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Aptamer optical switches: From biosensing to intracellular sensing

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ABSTRACT

Aptamers are the evidence of the power and versatility of DNA. In the biosensing field, if the important properties of aptamers together with all the many different conformations they can adopt are coupled to the proper transduction system, an unthinkable amount of possibilities is open up to construct biosensing methods for, in principle, any imaginable target. Inspired by classical molecular beacons, by using the intrinsic property of aptamers of changing their conformation when binding to the target and the possibility of labelling and coupling them to other DNA sequences, nanomaterials or polymers, a large variety of "turn on" or "turn off" optical biosensing systems, can be developed.

This review gives an overview of the recent works dealing with aptamers as optical switches in biosensing. Several optical approaches based on luminescence are considered, ranging from fluorescence, to chemi/bio luminescence, to plasmon field-enhanced fluorescence. The attractive combination of aptamer optical switches and nanomaterials is also discussed. Finally, the appealing and challenging application of aptamer optical switches to intracellular sensing and imaging is presented.

1. Introduction

DNA is a powerful molecule: with DNA, and RNA, fragments, researchers in the biosensing field not only give specificity to their analytical system, but they can build extraordinary tools, such as nanomachines [1–4], robots [5] and "plug and play" logic gates or nanocomputers [6]. With DNA, life inside single cells can be spied on and, after intracellular internalization, DNA molecules can function simultaneously as sensing and as therapeutic tools [7,8].

In this fascinating area, aptamers are the proof of the intrinsic power of DNA/RNA molecules. Researchers started playing with DNA-based combinatorial selection in 1990 [9,10] and these first successes have guided the research until the recent challenge of selecting aptamers for in vivo targets [11]. With the advancement and the consolidation of the systematic evolution of ligands by exponential enrichment (SELEX) process, a plethora of aptamers has been selected in 30 years [12–15], despite the tremendous potential of this technology has not been fully exploited yet [16,17]. Aptamers are short DNA or RNA fragments selected among a very complex library, to bind a specific target, which can range from a very small molecule, such as an ion [18], up to an entire microorganism, such as a bacterium [19] or from human proteins [20] up to pesticides [21] or explosives [22]. The wide range of targets for which aptamers can be generated is only one of the important features of these molecules, together with their chemical synthesis and modification which assures the overcoming of the batch-to-batch variation encountered in the production of antibodies, for example [23].

Beside this, the most fascinating property of aptamers is precisely their mechanism of binding the target molecule via a conformational change [24]: the definitive 3D conformations, such as hairpin [25], pseudoknot [26], bulge loop [27], G-quadruplex [28] etc., that aptamers can adopt binding the vast variety of possible different targets, allows their application in a huge choice of sensing methods [29,30]. This aspect is even more attractive when realizing that aptamers are acting exactly like many molecules in nature, which can achieve a selective and sensitive output or initiate a biological function when binding to a specific target [31]: they are biomimetic molecules.

If all these noteworthy characteristics of aptamers are coupled to an appropriate transduction tool, an unthinkable amount of possibilities is open up to construct biosensing methods for any imaginable target: inspired by the action of classical DNA molecular beacons (MBs), the same principle can be used with aptamers coupled to optical sensing systems [32]. In this sense, aptamers working as optical switches are sensitive and selective tools that can respond to specific molecular changes triggered by the binding to the target, combining the selectivity and sensitivity of MBs with the flexibility of aptamers. However, one important and giant difference between aptamer switches and MBs must be evidenced: although the two molecules have similar structures, classical MBs can recognize and bind via hybridization only other DNA or RNA molecules [33], whereas aptamers can trigger the generation of a signal upon binding to all the variety of possible targets for which they can be selected. In general, MBs are short oligonucleotidic sequences with a hairpin conformation, containing a loop and a stem formed by the two

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ends of the sequence hybridized with each other [34]. The hairpin is opened upon hybridization between the MB and its target, which is usually complementary to the loop portion of the beacon. If the two ends of the probe structure are labelled, for example, with a fluorophore and a quencher, these molecules are in close proximity in the intact hairpin form, i.e. in absence of the target sequence: in this situation, the sensor, constituted by the MB, is in its OFF stage. In presence of the complementary target, when the hairpin opens up, fluorophore and quencher are at a distance for which fluorescence emission can occur and the sensor switches to its ON stage. Based on this classical MB format, a vast variety of aptamer optical switches can be envisaged (Fig. 1). The simplest ones, rely on the intrinsic property of specific aptamers (those for adenosine triphosphate, aflatoxin B1 or thrombin, for example) of adopting a hairpin or a G-quadruplex structure when binding to the target (Fig. 1A): if properly labelled at the two ends of the sequence, this aptamer can alone function as optical switch by turning on or off its optical signal in presence of the target molecule [35,36]. In some cases, a bio-engineering step can be applied to specific aptamers for splitting them in two or more fragments [37]: these so called "split aptamers" (Fig. 1B) can re-arrange in the structure of the complete aptamer when binding to the target, cocaine, for example [38], turning on or off an optical signal if the two segments are properly labelled.

When the aptamer alone is not able to fold in a specific structure, it may be helped by the addition of other sequences: constructs can be built by coupling the aptamer to other oligonucleotidic fragments which, due to specific self-complementarity, can fold in hairpin structures in absence of the aptamer target molecule (Fig. 1C) [31]. Due to the stronger affinity of the aptamer towards its target, the molecular construct re-arranges in a more stable form with the aptamer folded to bind its specific molecule and not hybridized with the internal complementary fragment. Also in this case, with proper labelling, optical switches can be realized. A similar mechanism can be designed based on the hybridization of the aptamer, labelled for example with a fluorophore, with a complementary strand fixed onto gold nanoparticles (Fig. 1D): in this configuration, the fluorescence signal is quenched by the close vicinity of the nanoparticle. Upon binding to its target, the aptamer is released from the duplex and the fluorescence signal can be generated.

A last mechanism is based on very interesting two-dimensional materials, such as graphene oxide (GO) or other nanosheets, which have the property of adsorbing biomolecules and, at the same time, display optical features, such as fluorescence quenching (Fig. 1E) [39]. Aptamers labelled with fluorescent molecules, in absence of the target, and consequently in their unfolded and quasi-linear conformation, can interact with the nanosheet, which has the important property of adsorbing only single-stranded or unfolded DNA fragments. The binding to the target causes the folding of the aptamer which is in turn no more adsorbed onto the nanosheet, with the consequent turning on of the optical signal.

In all these methods, the optical mechanisms can be tuned by choosing the proper optically active labelling molecules or nanomaterials with the aptamer switch-based sensor functioning with a "turn on" or a "turn off" procedure. If sensitivity of these analytical systems can be greatly influenced by the optical detection, selectivity highly depends on the selectivity of the aptamer itself: aptamers have been demonstrated to be very selective, with the most famous cases being the anti-theophylline and the anti-l-arginine ones [23]. A selective binding of the aptamer to its target could be compromised by the introduction of additional short oligonucleotides or of functional moieties in the aptamer sequence or by the non-optimal binding conditions, which, in principle, should be as much as possible similar to the SELEX conditions: however, if all these important points are taken into consideration, the selectivity of the aptamer can be retained and very selective sensors can be obtained.

This review will give an overview of the very last works concerning aptamer optical switches; the biosensing methods will be subdivided on the basis of the luminescent-based optical approaches ranging from fluorescence, to chemi/bio luminescence, to plasmon field-enhanced fluorescence. In addition, the attractive features and biosensing possibilities offered by aptamer optical switches coupled to different nanomaterials will be also examined. Finally, the fascinating world of intracellular sensing will be considered with aptamers working not only as optical switches but also as targeting molecules coupled to classical MBs as sensing tool.

2. Luminescence-based aptamer switches

2.1. Fluorescence-based aptamer switches

The first developed aptamer switches for protein detection maintained the concept of donor/quencher pair, as it was commonly used for

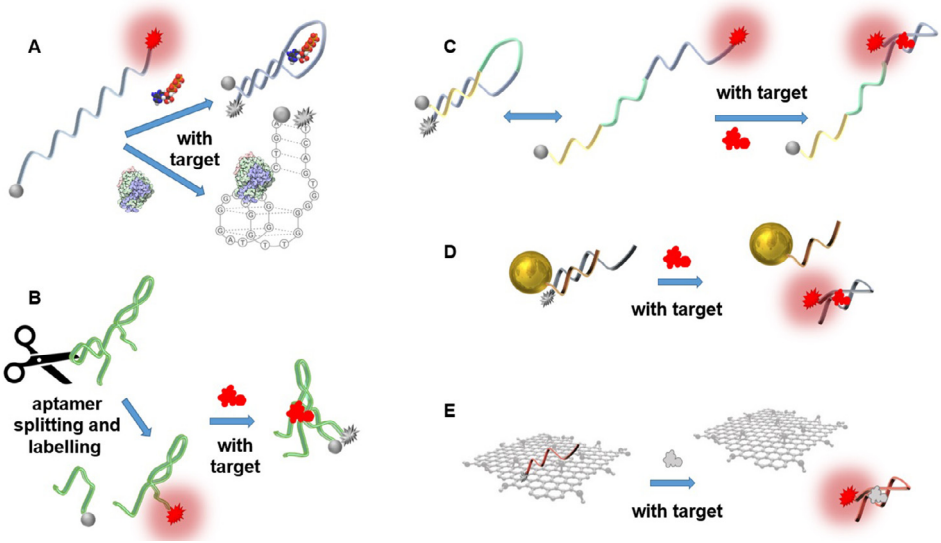

Fig. 1. Scheme of the different aptamer optical switch mechanisms. A) Aptamers adopting conformations of hairpin or G-quadruplex upon binding to the target; B) Split aptamers; C) Aptamer switches in more complex DNA constructs, changing conformation upon binding to the target; D) Aptamer hybridized to a complementary fragment fixed onto AuNPs and releasing from this probe once bound to its target; E) Aptamer adsorbed onto GO and released from it due to folding when binding to the target molecule.

MB application, applying the Förster Resonance Energy Transfer (FRET) principle [30]. In summary, FRET is a non-radiative process in which a donor molecule (usually a fluorophore) in its excited state transfers energy to a close acceptor molecule which is in its ground state. The acceptor has the characteristic to absorb energy at the emission wavelength(s) of the donor, but it can remit it not necessarily as fluorescence itself (quenching effect). The FRET phenomenon occurs when the two molecules (donor and acceptor) are in close proximity (10–100 Å).

Weihong Tan's group [40] developed an aptamer beacon, which formed a G-quadruplex structure upon the interaction with its target, the thrombin protein. The formation of G-quadruplex structure forced the fluorophore, 6- carboxyfluorescein (6-FAM), in close proximity to the quencher, 4-(4'-dimethylaminophenylazo)benzoic acid (DABCYL), reducing the fluorescence intensity. In order to overcome the fluorescence signal decrease upon target binding, Hamaguchi et al. [41] designed the aptamer sequence introducing additional nucleotides at the 5′ end, which allowed the formation of a stem in which fluorescein was quenched by the DABCYL and, consequently, the formation of G-quadruplex conferred a fluorescence signal increase to the formed complex. Moreover, they discovered the importance of having some metal ions (such as K^+ or Mg^{2+}) into the assay buffer, in order to optimize the aptamer conformation and the emitted fluorescence intensity.

The possibility to design aptamers as molecular switches, expanded the range of different potential targets, such as myotonic dystrophy kinase-related Cdc42-binding kinase α (MRCKα) using 5' FAM and 3' DABCYL as fluorophore/quencher pair [42], trans-activator of transcription (Tat-1) protein, a regulatory protein that increases the efficiency of human immunodeficiency virus type I (HIV-1) viral transcription, using fluorescein and DABCYL as fluorophore/quencher pair [43]. The working principle of this assay was based on the presence of a beacon containing the fluorophore/quencher pair and a non-functionalized duplex aptamer sequence. Only in presence of Tat-1, the functionalized aptamer beacon and its complementary oligonucleotide sequence were capable to form a ternary complex, in which the fluorescence intensity increases together with the increase of Tat-1 concentration.

In addition, targets such as chloramphenicol (CAP) were monitored using aptamer-switching molecules [44]. A limit of detection (LOD) of 0.987 pg/mL in buffer, was reached in a three-oligonucleotide aptaswitch sensor complex, in which the aptamer sequence is hybridized with two other sequences labelled respectively with the fluorophore and the quencher (FAM and BHQ, black hole quencher, respectively) in such a way that the two result in close proximity upon hybridization. The interaction of the aptamer with the target CAP determines a conformational change, which displaces the sequence carrying the quencher and the fluorescence is therefore switched on.

An aptamer switching molecule for kanamycin detection, was proposed by Ma et al. [45]. The assay is simply based on an aptamer specific for kanamycin, which is labelled with a fluorophore (FAM) and a complementary fragment bringing the quencher (DABCYL) close to the fluorophore during the complementary pairing of bases. In the presence of kanamycin, the aptamer, due to the high affinity with the target, changes its conformation for target binding and the fragment carrying the quencher is consequently released, giving rise to fluorescence enhancement. An LOD of 13.52 nM in milk samples has been reached.

Immunoglobulin E (IgE) has been detected using a pyrene-labeled aptamer [46]. In summary, the aptamer specific for IgE was labelled at the two distal ends (5' and 3') with two single pyrene molecules, which came into close proximity upon the aptamer/IgE binding. This event generates the excimer formation and a consequent distinct fluorescence emission peak. A LOD of 1.6 nM was reached.

Aptamer optical switches have been designed also for metal ions such as Pb^{2+} and Hg^{2+} [47] and in a very simple scheme a detection limit of 0.16 and of 0.36 nmol/L, respectively, has been reached. In particular, two specific MB probes, containing the specific aptamers, have been designed, that one for Hg^{2+} was labelled at the two side ends with FAM/BHQ-1, as fluorophore and quencher pair; while that one for Pb^{2+} was labelled with tetramethyl-6-carboxyrhodamine (TAMRA) and BHQ-2. Moreover, in both cases, the quenchers were linked to several guanines (G base), which contributed to the quenching effect. In presence of the metal ions, the aptamer construct is recovered and the stem-loop conformation is destroyed, the fluorescence is then switched on.

Alternative strategies of aptamer-beacon application, also for the detection of metal ions, have been proposed by Wang et al. [48], who designed a dual-aptamer-conjugated molecular modulator for the detection and inhibition of Zn^{2+}, involved in amyloid β (A β) aggregation. Briefly, the dual-aptamer-conjugated molecular modulator is composed by four parts, as showed in Fig. 2: aptamer called β55 (specific for A β), aptamer called Zn6m2, labelled with Texas Red (specific for Zn^{2+}), a peptide called TGN (which can penetrate the blood-brain barrier), a complementary DNA fragment, labelled with BHQ-2, which binds the β55 and TGN-Zn6m2 conjugate via complementary base pairing. In presence of Zn^{2+}, the aptamer Zn6m2 goes towards a conformational change, which causes the release of BHQ-2 labelled-DNA. At this point, the fluorescence signal is switched on. Moreover, Zn^{2+} is removed by this complex formation avoiding its triggering activity for Aβ-aggregate formation. A detection limit of 4.9 μM was determined.

Thompson et al. [49] have designed aptamer switches with programmable pH response within the broad range 5.5–7. Their approach has been a great improvement respect to what was already present [50–52]. In fact, the previous studies showed aptamers for very specific pH values, while, in this study, a general strategy capable to create a pH-responsive switch tunable under acidic, neutral, or alkaline conditions have been proposed. The entire strategy is based on the construct based on an aptamer, which is covalently linked to a partially complementary

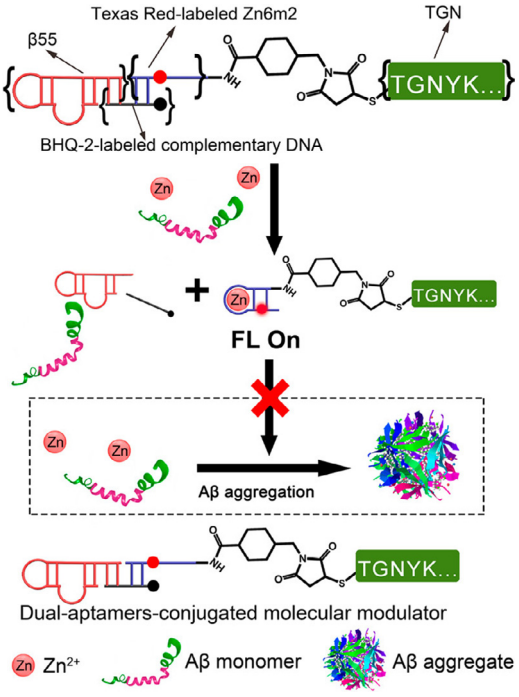

Fig. 2. Schematic representation of the dual-aptamer-conjugated molecular modulator activity. Reprinted with permission from [48]. The four parts forming the dual-aptamer-conjugated molecular modulator are showed: aptamer called β55 (specific for A β), aptamer called Zn6m2, labelled with Texas Red (specific for Zn^{2+}), a peptide called TGN (which can penetrate the blood-brain barrier), a complementary DNA fragment, labelled with BHQ-2, which binds the β55 and TGN-Zn6m2 conjugate via complementary base pairing. In presence of Zn^{2+}, the aptamer Zn6m2 goes towards a conformational change, which causes the release of BHQ-2 labelled-DNA. At this point, the fluorescence signal is switched on. Moreover, Zn^{2+} is removed by this complex formation avoiding its triggering activity for Aβ-aggregate formation.

displacement strand (DS) through an inert DNA linker. Introducing pH-responsive DNA motifs into either the linker or the DS, the aptamer pH dependence can be tuned. Using the same core aptamer sequence, an array of pH-sensitive aptamer switches, which preferentially bind or release their molecular target under various pH conditions, have been realized. As schematized in Fig. 3 (a), the construct is formed by an intramolecular triplex motif inserted into an aptamer sequence (for example specific for adenosine triphosphate (ATP)) and the DS. A duplexed structure is formed at neutral pH bringing the aptamer and the DS close together Fig. 3 (b, top), while at low pH the protonation of cytosine bases provokes Hoogsteen base pairing within CGC triplets in the linker (Fig. 3 (b), bottom).

2.2. Chemi/Bio luminescence-based aptamer switches

Despite the great majority of aptamer optical switches is based on fluorescence, it is possible to find some studies based on chemi/bioluminescence, which has the advantage to avoid the background coming from the light source and then to increase the signal-to-noise ratio [53]. Following this assumption, the chemiluminescence resonance energy transfer (CRET) is the technique applied by Zhang et al. [54], who developed a chemiluminescent (CL) aptasensor for ATP detection using a luminol-H_2O_2-horseradish peroxidase (HRP)-fluorescein system (Fig. 4). In particular, they have designed a structure-switching aptamer, the linking region of which (green sequence in Fig. 4) has an extension that can hybridize with binary DNA MBs (named CRET-BMBP). In presence of ATP, the aptamer switches conformation to the more energetically favorite aptamer-ATP complex, giving rise to the dissociation of CRET-BMBP probe from the aptamer. Since the CL intensity detected is due to the CRET-BMBP release, it is therefore proportional to the amount of ATP interacting with the aptamer. The LOD reached with this CRET-based aptasensor was 0.11 μM ATP. The presence of magnetic nanoparticles is due to their usefulness in the separation of reacted probes from unreacted ones, which can be conveniently achieved by simply using a permanent magnet.

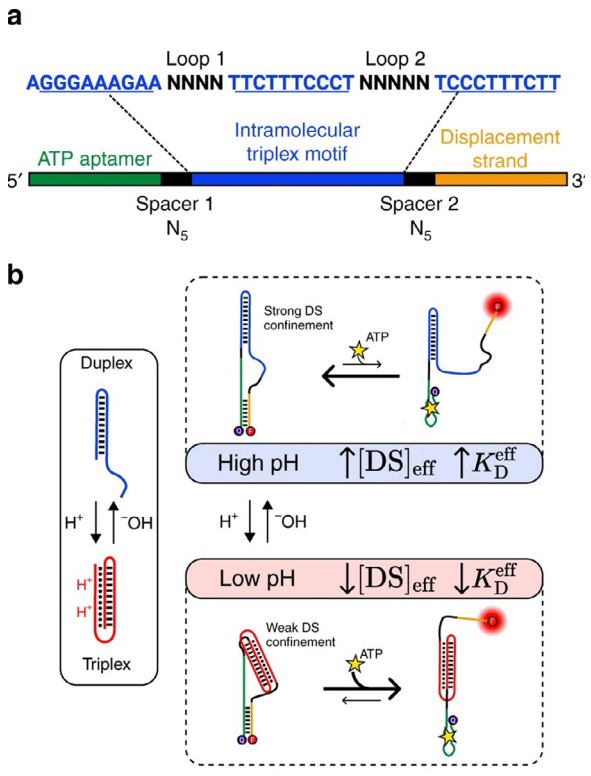

Fig. 3. (a) Design of an ATP aptamer for selective binding at low pH. (b) A simplified model for pH-dependent binding. pH dependence is achieved via conformational changes in the triplex motif, which increases the effective DS concentration near the aptamer at high pH, thereby inhibiting ATP binding and fluorescent signaling, where K_D^{eff} is the dissociation constant. Reprinted with permission from [49].

Another interesting approach is given by Davydova et al. [55], who recently (2020) proposed a novel bimodular aptamer construct with double functionality (recognition sequences for hemoglobin (Hb) and obelin, respectively). The bi-specific aptamer reported in this paper is based on two structural modules: (1) the molecular recognition region which is specific for the target analyte (2' -F-RNA aptamer H9t11 as the analyte-binding module for human Hb as proof of concept in this paper), and (2) the obelin-binding aptamer for a reporter-recruiting module. The event of the analyte binding confers a structural reorganization to the whole construct with the consequent disclosure of the obelin-binding region. The bioluminescent signal is then generated in presence of calcium ions. Fig. 5 illustrates the general scheme of bioluminescent (BL) detection assay for an analyte with bi-specific aptamer, in which it is clearly evidenced the possibility to generate bioluminescent signal only in presence of both the target analyte and the obelin, strictly in a sequential manner. The reached LOD was 6.25 nM Hb.

Freeman et al. [56,57] have previously described an aptasensor for vascular endothelial growth factor (VEGF), which is an important biomarker for different diseases and clinical disorders. The association of the protein to its aptamer forms a G-quadruplex, which can bind hemin, resulting in a catalytic hemin/G-quadruplex that mimics the activity of HRP. Then CL can be generated using this complex in presence of H_2O_2 and luminol. This method allowed reaching a LOD of 1.8×10^{-8} M. A better performing method, reaching a LOD of 8.75×10^{-10} M, was optimized by the same group by introducing in the CRET process a quantum dot (QD) at the 5' end of the aptamer (λ = 600 nm).

Moutsiopoulou et al. [58], focused their attention on the fact that even if, as mentioned before, the chemi/bio luminescence approach has the advantage to avoid the background coming from the light source and then to increase the signal-to-noise ratio with respect to the fluorescence approach, nevertheless, bioluminescence quenching is still comparatively inefficient, resulting in a signal-to-noise ratio that could still be improved. Their idea is based on the design of an aptamer switch for the detection of the immune-stimulating cytokine, interferon-γ (IFN-γ) by the use of the bioluminescent protein, Gaussia luciferase (GLuc), as replacement of the traditional fluorophore (bioconjugated at the 3'-azide). Moreover, in order to overcome the quenching inefficiency, an inhibitor of the enzyme/substrate relationship rather than a quencher has been introduced (conjugated to the 5'-carboxylic acid). The inhibitor molecule is a compound structurally very similar to the native substrate, which has the aim of totally eliminating the undesired triggering of the bioluminescent reaction. The inhibitor molecule is coelenteramine, which has a similar structure to the native substrate coelenterazine. This construct allowed reaching a LOD of 1 nM IFN-γ.

2.3. Plasmon field-enhanced fluorescence-based aptamer switches

Aptamer optical switches have been also used in plasmonically amplified fluorescence-based assays, where the interaction of fluorescent dyes with the plasmonically enhanced electromagnetic field allows an increase of the fluorescence intensity, the quantum yield of fluorescence and the photostability of the dye [59].

Actually, the great majority of plasmonically amplified fluorescence-based assays using an aptamer beacon as carrier of the fluorophore, are based on the localized surface plasmon (LSP), which takes the advantage of chemically synthesized metallic nanostructures (deeply reported in the specific Section 4 of this review).

Nevertheless, an aptamer beacon-based assay that is mediated by the propagating surface plasmons on metallic films, has been reported to give very high fluorescence intensity change, up to 18-fold higher than non-plasmonic surface, as consequence of the analyte binding [59].

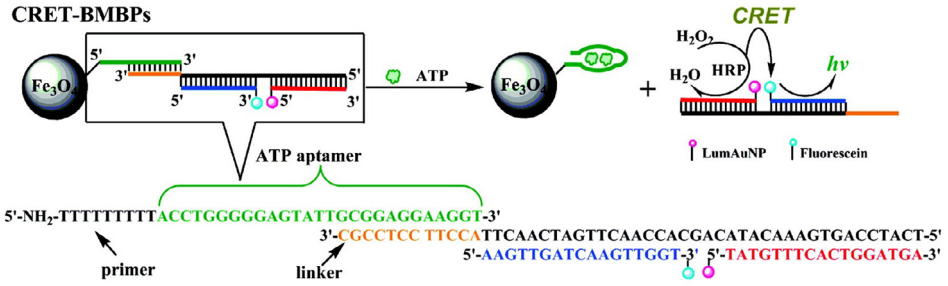

Fig. 4. Schematics of CRET-BMBPs and its application in the detection of ATP. Reprinted with permission from [54].

A necessary condition in order to reach the maximum plasmonic fluorescence enhancement is to keep the distance between the fluorophore and the metallic surface in the range of 15–20 nm. At shorter distances, the fluorescence signal is attenuated by the quenching effect given by the metal; while at longer distances, the fluorophore is moved outside the evanescent surface plasmon field [60]. In fact, the conformational change of the aptamer switch, immobilized onto the metal sensor surface, upon binding to a target, determines an increase in the distance between the fluorophore and the metal. Thus, the enhanced fluorescence emission is observed due to the interaction between the fluorophore and the metallic surface changing from a quenching effect to an enhancement effect [59,61,62].

In Xie et al. [62], an anti-thrombin 15-mer aptamer beacon was immobilized on a gold-thin-film (50 nm) deposited onto a glass slide. The reverse Kretschmann configuration was used for the surface plasmon-coupled fluorescence emission measurements and the signal at 45° was about six-fold greater than the spontaneous free space emission at 135° after the interaction of the aptamer beacon with 1 μM thrombin.

Sergelen et al. [59], on the same principle, developed a plasmon field-enhanced fluorescence energy transfer assay using an aptamer switch for small molecules, such as adenosine and ATP, reaching a LOD of 1 μM. In Fig. 6, a scheme of the aptamer beacon-based assay on the gold sensor surface is reported. The design of the aptamer beacon (composed not only by the stem and analyte specific sequences, but also by two different spacers) has been studied in order to achieve the suitable distance between the fluorophore and the metal surface sensor upon the interaction with the analyte and consequent opening of the structure.

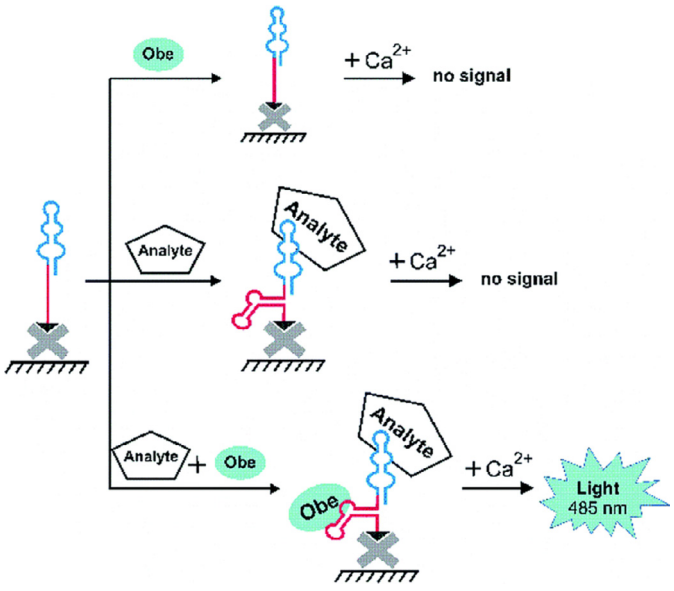

Fig. 5. A general scheme of bioluminescent detection assay for an analyte with a bi-specific aptamer. Reprinted with permission from [55].

To better compare the different biosensing mechanisms based on aptamer switches, a summary of the discussed optical detection methods is reported in Table 1 [40–49,54–56,58,59,62].

3. Aptamer switches coupled to nanomaterials

3.1. Nanoparticles, nanoclusters and nanosheets

The functionalization of aptamers with nanomaterials allows the exploitation of the different dynamic properties of these versatile molecules: this modification is often used to improve the sensitivity of the detection due to signal amplification or to allow the implementation of the detection assay in homogeneous format with the generation of the signal in the sample solution [63–65]. The use of nanometric-size material offers intriguing advantages, for example, in labelling aptamers: the interaction of aptamers with fluorescent nanoparticles such as quantum dots (QDs) or upconversion nanoparticles (UCNPs) can lead to highly efficient fluorescent aptamers which, at the same time, keep the conformation switching properties. On the other end, in combination with nanomaterials, the small dimensions of aptamers, for example with respect to antibodies, can assure a more efficient optical switch mechanism or a higher sensitivity in Förster Resonance Energy Transfer (FRET)-based methods [66].

In addition to NPs, among the different nanomaterials used in combination with switching aptamers, nanosheets have been frequently proposed as signal quenching substrate: this nanomaterial offers several advantages, which make it a promising substrate in aptasensors, such as the large specific surface area enabling the adsorption of biomolecules [67,68] and the excellent mechanical and optical properties [69,70].

A summary of the most recent aptamer-based sensing mechanisms coupled with different kinds of nanomaterials, is reported in Table 2 [67,71–87]. The combination of switching aptamers with graphene oxide (GO) will be discussed in details in the next section of this review.

When used in combination with nanosheets, optical aptasensors can be developed by exploiting their excellent adsorption onto this substrate and their intrinsic target binding induced switching conformation, which leads to the desorption of the aptamer from the nanosheet. In addition, due to the extraordinary quenching efficiency of several nanosheets, when using aptamers labelled with fluorescent molecules, versatile "turn on" aptasensors can be realized. To this aim, molybdenum disulfide (MoS_2) nanosheets have been extensively used in combination with DNA molecules in general [88] and with aptamers [89]. Recently, the quenching property of MoS_2 nanosheets have been reported in combination with switching aptamers labelled with the FAM fluorophore [86] and with MoS_2 QDs [73] for the detection of arsenic ions and dopamine, respectively.

The same scheme has been used by combining cobalt oxyhydroxide (CoOOH) nanosheets and a methamphetamine specific aptamer labelled with fluorescent carbon dots prepared by a one-step hydrothermal treatment from grape leaves [74].

A classical target-binding induced turn on method has been used for the detection of citrulline in combination with gold nanoparticles

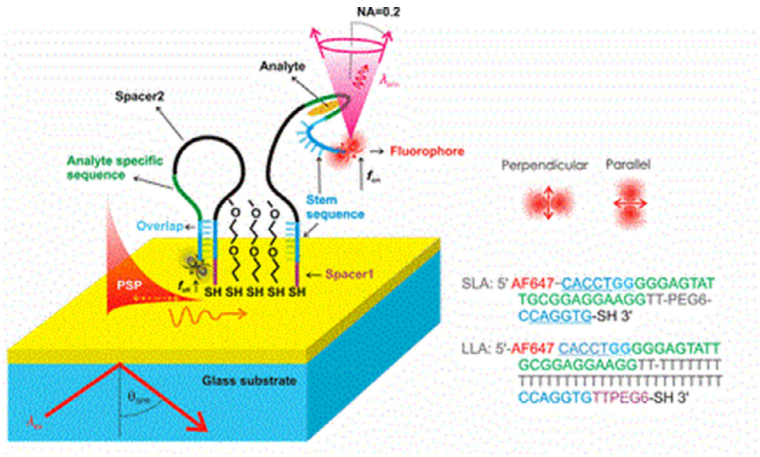

Fig. 6. Scheme of the aptamer-based assay on the gold sensor surface that utilizes a fluorophore coupled to surface plasmons (PSP). The aptamers were anchored onto the gold surface via a thiol group at the 3' end and polyethylene glycol-thiol was used to better distribute their density. The fluorescence light emitted by the used dye to the direction normal to the surface was collected by a lens (focal length 30 mm, numerical aperture of NA = 0.2). Reprinted with permission from [59].

(AuNPs) [80]. Due to the higher sensitivity assured by surface-enhanced Raman spectroscopy (SERS) with respect to fluorescence [90], the first detection method was applied to the detection of the small molecule. In particular, the citrulline specific aptamer was labelled at one end with Cy3 and at the opposite end with AuNPs (34 nm): these modifications of the aptamer cause a distance dependent change in the Raman signal due to the conformation switching of the aptamer upon binding with citrulline. Moreover, with the aim of developing a point-of-care (POC) system, a paper-based vertical flow platform has been optimized to perform the aptamer-based assay: the vertical configuration of the paper-based microfluidic system can lead to an enhanced sensitivity due to the smaller distance for the sample to pass over the different stages with a lower loss of analyte in the paper before the detection portion [91]. With the use of a handheld Raman spectrometer, a POC system for the detection of citrulline has been finally optimized with a LOD of 10 μM, which can be considered adequate for the diagnosis of intestinal diseases characterized by citrulline concentrations of about 30 μM.

AuNPs and a signal turn off mechanism based on aptamers, have been recently combined with another interesting nanomaterial, terbium (III) based metal-organic frameworks (Tb-MOF) [76,77] for the detection of ATP even in ATP-spiked human plasma samples. Tb-MOFs are porous crystalline materials formed by the coordination of organic ligands and terbium(III) ions, possessing several important characteristics, such as high surface area and luminescent properties [92]: the organic ligand-to-Tb^{3+} ion energy transfer can promote their characteristic green emission which can be quenched by dispersed AuNPs rather than the aggregated ones. In addition, it was observed that about 16% of quenching effect was due to the inner filter effect, which was coexisting with the dynamic quenching effect as demonstrated by the shift of the fluorescence lifetime of Tb-MOFs from 5.8 to 4.1 ns in presence of AuNPs coupled to the thiolated aptamer. This FRET mechanism has been used for the detection of ATP with its specific binding aptamer, which can protect AuNPs against salt-induced aggregation through multiple forces. The aptamer was complexed with AuNPs by two distinct methods, covalent immobilization via thiol-gold bonds or electrostatic interaction, leading to opposite repulsion forces between the AuNPs: the covalently immobilized aptamer, free from ATP, can protect AuNPs from aggregation in high salt solution due to steric hindrance enhancement whereas the same aptamer without ATP, only electrostatically interacting with AuNPs cannot protect them from aggregation. Only in presence of ATP, the aptamer switches in its characteristic folded conformation to bind to its target and this conformation change leads to increased proximity of AuNPs covalently modified with the thiolated aptamer, which aggregate. On the other hand, the folding of the free aptamer due to ATP binding, increases protection of AuNPs from aggregation. By playing with the combination of AuNPs aggregation, Tb-MOFs fluorescence quenching and immobilized or free aptamers, two sensors for ATP have been presented based on a turn on or a turn off mechanism, respectively [76,77]. Both the sensors reached a detection limit in the sub-micromolar range with a good selectivity and applicability to real sample analysis.

The FRET mechanism between a CAP Cy3-labelled aptamer and AuNPs has been interestingly considered to construct a plug-and-play molecular logic system with logic gates created by the modulation of the fluorescence emission by metal ions free or chelated [75].

Table 1
Aptamer optical switches based on luminescence and plasmonically-enhanced fluorescence.

Optical detection method	Target	Sensing mechanism	LOD	Ref.
Fluorescence decrease	Thrombin	G-quadruplex formation		[40]
Fluorescence increase	Thrombin	G-quadruplex formation		[41]
Fluorescence increase	MRCKα	Aptamer switch		[42]
Fluorescence increase	Tat-1	Ternary complex formation		[43]
Fluorescence increase	CAP	Three-oligonucleotide aptaswitch sensor complex	0.987 pg/mL	[44]
Fluorescence increase	Kanamycin	Aptamer switch	13.52 nM in milk sample	[45]
Excimer formation	IgE	Pyrene-labeled aptamer	1.6 nM	[46]
Fluorescence increase	Pb^{2+} and Hg^{2+}	Aptamer switch	0.16 and of 0.36 nM, respectively	[47]
Fluorescence increase	Zn^{2+}	Dual-aptamer-conjugated molecular modulator	4.9 μM	[48]
Fluorescence increase and decrease	H^{+}	pH-responsive tunable aptamer switch		[49]
CL	ATP	Structure-switching aptamer	0.11 μM	[54]
BL	Hb	Bimodular aptamer construct	6.25 nM	[55]
CL	VEGF	G-quadruplex formation	1.8×10^{-8} M	[56]
BL	IFN-γ	Aptamer switch with inhibitor instead of the quencher	1 nM	[58]
Plasmonically-enhanced fluorescence	Thrombin	Aptamer switch		[62]
Plasmonically-enhanced fluorescence	ATP	Aptamer switch	1 μM	[59]

Table 2
Biosensing systems based on aptamer switches and nanomaterials.

Aptamer target	Nanomaterial	Sensing mechanism	Analytical characteristics	Ref.
OTA	Molybdenum disulfide nanosheets + quantum dots	Target-binding induced turn on signal/fluorescence	LOD 1 ng/mL Real samples: spiked red wine	[67]
OTA	Colloidal cerium oxide nanoparticles + graphene quantum dots	Target-binding induced turn on signal/fluorescence	LOD 2.5 pg/mL Real samples: penauts	[71]
OTA	Gold nanorods + enzyme induced metallization	Target-binding induced turn on signal/colorimetric	LOD 3.6 ng/mL Real samples: grape juice	[72]
Dopamine	Molybdenum disulfide nanosheets and quantum dots	Target-binding induced turn on signal/fluorescence	LOD: 45 pM	
Real samples: human serum, dopamine hydrochloride injection samples	[73]			
Methamphetamine	Cobalt oxyhydroxide nanosheets and carbon dots	Target-binding induced turn on signal/fluorescence	LOD: 1 nM Real samples: human serum	[74]
CAP	Gold nanoparticles	Target-binding induced turn on signal/fluorescence	LOD: 81 ng/mL	[75]
ATP	Gold nanoparticles + terbium(III) based metal-organic framework	Target-binding induced turn off signal/fluorescence	LOD: 23 nM Real samples: human plasma	[76, 77]
Small molecules (adenosine, cocaine, 17β-estradiol)	DNA-Silver nanoclusters	Split aptamer/fluorescence	LOD: adenosine 40 pM, cocaine 35 pM, 17β-estradiol 15 pM) Real samples: human serum	[78]
Cocaine	Silica nanoparticles	Split aptamer/fluorescence	LOD: 84 pM Real samples: human serum	[79]
Citrulline	Gold nanoparticles	Target-binding induced turn on signal/SERS	LOD: 10 μM	
	[80]			
CEA	Hollow gold nanoparticles + magnetic beads	Target-binding induced turn on signal/colorimetric	LOD: 0.78 ng/mL Real samples: human serum	[81]
Aflatoxin B1	CdZnTe quantum dots + Gold nanoparticles	Target-binding induced turn off signal/fluorescence	LOD: 20 pg/mL Real samples: peanuts	[82]
Zearalenone	DNA-Silver nanoclusters	Aptamer-trigger sequence/ fluorescence	LOD: 0.32 pg/mL Real samples: maize, beer	[83]
Kanamycin	Platinum nanoparticles	Hairpin aptamer construct/colorimetric detection	LOD: 0.2 pg/mL Real samples: milk	[84]
Acetamiprid	Copper nanoparticles	Hairpin aptamer construct/ fluorescence	LOD: 2.37 nM Real samples: apple, ginger	[85]
Arsenic ions	Molybdenum disulfide nanosheets	Target-binding induced turn on signal/fluorescence	LOD: 18 nM Real samples: tap and lake water	[86]
Mercury ions	Upconversion nanoparticles + Gold nanoparticles	Target-binding induced turn off signal/fluorescence	LOD: 60 nM Real samples: tap water, milk	[87]

In addition, a colorimetric detection based on gold nanorods or hollow AuNPs, has been reported in combination with conformation switching aptamers for the detection of ochratoxin A (OTA) and carcinoembryonic antigen CEA, respectively [72,81].

Increased sensitivity in aptamer-based detection has been achieved by coupling the use of AuNPs with UCNPs which offer important advantages with respect to other fluorescent nanomaterials often coupled to AuNPs in FRET-based detection: UCNPs possess high Stokes shift, they are resistant to photobleaching and they can provide higher signal-to-background ratios due to their weak autofluorescence background [93]. These interesting features have been used for the determination of mercury ions (Hg^{2+}) in combination with a structure switching aptamer modified with NaYF4:Tm^{3+},Yb^{3+} UCNPs and initially hybridized with a complementary sequence fixed onto AuNPs [87]. In this configuration, UCNPs fluorescence is quenched by the proximity of AuNPs, but with the addition of Hg^{2+}, the aptamer binds to its target switching its structure and releasing the complementary strand with the AuNPs. In this form, the fluorescence of the UCNPs is restored. The sensor could achieve a LOD of 60 nM, a concentration well below the maximum level of Hg^{2+} in food of 10 μM.

Very interesting nanomaterials-modified DNA sequences have been used in combination with aptamers to build turn-on fluorescence biosensors: these sequences, termed DNA-silver nanoclusters (DNA-AgNCs) are formed by cytosine-rich DNA modified by reducing Ag^{+} on the nucleation region of the DNA molecules leading to the formation of a fluorescent DNA nanomaterial (diameter around 2 nm) [94]. When placed in the immediate proximity of guanine-rich DNA sequences, the DNA-AgNCs increase their fluorescence emission due to the transition of electronic states between the surface state and the ground state, leading to enhancements up to 500 fold and signal to background ratio decrease [95]. This mechanism has been used in combination with aptamers promoting a change in conformation upon binding to the target molecule [78]. In particular, in a first example [83], the aptamer for zearalenone was strongly hybridized to a complementary DNA strand, an inhibitor strand, which prevents the binding of the aptamer with other DNA sequences; in presence of zearalenone, the aptamer binds to it switching to a conformation in which it is not hybridized to the inhibitor sequence any more. The availability of the aptamer triggers a series of hybridization and conformational changes in the aptamer together with other hairpin shaped DNA sequences containing the AgNCs probe: at the end of this process, the AgNCs probe, at low fluorescence when single, results in close proximity to a specific G-rich-strand with the consequent dramatic enhancement of the fluorescence signal.

A similar protocol has been adopted in [78] for the detection of small molecules, such as adenosine, cocaine, and 17β-estradiol: in this case, the DNA-AgNCs and the G-rich sequence were connected to different segments of a specific aptamer (split aptamer), which, upon binding to the target, re-organizes its original structure bringing the DNA-AgNCs and the G-rich sequence in close proximity with the consequence enhancement of the fluorescence signal. The versatility of the aptasensor was also demonstrated by the detection of bigger molecules such as thrombin with the insertion of a linker sequence between the split aptamer segments and DNA-AgNCs/G-rich sequence to avoid steric hindrance.

Split aptamers have been also used in combination with silica nanoparticles (SiNPs) for the detection of cocaine [79]. To this aim, one of the two segments of the cocaine split aptamer was labelled with BHQ-1 and fixed onto SiNPs and the other was labelled with the two fluorophores FAM and TAMRA at the two ends: binding of the two segments to cocaine generates the re-folding of the aptamer in its original structure increasing the fluorescence signal of TAMRA due to the close proximity to SiNPs, and the decrease of the fluorescence of FAM due to the vicinity of the BHQ-1 quencher.

Aptamer-based colorimetric detection coupled to the use of nanomaterials have attracted researcher attentions in the last years due to several advantages such as practicability, low cost and naked eye detection [96]. In addition, several enzymatic amplification strategies have been recently coupled to aptamer-based colorimetric assays in order to achieve a sensitivity enhancement: restriction endonuclease, lambda exonuclease and exonucleases, mainly exonuclease I, have been used in combination with aptameric constructs to improve the sensitivity of colorimetric biosensors [97]. Coupling a DNA nicking endonuclease with an aptamer-based construct having a hairpin conformation, a colorimetric kanamycin aptasensor has been realized [84]. In particular, the hairpin construct was formed by the kanamycin aptamer and a target DNA and extended DNA (Fig. 7): the binding between the aptamer and kanamycin leads to an aptamer change in conformation which switches the construct format from a hairpin to a partially linear one with the target DNA available for the hybridization with a complementary strand labelled with platinum nanoparticles (PtNPs) and magnetic beads. In the cycling amplification process, the nicking enzyme cleaves the double stranded DNA releasing the labelled strand: after magnetic separation the colorimetric signal (blue color) was obtained by the reaction between 3,3′,5,5′-tetramethylbenzidine (TMB) and H_2O_2 catalyzed by the PtNPs. The signal is highly amplified by the continuous enzyme cleavage which leaded to a detection limit as low as 0.2 pg/mL for kanamycin in milk.

3.2. Graphene oxide

Graphene oxide (GO) is a widely used nanomaterial in DNA-based sensing methods [98]. GO, being a chemically modified graphene with oxygen functionalities, overcomes many of the drawbacks of pristine graphene, such as its hydrophobic properties, which make it hardly dispersed in water [99]. On the contrary, GO can be dissolved in water and other solvents due to the exposure of some functional groups, such as ester, epoxide, hydroxyl and carboxyl groups. Moreover, its tunable electrical conductivity and optical properties make GO a very good substrate for the development of electrochemical and optical biosensors [100–103]: most of the GO-based optical biosensors are based on the FRET mechanism in which GO can function as a donor or an acceptor molecule having at the same time quenching [101] and luminescence properties [104].

DNA-based biosensors using GO as substrate, take advantage of its optical properties and of its large surface area and abundant π electrons to provide an excellent solid support capable of interacting with the nucleobases of single-stranded DNA via π-π stacking and hydrogen bonding [98]. This format has been also implemented with aptamers to create DNA-based biosensors to detect food allergens [39], antibiotics [105,106], pesticides [107], cancer biomarkers [108,109] and a plethora of other different targets [65]. Less common are GO-based biosensors with RNA or RNA aptamers as functional molecule due to the low stability of RNA [110]. The property of GO of preferentially interacting with single-stranded DNA or, at least, unstructured DNA, opened the possibility of realizing optical biosensors with a target binding induced signal generation [111]: this can be particularly significant for those aptamers having intrinsic switching structures, such as the ATP [112] and the platelet-derived growth factor (PDGF) aptamers [113], which can strongly adsorb onto GO when target-free and unstructured, whereas a desorption from GO is caused by their structuring in a more complex format due to the binding with the target [114–118]. A similar mechanism can be used for GO-based biosensor with the 15-mer thrombin aptamer which is known to switch to an intramolecular guanine quadruplex (G-quadruplex) [119] structure with two layers of guanine tetrads stacking on each other through $\pi-\pi$ interactions when binding to its target [120,121]. The G-quadruplex conformation can be also adopted by the Hg^{2+} specific aptamer [119]: this feature has been again applied in combination with GO and with the addition of a further DNA sequence in order to form a T–Hg^{2+}–T structure [122]. In absence of the mercury ion, the specific aptamer is in its unstructured form and GO can function as a quencher of the fluorescence of free acridine orange which can interact with GO via $\pi-\pi$ stacking and electrostatic interactions. When binding to Hg^{2+} the aptamer switches in its G-quadruplex conformation forming the T–Hg^{2+}–T structure and acridine orange is complexed in the G-quadruplex aptamer portion releasing from GO with the consequent switching on of the fluorescence signal. Excellent LOD of 0.17 nM was achieved with the sensing method with good selectivity and the demonstration of applicability to the analysis of real samples such as tap water.

Among the works dealing with the aptamer switching mechanism, an aptasensor based on GO was reported for the detection of a tuberculosis biomarker (TB7.7, also known as Rv2654c) [123]: the TB7.7 21-mer

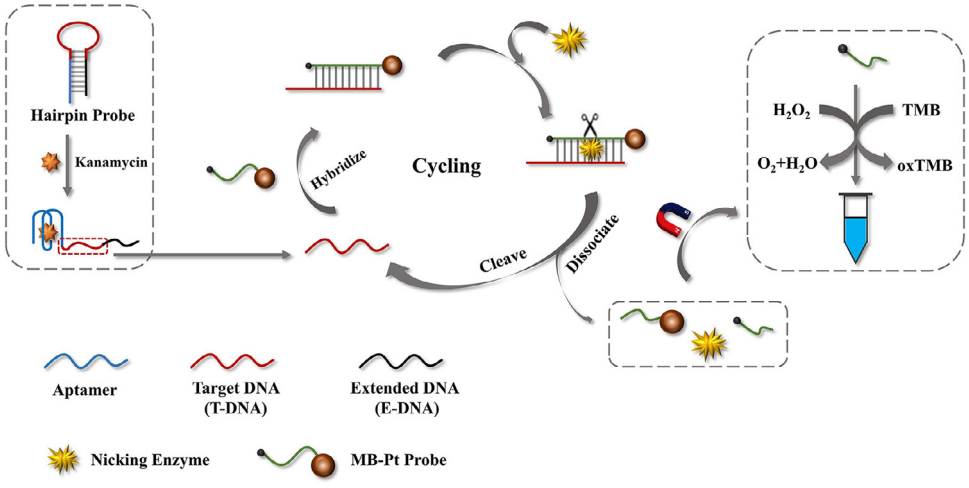

Fig. 7. Scheme of the aptamer switch assay for kanamycin based on colorimetric detection and an enzyme-assisted signal amplification. Reprinted with permission from [84].

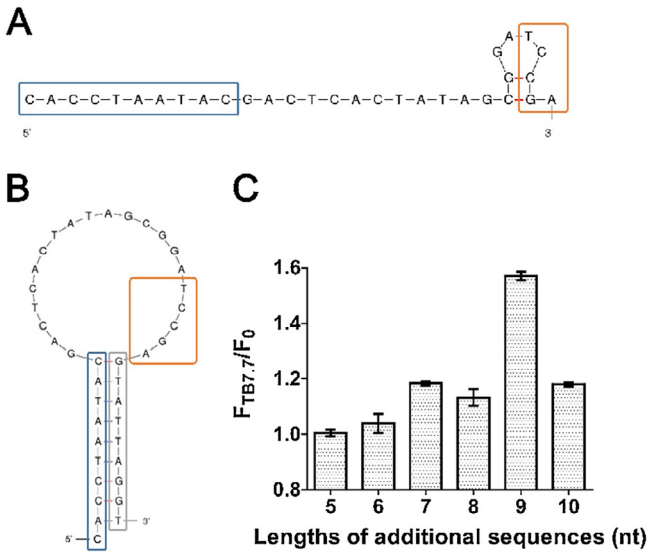

Fig. 8. Design and optimization of the aptamer beacon for TB7.7. (A) Secondary structure of the final aptamer. Blue and yellow boxes indicate the sequences implicated in the interaction with TB7.7. (B) Design of aptamer beacon by adding 9 nucleotides at the 3′-end (grey box) of the aptamer. (C) Optimization of the additional sequence (5–10 nt) of the aptamer beacon by using 30 nM TB7.7. Reprinted with permission from [123].

aptamer was engineered to display a beacon structure by flanking a complementary sequence, optimized in length, to its 5'-end to create the stem and by using the last 5 nucleotides of the 3'-end, in the hairpin, as initiator of the ligand binding process (Fig. 8). This hairpin structure labelled with 5′-FAM and 3′-BHQ1, produces a fluorescence signal only when opening due to the binding with TB7.7. GO was here used as a background noise reducer in order to obtain an enhanced sensitivity of the aptasensor: low LODs of 0.8779 nM and 0.7089 nM were obtained in buffer and serum, respectively.

A very interesting work has been recently published on the exploitation of a similar mechanism for the detection of surface protein patterns of tumor-derived exosomes [124]: GO served as quencher of the fluorescence generated by the aggregation-induced emission luminogen (AIEgens) tertiary amine-containing tetraphenylethene (TPE-TA) which can bind in high number a single aptamer resulting in an amplified fluorescence signal. In absence of tumor-derived exosomes, the TPE-TA/aptamer complex is strongly adsorbed onto GO, resulting in a quenched signal; when interacting with specific target proteins on the surface of the tumor-associated exosomes, the florescence signal is restored due to the binding of the aptamer to its target on the exosome, which causes the TPE-TA/aptamer complex detachment from GO (Fig. 9). The biosensor can detect breast cancer-derived exosomes by testing three different tumor-associated protein biomarkers on exosomes, epidermal growth factor receptor (EGFR), epithelial cell adhesion molecule (EpCAM), and human epidermal growth factor receptor 2 (HER2).

Original long DNA nanostructures termed as "nanoladders" in combination with GO have been recently presented for the detection of OTA [125]: the nanoladder was composed of repeated units formed by the OTA-specific aptamer and a rhodamine X (ROX) fluorophore labelled DNA strand complementary to the aptamer. The biosensor was based on a "turn-off" mechanism with GO acting as quencher of the multiple fluorophores labelling the nanoladder structure: in absence of OTA, the nanoladder is intact in its double-stranded form, which prevents its adsorption onto GO and, consequently, without the interaction with the quencher, the fluorescence signal is on the ON state. Upon the binding of the aptamers in the nanoladder with OTA, the aptamers change their conformation separating from the labelled strand, which, being now in its single-stranded form, is adsorbed onto GO. Having GO in close proximity, the fluorescence of the ROX fluorophores of the nanoladder is quenched turning the sensor in its OFF state. A detection limit for OTA of 0.1 nM was achieved by further enhancing the sensitivity with the creation of frail nanoladders, which can be easily disassembled by OTA.

4. Aptamer optical switches for intracellular sensing

Recently, the use of nucleic acids for sensing molecules in the intracellular environment has been exhaustively reviewed by Samanta et al. (2020) [126]: nucleic acid-based probes offer several advantages for imaging and detecting different intracellular molecules, such as low cost of production, variability in their sequence and possibility of chemical modification in order to improve their selectivity and stability. Nucleic acid probes can be used to detect other nucleic acids through pairing via complementarity: this property has been widely utilized, coupled to proper labeling with optical signal-generating molecules, to create intracellular imaging tools to detect and image other nucleic acids such as messenger RNA (mRNA). More interestingly, aptamer-based probes have been used to detect molecules different from nucleic acids such as small molecules or proteins inside cells [30,127]. In the past years, several works appeared in the literature based on aptamers both as

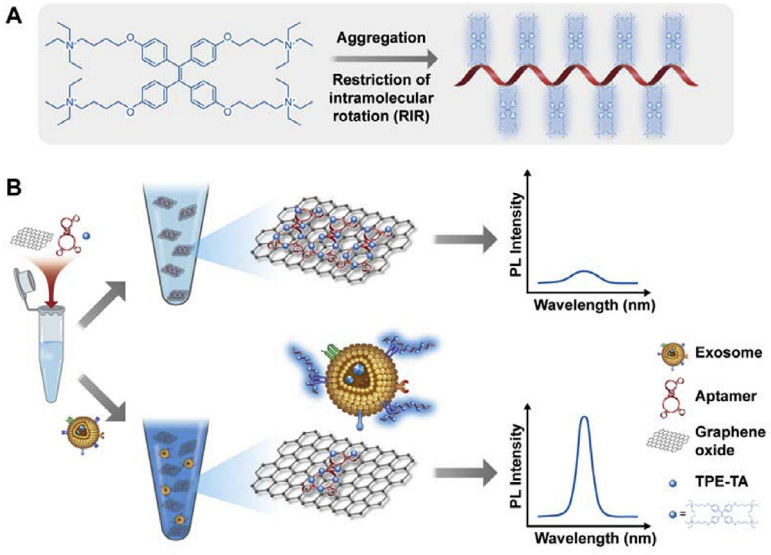

Fig. 9. Scheme of (A) chemical structure and luminous mechanism of TPE-TAs aggregated on nucleic acid oligomers; (B) the aptamer fluorescence switch sensor for profiling exosome surface proteins. Reprinted with permission from [124].

Table 3
Aptamer optical switches for intracellular sensing or cell targeting.

Aptamer target	Aptamer role	Sensing mechanism	Cell type	Ref
ATP	Sensing molecule	Molecular-beacon-type switch aptamer/fluorescence	Yeast cells	[128]
HIV-1 reverse transcriptase	Sensing molecule	Molecular-beacon-type switch aptamer/fluorescence	HeLa cells	[129]
Nucleolin	Cell targeting molecule	Molecular beacon/fluorescence	MCF-7 cells	[130]
ATP	Sensing molecule	Split aptamer coupled to DNA nanostructures/FRET	Hela cells	[131]
ATP	Sensing molecule	Aptamer/AgCNs complex/fluorescence	Hela cells	[132]
Nucleolin	Cell targeting molecule	Molecular beacon and RNA-cleaving DNAzyme	Human A549 lung cancer cells and MCF-7 breast cancer cells	[133]
Protein tyrosine kinase 7 (PTK7)	Sensing molecule	Aptamer + MnO2 nanosheets/fluorescence	Human T cell acute lymphoblastic leukemia cells	[134]
ATP	Sensing molecule	Molecular-beacon-type switch aptamer/fluorescence	HeLa cells	[135]
Ca^{2+} ions	Sensing molecule	Switching aptamer coupled to QDs and AuNPs	Mouse pre-osteocyte cells	[149]

signaling molecules due to a switching process (change in conformation) and as targeting molecules, to direct the sensing tools towards specific cells such as cancer cells; several examples are reported in Table 3 [128–135] and the more recent ones are detailed in this section.

Among small molecules of particular interest due to their role in cell metabolism and function, ATP has been denoted both as indicator of several diseases and as important factor in maintaining the cell function [136] and the biological processes of the organism [137]. Regarding the analysis of intracellular ATP, many *in vitro* methods have been developed, most based on ATP extraction and subsequent detection [54,138]. Several other works have been presented on the bioimaging of intracellular ATP [139–141], whereas simultaneous imaging and quantitative detection have been rarely achieved [142,143]. This kind of analysis is more challenging mostly due to the background interference generated by the complex matrix [144]. Also in this field, aptamers can play an important role and, in particular, the ATP specific aptamer due to its tertiary structure [145]: several studies have demonstrated that the ATP-aptamer exists in a random configuration at low ionic strength and that it adopts a rigidified double-loop hairpin conformation in high-ionic-strength conditions or upon binding to ATP [146]. Very interestingly, by taking advantage of this property, an aptamer-based nanoprobe has been developed [132] with the aptamer serving as ATP capturing element and as template to complex AgNCs. In particular, the sensing mechanism (Fig. 10) was based on the interaction between the ATP aptamer (5'-CCTCCTTCCTCCTACCTGGGGGAGTATTGCGGAGGAAGGT-3') complexed with AgNCs and transition metal dichalcogenides (TMDCs) nanosheets made of molybdenum disulfide (MoS_2): the vicinity between the nanosheets and the aptamer/AgNCs simply loaded onto the nanosheets by electrostatic forces, quenches the AgNCs fluorescence. The complex (MoS_2 – aptamer/AgNCs) was then incubated with cells (HeLa cells) after coating the nanosheets with lipoic acid polyethylene glycol folate (LA-PEG-FA) to promote internalization into cells. In the cells, due to the interaction between the aptamer and ATP, the aptamer/AgNCs/ATP complex is released by the nanosheets causing the restoring of the AgNCs fluorescence signal. After this intracellular imaging of ATP, cell lysis was conducted and the released aptamer/AgNCs/ATP (few nanometers) was separated by the MoS_2 nanosheets (about 150 nm) by centrifugation: the complex containing ATP was then analyzed by inductively coupled plasma mass spectrometry (ICP-MS) and the ATP concentration was derived by the detected amount of ^{107}Ag. High reliability was demonstrated by the method due to the use of AgNCs, which are at the same time a good fluorophore and a tag, which can be detected by ICP-MS: a concentration of ATP in HeLa cells of 24.6 ± 1.7 nM was detected, which was in good agreement with a commercial ATP detection kit. Moreover, the complex nanoprobe was demonstrated to be biocompatible, selective, without background interferences by the biological complex matrix, and simple to prepare.

Another research group has reported the realization of a switchable aptamer-based complex for the intracellular imaging of ATP, which can also serve for photodynamic therapy [147]. In this work, the ATP aptamer is immobilized onto PEGylated AuNPs and hybridized with a complementary strand labelled with methylene blue: in this condition, the photodynamic properties of methylene blue are inhibited by the close proximity to the AuNP (OFF state). Once internalized into cells, the aptamer binds to ATP changing its conformation and inducing the release of the methylene blue-labelled strand from the AuNP with the consequent activable generation of singlet oxygen under light irradiation bringing the system in the ON state.

In intracellular sensing, aptamers have been also used as targeting agent, to direct the sensing tool only towards specific cells, often coupled to molecular beacons as sensing molecules. In particular, the anti-nucleolin aptamer AS1411 has been widely employed with this aim due to its particular G-quadruplex structure and to its very specific binding to nucleolin, a multifunctional protein overexpresses on the surface of cancer cells but absent on most of the normal cells [148]. This aptamer was recently coupled to a MB specific for survivin mRNA engineered to contain in the loop sequence a RNA-cleaving DNAzyme [133]: the complex structure was internalized into cancer cells thanks to the presence and specificity of the aptamer and the MB, labelled with a FAM fluorophore and a dark quencher, served as sensing molecule due to its opening upon hybridizing with the target mRNA. At the same time, the opening of the MB and its conformation change generate a typical DNAzyme

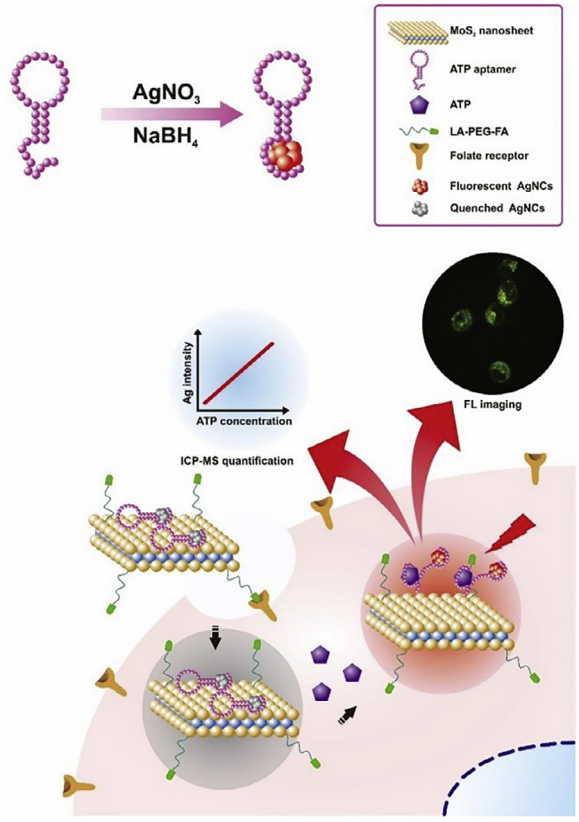

Fig. 10. Scheme of the aptamer switch probe for imaging and quantification of intracellular adenosine triphosphate based on molybdenum disulfide nanosheets and silver nanoclusters. Reprinted with permission from [132].

structure, which cleaves survivin mRNA preventing the further production of the protein and leading to an ultimate theranostic effect.

5. Conclusions and future trends

Aptamers were discovered in the 1990s and, since that moment, a certain number of molecular constructs with good affinity for their specific targets, were designed and developed via SELEX processes. In fact, aptamers have the advantage that, even if based on oligonucleotidic sequences, their definitive 3D conformations (hairpin, pseudoknot, bulge loop, G-quadruplex, etc.) make it possible the binding with a vast variety of different targets (proteins, small molecules, ions, etc.), allowing to compare them to antibodies. Anyhow, notwithstanding the great enthusiasm of the scientific community for aptamers, the gap between their potentialities and the real applications in clinical environment was never filled. This is quite in contrast with the large use of antibodies in that field. In fact, the latter are much more laborious, time-consuming, and expensive to be produced, while aptamers are chemically more stable, biologically non-toxic and easy to be manipulated. Among all the possible reasons for that, a limitation of aptamers expansion could be a well-developed commercial infrastructure, which is still missing, while it is well established for antibodies production and commercialization; together with sensitivity and specificity performances, which are quite below what is currently achievable with antibodies.

In this context, the success of developing structure-switching aptamers resides in creating the possibility for real-time detection and imaging of the analyte of interest. Such idea was already implemented by the use of molecular beacons (for which, for example, commercial kits are already available for real time polymerase chain reaction (RT-PCR) assays), but with the aptamer optical switches the selectivity and sensitivity of molecular beacons together with aptamer technologies (antibody-like selective recognition of an immense variety of target molecules) have been combined, giving the possibility for new future perspectives, but for which commercial kits are not available yet.

It is important to notice that aptamers can be combined not only with fluorescent systems but also with a variety of nanomaterials, which have improved a lot their performances and expectations. Their application in real samples (i.e. human serum/plasma, milk, red wine, grape juice, peanuts, etc.) has been also demonstrated and here reported in details in Table 2.

Moreover, those constructs can not only work in a system with the configuration of a classical bioassay, but also can be internalized in cells bringing the scientists to the challenging world of the *in vitro* real-time biosensing in living cells.

The expanding developments of nanotechnology will provide unique strategies to address sensitivity and specificity limitations bringing the aptamer optical switches to further fascinating applications, such as improvements in the early detection of severe diseases accompanied by possible therapeutic effects (theranostics) and more controlled outcomes during pharmaceutical treatments.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] N. Wu, K. Wang, Y. Wang, M. Chen, X. Chen, T. Yang, J. Wang, Three-dimensional DNA nanomachine biosensor by integrating DNA walker and rolling machine cascade amplification for ultrasensitive detection of cancer-related gene, Anal. Chem. 92 (2020) 11111–11118.
[2] C. Angell, M. Kai, S. Xie, X. Dong, Y. Chen, Bioderived DNA nanomachines for potential uses in biosensing, diagnostics, and therapeutic applications, Adv. Healthc. Mater. 7 (2018).
[3] M. Beissenhirtz, I. Willner, DNA-based machines, Org. Biomol. Chem. 4 (2006) 3392–3401.
[4] F. Wang, B. Willner, I. Willner, A. Credi, S. Silvi, M. Venturi, DNA-based machines, Mol. Mach. Motors 354 (2014) 279–338.
[5] S. Nummelin, B. Shen, P. Piskunen, Q. Liu, M. Kostiainen, V. Linko, Robotic DNA nanostructures, ACS Synth. Biol. 9 (2020) 1923–1940.
[6] R. Yue, Z. Li, G. Wang, J. Li, N. Ma, Logic sensing of microRNA in living cells using DNA-programmed nanoparticle network with high signal gain, ACS Sens. 4 (2019) 250–256.
[7] B. Adinolfi, M. Pellegrino, A. Giannetti, S. Tombelli, C. Trono, G. Sotgiu, G. Varchi, M. Ballestri, T. Posati, S. Carpi, P. Nieri, F. Baldini, Molecular beacon-decorated polymethylmethacrylate core-shell fluorescent nanoparticles for the detection of survivin mRNA in human cancer cells, Biosens. Bioelectron. 88 (2017) 15–24.
[8] Y. Ma, Z. Wang, M. Zhang, Z. Han, D. Chen, Q. Zhu, W. Gao, Z. Qian, Y. Gu, A telomerase-specific doxorubicin-releasing molecular beacon for cancer theranostics, Angew. Chem.-Int. Ed. 55 (2016) 3304–3308.
[9] A. Ellington, J. Szostak, In vitro selection of RNA molecules that bind specific ligands, Nature 346 (1990) 818–822.
[10] C. Tuerk, L. Gold, Systematic evolution of ligands by exponential enrichment – RNA ligands to bacteriophage-t4 DNA-polymerase, Science 249 (1990) 505–510.
[11] M. Sola, A. Menon, B. Moreno, D. Meraviglia-Crivelli, M. Soldevilla, F. Carton-Garcia, F. Pastor, Aptamers against live targets: is in vivo SELEX finally coming to the edge? Mol. Ther.-Nucleic Acids 21 (2020) 192–204.
[12] R. Cataldo, G. De Nunzio, J. Millithaler, E. Alfinito, Aptamers which target proteins: what proteotronics suggests to pharmaceutics, Curr. Pharm. Des. 26 (2020) 363–371.
[13] Y. Wu, I. Belmonte, K. Sykes, Y. Xiao, R. White, Perspective on the future role of aptamers in analytical chemistry, Anal. Chem. 91 (2019) 15335–15344.
[14] M. Mascini, I. Palchetti, S. Tombelli, Nucleic acid and peptide aptamers: fundamentals and bioanalytical aspects, Angew. Chem. – Int. Ed. 51 (2012) 1316–1332.
[15] T. Wang, C. Chen, L. Larcher, R. Barrero, R. Veedu, Three decades of nucleic acid aptamer technologies: lessons learned, progress and opportunities on aptamer development, Biotechnol. Adv. 37 (2019) 28–50.
[16] E. Oteng, W. Gu, M. McKeague, High-efficiency enrichment enables identification of aptamers to circulating Plasmodium falciparum-infected erythrocytes, Sci. Rep. 10 (2020) 9706.
[17] N. Komarova, A. Kuznetsov, Inside the black box: what makes SELEX better? Molecules 24 (2019) 3598.
[18] H. Wang, H. Cheng, J. Wang, L. Xu, H. Chen, R. Pei, Selection and characterization of DNA aptamers for the development of light-up biosensor to detect Cd(II), Talanta 154 (2016) 498–503.
[19] M. Saad, D. Chinerman, M. Tabrizian, S. Faucher, Identification of two aptamers binding to Legionella pneumophila with high affinity and specificity, Sci. Rep. 10 (2020) 9145.
[20] J. Han, L. Gao, J. Wang, J. Wang, Application and development of aptamer in cancer: from clinical diagnosis to cancer therapy, J. Cancer 11 (2020) 6902–6915.
[21] M. Liu, A. Khan, Z. Wang, Y. Liu, G. Yang, Y. Deng, N. He, Aptasensors for pesticide detection, Biosens. Bioelectron. 130 (2019) 174–184.
[22] M.Shorie Priyanka, V. Bhalla, P. Pathania, C. Suri, Nanobioprobe mediated DNA aptamers for explosive detection, Chem. Commun. 50 (2014) 1080–1082.
[23] S. Tombelli, A. Minunni, A. Mascini, Analytical applications of aptamers, Biosens. Bioelectron. 20 (2005) 2424–2434.
[24] C. Roxo, W. Kotkowiak, A. Pasternak, G-Quadruplex-forming aptamers-characteristics, applications, and perspectives, Molecules 24 (2019) 3781.
[25] J. Li, H. Fu, L. Wu, A. Zheng, G. Chen, H. Yang, General colorimetric detection of proteins and small molecules based on cyclic enzymatic signal amplification and hairpin aptamer probe, Anal. Chem. 84 (2012) 5309–5315.
[26] B. Jiang, F. Li, C. Yang, J. Xie, Y. Xiang, R. Yuan, Aptamer pseudoknot-functionalized electronic sensor for reagent less and single-step detection of immunoglobulin E in human serum, Anal. Chem. 87 (2015) 3094–3098.
[27] F. Darfeuille, S. Reigadas, J. Hansen, H. Orum, C. Di Primo, J. Toulme, Aptamers targeted to an RNA hairpin show improved specificity compared to that of complementary oligonucleotides, Biochemistry 45 (2006) 12076–12082.
[28] K. Leung, B. He, C. Yang, C. Leung, H. Wang, D. Ma, Development of an aptamer-based sensing platform for metal ions, proteins, and small molecules through terminal deoxynucleotidyl transferase induced G-quadruplex formation, ACS Appl. Mater. Interfaces 7 (2015) 24046–24052.
[29] C. Cao, F. Zhang, E. Goldys, F. Gao, G. Liu, Advances in structure-switching aptasensing towards real time detection of cytokines, TRAC-Trends Anal. Chem. 102 (2018) 379–396.
[30] A. Moutsiopoulou, D. Broyles, E. Dikici, S. Daunert, S. Deo, Molecular aptamer beacons and their applications in sensing, imaging, and diagnostics, Small 15 (2019) 1902248.
[31] B. Wilson, A. Hariri, I. Thompson, M. Eisenstein, H. Soh, Independent control of the thermodynamic and kinetic properties of aptamer switches, Nat. Commun. 10 (2019) 5079.
[32] A. Giannetti, S. Tombelli, F. Baldini, Oligonucleotide optical switches for intracellular sensing optical nanosensing in cells, Anal. Bioanal. Chem. 405 (2013) 6181–6196.
[33] S. Tyagi, F. Kramer, Molecular beacons: Probes that fluoresce upon hybridization, Nat. Biotechnol. 14 (1996) 303–308.

[34] A. Tsourkas, M. Behlke, S. Rose, G. Bao, Hybridization kinetics and thermodynamics of molecular beacons, Nucleic Acids Res. 31 (2003) 1319–1330.

[35] B. Deng, Y. Lin, C. Wang, F. Li, Z. Wang, H. Zhang, X. Li, X. Le, Aptamer binding assays for proteins: the thrombin example-a review, Anal. Chim. Acta 837 (2014) 1–15.

[36] C. Wang, L. Sun, Q. Zhao, A simple aptamer molecular beacon assay for rapid detection of aflatoxin B1, Chin. Chem. Lett. 30 (2019) 1017–1020.

[37] A. Chen, M. Yan, S. Yang, Split aptamers and their applications in sandwich aptasensors, TRAC-Trends Anal. Chem. 80 (2016) 581–593.

[38] M. Stojanovic, P. de Prada, D. Landry, Fluorescent sensors based on aptamer self-assembly, J. Am. Chem. Soc. 122 (2000) 11547–11548.

[39] X. Weng, S. Neethirajan, A microfluidic biosensor using graphene oxide and aptamer-functionalized quantum dots for peanut allergen detection, Biosens. Bioelectron. 85 (2016) 649–656.

[40] J. Li, X. Fang, W. Tan, Molecular aptamer beacons for real-time protein recognition, Biochem. Biophys. Res. Commun. 292 (2002) 31–40.

[41] N. Hamaguchi, A. Ellington, M. Stanton, Aptamer beacons for the direct detection of proteins, Anal. Biochem. 294 (2001) 126–131.

[42] J. Tok, J. Lai, T. Leung, S. Li, Molecular aptamer beacon for myotonic dystrophy kinase-related Cdc42-binding kinase alpha, Talanta 81 (2010) 732–736.

[43] R. Yamamoto, P. Kumar, Molecular beacon aptamer fluoresces in the presence of Tat protein of HIV-1, Genes Cells 5 (2000) 389–396.

[44] R. Sharma, U. Akshath, P. Bhatt, K. Raghavarao, Fluorescent aptaswitch for chloramphenicol detection – quantification enabled by immobilization of aptamer, Sens. Actuators B-Chem. 290 (2019) 110–117.

[45] X. Ma, S. Qiao, H. Sun, R. Su, C. Sun, M. Zhang, Development of structure-switching aptamers for kanamycin detection based on fluorescence resonance energy transfer, Front. Chem. 7 (2019) 29.

[46] Y. Bai, Q. Zhao, Rapid fluorescence detection of immunoglobulin E using an aptamer switch based on a binding-induced pyrene excimer, Anal. Methods 9 (2017) 3962–3967.

[47] Z. Lu, W. Xiong, P. Wang, X. Li, K. Zhai, R. Shi, D. Xiang, Simultaneous detection of lead (II) and mercury (II) ions using nucleic acid aptamer molecular beacons, Int. J. Environ. Anal. Chem. (2019), doi: 10.1080/03067319.2019.1691183.

[48] J. Wang, Y. Wang, X. Hu, C. Zhu, Q. Ma, L. Liang, Z. Li, Q. Yuan, Dual-aptamer-conjugated molecular modulator for detecting bioactive metal ions and inhibiting metal-mediated protein aggregation, Anal. Chem. 91 (2019) 823–829.

[49] I. Thompson, L. Zheng, M. Eisenstein, H. Soh, Rational design of aptamer switches with programmable pH response, Nat. Commun. 11 (2020) 2946.

[50] A. Porchetta, A. Idili, A. Vallee-Belisle, F. Ricci, General strategy to introduce pH-induced allostery in DNA-based receptors to achieve controlled release of ligands, Nano Lett. 15 (2015) 4467–4471.

[51] E. McConnell, R. Bolzon, P. Mezin, G. Frahm, M. Johnston, M. DeRosa, pHAST (pH-Driven Aptamer Switch for Thrombin) catch-and-release of target protein, Bioconjug. Chem. 27 (2016) 1493–1499.

[52] L. Li, Y. Jiang, C. Cui, Y. Yang, P. Zhang, K. Stewart, X. Pan, X. Li, L. Yang, L. Qiu, W. Tan, Modulating aptamer specificity with pH-responsive DNA bonds, J. Am. Chem. Soc. 140 (2018) 13335–13339.

[53] S. Ng, H. Lim, Q. Ma, Z. Gao, Optical aptasensors for adenosine triphosphate, Theranostics 6 (2016) 1683–1702.

[54] S. Zhang, Y. Yan, S. Bi, Design of molecular beacons as signaling probes for adenosine triphosphate detection in cancer cells based on chemiluminescence resonance energy transfer, Anal. Chem. 81 (2009) 8695–8701.

[55] A. Davydova, V. Krasitskaya, P. Vorobjev, V. Timoshenko, A. Tupikin, M. Kabilov, L. Frank, A. Venyaminova, M. Vorobyeva, Reporter-recruiting bifunctional aptasensor for bioluminescent analytical assays, RSC Adv. 10 (2020) 32393–32399.

[56] R. Freeman, J. Girsh, A. Jou, J. Ho, T. Hug, J. Dernedde, I. Willner, Optical aptasensors for the analysis of the Vascular Endothelial Growth Factor (VEGF), Anal. Chem. 84 (2012) 6192–6198.

[57] S. Sahin, M. Caglayan, Z. Ustundag, Recent advances in aptamer-based sensors for breast cancer diagnosis: special cases for nanomaterial-based VEGF, HER2, and MUC1 aptasensors, Microchim. Acta 187 (2020) 549.

[58] A. Moutsiopoulou, D. Broyles, H. Joda, E. Dikici, A. Kaur, A. Kaifer, S. Daunert, S. Deo, Bioluminescent protein-inhibitor pair in the design of a molecular aptamer beacon biosensing system, Anal. Chem. 92 (2020) 7393–7398.

[59] K. Sergelen, S. Fossati, A. Turupcu, C. Oostenbrink, B. Liedberg, W. Knoll, J. Dostalek, Plasmon field-enhanced fluorescence energy transfer for hairpin aptamer assay readout, ACS Sens. 2 (2017) 916–923.

[60] A. Wood, S. Basuray, S. Bok, K. Gangopadhyay, S. Gangopadhyay, S. Grant, Enhanced DNA detection through the incorporation of nanocones and cavities into a plasmonic grating sensor platform, IEEE Sens. J. 16 (2016) 3403–3408.

[61] K. Sergelen, B. Liedberg, W. Knoll, J. Dostalek, A surface plasmon field-enhanced fluorescence reversible split aptamer biosensor, Analyst 142 (2017) 2995–3001.

[62] T. Xie, Q. Liu, W. Cai, Z. Chen, Y. Li, Surface plasmon-coupled directional emission based on a conformational-switching signaling aptamer, Chem. Commun. (2009) 3190–3192.

[63] Q. Ren, L. Ga, Z. Lu, J. Ai, T. Wang, Aptamer-functionalized nanomaterials for biological applications, Mater. Chem. Front. 4 (2020) 1569–1585.

[64] Z. Pehlivan, M. Torabfam, H. Kurt, C. Ow-Yang, N. Hildebrandt, M. Yuce, Aptamer and nanomaterial based FRET biosensors: a review on recent advances (2014–2019), Microchim. Acta 186 (2019) 563.

[65] D. Lu, L. He, G. Zhang, A. Lv, R. Wang, X. Zhang, W. Tan, Aptamer-assembled nanomaterials for fluorescent sensing and imaging, Nanophotonics 6 (2017) 109–121.

[66] C. Yang, S. Jockusch, M. Vicens, N. Turro, W. Tan, Light-switching excimer probes for rapid protein monitoring in complex biological fluids, Proc. Natl. Acad. Sci. USA 102 (2005) 17278–17283.

[67] Z. Lu, X. Chen, W. Hu, A fluorescence aptasensor based on semiconductor quantum dots and MoS2 nanosheets for ochratoxin A detection, Sens. Actuators B-Chem. 246 (2017) 61–67.

[68] Y. Yang, Y. Cen, W. Deng, R. Yu, T. Chen, X. Chu, An aptasensor based on cobalt oxyhydroxide nanosheets for the detection of thrombin, Anal. Methods 8 (2016) 7199–7203.

[69] X. Zhang, Z. Lai, C. Tan, H. Zhang, Solution-processed two-dimensional MoS2 nanosheets: preparation, hybridization, and applications, Angew. Chem.-Int. Ed. 55 (2016) 8816–8838.

[70] G. Li, W. Kong, M. Zhao, S. Lu, P. Gong, G. Chen, L. Xia, H. Wang, J. You, Y. Wu, A fluorescence resonance energy transfer (FRET) based "Turn-On" nanofluorescence sensor using a nitrogen-doped carbon dot-hexagonal cobalt oxyhydroxide nanosheet architecture and application to alpha-glucosidase inhibitor screening, Biosens. Bioelectron. 79 (2016) 728–735.

[71] J. Tian, W. Wei, J. Wang, S. Ji, G. Chen, J. Lu, Fluorescence resonance energy transfer aptasensor between nanoceria and graphene quantum dots for the determination of ochratoxin A, Anal. Chim. Acta 1000 (2018) 265–272.

[72] F. Tian, J. Zhou, R. Fu, Y. Cui, Q. Zhao, B. Jiao, Y. He, Multicolor colorimetric detection of ochratoxin A via structure-switching aptamer and enzyme-induced metallization of gold nanorods, Food Chem. 320 (2020) 126607.

[73] J. Chen, Y. Li, Y. Huang, H. Zhang, X. Chen, H. Qiu, Fluorometric dopamine assay based on an energy transfer system composed of aptamer-functionalized MoS2 quantum dots and MoS2 nanosheets, Microchim. Acta 186 (2019) 58.

[74] Z. Saberi, B. Rezaei, H. Faroukhpour, A. Ensafi, A fluorometric aptasensor for methamphetamine based on fluorescence resonance energy transfer using cobalt oxyhydroxide nanosheets and carbon dots, Microchim. Acta 185 (2018) 303.

[75] Y. Zhang, C. Li, L. Zhou, Z. Chen, C. Yi, Plug and Play" logic gate construction based on chemically triggered fluorescence switching of gold nanoparticles conjugated with Cy3-tagged aptamer, Microchim. Acta 187 (2020) 437.

[76] F. Qu, C. Sun, X. Lv, J. You, A terbium-based metal-organic framework@gold nanoparticle system as a fluorometric probe for aptamer based determination of adenosine triphosphate, Microchim. Acta 185 (2018) 359.

[77] C. Sun, S. Zhao, F. Qu, W. Han, J. You, Determination of adenosine triphosphate based on the use of fluorescent terbium(III) organic frameworks and aptamer modified gold nanoparticles, Microchim. Acta 187 (2020) 34.

[78] T. Suo, M. Sohail, Y. Ma, B. Li, Y. Chen, X. Zhang, X. Zhou, A versatile turn-on fluorometric biosensing profile based on split aptamers-involved assembly of nanocluster beacon sandwich, Sens. Actuators B-Chem. 324 (2020) 128586.

[79] K. Abnous, N. Danesh, M. Ramezani, S. Taghdisi, A. Emrani, A novel amplified double-quenching aptasensor for cocaine detection based on split aptamer and silica nanoparticles, Anal. Methods 10 (2018) 3232–3236.

[80] A. Locke, S. Belsare, N. Deutz, G. Cote, Aptamer-switching optical bioassay for citrulline detection at the point-of-care, J. Biomed. Opt. 24 (2019) 1–6.

[81] Q.-L. Wang, H.-F. Cui, C.-L. Li, X. Song, Q.-Y. Lv, Z.-Y. Li, A multimode aptasensor based on hollow gold nanoparticles and structure switching of aptamer: fast and sensitive detection of carcinoembryonic antigen, Sens. Actuators Rep. 2 (2020) 100021.

[82] X. Lu, C. Wang, J. Qian, C. Ren, K. An, K. Wang, Target-driven switch-on fluorescence aptasensor for trace aflatoxin B1 determination based on highly fluorescent ternary CdZnTe quantum dots, Anal. Chim. Acta 1047 (2019) 163–171.

[83] N. Yin, S. Yuan, M. Zhang, J. Wang, Y. Li, Y. Peng, J. Bai, B. Ning, J. Liang, Z. Gao, An aptamer-based fluorometric zearalenone assay using a lighting-up silver nanocluster probe and catalyzed by a hairpin assembly, Microchim. Acta 186 (2019) 765.

[84] M. Liu, Z. Yang, B. Li, J. Du, Aptamer biorecognition-triggered hairpin switch and nicking enzyme assisted signal amplification for ultrasensitive colorimetric bioassay of kanamycin in milk, Food Chem. 339 (2021) 128059.

[85] K. Fan, W. Kang, S. Qu, L. Li, B. Qu, L. Lu, A label-free and enzyme-free fluorescent aptasensor for sensitive detection of acetamiprid based on AT-rich dsDNA-templated copper nanoparticles, Talanta 197 (2019) 645–652.

[86] A. Ravikumar, P. Panneerselvam, K. Radhakrishnan, A. Christus, S. Sivanesan, MoS2 nanosheets as an effective fluorescent quencher for successive detection of arsenic ions in aqueous system, Appl. Surf. Sci. 449 (2018) 31–38.

[87] Y. Li, Q. Ouyang, H. Li, M. Chen, Z. Zhan, Q. Chen, Turn-on fluoresence sensor for Hg2 + in food based on FRET between aptamers-functionalized upconversion nanoparticles and gold nanoparticles, J. Agric. Food Chem. 66 (2018) 6188–6195.

[88] C. Zhu, Z. Zeng, H. Li, F. Li, C. Fan, H. Zhang, Single-layer MoS2-based nanoprobes for homogeneous detection of biomolecules, J. Am. Chem. Soc. 135 (2013) 5998–6001.

[89] P. Singh, R. Gupta, M. Sinha, R. Kumar, V. Bhalla, MoS2 based digital response platform for aptamer based fluorescent detection of pathogens, Microchim. Acta 183 (2016) 1501–1506.

[90] T. Vo-Dinh, A. Fales, G. Griffin, C. Khoury, Y. Liu, H. Ngo, S. Norton, J. Register, H. Wang, H. Yuan, Plasmonic nanoprobes: from chemical sensing to medical diagnostics and therapy, Nanoscale 5 (2013) 10127–10140.

[91] O. Clarke, B. Goodall, H. Hui, N. Vats, C. Brosseau, Development of a SERS-based rapid vertical flow assay for point-of care diagnostics, Anal. Chem. 89 (2017) 1405–1410.

[92] G. Ji, J. Liu, X. Gao, W. Sun, J. Wang, S. Zhao, Z. Liu, A luminescent lanthanide MOF for selectively and ultra-high sensitively detecting Pb^{2+} ions in aqueous solution, J. Mater. Chem. A 5 (2017) 10200–10205.

[93] W. Xu, X. Chen, H. Song, Upconversion manipulation by local electromagnetic field, Nano Today 17 (2017) 54–78.

[94] Y. Chen, M. Phipps, J. Werner, S. Chakraborty, J. Martinez, DNA templated metal nanoclusters: from emergent properties to unique applications, Acc. Chem. Res. 51 (2018) 2756–2763.

[95] S. Yau, N. Abeyasinghe, M. Orr, L. Upton, O. Varnavski, J. Werner, H. Yeh, J. Sharma, A. Shreve, J. Martinez, T. Goodson, Bright two-photon emission and ultra-fast relaxation dynamics in a DNA-templated nanocluster investigated by ultra-fast spectroscopy, Nanoscale 4 (2012) 4247–4254.
[96] R. Gupta, A. Kumar, S. Kumar, A.K. Pinnaka, N.K. Singhal, Naked eye colorimetric detection of Escherichia coli using aptamer conjugated graphene oxide enclosed Gold nanoparticles, (2020) 129100.
[97] M. Debiais, A. Lelievre, M. Smietana, S. Muller, Splitting aptamers and nucleic acid enzymes for the development of advanced biosensors, Nucleic Acids Res. 48 (2020) 3400–3422.
[98] B. Liu, S. Salgado, V. Maheshwari, J. Liu, DNA adsorbed on graphene and graphene oxide: fundamental interactions, desorption and applications, Curr. Opin. Colloid Interface Sci. 26 (2016) 41–49.
[99] X. Wu, F. Mu, Y. Wang, H. Zhao, Graphene and graphene-based nanomaterials for DNA detection: a review, Molecules 23 (2018) 2050.
[100] M. Thangamuthu, K. Hsieh, P. Kumar, G. Chen, Graphene- and graphene oxide-based nanocomposite platforms for electrochemical biosensing applications, Int. J. Mol. Sci. 20 (2019) 2975.
[101] M. Pumera, Graphene in biosensing, Mater. Today 14 (2011) 308–315.
[102] M. Rahaie, S. Noroozi, A nanobiosensor based on graphene oxide and DNA binding dye for multi-microRNAs detection, Biosci. Rep. 39 (2019) BSR20181404.
[103] A. Huang, L. Zhang, W. Li, Z. Ma, S. Shuo, T. Yao, Controlled fluorescence quenching by antibody-conjugated graphene oxide to measure tau protein, R. Soc. Open Sci. 5 (2018) 171808.
[104] Q. Mei, B. Liu, G. Han, R. Liu, M. Han, Z. Zhang, Graphene oxide: from tunable structures to diverse luminescence behaviors, Adv. Sci. 6 (2019) 1900855.
[105] H. Youn, K. Lee, J. Her, J. Jeon, J. Mok, J. So, S. Shin, C. Ban, Aptasensor for multiplex detection of antibiotics based on FRET strategy combined with aptamer/graphene oxide complex, Sci. Rep. 9 (2019) 7659.
[106] H. Zhao, S. Gao, M. Liu, Y. Chang, X. Fan, X. Quan, Fluorescent assay for oxytetracycline based on a long-chain aptamer assembled onto reduced graphene oxide, Microchim. Acta 180 (2013) 829–835.
[107] Y. Rong, H. Li, Q. Ouyang, S. Ali, Q. Chen, Rapid and sensitive detection of diazinon in food based on the FRET between rare-earth doped upconversion nanoparticles and graphene oxide, Spectrochim. Acta Part A-Mol. Biomol. Spectrosc. 239 (2020) 118500.
[108] W. Xiang, Z. Zhang, W. Weng, B. Wu, J. Cheng, L. Shi, H. Sun, L. Gao, K. Shi, Highly sensitive detection of carcinoembryonic antigen using copper-free click chemistry on the surface of azide cofunctionalized graphene oxide, Anal. Chim. Acta 1127 (2020) 156–162.
[109] Y. Wang, Z. Wei, X. Luo, Q. Wan, R. Qiu, S. Wang, An ultrasensitive homogeneous aptasensor for carcinoembryonic antigen based on upconversion fluorescence resonance energy transfer, Talanta 195 (2019) 33–39.
[110] K. Ling, H. Jiang, Y. Li, X. Tao, C. Qiu, F. Li, A self-assembling RNA aptamer-based graphene oxide sensor for the turn-on detection of theophylline in serum, Biosens. Bioelectron. 86 (2016) 8–13.
[111] S. Manochehry, M. Liu, D. Chang, Y. Li, Optical biosensors utilizing graphene and functional DNA molecules, J. Mater. Res. 32 (2017) 2973–2983.
[112] D. Huizenga, J. Szostak, A DNA aptamer that binds adenosine and ATP, Biochemistry 34 (1995) 656–665.
[113] L. Green, D. Jellinek, R. Jenison, A. Ostman, C. Heldin, N. Janjic, Inhibitory DNA ligands to platelet-derived growth factor B-chain, Biochemistry 35 (1996) 14413–14424.
[114] X. Cheng, Y. Cen, G. Xu, F. Wei, M. Shi, X. Xu, M. Sohail, Q. Hu, Aptamer based fluorometric determination of ATP by exploiting the FRET between carbon dots and graphene oxide, Microchim. Acta 185 (2018) 144.
[115] M. Wang, Z. Lin, Q. Liu, S. Jiang, H. Liu, X. Su, DNA-hosted copper nanoclusters/graphene oxide based fluorescent biosensor for protein kinase activity detection, Anal. Chim. Acta 1012 (2018) 66–73.
[116] Y. Ning, K. Wei, L. Cheng, J. Hu, Q. Xiang, Fluorometric aptamer based determination of adenosine triphosphate based on deoxyribonuclease I-aided target recycling and signal amplification using graphene oxide as a quencher, Microchim. Acta 184 (2017) 1847–1854.
[117] M. Lin, Y. Lu, A. Grumezescu, F. Ho, Y. Kao, Y. Yang, C. Yang, Tumor marker detection by aptamer-functionalized graphene oxide, Curr. Org. Chem. 17 (2013) 132–136.
[118] Y. Lou, Y. Peng, X. Luo, Z. Yang, R. Wang, D. Sun, L. Li, Y. Tan, J. Huang, L. Cui, A universal aptasensing platform based on cryonase-assisted signal amplification and graphene oxide induced quenching of the fluorescence of labeled nucleic acid probes: application to the detection of theophylline and ATP, Microchim. Acta 186 (2019) 494.
[119] H. Yang, Y. Zhou, J. Liu, G-quadruplex DNA for construction of biosensors, TRAC-Trends Anal. Chem. 132 (2020) 116060.
[120] R. Macaya, P. Schultze, F. Smith, J. Roe, J. Feigon, Thrombin-binding DNA aptamer forms a unimolecular quadruplex structure in solution, Proc. Natl. Acad. Sci. USA 90 (1993) 3745–3749.
[121] Z. Huang, Z. Luo, J. Chen, Y. Xu, Y. Duan, A Facile, Label-free, and universal biosensor platform based on target-induced graphene oxide constrained DNA dissociation coupling with improved strand displacement amplification, ACS Sens. 3 (2018) 2423–2431.
[122] H. Guo, J. Li, Y. Li, D. Wu, H. Ma, Q. Wei, B. Du, A turn-on fluorescent sensor for Hg2+ detection based on graphene oxide and DNA aptamers, New J. Chem. 42 (2018) 11147–11152.
[123] J. Jeon, J. Lee, J. So, H. Lee, Y. Chang, S. Shin, J. Jo, C. Ban, Homogeneous fluorescent aptasensor for active tuberculosis diagnosis by direct quantification of circulating TB7.7 based on aptamer beacon with graphene oxide, Sens. Actuators B-Chem. 317 (2020) 128126.
[124] B. Li, C. Liu, W. Pan, J. Shen, J. Guo, T. Luo, J. Feng, B. Situ, T. An, Y. Zhang, L. Zheng, Facile fluorescent aptasensor using aggregation-induced emission luminogens for exosomal proteins profiling towards liquid biopsy, Biosens. Bioelectron. 168 (2020) 112520.
[125] X. Shao, L. Zhu, Y. Feng, Y. Zhang, Y. Luo, K. Huang, W. Xu, Detachable nanoladders: a new method for signal identification and their application in the detection of ochratoxin A (OTA), Anal. Chim. Acta 1087 (2019) 113–120.
[126] D. Samanta, S. Ebrahimi, C. Mirkin, Nucleic-acid structures as intracellular probes for live cells, Adv. Mater. 32 (2020) 1901743.
[127] L. Truong, A. Ferre-DAmare, From fluorescent proteins to fluorogenic RNAs: tools for imaging cellular macromolecules, Protein Sci. 28 (2019) 1374–1386.
[128] L. Nielsen, L. Olsen, V. Ozalp, Aptamers embedded in polyacrylamide nanoparticles: a tool for in vivo metabolite sensing, ACS Nano 4 (2010) 4361–4370.
[129] Y. Liang, Z. Zhang, H. Wei, Q. Hu, J. Deng, D. Guo, Z. Cui, X. Zhang, Aptamer beacons for visualization of endogenous protein HIV-1 reverse transcriptase in living cells, Biosens. Bioelectron. 28 (2011) 270–276.
[130] L. Qiu, C. Wu, M. You, D. Han, T. Chen, G. Zhu, J. Jiang, R. Yu, W. Tan, A Targeted, Self-delivered, and photocontrolled molecular beacon for mRNA detection in living cells, J. Am. Chem. Soc. 135 (2013) 12952–12955.
[131] X. Zheng, R. Peng, X. Jiang, Y. Wang, S. Xu, G. Ke, T. Fu, Q. Liu, S. Huan, X. Zhang, Fluorescence resonance energy transfer-based DNA nanoprism with a split aptamer for adenosine triphosphate sensing in living cells, Anal. Chem. 89 (2017) 10941–10947.
[132] Y. Xu, Q. Kang, B. Yang, B. Chen, M. He, B. Hu, A nanoprobe based on molybdenum disulfide nanosheets and silver nanoclusters for imaging and quantification of intracellular adenosine triphosphate, Anal. Chim. Acta 1134 (2020) 75–83.
[133] S. Wang, J. Ding, W. Zhou, An aptamer-tethered, DNAzyme-embedded molecular beacon for simultaneous detection and regulation of tumor-related genes in living cells, Analyst 144 (2019) 5098–5107.
[134] Z. Zhao, H. Fan, G. Zhou, H. Bai, H. Liang, R. Wang, X. Zhang, W. Tan, Activatable fluorescence/MRI bimodal platform for tumor cell imaging via MnO_2 nanosheet-aptamer nanoprobe, J. Am. Chem. Soc. 136 (2014) 11220–11223.
[135] C. Wu, T. Chen, D. Han, M. You, L. Peng, S. Cansiz, G. Zhu, C. Li, X. Xiong, E. Jimenez, C. Yang, W. Tan, Engineering of switchable aptamer micelle flares for molecular imaging in living cells, ACS Nano 7 (2013) 5724–5731.
[136] J. Zhang, X. Han, Y. Lin, Dissecting the regulation and function of ATP at the single-cell level, PLoS Biol. 16 (2018) e3000095.
[137] Z. Wang, J. Wang, W. Shan, F. Zheng, C. Niu, C. Liu, Q. Li, Intracellular Adenosine Triphosphate (ATP) content sensitively reflects subtle differences in yeast physiology, J. Am. Soc. Brew. Chem. 77 (2019) 92–98.
[138] X. Xue, F. Wang, J. Zhou, F. Chen, Y. Li, J. Zhao, Online cleanup of accelerated solvent extractions for determination of Adenosine 5 '-Triphosphate (ATP), Adenosine 5 '-Diphosphate (ADP), and Adenosine 5 '-Monophosphate (AMP) in royal jelly using high-performance liquid chromatography, J. Agric. Food Chem. 57 (2009) 4500–4505.
[139] L. Wang, L. Yuan, X. Zeng, J. Peng, Y. Ni, J. Er, W. Xu, B. Agrawalla, D. Su, B. Kim, Y. Chang, A multisite-binding switchable fluorescent probe for monitoring mitochondrial ATP level fluctuation in live cells, Angew. Chem.-Int. Ed. 55 (2016) 1773–1776.
[140] K. Tan, C. Li, Y. Li, J. Fei, B. Yang, Y. Fu, F. Li, Real-time monitoring ATP in mitochondrion of living cells: a specific fluorescent probe for ATP by dual recognition sites, Anal. Chem. 89 (2017) 1749–1756.
[141] Z. Wang, Y. Zhang, J. Yin, M. Li, H. Luo, Y. Yang, X. Xu, Q. Yong, S. Wang, An easily available camphor-derived ratiometric fluorescent probe with AIE feature for sequential Ga^{3+} and ATP sensing in a near-perfect aqueous media and its bio-imaging in living cells and mice, Sens. Actuators B-Chem. 320 (2020) 128249.
[142] T. Yoshida, A. Kakizuka, H. Imamura, BTeam, a Novel BRET-based biosensor for the accurate quantification of ATP concentration within living cells, Sci. Rep. 6 (2016) 39618.
[143] Z. Wu, M. Liu, Z. Liu, Y. Tian, Real-time imaging and simultaneous quantification of mitochondrial H_2O_2 and ATP in neurons with a single two-photon fluorescence-lifetime-based probe, J. Am. Chem. Soc. 142 (2020) 7532–7541.
[144] J. Dong, M. Zhao, In-vivo fluorescence imaging of adenosine 5 '-triphosphate, TRAC-Trends Anal. Chem. 80 (2016) 190–203.
[145] Y. Biniuri, G. Luo, M. Fadeev, V. Wulf, I. Willner, Redox-switchable binding properties of the ATP-aptamer, J. Am. Chem. Soc. 141 (2019) 15567–15576.
[146] T. Xia, J. Yuan, X. Fang, Conformational dynamics of an ATP-binding DNA aptamer: a single-molecule study, J. Phys. Chem. B 117 (2013) 14994–15003.
[147] B. Liu, R. Ma, J. Zhao, Y. Zhao, L. Li, A smart DNA nanodevice for ATP-activatable bioimaging and photodynamic therapy, Sci. China-Chem. 63 (2020) 1490–1497.
[148] J. Carvalho, A. Paiva, M. Campello, A. Paulo, J. Mergny, G. Salgado, J. Queiroz, C. Cruz, Aptamer-based targeted delivery of a G-quadruplex ligand in cervical cancer cells, Sci. Rep. 9 (2019) 7945.
[149] S. Ghosh, Y. Chen, A. George, M. Dutta, M.A. Stroscio, Fluorescence resonant energy transfer-based quantum dot sensor for the detection of calcium ions, Front. Chem. 8 (2020) 594.