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Functional DNA Nanomaterials for Sensing and Imaging in Living Cells

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Abstract

Recent developments in integrating high selectivity of functional DNA, such as DNAzyme and aptamers, with efficient DNA delivery into cells by gold nanoparticles or superior near-infrared optical properties of upconversion nanoparticles are reviewed. Their applications in sensing and imaging small organic metabolites, toxins, metal ions, pH, DNA, RNA, proteins, and pathogens are summarized. The advantages and future directions of these functional DNA materials are discussed.

Introduction

The past decade of biotechnology has witnessed exciting development and integration of biology with nanotechnology and is beginning to enjoy the fruits of this integration as a result of its applications in sensing and imaging. Nanomaterials possess many unique properties, such as surface plasmon effect for gold nanoparticles (AuNP) and non-linear optical processes of upconversion nanoparticles (UCNP), making them superior choices for signal transductions in sensing and imaging applications. However, these nanomaterials do not have any selectivity toward targets of interests, which can be complemented by biomolecules with high selectivity very well. Therefore a number of nanomaterials have been combined with biomolecules [1–7], among which functional DNA (FDNA) has shown the most promise.

FDNAs are short single-stranded DNA molecules with enzymatic function (called DNAzymes or deoxyribozymes), biorecognition function (called aptamers) or both (called aptazymes). They are obtained by *in vitro* selection or Systematic Evolution of Ligands by Exponential Enrichment (SELEX) [8–10]. One advantage of SELEX is that, in principle, the process can be tailored to obtain FDNAs selective for almost any target of interest, ranging from metal ions, small organic metabolites to large proteins and even whole cells [11].

FDNAs are also much more stable than other biomolecules and easier to be conjugated to

nanomaterials. These properties make FDNA an ideal choice to integrate them with nanomaterials for sensing and imaging applications, with the majority of success outside cellular environment, such as environmental monitoring, when sample matrix is not very complex [2,12–15]. To realize its full potentials, FDNA nanomaterials have been developed for applications in living cells. This review focuses on these recent achievements by highlighting the impact of FDNA-functionalized AuNPs and luminescent UCNP in sensing and imaging in living cells.

Functional DNA and Gold Nanoparticles

AuNPs are among the most extensively studied nanomaterials because they are stable, and easy to synthesize and functionalize. The unique properties, such as distance dependent surface plasmon effects that can result in dramatic color changes with extinction coefficients that are several hundred times higher than the best organic dyes, make them excellent sensing platform [6,16], which has been reviewed elsewhere [5,17]. For sensing and imaging in living cells, the AuNPs have been shown to confer greater stability of DNAs against degradation [18,19] and allow delivery of DNA into cells [20]. The first such demonstration is intracellular ATP detection in HeLa cells using DNA aptamer-functionalized AuNPs (Figure 2a) [21]. The presence of a dense layer of the aptamer on the surface of the AuNPs was shown to enhance aptamer stability inside the cell and provides a mechanism for cellular uptake (Figure 2b) [21].

In addition to organic metabolites, such as ATP, metal ions play important roles in cells and yet sensing and imaging agents for metal ions in living cells are quite limited. While DNAzymes specific a wide variety of metal ions have been obtained and converted to fluorescent, colorimetric, electrochemical sensors and magnetic resonance imaging (MRI) contrast agents outside cells [22–28], intracellular metal sensing and imaging was reported only recently [29]. In this work, 13 nm AuNPs were functionalized with the uranyl-specific 39E DNAzyme, and fluorophore-modified substrate was quenched by both the AuNP and a molecular quencher (Figure 2c). The DNAzyme-AuNP nanoprobe was shown to cleave the substrate in the presence of uranyl and subsequently resulted in release of a shorter, Cy3-modified product and thus increase in fluorescence signal. It was demonstrated that the DNAzyme-AuNP probe can efficiently enter HeLa cells and serve as an intracellular metal ion nanosensor (Figure 2d) [29].

The DNAzyme-AuNPs can not only detect metal ions, but also target mRNA in cells. The 10–23 DNAzyme functionalized AuNPs is able to specifically target and cleave cancer-associated mRNA in breast cancer cells. AuNPs were shown to protect the DNAzyme from hydrolytic cleavage by DNAases and were able to reduce GDF15 gene expression by ~ 60% [30]. In this work, selective targeting of the desired mRNA was achieved by changing the sequence of the 10–23 DNAzyme binding arms to target a specific RNA sequence. DNAzymes have the advantage of being more stable in comparison to short interfering RNAs (siRNA). Furthermore, DNAzyme-based gene regulation mechanism is independent of cellular machineries like RNA-induced silencing complex (RISC). Dual functionalization of AuNP with both DNAzyme and siRNA is of special interest specially in targeting genes that regulate RISC function. In addition, detection of cancer-related genes and DNA

biomarkers has also been demonstrated using a DNA-AuNP system. AuNPs functionalized with hairpin DNA in a molecular beacon (MB) format was used for intracellular detection of a cancer associated gene, signal transducer and activator of transcription 5B (STAT5B), expression level in MCF-7 cancer cells [31]. Similarly, expression of tyrosinase, a melanoma-associated gene, was detected in melanoma cells using hairpin DNA-functionalized AuNPs [32]. It was shown that DNA functionalized AuNPs could be used in combination with specific antibody to target desired cells. This system showed cell selective uptake while conferring significant gene targeting and knockdown [33].

In order to prevent false positive results to improve the reliability of detection, a multicolor fluorescent nanoprobe has been developed to detect cancer [34]. AuNPs were functionalized with recognition DNA sequences for three specific tumor-related mRNA transcripts. Each recognition sequence was hybridized to a fluorophore-functionalized complement DNA strand, whose fluorescence was quenched by the AuNP. In the presence of the target mRNA, release of the reporter caused fluorescence signal to increase. This platform was used to simultaneously detect three different tumor-related mRNAs in living cells [34]. Similarly, AuNPs functionalized with two recognition DNA sequences, one as internal control (targeting actin mRNA) and the other one for designated target mRNA, was shown to improve precision of determining mRNA levels in individual cells and correct cell-to-cell variations [35]. AuNPs functionalized with both cancer-targeting and drug-carrier DNAs were also prepared, to improve cancer cell imaging and therapy. In this design, the cancer-targeting DNA was modified with folic acid and the drug-carrier DNA was a molecular beacon (MB) loaded with Doxorubicin. The MB was designed to release the drug only in presence of tumor mRNA [36].

Intracellular sensing based on FDNA and AuNPs is not limited to small molecules or biomarkers. Recently, core-satellite assembly of two different sizes of AuNPs prepared by an i-motif DNA linker was used to detect pH changes in the endocytic pathway (Figure 3a) [37]. The i-motif consists of cytosine-rich single stranded DNA that adopts a quadruplex structure under acidic conditions (pH ~ 5.0). In this study 14 nm satellite AuNPs were functionalized with i-motif ssDNA. Satellite AuNPs were assembled on the 50 nm core AuNP through hybridization with i-motif complementary DNA on the core particle to form a core-satellite nanostructure (CSNS). RAW 264.7 macrophage cells were used to study the well-known drop of pH in the endocytic pathway from 5.9–6.2 in early endosomes to 4.7–5.5 in lysosomes (Figure 3b). Bright yellow CSNSs were taken up efficiently by macrophages through endocytosis. Due to the pH drop and i-motif formation that led to disassembly of CSNSs, the yellow scattering signal became green (Figure 3c) [37]. In a separate study, cellular imaging and endocytic trafficking of proteins were demonstrated using nucleolin and prion aptamers in combination with AuNPs [38].

FDNA and AuNPs have been also applied for cancer cell targeting and imaging in combination of other nanomaterials such as iron oxide nanoparticles [39]. In this study gold-coated iron oxide nanoroses functionalized with sgc8 aptamers were shown to specifically recognize CCRF-CEM leukemia cells and enter them through endocytosis process. Such a combination of gold and iron oxide nanomaterials with FDNA provided a multifunctional

theranostic tool for MRI (due to iron oxide inner core) and photothermal therapy (due the gold shell) [39].

Functional DNA and Luminescent Upconversion Nanoparticles

Luminescent upconversion nanoparticles (UCNPs) have distinctive properties that make them ideal nanoprobe for targeted imaging and selective sensing. Unlike most other optical probes, UCNPs can convert near-infrared (NIR) excitation light into shorter wavelength visible luminescence. This property, in addition to their high photostability and low cytotoxicity, make them suitable for deep tissue bioimaging [40,41]. A major drawback of UCNPs is lack of general methods to make them water soluble and functionalizable. To overcome this issue, several approaches such as silica coating [42,43], oxidation of capped ligands [44,45], and ligand-free treatments [46] have been reported. Recently, our lab has developed a simple and general method to engineer UCNPs whose surface has been coated with a monolayer of PEGylated phospholipids. Such UCNPs have been shown to be water soluble and biocompatible and could be functionalized by a number of targeting molecules for a wide range of applications including selective sensing or imaging. As a proof of concept, PEGylated phospholipids conjugated with folate were used to functionalize UCNPs, which were then tested successfully for imaging cancer cells with high specificity [47]. To expand the targeting ability further, our group developed a one-step strategy to prepare uniform DNA-modified UCNPs (Figure 4a) [48]. It was shown that DNA molecules remained functional on the surface of UCNPs and were able to assemble into hybrid structures such as AuNP-UCNP nanostructures. More importantly, DNA aptamer-functionalized UCNPs were able to specifically target cancer cells and cross the cell membrane with excellent internalization efficiency without the use of any transfection agents (Figure 4b). Therefore DNA-UCNPs are excellent choices for DNA delivery and targeted imaging of live cells (Figure 4c) [48]. Similarly, UCNPs functionalization with DNA was demonstrated using click chemistry [49]. It was shown that DNA remained functional after attachment to UCNPs and also enhanced water solubility of the nanoparticles. Confocal microscopy was used to demonstrate the ability of DNA functionalized UCNPs for live cell imaging [49].

Owing to the beneficial properties of UCNPs, many groups have utilized them in a variety of biomolecule sensing platforms. UCNPs in a FRET sensor platform have been combined with graphene oxide (GO) as an acceptor to detect ssDNA in human serum [50]. Similarly, fluorescent silver clusters, which have upconversion properties, have been used in DNA biosensing and nucleobase recognition [51]. Multicolor UCNPs functionalized with aptamers have been reported for multiplexed and simultaneous sensing of mycotoxins. In this study, functionalized UCNPs with ochratoxin A (OTA)-aptamers and fumonisin B1 (FB1)-aptamers were quenched by GO due to the interaction of GO with nucleobases of the aptamers. In the presence of OTA and FB1, and upon release of GO, the fluorescence intensity of UCNPs were shown to have a concentration-dependent increase. The limits of detection (LOD) were 0.02 ng/mL for OTA and 0.1 ng/mL for FB1 [52]. In other similar examples, ATP [53,54], enterotoxin B (EB) [55] and, thrombin [56] nanosensors have been reported using UCNPs (as FRET donor) functionalized with an ATP, EB or thrombin aptamer in combination with either GO, Black Hole Quencher-3 or carbon nanoparticles (as

a FRET acceptor), respectively. Metal ion detection has been also demonstrated using UCNPs and gold nanorods in combination with mercury(II) aptamer. This system was tested for detecting mercury in human serum [57].

While current LODs may be sufficient to detect certain targets such as small organic molecules or metal ions, they may not be sufficient for detection of other targets, such as pathogens. To address this issue, aptamer-functionalized UCNPs were combined with DNA aptamer-modified magnetic nanoparticles in order to concentrate and detect bacterial pathogens [58]. Two different types of UCNPs with different colors were functionalized with aptamers for *Salmonella typhimurium* or *Staphylococcus aureus* and were used to selectively capture and detect these pathogens, with a LOD of <10 cfu ml⁻¹.

Conclusions and Perspectives

Nanomaterials have brought a new and powerful paradigm in biosensing. Along with advances in the field of nanomaterials, the combination of novel nanomaterials with functional nucleic acids as biological recognition and targeting elements has enabled functionalized nanomaterials to be used in life science research. Despite the advances recently made with application of functional nanomaterials in biosensing, there are still many challenges that remain to be addressed. First, more reliable and efficient molecular and cellular markers need to be made available. For example, prostate-specific antigen (PSA), a well known protein-based prostate cancer marker, is a poor disease marker with high rates of false positive and false negatives [59]. Both knowledge of reliable and effective biomarkers and selective and specific recognition elements for those biomarkers are required for successful applications in a sensor platform. In addition, despite tremendous progress in detecting nucleic acids and proteins *in vivo*, detection of small molecules and metal ions as a disease marker or biochemical pathway cofactors has lagged behind. By utilizing SELEX or *in vitro* selection, it is possible in principle to select functional nucleic acids for any target, whether aptamer for small molecular target or DNAzyme for metal ion cofactor. Therefore, selection for more aptamers and DNAzymes needs to be carried out in order to provide efficient recognition elements for different biosensing applications, especially for small molecules and metal ions.

Furthermore, new nanomaterials with improved biocompatibility and better optical properties and lower cytotoxicity are expected to advance their usage in intracellular and biological sensing. Utilizing two or more nanomaterials in a hybrid format can produce better nanocomposite materials with desired properties, such as multiple functionalities and controlled spatial positioning. To utilize the beneficial properties of different nanomaterials, better strategies need to be developed for functionalization of nanomaterials with recognition elements, such as DNA aptamers, to generate effective biosensing platforms. Overcoming these challenges will allow functional DNA nanomaterials to be effective sensors and imaging agents not only in living cells, but also in animals and human for better medical diagnostics and personal medicine.

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Highlights

Functional DNA and nanomaterials complement each other well for intracellular sensing

Gold nanoparticles allow efficient delivery of functional DNA into cells for sensing

Upconversion nanoparticles allow near-infrared imaging by selective functional DNA

Figure 1.
General representation of functional DNA based nanomaterials and their application in intracellular sensing and imaging.

Figure 2.

a) Schematic representation of intracellular ATP probe based on aptamer nano-flare system; b) Fluorescence microscopy images of HeLa cells incubated with aptamer nano-flares (top) and control particles (bottom). Adapted from [21**]; c) Schematic representation of the first intracellular DNAzyme based metal ion probe for detecting uranyl ion in living cells; d) Fluorescence microscopy images of HeLa cell treated with (top) or without (bottom) uranyl. Adopted from [29**].

Figure 3.

a) Schematic representation of the formation and pH-induced disassembly of the core-satellite nanostructures (CSNS); b) Proposed endocytosis mechanism for i-motif based pH detection; c) Overlaid dark field and fluorescence images of RAW264.7 cells incubated with i-motif and control CSNS after 15 min incubation and 30 min post-incubation. Framed areas are magnified in insets. Adopted from [37**].

Figure 4.

a) Schematic illustration of functionalizing UCNP with cancer specific DNA aptamer for cancer cell targeting; b) Fluorescence microscopy images of MCF-7 breast cancer cells treated with aptamer modified UCNP (apt-UCNP, top) and random DNA modified UCNP (Rnd-UCNP, bottom). Adapted from [48*]; c) Schematic representation of the "click"-conjugation of an alkyne-modified oligonucleotide (cU) to an azidomodified UCNP; d) Bright UCNP DNA as "nano-sized" lamp for live cell imaging in the near-IR range. Adapted from [49*]