and Pb^{2+} [259]. Finally, DNAzyme-functionalized AuNPs were also incorporated with electrochemically active substances for biosensor applications. Electrochemical sensors for Pb^{2+} were reported using Pb^{2+}-induced cleavage of nucleic acid substrates on AuNPs by 8–17 DNAzymes to enable the detachment (Fig. 7c) [249] or attachment [258] of AuNPs onto a DNA-coated electrode. The DNA on AuNPs permits attachment of large amounts of

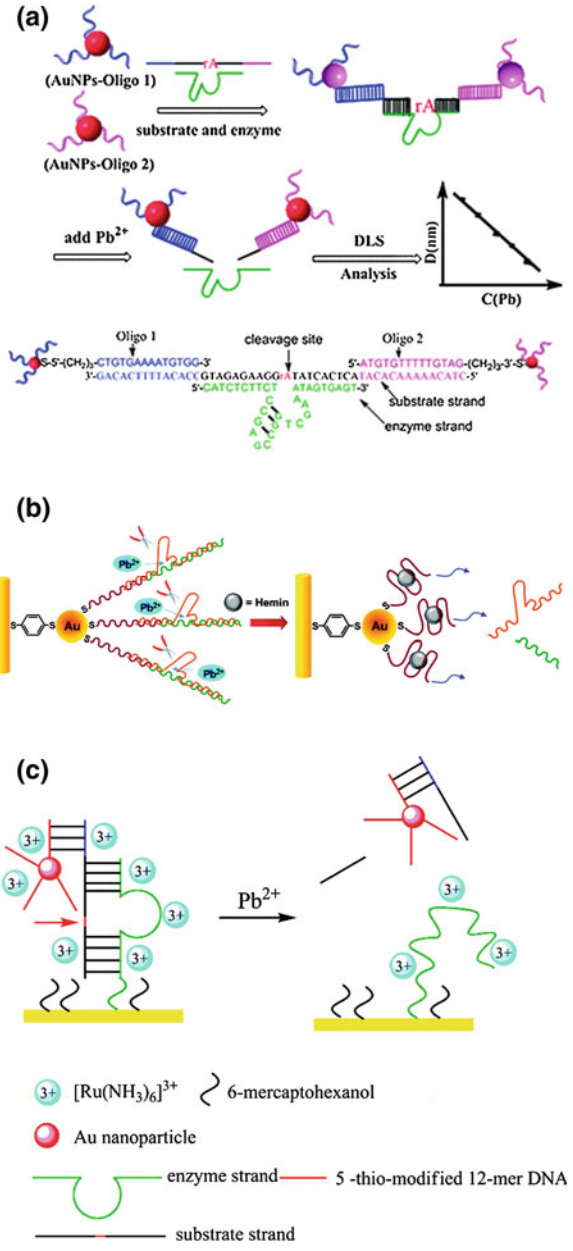

Fig. 7 DNAzyme-functionalized AuNPs for the detection of Pb^{2+} using (**a**) dynamic light scattering, (**b**) SPR, and (**c**) electrochemistry (Adapted from Ref. [249, 263, 275])

electrochemically active metal complexes, thus providing a large signal enhancement on the electrode in the presence of Pb^{2+} [249, 258]. Graphene sheets decorated by DNAzyme-functionalized AuNPs were also used to detect L-histidine and Pb^{2+} as the cofactors of two DNAzymes, respectively [261, 278].

2.2.2 Peroxidase-mimicking DNAzymes and AuNPs for Biosensing

Unlike the nucleic acid cleaving or ligating DNAzymes mentioned above that could recognize the target molecules and transform the recognition event into changes of AuNPs, peroxidase-mimicking DNAzymes [28, 29, 40, 169] usually serve as signal generators or enhancers by catalyzing the production of optically active substances, with AuNPs as carriers of many such DNAzymes for signal amplification. Willner and coworkers developed a telomerase activity assay using DNAzyme-functionalized AuNPs (Fig. 8a). In their method, the DNA immobilized on a surface was extended with telomere repeat units to capture the AuNPs via DNA hybridization. Then, the DNAzymes on the surface-bound AuNPs catalyzed the production of chemiluminescence. The more telomerase activity was present in samples, the more DNAzymes and AuNPs were immobilized, generating more intense chemiluminescence [221].

A similar design using magnetic particles as the surface was reported for the colorimetric detection of nucleic acids, where the target-induced immobilization of DNAzyme-functionalized AuNPs catalyzed the production of colored $ABTS^+$ from H_2O_2 and ABTS (Fig. 8b) [253]. By functionalizing AuNPs with both DNAzymes and antibodies, sandwich immunoassays were successfully achieved using the DNAzymes on AuNPs to generate color [254], electrochemiluminescence (Fig. 8c) [262], and chemiluminescence [264] signals for the detection of α-fetoprotein and carcinoembryonic antigen. Pb^{2+} detection using Pb^{2+}-specific DNAzymes [275] or Pb^{2+}-binding G-quarduplex [270] and AuNPs as carriers for peroxidase-mimicking DNAzymes were also achieved by electrochemistry [275] and fluorescence [270] measurements. Another study used AuNPs as carriers of two halves of a peroxidase-mimicking DNAzyme. The two halves were released when target nucleic acids or small molecules interacted with the DNA or aptamers on the surface of the AuNPs, respectively. Then the released halves formed active DNAzymes in solution and generated chemiluminescence for sensitive detection [276].

2.2.3 DNAzyme-Functionalized AuNPs for Intracellular Biosensing

AuNPs were first found by Mirkin and coworkers as an efficient material for the delivery of DNA into cells for intracellular gene regulation and the detection of mRNA and ATP [230, 279, 280]. By functionalizing AuNPs with DNAzymes, delivery of DNAzymes into cells to sequence-specifically cleave mRNA [281] and perform RNAi-independent gene regulation [282] were achieved. Despite these achievements, there are still very few studies using DNAzyme-functionalized AuNPs for intracellular biosensing.

Recently, our group has developed a new method to attach fluorophore-labeled UO_2^{2+}-specific DNAzymes and substrates onto AuNPs for intracellular UO_2^{2+} detection (Fig. 9). The UO_2^{2+}-specific 39E DNAzyme was conjugated to the AuNP through a thiol tag, and the substrate strand was modified with a Cy3

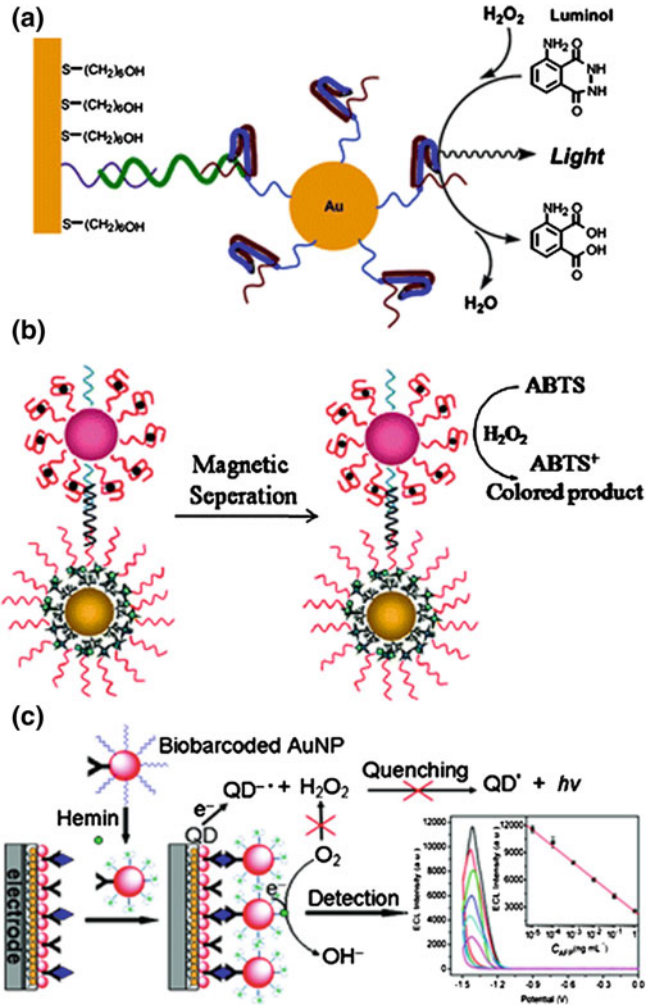

Fig. 8 Detection of (**a**) telomerase activity, (**b**) nucleic acid, and (**c**) protein biomarker using AuNPs as support for peroxidase-mimicking DNAzymes (Adapted from Ref. [211, 253, 262])

fluorophore and a molecular quencher to reduce background. In the absence of UO_2^{2+}, the fluorescence of the Cy3 was quenched by both the AuNP and the molecular quencher. In the presence of UO_2^{2+}, the DNAzyme cleaved the fluorophore-labeled substrate strand, resulting in the release of the shorter ssDNA containing the Cy3, and accompanied by fluorescence enhancement. We demonstrated that this DNAzyme–AuNP biosensor could readily enter cells and serve as a UO_2^{2+} sensor within a cellular environment, making it the first demonstration of DNAzymes as intracellular metal ion sensors [283].

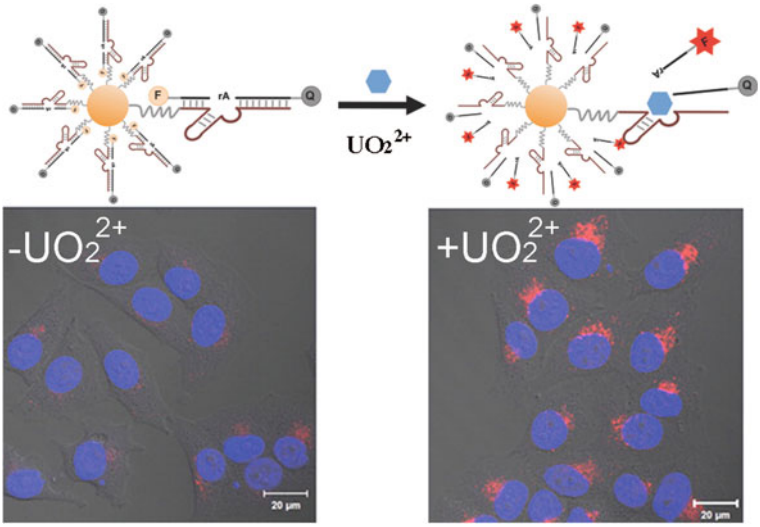

Fig. 9 Fluorescent UO_2^{2+}-specific DNAzyme immobilized onto AuNP as selective turn-on UO_2^{2+} sensors inside live cells. The scale bar is 20 μm (Adapted from Ref. [283])

3 Summary and Outlook

Since the discovery of the first DNAzyme that catalyzed the cleavage of RNA in 1994, more and more DNAzymes with diverse functions have been obtained by the in vitro selection technique and are being actively pursued for many applications including biosensing, especially for metal ion detection in both the environment and biology. AuNPs, on the other hand, are ideal materials for biosensing due to their prominent properties as efficient signal reporters, DNA carriers, and cellular delivery materials. Therefore, intensive research has been carried out in recent years to utilize DNAzyme-functionalized AuNPs for biosensing and other applications, and there is no doubt that the future work and impact of this field will continue to grow.

One challenge in this field is the discovery of new DNAzymes to cover more metal ions and other target molecules. DNAzymes specific for Mg^{2+}, Zn^{2+}, Pb^{2+}, Cu^{2+}, Co^{2+}, Hg^{2+}, and UO_2^{2+} have been selected from random DNA libraries and biochemically characterized [3, 4, 10–20]. However, many other important molecules and especially metal ions, such as iron (Fe^{2+} and Fe^{3+}), Cr^{3+}, Ni^{2+}, and Mn^{2+} in biology, still do not have a DNAzyme to selectively recognize each of them. Introducing modified DNA bases or backbone into random DNA libraries is one promising method to extend the possibility of obtaining these candidates. However, the available techniques for amplifying DNA with artificial modifications for in vitro selection are still limited and require active collaboration from many disciplines such as chemistry, chemical biology, and biochemistry.

On the other hand, although AuNPs provide many unique properties for signal amplification, one hurdle for wide adoption in commercial products is batch-to-batch variations and quality controls. Overcoming this hurdle is critical to translate the technology from bench to the field, clinics, and home.

Another challenge is the application of DNAzyme-functionalized AuNPs in intracellular biosensing. Although AuNPs can efficiently deliver DNAzymes and substrates into cells and serve as part of the sensor systems, such as signal reporters for light scattering and SERS or quenchers for fluorescence, the intracellular localization and activation of such sensors are still difficult and demand more general methods to be developed because many intracellular targets are not evenly distributed in cells. In addition, most DNAzymes currently available are not selected under cellular conditions, so they may need optimization and reselection to ensure their activities are still viable inside cells and on the surface of AuNPs to recognize specific targets. Stability of DNAzymes in a cellular environment has not been well understood and DNA modifications are required to make DNAzymes more resistant to enzymatic degradation while still retaining their activities.

Finally, the cytotoxicity and biological effects of nanomaterials such as AuNPs on different cells are not well understood currently, and how DNAzyme functionalization will affect these properties of AuNPs is still an open question to be answered. These are critical for the future applications of DNAzyme-functionalized AuNPs in cellular and in vivo biosensing. Given the progress made in this area in the past 10 years, more efficient DNAzyme–AuNP-based sensors will be developed in the near future.

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