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Functional DNA nanomaterials for sensing and imaging in living cells

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Recent developments in integrating high selectivity of functional DNA, such as DNAzymes 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.

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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.

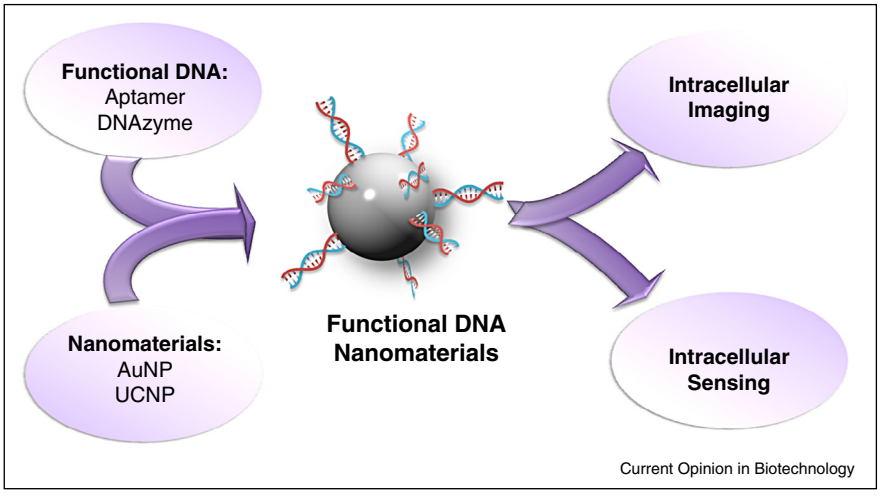
FDNAs are short single-stranded DNA molecules with enzymatic function (called DNAzymes or deoxyribozymes), biorecognition function (called aptamers) or both (called aptazymes). They are obtained by *in vitro* selection or Systematic Evolution of Ligands by Exponential Enrichment (SELEX) [8–10]. One advantage 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 FDNAs 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 UCNPs in sensing and imaging in living cells (Figure 1).

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. Although DNAzymes specific for a wide variety of metal ions have been obtained and converted to fluorescent, colorimetric, electrochemical sensors and magnetic resonance imaging (MRI) contrast agents outside the cells [22–28], intracellular metal sensing and imaging was reported only recently [29••]. In this work, 13 nm AuNPs were functionalized with a uranyl-specific 39E DNAzyme, and a fluorophore-modified substrate was quenched by both the AuNPs and a molecular quencher (Figure 2c). The DNAzyme-AuNP nanoprobe was shown to cleave the substrate in the presence of uranyl and

Figure 1

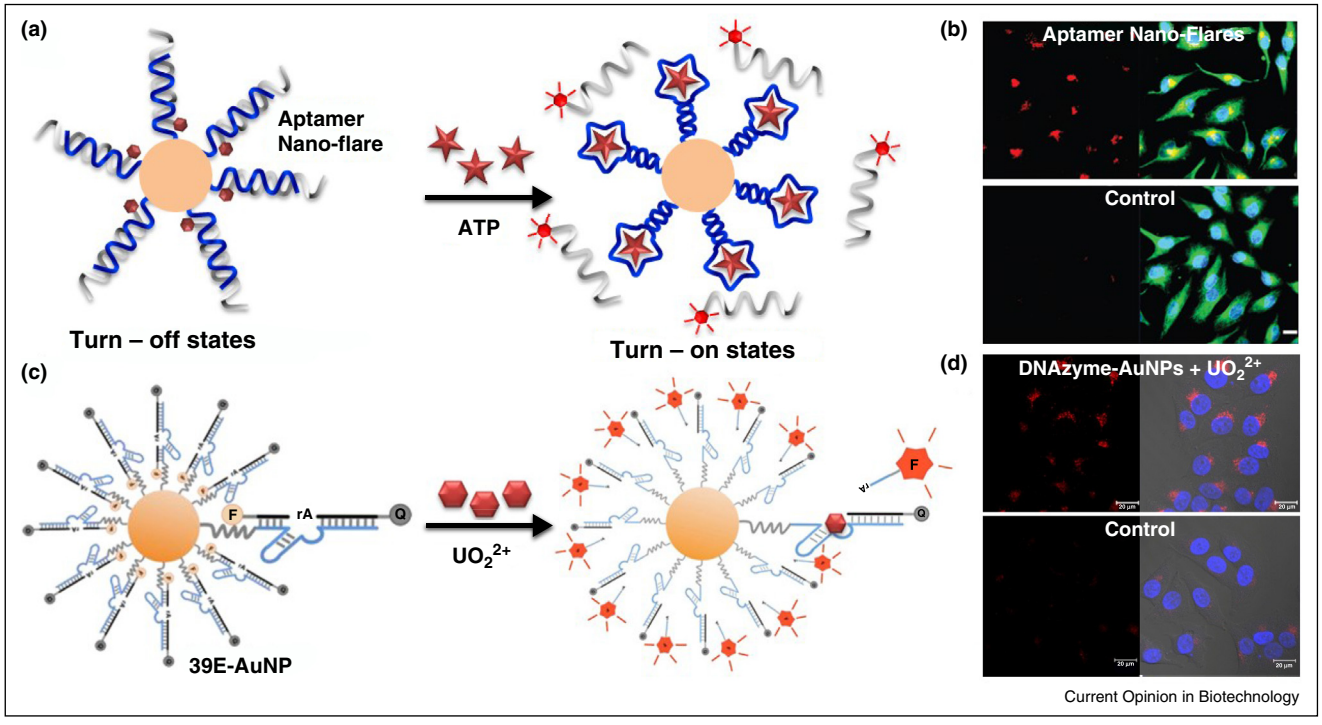


General representation of functional DNA based nanomaterials and their application in intracellular sensing and imaging.

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. Such AuNPs functionalized with a 10-23 DNAzyme are able to specifically target and cleave cancer-associated mRNA in breast cancer cells. The AuNPs were shown to protect the DNAzyme

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 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••].

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