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Functional DNA directed assembly of nanomaterials for biosensing†

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Abstract

This review summarizes recent progress in the development of biosensors by integrating functional DNA molecules with different types of nanomaterials, including metallic nanoparticles, semiconductor nanoparticles, magnetic nanoparticles, and carbon nanotubes. On one hand, advances in nanoscale science and technology have generated nanomaterials with unique optical, electrical, magnetic and catalytic properties. On the other hand, recent progress in biology has resulted in functional DNAs, a new class of DNAs that can either bind to a target molecule (known as aptamers) or perform catalytic reactions (known as DNAzymes) with the ability to recognize a broad range of targets from metal ions to organic molecules, proteins and cells specifically. By taking advantage of the strengths in both fields, the physical and chemical properties of nanomaterials have been modulated by the target recognition and catalytic activity of functional DNAs in the presence of a target analyte, resulting in a large number of colorimetric, fluorescent, electrochemical, surface-enhanced Raman scattering and magnetic resonance imaging sensors for the detection of a broad range of analytes with high sensitivity and selectivity.

1. Introduction

The rapid development of nanoscale science and technology has resulted in the successful synthesis and characterization of a variety of nanomaterials including metallic nanoparticles,[1,2] semiconductor nanocrystals (quantum dots),[3–5] carbon nanotubes (CNTs),[6–8] nanorods[9] and nanoshells.[10,11] These nanomaterials have been shown to possess unique optical,[12,13] electronic,[14] magnetic[15,16] and catalytic properties,[17] making them ideal candidates for signal generation and transduction in sensing. However, a sensing system requires at least two components: a target recognition element and a signal transduction element. The nanomaterials do not intrinsically possess the target recognition abilities necessary for selective binding and sensing.

DNA is among one of the most important classes of biopolymers and has been known as a carrier of genetic information.[18,19] Since the early 1990s, however, certain DNA molecules have also been shown to perform catalytic reactions (called DNAzymes, or

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deoxyribozymes[20–22]) like protein enzymes, or bind to a specific target molecule (called aptamers) like antibodies.[23] DNAzymes, aptamers and their combination (called aptazymes or allosteric DNAzymes[24]) are now collectively called functional DNA. These functional DNA molecules have not been discovered in nature thus far, instead they have been isolated *via* a combinatorial biology technique known as *in vitro* selection,[25] or a process also known as systematic evolution of ligands by exponential enrichment (SELEX).[26,27]

A unique feature of functional DNA and related functional RNA molecules (ribozymes and RNA aptamers) is their ability to specifically bind a broad range of analytes including inorganic, organic, and biomolecules, as well as bacteria, viruses, and cancer cells.[28–32] The binding affinity of these functional DNA/RNA molecules to specific targets can rival that of protein antibodies. In addition to their ability to recognize a large variety of targets, functional DNAs offer a number of competitive advantages over other molecules such as antibodies.[33] First, functional DNAs are isolated by *in vitro* selection and can be chemically synthesized and engineered in test tubes after their sequences are determined, whereas antibody preparation often requires animals or cell cultures. Second, functional DNAs can stand much harsher conditions, such as the high temperatures and non-aqueous solvents that are often encountered in materials synthesis and engineering; even if the DNAs are denatured, they can be refolded to their native active conformation, while denatured antibodies usually cannot be refolded. Finally, these functional DNAs induce less or no immunogenicity in clinical applications compared to those protein antibodies. Despite these advantages, there is still a lack of general methods to transform the selective binding of these functional DNAs into physically detectable signals such as fluorescence or color changes. Therefore, integration of functional DNA with nanomaterials provides new hybrid systems that combine the specific molecular recognition or catalytic properties of functional DNA with the diverse and strong signal transduction of nanomaterials. This novel combination has yielded stimuli-responsive nanomaterial assemblies, and various types of sensors for selective and sensitive detection of a wide range of analytes.[34–42] In this review, we will discuss recent advances in sensing through the integration of functional DNA with different nanomaterials including metallic nanoparticles, semiconductor nanoparticles, magnetic nanoparticles, and carbon nanotubes.

2. Functional DNA directed assembly of metallic nanoparticles for biosensing

2.1. Metallic nanoparticles as color reporters in colorimetric detection

Noble metal nanoparticles such as gold and silver nanoparticles display unique size, composition and distance-dependent optical properties.[43] Rapid progress in the preparation of size- and shape-controlled metallic nanoparticles and the development of different surface modifications for better stability, solubility and biocompatibility make it possible to explore applications of noble metal nanoparticles in the sensing and biomedical fields.[44,45] For example, gold nanoparticle (AuNP) colloids are red in the dispersed state but change to blue upon aggregation due to interparticle plasmon coupling, and their extinction coefficients are several orders of magnitude higher than organic dyes.[46,47] Therefore, these metallic nanoparticles are ideal color reporters in colorimetric sensing.

Oligonucleotide-mediated AuNP assembly was first reported by both the Mirkin group and the Alivisatos group.[48,49] AuNPs modified with single strand DNA (ssDNA) would aggregate upon addition of a complementary target DNA, resulting in a color change from red to blue. By carefully controlling the temperature at which the aggregation occurred, this method could detect oligonucleotides down to sub-picomolar levels with single base mismatch resolution.[50]

In addition to oligonucleotides, there are many other analytes such as metal ions, organic molecules, proteins, carbohydrates, bacteria, and viruses in biological systems and in the environment. Therefore, it would be desirable to use AuNPs as a method of signal transduction for the detection of even broader targets beyond oligonucleotides. Toward this goal, our group first reported a colorimetric biosensor for lead (Pb^{2+}) based on the DNAzyme directed assembly of AuNPs in response to Pb^{2+} as a stimulus.[34] The DNAzyme consisted of an enzyme strand and a substrate strand which had a single RNA base as the cleavage site (Fig. 1a, b). The substrate strand was extended on both ends to facilitate hybridization with DNA-modified AuNPs in a head-to-tail configuration (Fig. 1c). Because there are a number of DNA molecules on each AuNP, these AuNPs can be linked together by the substrate DNAs, forming AuNP aggregates that appear blue. Upon heating the system to 50 °C, the AuNPs and DNAzyme disassembled and they could be reassembled again when cooled down to room temperature which allows DNA annealing. However, in the presence of Pb^{2+}, the substrate was cleaved, thereby inhibiting reassembly and resulting in a red color. The color change was conveniently monitored by UV-vis spectroscopy. The ratio of the extinction at 522 and 700 nm was chosen to quantify the color change of the AuNPs (Fig. 1d). A lower ratio is associated with aggregated nanoparticles of blue color, while a higher ratio is associated with dispersed nanoparticles of red color. This sensor had a detection limit of 100 nM and was shown to be able to detect lead in paint (Fig. 1f).

A unique feature of the DNAzyme-AuNP sensor described above is tunable dynamic range, which is required for the sensor to match the detection level required for different applications. For example, the maximum contamination level (MCL) for lead in water is defined by the US Environmental Protection Agency to be 75 nM, while the MCL for lead in paint is 2 μM. To tune the same sensor to different dynamic ranges of detection, our group took advantage of a finding that a single base mutation in the enzyme strand completely inactivates the Pb^{2+}-dependent activity toward the same substrate strand.[51] The detection range of the sensor could then be tuned from 100 nM–2 μM when using all native DNAzyme, to 10–200 μM by replacing 95% of DNAzymes with the inactive mutant DNAzyme (Fig. 1e), because the latter system requires more Pb^{2+} to achieve the same degree of de-aggregation and thus color change.[34] By mixing different percentages of active and inactive DNAzymes, the dynamic detection range can be tailored to almost any desirable level.

Since annealing was required for the head-to-tail aggregate formation and for sensor operation (Fig. 1c), an improved design using tail-to-tail alignment in the AuNP aggregates was developed to facilitate the nanoparticle assembly and disassembly (Fig. 1g).[52] Due to less steric hindrance in comparison to the head-to-tail alignment, aggregation at room temperature was observed. However, the assembly kinetics of this system were still relatively slow, as it takes more than one hour for the aggregation to reach completion. To overcome this limitation, we explored the effect of the size of the AuNPs. As the optical properties of nanoparticle aggregates are determined by the size of the aggregates rather than the number of nanoparticles in the aggregates,[47] AuNPs with a larger size were employed and a clear color change was observed in 5 min (Fig. 1h). This optimal design allowed fast detection of Pb^{2+} at ambient temperature.[31,52–54]

While the DNAzyme-AuNP system is powerful for the detection of metal ions because of the high metal-binding specificity of the DNAzymes, aptamers can be used for detecting many other targets because they can bind to a variety of molecules with high affinity and specificity. Therefore, this binding property of aptamers has been applied to control the assembly of AuNPs for sensing applications. Chang and co-workers have functionalized platelet-derived growth factor (PDGF) aptamers onto AuNPs for sensitive and selective detection of PDGF.[55] Since each PDGF could bind two aptamers, AuNPs were crosslinked

upon addition of PDGF and a red to purple color change was observed (Fig. 2a).[55] This sensor design required the target molecule to bind at least two aptamers, making it difficult for application to other target molecules because most aptamers have only a single binding site for their targets. To apply the nanoparticle based sensing method to a wide range of targets, our group developed a general sensor design based on the disassembly of aggregated AuNPs, induced by structure switching of the aptamer upon binding to the target molecule.[38] Two different ssDNAs were attached to two different batches of AuNPs, and a linker DNA containing the aptamer sequence was used to crosslink these two ssDNAs and aggregate the AuNPs (Fig. 2b). In the presence of the target molecule, the folding of the aptamer caused by target binding resulted in fewer base pairs being used for linking the AuNPs, and thus disassembled the aggregates, with an accompanying blue to red color change (Fig. 2b). This method has been extended to detect adenosine, cocaine, and potassium ions, as well as multi-analytes with controllable cooperativity.[56]

Different from the sensor system based on analyte induced disassembly of AuNP aggregates, Li and co-workers developed a new type of sensing method based on non-crosslinking DNA-AuNP conjugates.[39] They functionalized AuNPs with a moderate number of short alkane thiol-modified DNA and hybridized an adenosine aptamer to the DNA. Addition of adenosine induced the structure switching of the aptamer and dissociated the aptamer strands from the AuNPs (Fig. 2c). The salt stability of the AuNPs then decreased due to the lesser number of DNA strands on the nanoparticle surface (to protect them), resulting in a red to purple color change in the presence of a 35 mM $MgCl_2$ salt solution. This sensor had a detection limit of 10 μM for adenosine. It was later found that folding of the aptamer tethered on AuNPs could stabilize dispersed AuNPs. The Li group[41] and Chang group[57] independently reported colorimetric sensing of adenosine based on this phenomenon (Fig. 2d).

Other than the aptamer based sensor, Mirkin and co-workers reported a colorimetric sensor for Hg^{2+} detection based on thymidine–Hg^{2+}–thymidine coordination chemistry.[58] Hg^{2+} was shown to specifically bind to two DNA thymine bases (T) and stabilize T–T mismatches in a DNA duplex.[59,60] With two complementary DNAs containing T–T mismatches attached to different AuNPs, Hg^{2+} was able to stabilize the inter-particle DNA hybridization, leading to AuNP assembly and an increased melting temperature of the aggregates. Therefore, Hg^{2+} concentration was correlated with the melting temperature of the aggregates and a detection limit of 100 nM was reported.[58] Liu and co-workers made a significant improvement in sensor design and made the sensor work at ambient temperature, with a detection limit of 1 μM.[61]

The colorimetric sensors discussed above, all require chemical modifications on the DNA as well as surface functionalization of the AuNPs. It is also possible to make sensors without the need to use chemical modifications, which can be called label-free colorimetric sensors. In 2004, Rothberg and Li discovered that non-thiolated short ssDNA could be easily absorbed onto citrate coated AuNPs and stabilize the AuNPs from salt induced aggregation, while dsDNA could not bind to AuNPs and thus could not prevent aggregation of the AuNPs caused by salt.[62] Based on this phenomenon, they reported hybridization assays to detect specific DNA[63] or RNA sequences[64] using unmodified AuNPs and DNA.

Aptamers were firstly applied in this label-free colorimetric sensing system. Unstructured aptamer without target and folded aptamer bound to target have different interactions with AuNPs (Fig. 3a). Fan and co-workers demonstrated the colorimetric detection of potassium[65] and adenosine triphosphate (ATP),[66] and the Dong group reported thrombin detection.[67] Based on a similar principle, a label-free colorimetric method for the sensitive and selective detection of Hg^{2+} was also reported.[68–70]

The introduction of DNAzyme into such a system poses additional challenges, as its function requires not only binding, like aptamers, but also the reaction and release of products. Our group reported label free colorimetric sensing of Pb^{2+} using AuNPs and DNAzymes.[42] In the sensor design, the DNAzyme was extended by 8 bases at the 5′ end for stable hybridization of the enzyme-substrate complex. In the presence of Pb^{2+}, the DNAzyme complex was cleaved and hence released short ssDNA that would adsorb onto AuNPs, thereby preventing aggregation under high ionic strength conditions. In the absence of Pb^{2+}, the uncleaved complex could not stabilize the AuNPs, resulting in purple-blue AuNP aggregates (Fig. 3b). This sensor was highly sensitive with a detection limit of 3 nM, which is much lower than those of labeled methods using the same DNAzyme and AuNPs (100 nM),[34] and even lower than the DNAzyme-based fluorescent sensor for lead (10 nM).[71] This sensor also showed good selectivity and a tunable dynamic range by adjusting pH. A similar strategy using unmodified AuNPs and DNAzyme was also reported by Wang and co-workers.[72]

Both labeled and label-free methods have been shown to successfully detect a broad range of analytes, even though they are based on different principles. A systematic comparison of these two systems using the same uranyl dependent DNAzyme and AuNPs was carried out recently.[73] The results showed that the labeled sensor took more time and effort for preparation, but was easier to operate once prepared. The label-free sensor had better sensitivity, shorter operation time and lower costs, but was more vulnerable to ionic strength and other variables.

2.2. Metallic nanoparticles as fluorescence quenchers in fluorescent detection

In addition to playing a major role as color reporters in colorimetric sensing, metallic nanoparticles can also be used in fluorescent sensing. Gold nanoparticles have been shown to be excellent quenchers for organic dyes in their proximity, due to an increase in their nonradiative rate and a decrease in the dye's radiative rate.[74] This quenching property of AuNPs makes them exceptional materials in fluorophore/quencher based biosensors. As an example, AuNPs have been used in molecular beacons for the sensing of DNA with 100-fold better sensitivity than organic quenchers.[75]

Chang and co-workers designed a "turn-on" fluorescent sensor for PDGFs using aptamer functionalized AuNPs.[76] *N*,*N*-dimethyl-2,7-diazapyrenium dication (DMDAP) was used as the fluorescence signaling molecule that intercalated with the aptamers and was quenched by the AuNPs. PDGF bound to the aptamers and released DMDAP, causing a significant fluorescence increase. This method was highly sensitive, with a detection limit of 8 pM. Instead of DMDAP, the same group also used photoluminescent Au nanodots as fluorescent labels and reported a protein sensing assay based on a similar strategy.[77] In a different design, Zhao and co-workers reported a thrombin biosensor based on structure-switch signaling of aptamers.[78] Dye-labeled DNA was hybridized with aptamer functionalized AuNPs and upon recognition of the target by the aptamers the fluorescent DNA strand was released and the fluorescence was recovered.

2.3. Metallic nanoparticles for signal generation and amplification in biodetection

As shown in the above examples, a sensor normally requires two components: a target recognition element for interacting with the target and a signal transduction element for readout. Since the analyte level in real biological samples can be very low, high sensitivity is desirable for a biosensor in practical applications. To improve the sensitivity of a biosensor, a signal amplification process is required. Metallic nanoparticles composed of gold or platinum have high catalytic activities in many chemical reactions and can thus be integrated into a biosensor for signal generation and amplification.

One method using metallic nanoparticles for signal amplification is based on their catalytic function of reducing metal ions to grow NPs of identical composition or core-shell structures. This has been utilized by Mirkin and co-workers to detect DNA with very high sensitivity.[79] Willner and co-workers reported the use of aptamer functionalized AuNPs as a catalytic label for the amplified detection of thrombin (Fig. 4a).[80] Since thrombin has two binding sites for aptamers, by attaching one aptamer to a glass surface and another to AuNP, the presence of thrombin would link the nanoparticle to the glass surface. This was then followed by gold reduction and the absorbance of the functionalized glass slides was used to determine thrombin concentration. The optimized sensor achieved a detection limit of 2 nM. Instead of using aptamers chemically linked to the solid support, Ying and Jana recently reported a dot-plot assay to detect thrombin by non-covalent adsorption of thrombin on a nitrocellulose membrane.[81] The immobilized thrombin could bind to the aptamer, which was attached to silica-gold core-shell nanoparticles, and a red color change representing the thrombin concentration after gold reduction could be read directly by eye. A similar strategy was also reported by Dong and co-workers, and the thrombin detection was tested in human plasma.[82]

Other than AuNPs, platinum NPs have also been used to amplify the electrochemical detection of DNA and thrombin by catalyzing the reduction of H_2O_2 as output signals, and detection limits of 10 pM for DNA and 1 nM for thrombin were obtained.[83] Pt NPs were also shown to catalyze the generation of chemiluminescence from luminol/H_2O_2, and this property was utilized by the Willner group to design a chemiluminescent sensor for thrombin detection (Fig. 4b).[84]

2.4. Metallic nanoparticle based SERS detection

Surface-enhanced Raman scattering (SERS) spectroscopy is a very sensitive technique based on the large enhancement of Raman scattering of molecules residing at or near the surface of certain nanostructured metals, such as gold and silver. The enhancement factor can be as much as 10^{14}–10^{15}, suggesting the possibility of developing ultrasensitive detection methods based on SERS.[85,86] A key step in developing a SERS sensing platform is the choice of an appropriate surface to provide the desired and reproducible enhancement. Silver or gold nanoparticles are good choices because they can be easily synthesized and functionalized, and they provide enough Raman signal enhancement.[87] Mirkin and co-workers first applied SERS to DNA detection by using gold nanoparticles labeled with oligonucleotides and Raman-active dyes, and a 20 femtomolar detection limit of target DNA was obtained.[88]

To detect analytes beyond DNA, Dong and co-workers designed a SERS based aptasensor for protein detection.[89] Thrombin aptamers were attached to a gold substrate and to Raman reporter labeled AuNPs. Since thrombin has two aptamer binding sites (Fig. 5a), AuNPs would be captured on the gold substrate upon the addition of thrombin, resulting in an enhanced SERS signal. A more general design for a SERS based aptasensor was reported by Yu and co-workers (Fig. 5b).[90] Silver-gold core-shell nanoparticles modified with thiolated capture DNA were deposited on gold film for SERS enhancement. Another SERS reporter labeled DNA strand complementary to the captured DNA was first hybridized with extended adenosine aptamer strand, thus inhibiting the capture of this DNA complex on the substrate. Addition of adenosine would cause structure switching of the aptamer and release the reporter labeled DNA, allowing its capture on the substrate. This design resulted in an enhanced SERS signal that correlated to adenosine concentration. This sensor was a “turn on” sensor and the detection limit was reported to be 10 nM.

3. Functional DNA modified quantum dots for biosensing

Quantum dots (QDs) are semiconductor nanoparticles that have a number of attractive optical properties such as broad absorption spectra and narrow symmetrical photoluminescence (PL) spectra. The PL emission wavelength can be tuned by the size and composition of the QD. In addition, multiplex PL emissions can be excited using a single wavelength light source and fit into a given spectral region. Furthermore, QDs are much more resistant to photobleaching than conventional organic fluorophores. Due to these attractive properties, QDs were extensively investigated for imaging applications.[13,91,92] Instead of being simply used as labels or stains for imaging, QDs have recently been applied for the quantitative detection of a number of targets including maltose,[93] protease,[94] and specific DNA[95–97] based on fluorescence resonance energy transfer (FRET), a technique widely used to quantify molecular dynamics in biochemistry and biophysics.[98,99]

Ellington and co-workers reported the first aptamer based QD FRET biosensor for protein detection (Fig. 6a).[36] Through a streptavidin-biotin interaction, QDs were modified with aptamers which were then hybridized with a short DNA strand labeled with an acceptor. The hybridization event brought the acceptor into close proximity with the QD surface and quenched its fluorescence. Binding of the target would displace the acceptor strand and produce enhanced fluorescence (Fig. 6a).

In addition to energy transfer, charge transfer between QDs and specific molecules has also been used to design sensors. Benson and co-workers developed a protein based QD sensor for maltose by modulating the interaction between a ruthenium compound linked maltose binding protein (MBP) and QDs.[100] The ruthenium compound acted as an electron donor and could quench the fluorescence of QDs in a distance-dependent manor. The binding of maltose to the MBP would cause its conformation to change and pull the ruthenium compound away from the QD surface, resulting in an increased emission. Strano and co-workers found that the fluorescence of thrombin aptamer functionalized PbS QDs could be selectively quenched upon binding to thrombin (Fig. 6b).[101] They attributed this quenching to the charge transfer from thrombin to the QDs and reported the detection limit to be 1 nM.

QDs can also be used for multiplex sensing since a single wavelength light source can be used to excite QDs with different PL emissions. Our group utilized QDs emitting at 525 and 585 nm to encode aptamer-linked nano-assemblies sensitive to adenosine and cocaine respectively.[102] The nano-assemblies contained gold nanoparticles that served as quenchers. The addition of target analytes disassembled the nanostructures, resulting in an increased emission (Fig. 7a, b). This design allowed the detection of both analytes in one-pot, and can be applied to the detection of more analytes simultaneously.

Instead of using their tunable PL properties, the different chemical compositions of QDs can also be used for multiplex detection. Wang and co-workers demonstrated a multi-analyte electrochemical aptamer biosensor using QD tagged proteins.[103] As shown in Fig. 7c, CdS QD labeled thrombin and PbS QD labeled lysozyme were captured by the corresponding aptamers immobilized on gold substrate. Displacement of the QDs upon addition of target analytes was monitored by the electrochemical stripping detection method (Fig. 7c). This method was shown to be very sensitive and selective, allowing the simultaneous quantification of picomolar levels of target proteins.

4. Functional DNA modified magnetic nanoparticles for biosensing

Bulk ferromagnetic materials exhibit permanent magnetization in the absence of an external magnetic field. When their sizes decrease to the nanometer scale, thermal energy at room temperature is sufficient to flip the electron spin directions and quickly randomize the

magnetic dipoles.[104] Such small nanoparticles that exhibit the magnetization property only in the presence of an external magnetic field, are called superparamagnetic (SP) nanoparticles.[105] Due to their unique magnetic properties, SP nanoparticles hold great potential in the biomedical field and can be used for labeling and separation of biological species, drug delivery and cancer treatment, as well as magnetic resonance imaging (MRI).[106–109]

Weissleder and co-workers found that the assembly-disassembly state of SP nanoparticles affects the magnetic relaxation of surrounding water protons, and they have used these nanoparticles as magnetic relaxation switches (MRS) for MRI based detection.[110,111] Our group has recently demonstrated smart MRI contrast agents responsive to stimuli using aptamer-functionalized SP iron oxide nanoparticles.[112,113] As a "turn-off" MRI sensor for thrombin, the nanoparticles assembled into an aggregated structure upon the addition of thrombin, due to multiple aptamer binding sites in each thrombin molecule. The assemblies resulted in a darker MRI image, indicating a smaller spin-spin relaxation time (T2) (Fig. 8a).[112] In a "turn-on" design, adenosine caused the disassembly of pre-formed nanoparticle aggregates crosslinked by aptamer strands, resulting in a brighter image and larger T2 (Fig. 8b).[113] These sensors worked well in both buffer solution and human serum.

Other than detection based on the MRS technique, the high-throughput separation capabilities of magnetic nanoparticles (or magnetic spheres) have also been used for sensitive detection of specific nucleic acid sequences (DNA or RNA) combined with other signal readout methods.[114,115] Tan and co-workers functionalized magnetic nanoparticles and fluorescent silica nanoparticles with aptamers that could recognize certain type of leukemia cells. The aptamer modified magnetic nanoparticles allowed for the rapid extraction of target cells while the fluorescent nanoparticles were simultaneously added for sensitive cell detection.[116] This method was further extended to detect multiple types of cancer cells.[117]

5. Functional DNA modified nanotubes for biosensing

Carbon nanotubes (CNTs) have attracted substantial research interest since they were discovered due to their unique 1D quantum confinement properties.[7,118,119] In recent years, the unique electronic and optical properties of CNTs have been found to be useful for sensing applications.

One promising direction is to incorporate CNTs into field effect transistors (FETs) for the efficient detection of a variety of biomolecules.[36,120–122] CNTs act as conduction channels in the FET and their conductivity can be modulated through interaction with chemical and biological species. The first aptamer-based single walled carbon nanotube field effect transistor (SWNT-FET) was demonstrated by Lee and co-workers.[37] As shown in Fig. 9a, a 15-mer thrombin aptamer was covalently attached to Tween 20 that was bound to the CNT side wall through hydrophobic interactions. Binding of thrombin to the aptamer induced a sharp drop in the conductance, while the addition of elastase as a control molecule did not affect conductance. A 10 nM detection limit was reported in this work (Fig. 9b, c). Based on a similar design, Tamiya and co-workers reported immunoglobulin E (IgE) CNT-FET biosensors using both aptamers and antibodies against IgE.[123] They further showed that the performance of the aptamer-based CNT-FET is better than that of the antibody-based sensors under similar conditions.

Other than electronic detection, the optical properties of CNTs are also useful for biodetection. Carbon nanotubes were shown to have photostable near-infrared (NIR) fluorescence.[124,125] This NIR emission is responsive to changes in the local dielectric function and this can be applied to design biosensors. Strano and co-workers demonstrated

DNA detection using the NIR fluorescence of SWCNTs.[126] Unmodifed 24-mer DNA was immobilized on nanotubes *via* dialysis. Hybridization of this DNA to its complementary strand induced a shift of the NIR emission, while non-complementary DNA did not show such a shift. The detection limit of this system was 6 nM. The same group also discovered that the change of dsDNA conformation from the right handed B-form to the left handed Z-form could also cause a shift of the CNTs' emission. Since Hg^{2+} was found to specifically induce this conformational transition, this system allowed the detection of Hg^{2+} even in blood and black dye solutions.[127] The integration of the target recognition properties of functional DNAs with NIR fluorescent CNTs should make it possible to design sensors for a wide range of analytes. As NIR light has good penetration and reduced autofluorescent background in tissue and blood media, functional DNA-CNT based NIR sensors are potentially useful for *in vivo* applications.

In addition to detection based on the electronic and optical properties of CNTs, they can also be carriers of DNAzyme or aptamer sensors. In a collaboration between the Lu, Kane and Dordick groups, a Pb^{2+} specific DNAzyme was immobilized onto multiple walled nanotubes while maintaining high activity.[128] If combined with a CNT-FET, it may allow the electrical detection of Pb^{2+} with high sensitivity and selectivity.

6. Conclusion and future directions

In this article, we have reviewed the recent developments in combining functional DNAs with nanomaterials as a method of designing biosensors for a diverse range of analytes. The unique optical, electrical, and magnetic properties of nanomaterials have been modulated by highly selective functional DNAs. The target recognition and catalytic properties of functional DNAs have enabled the controlled assembly of these nanomaterials in response to external chemical and biological stimuli. Table 1 summarizes the recently reported examples of integrating different types of nanomaterials with functional DNAs for sensing applications. The combination of functional DNA with other types of nanomaterials such as nanowires for biosensing applications is currently less explored.[129] However, due to the unique electronic and optical properties of nanowires and nanobarcodes,[130,131] we envision that the integration of functional DNA with nanowires as well as with the nanobarcodes technique,[132] could generate new biosensors, allowing single or multiplex analyte detection with high performance.

Most of the sensors mentioned in this review have been demonstrated in model systems as a proof of concept and tested mostly in buffer solution. For real medical diagnosis and environmental monitoring applications, significant sample matrix effects need to be carefully evaluated since the performance of a functional DNA sensor may differ considerably in biological fluids. More research and technological effort should be taken to optimize the sensors and minimize matrix effects in the analysis of biological samples. A further step would be the integration of individual sensors into sensor arrays, allowing simultaneous detection of multi-analytes while retaining good sensitivity and selectivity. Another important issue is that most of the currently developed functional DNA sensors are only for *in vitro* detection. To realize *in vivo* sensing, novel methods and materials combining nanomaterials and functional DNA for signal transduction are needed. For example, QDs, CNTs and other materials that have near IR emission can be used. Magnetic resonance imaging (MRI)[133] and surface-enhanced Raman scattering (SERS)[134] have also shown great potential for *in vivo* detection and could be integrated with functional DNA to sense a broad range of targets *in vivo*. Meanwhile, the toxicity and long-term health effects of nanomaterials should also be addressed before *in vivo* sensing can be realized.

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Biographies

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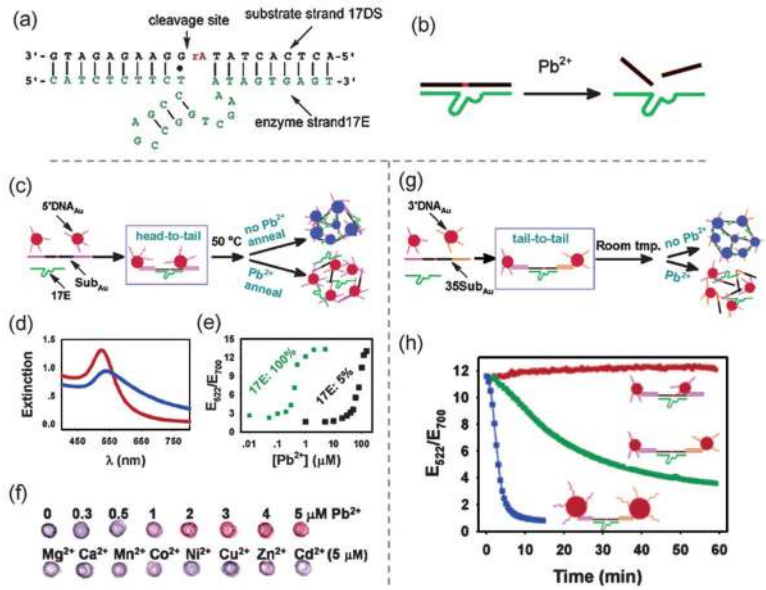

Fig. 1.

Colorimetric Pb^{2+} sensors based on DNAzyme functionalized AuNP. (a) Secondary structure of the Pb^{2+}-specific DNAzyme. (b) The substrate is cleaved into two pieces in the presence of Pb^{2+}. (c) Pb^{2+} directed assembly of DNAzyme-linked AuNPs aligned in a head-to-tail configuration; (d) UV-vis spectra of disassembled (red) and aggregated (blue) DNAzyme-AuNPs; (e) Pb^{2+} colorimetric sensing with tunable dynamic range, monitored by the extinction ratio at 522 and 700 nm; (f) color of the AuNPs in the presence of the different divalent metal ions shown in a TLC (thin layer chromatography) plate. Reprinted with permission of ref. 34, copyright 2003 American Chemical Society. (g) Pb^{2+} directed assembly of DNAzyme-linked AuNPs aligned in a tail-to-tail configuration; (h) effect of nanoparticle alignment and size on the rate of color change. The small red ball represents 13 nm AuNP and the big red ball represents 42 nm AuNP. Reprinted with permission of ref. 52, copyright 2004 American Chemical Society.

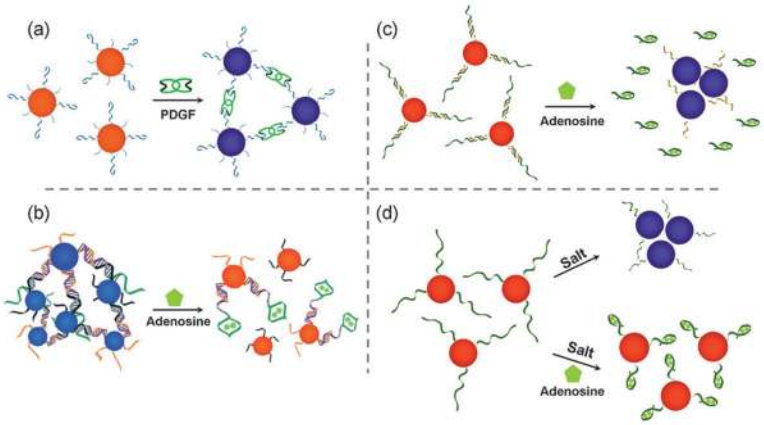

Fig. 2.

Schematics of colorimetric sensors based on aptamer directed AuNP assembly or disassembly. (a) Assembly of aptamer-functionalized AuNPs by target protein (PDGF) that can bind two aptamer molecules. (b) Disassembly of AuNPs linked by an adenosine aptamer. (c) Release of aptamer induced by addition of target molecule (adenosine) destabilized AuNPs, resulting in a red to blue color change. (d) Folding of aptamer upon binding to target molecule (adenosine) stabilized AuNPs from salt induced aggregation.

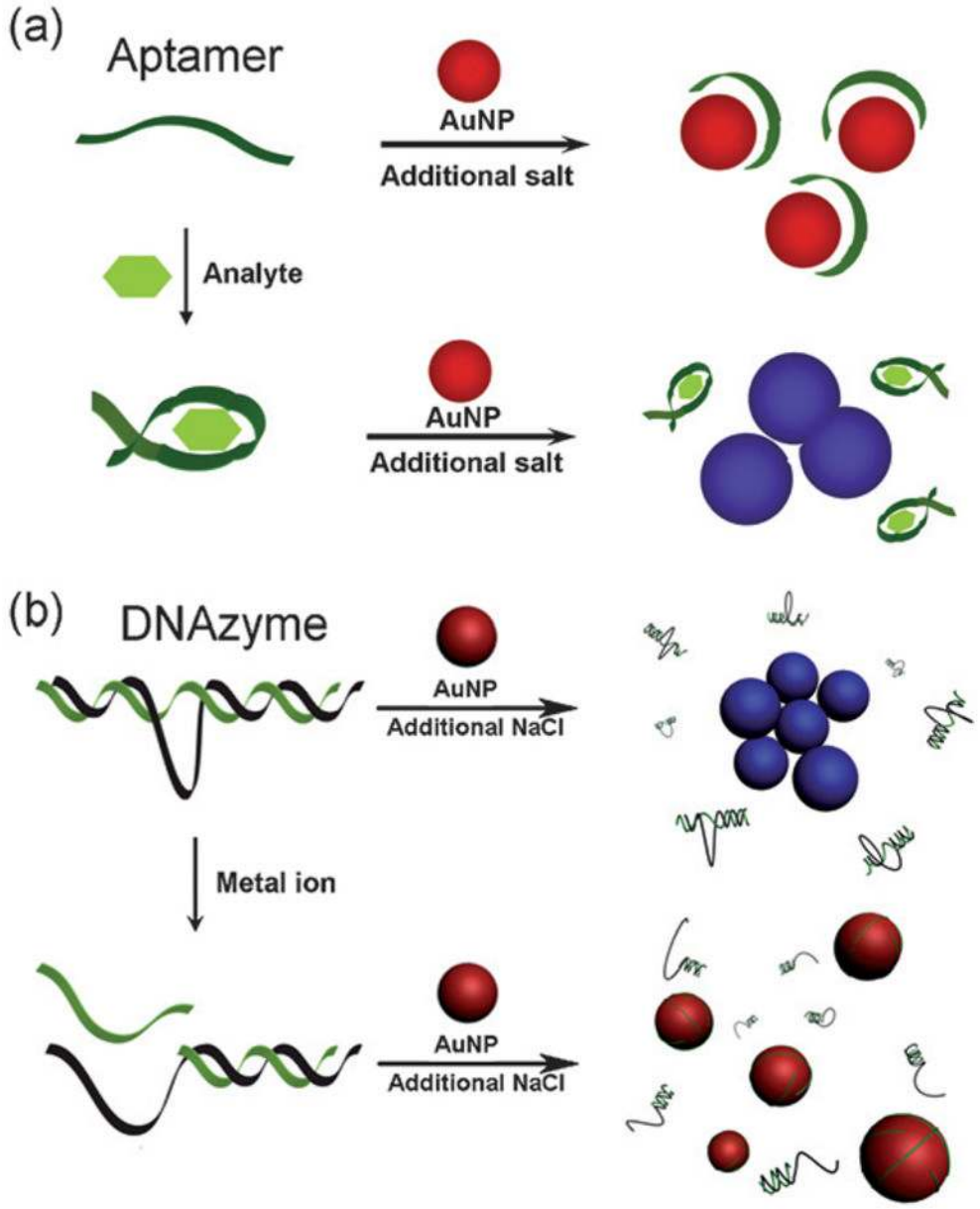

Fig. 3.

Label free colorimetric sensors based on functional DNA and AuNPs. (a) Schematic of aptamer based label free sensor. Folding of aptamer upon target binding would inhibit the adsorption of aptamer on the AuNPs. The AuNPs remained dispersed in the absence of target molecule but aggregated in the presence of target molecule. (b) Schematic of DNAzyme based label free sensor. Pb^{2+} induced the cleavage of DNAzyme complex and released a short ssDNA. The AuNPs aggregated in the absence of lead but remained dispersed in the presence of lead. Reproduced with permission of ref. 42, copyright 2008 Wiley-VCH.

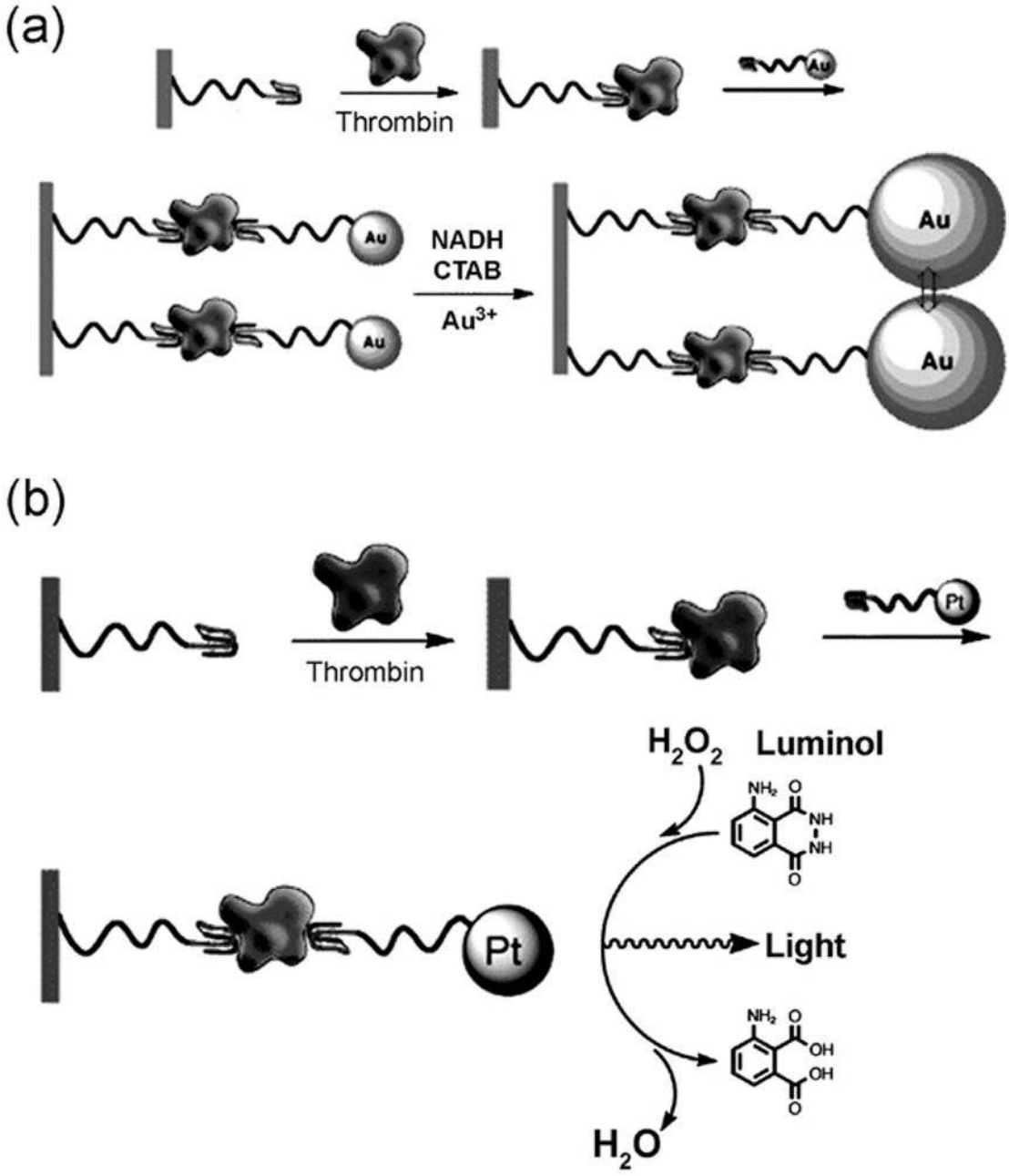

Fig. 4.

(a) Amplified detection of thrombin by Au^{3+} reducing enlargement of thrombin aptamer-modified AuNPs. Reprinted with permission of ref. 80, copyright 2004 American Chemical Society. (b) Amplified detection of thrombin by aptamer modified Pt NPs acting as catalytic labels for the generation of chemiluminescence. Reproduced with permission of ref. 84, copyright 2006 Wiley-VCH.

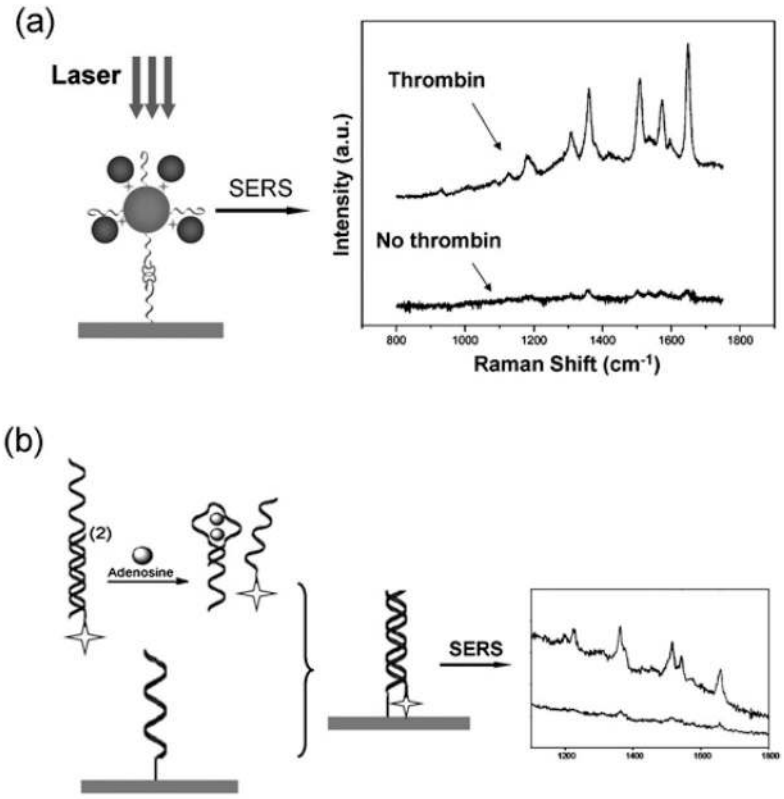

Fig. 5.

(a) Left: schematic of SERS based aptamer sensor for thrombin using Raman reporter labeled AuNPs functionalized with aptamers. The absorption of silver nanoparticles would enhance the SERS signal by forming hot spots. Right: SERS spectra of Raman reporters in the presence of 120 nM thrombin and in the absence of thrombin. Reproduced with permission of ref. 89, copyright of The Royal Society of Chemistry. (b) Schematic of SERS based aptamer sensor for adenosine using structure switching of aptamer. Upon addition of adenosine, folding of the aptamer would release the ssDNA labeled with Raman reporter. Hybridization of the ssDNA on the silver coated AuNP aggregate substrate generated a SERS signal. Reproduced with permission of ref. 90, copyright of Elsevier.

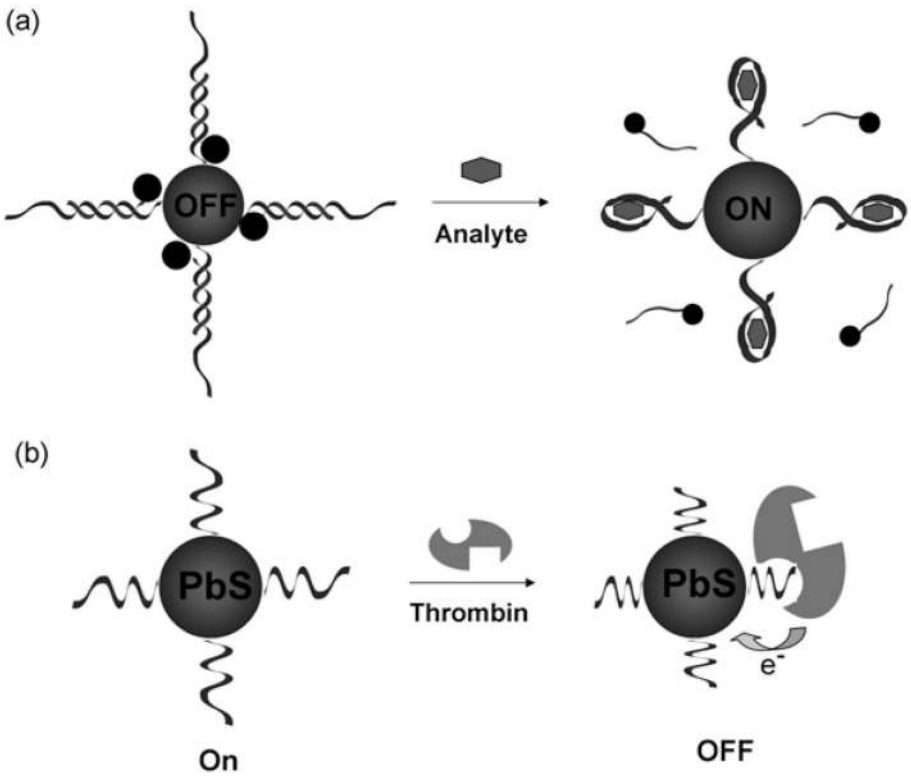

Fig. 6.

(a) Schematic of a QD FRET based aptamer sensor for thrombin. Binding of the target displaced the DNA strand labeled with a quencher and enhanced the fluorescence of the QDs. (b) Schematic of fluorescent thrombin sensor based on the selective quenching of thrombin on PbS QDs capped with aptamers.

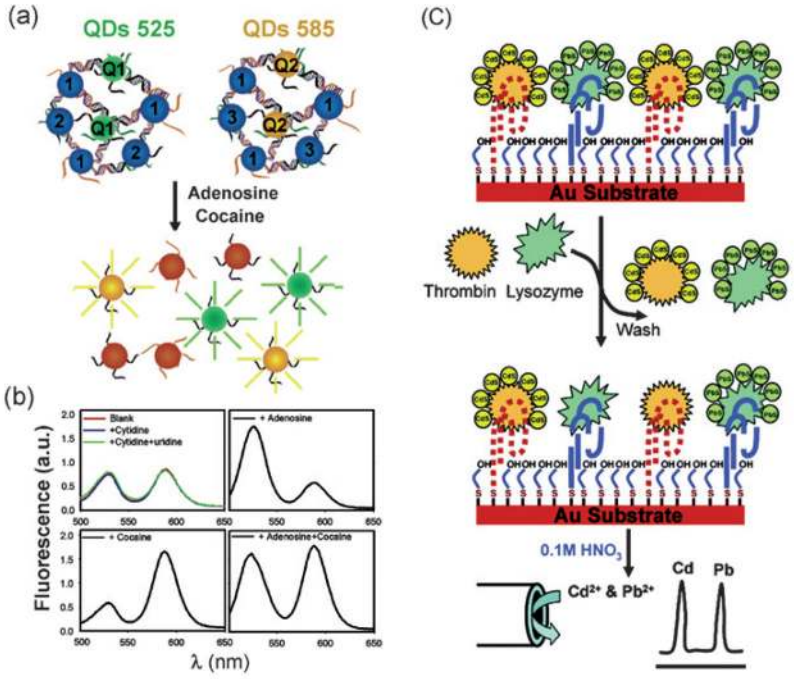

Fig. 7.

(a) Schematic of QD encoded aptamer linked nanostructures for the one-pot simultaneous detection of adenosine and cocaine. AuNP 1, AuNP 2 and QDs Q1 were assembled by adenosine aptamer DNA, while AuNP 1, AuNP 3 and QDs Q2 were assembled by cocaine aptamer. The QDs' fluorescence was quenched by nearby AuNPs. The addition of adenosine and cocaine disassembled the aggregates and increased the fluorescence. Reprinted with permission of ref. 102, copyright 2007 American Chemical Society. (b) Steady-state fluorescence emission spectra of mixed nanoparticles aggregated in response to target molecules or control molecules. (c) Operation of the aptamer-QD based dual biosensor for thrombin and lysozyme. Target analytes displaced the QD tagged proteins, and the displacement was monitored by electrochemical-stripping detection. Reprinted with permission of ref. 103, copyright 2006 American Chemical Society.

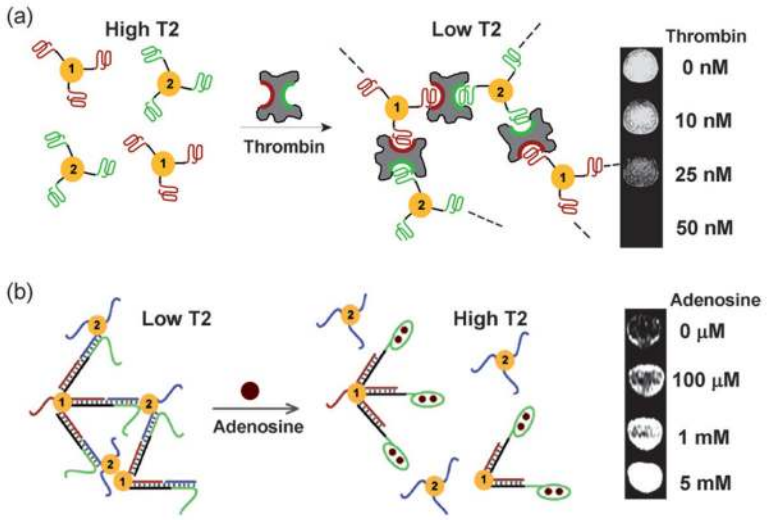

Fig. 8.

(a) Schematic of MRI "turn-off" detection of thrombin with aptamer functionalized superparamagnetic iron oxide nanoparticles. Aggregation of superparamagnetic nanoparticles induced by thrombin reduced the T2 relaxation time. The right panel shows the contrast change in a T2-weighted MR image at different thrombin concentrations. (b) Schematic of MRI "turn-on" detection of adenosine. Addition of adenosine disassembled the nanoparticle aggregates and increased the T2 relaxation time. The right panel shows the contrast change in a T2-weighted MR image at different adenosine concentrations.

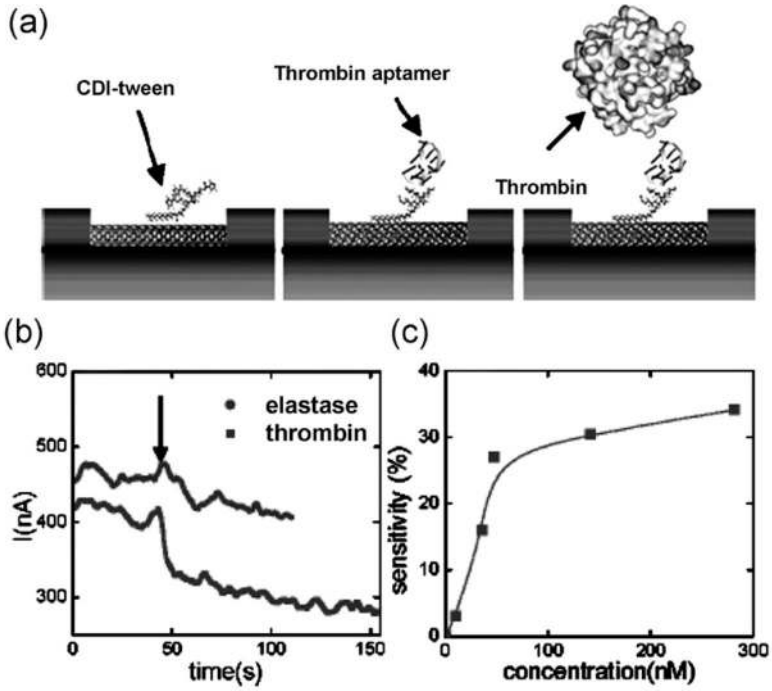

Fig. 9.

(a) Schematic of the binding of thrombin on a SWNT-FET based aptamer sensor. (b) Real-time conductance measurements from the SWNT-FET based aptamer sensor upon addition of thrombin (bottom curve) or elastase (top curve). (c) The sensitivity of the sensor as a function of thrombin concentration. Reprinted with permission of ref. 37, copyright 2005 American Chemical Society.

Table 1

Some recently reported examples of the integration of different types of nanomaterials with functional DNAs for sensing applications

Type of the nanomaterial	Property utilized for sensing	Signal readout method	Functional DNA integrated	Target analytes
Metallic nanoparticles	Optical	Colorimetric	DNAzymes, aptamers, aptazymes	Pb^{2+},[34,42,52,54,72] UO_2^{2+},[73] PDGF,[55] adenosine,[38,39,41,57] cocaine,[38] K^+,[65] throm
	Fluorescence quenching	Fluorescent	Aptamers	PDGF,[76,77] thrombin.[78]
	Catalytic	Signal amplification	DNAzymes, aptamers	Thrombin[80–84]
	Raman signal enhancement	SERS	Aptamers	Thrombin,[89] adenosine[90]
Quantum dots	Fluorescence	FRET	Aptamers	Thrombin,[36] adenosine,[102] Cocaine[102]
		Charge transfer	Aptamers	Thrombin[101]
	Chemical composition	Electrochemical stripping detection	Aptamers	Thrombin,[103] lysozyme[103]
Magnetic nanoparticles	Superparamagnetic	MRI	Aptamers	Thrombin,[112] adenosine[113]
	Magnetic separation	Combined readout method	Aptamers	Leukemia cells,[116] cancer cells[117]
Carbon nanotubes	Electrical	FET	Aptamers	Thrombin,[37] IgE[123]
	NIR fluorescence	Fluorescent	N/A	N/A
	Carrier	Fluorescent	DNAzyme	Pb^{2+}[128]