DE GRUYTER BioNanoMat 2017; 18(1-2): 20160012

Review Open Access

Katharina Urmann[a], Julia Modrejewski[a], Thomas Scheper and Johanna-G. Walter*

Aptamer-modified nanomaterials: principles and applications

DOI 10.1515/bnm-2016-0012
Received May 31, 2016; accepted July 27, 2016; previously published online August 31, 2016

Abstract: Aptamers are promising alternative binders that can substitute antibodies in various applications. Due to the advantages of aptamers, namely their high affinity, specificity and stability, along with the benefits originating from the chemical synthesis of aptamers, they have attracted attention in various applications including their use on nanostructured material. This necessitates the immobilization of aptamers on a solid support. Since aptamer immobilization may interfere with its binding properties, the immobilization of aptamers has to be investigated and optimized. Within this review, we give general insights into the principles and factors controlling the binding affinity of immobilized aptamers. Specific features of aptamer immobilization on nanostructured surfaces and nanoparticles are highlighted and a brief overview of applications of aptamer-modified nanostructured materials is given.

Keywords: applications; aptamer; immobilization; nanomaterial; nanoparticle.

Introduction

Aptamers are synthetic short single stranded oligonucleotides composed of DNA or RNA. Based on their unique three-dimensional structure, aptamers exhibit specific binding to their corresponding target molecule, which can be a small molecule, a macromolecule, or a complete cell. Due to this specificity and their high affinity, aptamers can be used to substitute antibodies in different applications. In comparison to antibodies, aptamers offer several advantages which are mainly based on their in-vitro generation and their oligonucleotide nature: aptamers are selected in an in-vitro process termed systematic evolution of ligands by exponential enrichment (SELEX) [1]. Due to this animal-free process, aptamers can be selected to exhibit binding of the target under non-physiological conditions and the selection of aptamers is also possible for highly toxic or non-immunogenic molecules [2]. Once aptamers are selected and their sequence is revealed, they can be produced by chemical synthesis, a process not only resulting in high and consistent product quality, but also facilitating the precise introduction of labels or other modifications at defined positions within the aptamer sequence.

Aptamers have already been applied successfully e.g. for the detection of proteins and small molecules [3, 4], the purification of proteins [5–7] and depletion of small molecules [8], as well as in cell targeting and drug delivery [9–11]. In most of the developed aptamer-based methods, the aptamer has to be immobilized on a solid support, which might be a nano-structured surface. Aptamer binding to the corresponding target molecule depends on the correct three-dimensional folding of the aptamer [12]. Therefore, it is crucial to immobilize aptamers without affecting their ability to fold into this binding-competent structure. Within the first section of this review article we will highlight factors that may interfere with correct folding of aptamers on solid supports and give general suggestions for the immobilization of functional aptamers.

Immobilization of aptamers

As mentioned before, functional groups can be incorporated into the aptamer sequence and can subsequently be used for the immobilization of the aptamer on a solid support.

[a]**Katharina Urmann and Julia Modrejewski:** These authors contributed equally to this work.
***Corresponding author: Johanna-G. Walter,** Institut für Technische Chemie, Leibniz Universität Hannover, Callinstrasse 5, 30167 Hannover, Germany, e-mail: walter@iftc.uni-hannover.de
Katharina Urmann: Institut für Technische Chemie, Leibniz Universität Hannover, Callinstrasse 5, 30167 Hannover, Germany; and Department of Biotechnology and Food Engineering, Technion – Israel Institute of Technology, 32000 Haifa, Israel
Julia Modrejewski and Thomas Scheper: Institut für Technische Chemie, Leibniz Universität Hannover, Callinstrasse 5, 30167 Hannover, Germany

For different types of materials, different modifications can be utilized, for example the introduction of terminal thiol groups is allowing for the straight-forward immobilization of aptamers on gold surfaces. Since the chemical synthesis enables precise control of the position of functional groups, the aptamer can be immobilized in a highly controlled orientation, i.e. via one of the termini of the aptamer. This controlled orientation facilitates high binding activity by avoiding a loss of functionality resulting from immobilization in random orientation. Nonetheless, several factors influencing aptamer folding have to be carefully considered during the immobilization of aptamers and in many cases, optimization of aptamer conjugation has to be performed to obtain functional aptamer-modified surfaces [13].

Effects of immobilization to aptamer performance

In order to immobilize aptamers in a functional manner, the conjugation process must not interfere with aptamer folding [14, 15]. Here, the user has to consider that during most of the selection processes, aptamers are present free in solution. Thus, aptamers can adopt their binding-competent folding while they are in solution but might lose their binding competence after immobilization mainly due to three different factors [13]:

First, the surface may directly interfere with aptamer folding. This is especially problematic when truncated versions of the aptamer sequence are used. To overcome steric hindrance caused by too close proximity of aptamer and surface, the use of spacer molecules can be recommended. Here, rather simple spacers like polyethylenglycol moieties can be used and either be provided on the surface or fused between the aptamer sequence and the aptamer modification used for immobilization chemistry [16]. Also the elongation of the aptamer sequence, e.g. by introduction of several thymin bases, can provide additional space to allow for proper aptamer folding. One other factor that might interfere with correct folding of the aptamer is its orientation. Therefore, a screening of different aptamer orientations (3′ terminal versus 5′ terminal immobilization) may be useful to optimize aptamer performance.

The second feature of aptamers that has to be considered is their highly negative charge. Immobilizing aptamers on positively charged surfaces may result in complete unfolding of aptamers – which interact with the surface electrostatically. This can be prevented by capping of the surface [13].

Finally, the third factor influencing the folding of conjugated aptamers is the immobilization density. While generally, high immobilization densities are desired to guarantee high binding capacity for the aptamer target, too high aptamer density may prevent formation of the correct three-dimensional structure. Here, one has to consider that the immobilized aptamer must be provided with sufficient space to fold encountering no steric interference caused by neighboring aptamers. Moreover, the negative charge of aptamers can provoke electrostatic repulsion of neighboring aptamers, thereby forcing the aptamers to erect into a rather linear conformation not able to bind the target molecule. Therefore, the aptamer density, which can be easily influenced by the aptamer concentration applied during the immobilization process, has to be optimized experimentally.

Methods to investigate immobilized aptamers

As elaborated briefly in the previous subsection, several parameters including the aptamer density, aptamer orientation, surface charge, and the presence of spacers influence the performance of immobilized aptamers. Thus, methods for the investigation and optimization of aptamer conjugation are needed. Surface plasmon resonance (SPR) measurements allow for the quantitative investigation of the binding affinities of immobilized aptamers. SPR measurements are especially useful to reveal immobilization-induced reduction of aptamer affinity when they are compared with immobilization-free methods for the determination of dissociation constants such as isothermal titration calorimetry (ITC) or microscale thermophoresis (MST) [17]. The comparison of dissociation constants obtained by different methods may uncover negative effects evoked by immobilization. Nonetheless, SPR measurements suffer from a limited degree of parallelization, thus require a large set of experiments to screen different immobilization conditions and additionally require rather large amounts of aptamer and target. In our group, aptamer microarrays have shown to be a suitable alternative for the systematic investigation and optimization of aptamer immobilization [13, 16, 18]. Here, many different immobilization conditions (e.g. different aptamer orientations and immobilization densities, as well as different spacer moieties) can be screened in parallel on one single microarray. When aptamers are utilized as a receptor probe in a biosensing scheme, depending on the type and complexity, optimization of aptamer-conjugation directly within the biosensing platform may be the most suitable approach. Aptamer performance can be set in relation with the output signal and optimized accordingly.

Aptamer-modified nanostructured surfaces

Many different materials are accessible to a wide variety of surface chemistries for the attachment of biomolecules, such as aptamers. One reason for immobilization of aptamers to nanostructured surfaces specifically can be to increase the aptamer-density on the material due to higher surface area of such materials and thus increased area of interaction between aptamer and target analyte [19, 20]. Another main reason are the desirable intrinsic properties of nanostructured materials in combination with the binding characteristics of the immobilized aptamers which are opening possibilities for a variety of applications. In the following chapter, we will discuss some of the main considerations when conjugating aptamers to nanomaterials and present a number of applications with their corresponding materials, where such concepts were realized in an outstanding manner.

Special considerations for aptamer immobilization on nanostructured surfaces

Nanomaterials and nanostructured materials of different kinds have recently gained increased attention for their application in concert with aptamer-receptors tethered to their surface [21–23]. Applications thereof, see Tables 1 and 2, can mainly be found in the field of biosensors and for the capture and purification of cellular targets (e.g. cancer cells, bacteria cells). However, in contrast to immobilization of oligonucleotides on planar surfaces, aptamer-conjugation to nanomaterials requires a number of additional considerations which are discussed in the following.

Increased immobilization-density of aptamers conjugated to a surface (i.e. by means of larger surface area in nanomaterials), also brings the risk of higher steric hindrance effects, commonly occurring [4, 13, 38, 39]. This phenomenon was recently studied by Daniel et al. on a planar gold-coated prism for SPR measurements with the thrombin-binding aptamer as model [39]. The researchers conducting the study consequently compared binding affinities of the thrombin to surface-immobilized aptamers and in a competitive mode when additional aptamers are present in solution. They varied grafting-density as well as concentrations of free aptamer and found that increasing grafting-density has a negative effect on the binding affinity (K_D) of the surface-conjugated aptamer, while it has no effect on the K_D of aptamer in solution. In order to ensure sufficient spacing and thus maintain aptamer-functionality, even on this planar surface, additional spacing between aptamer and surface had to be applied.

Nanoscale surface features (e.g. roughness, groves, pores) and spatial confinement of aptamers when immobilized on nanomaterials adds another dimension to the challenge of controlling steric hindrance effects. Even though close proximity of capture probe and target supported by nanostructure architecture (e.g. in a porous matrix) can enhance their interaction [40], high grafting-density and crowding within the nanostructures can hamper aptamer-functionality and accessibility of the target-binding sites [38, 41]. Herein, also electrostatic interactions can have a particular effect: high amounts of negative charges accumulated by conjugated aptamers on a surface can prevent access of target analytes to the binding sites, which is enhanced by spatial confinement and limited free surface. Hence, besides reduced crowding, reduced negative charges can be a reason for better capture efficiency at

Table 1: Aptamer-modified nanostructured surfaces for cell capture.

Aptamer-target	NS material	Immobilization	Application	Comments	References
Antiepithelial cell adhesion molecule	Nano-structured glass slides	Phenyldiisothio-cyanate – NH2 aptamer	Circulating tumor cell capture		[24]
EGFR (RNA aptamer)	Nanotextured PDMS	Silanization, isothiocyanate groups, NH2-DNA, prehybridization with salmon sperm, hybridization of RNA aptamer	Circulating tumor cell capture		[25]
T lymphocyte	Silicon nanowires	MPTMS, heterobifunctional linker (GMBS), DNA aptamer	Cell capture	Release mechanism	[26]
TD05	Polymer-modified silicon nanowires	Click chemistry [copper-catalysed azide–alkyne cycloaddition (CuAAC)]	Circulating tumor cell capture		[27]
Lactobacillus acidophilus	Porous SiO_2	Acrydite-coupling on SH-surface	Optical biosensor		[28]

Table 2: Aptamer-modified nanostructured surfaces for sensing applications.

Aptamer-target	NS material	Immobilization	Application	Comments	References
Dopamine	Au-hexagons on fused silica	SH-oligos on Au layer	SERS sensor		[29]
Vasopressin	Au-layer deposited on silicon pillars	SH-oligos on Au layer	SERS sensor	Target analyte was labeled with TAMRA (5-carboxytetramethylrhodamine)	[30]
Thrombin	Single wall carbon nanotubes on FET	Carbodiimidazole-activated Tween 20 onto SWCNTs, NH_2 aptamer	Field effect transistor (FET)		[31]
Thrombin	Carboxylic-acid-functionalized polypyrrole (CPPy) nanotubes on glass substrate	Condensation reaction between carboxylic acid and amine groups (substrate or aptamers) mediated by 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride	Field effect transistor (FET)		[32]
His-tagged protein	Porous SiO_2	EDC mediated coupling of NH_2 aptamer	Optical biosensor		[4]
Adenosin	Porous SiO_2	Avidin/biotin	Fluorescence-quenching	TID of cDNA with carboxy-tetramethyl-rhodamine/CTMR-label	[33]
Thrombin	Multiwalled carbon nanotubes on glassy carbon electrodes/gold-coated quartz crystal	Avidin/biotin, electrostatic adsorption	EQCM, amperometric, impedimetric	Methylene blue composites	[34, 35]
Human cellular prions	Multiwalled carbon nanotubes on gold electrodes	Avidin/biotin sandwich with conjugated ferrocenes	Amperometric		[36]
OTA	AuNPs/$MoSe_2$ modified glassy carbon electrode	Electrodeposition of AuNPs on $MoSe_2$, SH-oligos on AuNPs	Electrochemical sensing	TID of cDNA from aptamer upon target binding, dyeing of ssDNA with methylene blue	[37]

lower aptamer immobilization densities [28, 38]. Furthermore, while enhanced surface roughness due to nanoscale features on the surface can improve interaction of the target (i.e. cells) with the substrate, it may also render it prone to unspecific adsorption (e.g. matrix components) [42, 43]. Thus, when nanomaterials are functionalized with aptamers, special attention has to be paid to careful optimization of spacer-arms and immobilization density as well as to orientation of the aptamer (see Section "Effects of immobilization to aptamer performance").

Exemplary applications of aptamer-tethering to nanostructured surfaces are presented in the following.

Application of aptamer-modified nanostructured surfaces

Cell capture

Circulating tumor cells (CTCs) are an interesting target for the early detection, understanding and therapy of different cancer types [44, 45]. Since they occur in low numbers in the blood stream of patients with solid tumors, there is a strong need for effective methods to enrich and isolate these cells [46–48]. Over the past few years, efficient approaches have been developed, many of which take advantage of highly specific aptamers that have been selected for targeted capture of such cells with high affinity. Combination of aptamer capture probes and nanostructured materials has brought forth a number of excellent studies taking advantage of increased surface roughness and receptor-density by means of the used nanomaterials or by appropriate treatment of substrates in order to create nanoscale features [49].

One example was presented by Wang et al. [24], demonstrating an increase of target cell capture by almost 50% with only one additional step of nanostructuring their glass slides prior to functionalization with anti-EpCAM (epithelial cell adhesion molecule) aptamers for the specific capture of EpCAM-expressing PC3 cells. Aiming to mimic the surface roughness of extracellular matrix (with feature sizes between 260 and 410 nm [50]), Wang and colleagues exposed borosilicate glass to a reactive ion etching (REI) process yielding average features of 374.3 nm under optimized conditions. Such homogeneously nanostructured glass slides were then subject to further functionalization and finally conjugation with aptamers before being studied for the effectiveness of cell capture onto them. As a result, the group showed a 76% cell-capture efficiency for the nanostructured slides in comparison to only 30% of PC3 cells captured on the planar slides.

In a similar study, Wan et al. [25] compared the specific capture of human glioblastoma and meninges-derived primary fibroblast cells (hGBM) by a RNA-aptamer, targeting cell membrane overexpressed epidermal growth factor receptors (EGFRs) on planar and nanotextured polydimethylsiloxane (PDMS) substrate. While a treatment with NaOH on the PDMS template resulted in an increased PDMS surface roughness (with feature sizes of about 289 nm after complete functionalization with RNA capture probes), untreated templates resulted in a PDMS substrate with minor features of 22 nm. Herein, the authors draw a direct connection of the surface roughness and the amount of subsequently immobilized aptamers. Thus, enhanced cell capture on the nanotextured PDMS was concluded to be a synergistic effect of increased aptamer density and simulation of basement membrane structure through appropriate roughness on the substrate, both promoting cell attachment.

Besides their excellent biocompatibility [51], silicon nanowires (SiNWs) have also shown high efficiency in cell capturing, especially when functionalized with specific aptamer-capture probes [26, 27]. Significantly increasing the aptamer density on the exposed substrate surface and through their topography preferred by cells, the nanowire structures facilitate the contact between capture probe and cell receptors and provide a suitable 3D structure for cell contacts [52–55], see Figure 1C. Nanowires have demonstrated capture efficiencies two orders of magnitude higher, in comparison to planar silicon substrates, and enable controlled release of captured cells through reversible aptamer-folding [26]. While such a SiNW-system has been demonstrated for the capture of T-lymphocyte cells [26], an example of SiNWs grafted with Ramos-cell specific aptamers and glycopolymers, showed high capturing-efficiency at notably low cell-concentrations and directly in serum-containing medium [27]. Herein, the utilized glycopolymer has a cell-affinity itself, binding glucose transporter proteins on the cell membrane, but only the combination with cell-specific aptamers created a highly efficient multifunctional surface for the capture of CTCs.

In the aforementioned examples, the aptamer-functionalized nanostructures and their high surface to volume ratio, had the main purpose to promote cell attachment through cell-compatible roughness and nanoscale features. Not in all applications, where cells are captured, their detection based on labeling or cell staining is suitable. Instead, depending on the nature of the utilized nanomaterial, it can be additionally exploited as the signal transducer: visible white light reflection from Faby-Pérot thin films can easily be recorded with a spectrometer. Porous silicon thin films display such

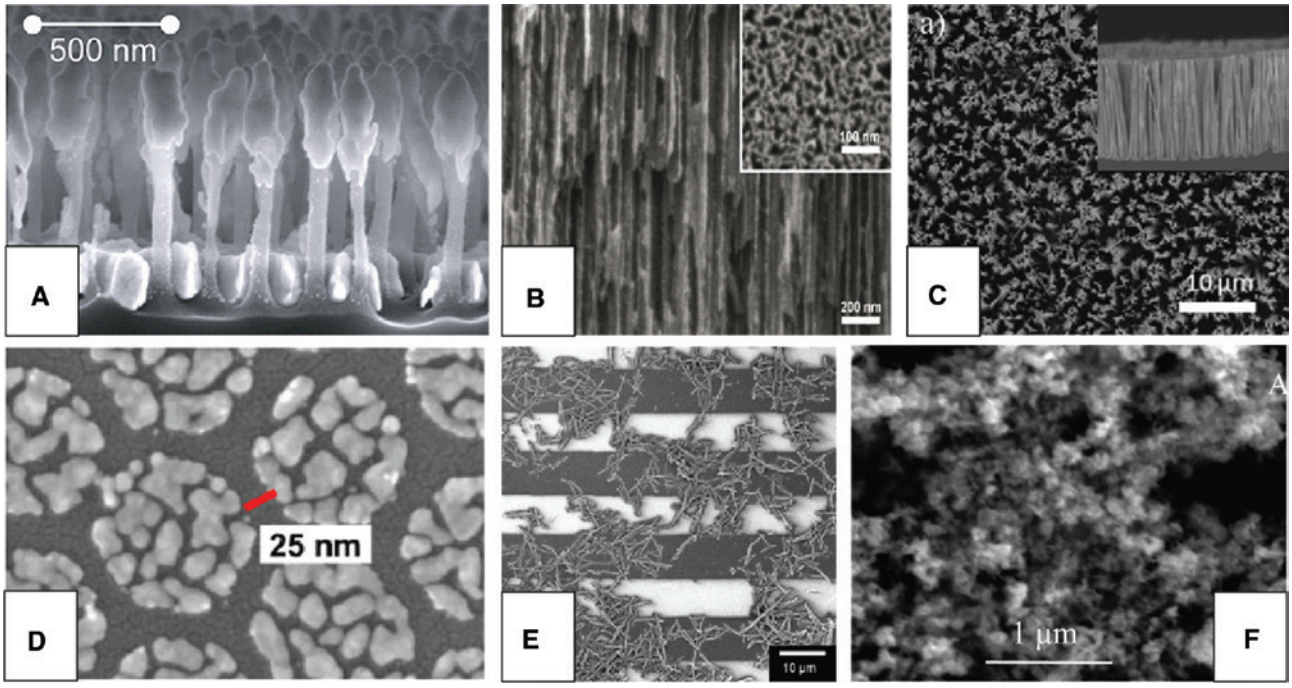

Figure 1: SEM micrographs of different nanomaterials.
(A) Au-capped nanopillars [30]. (B) Porous silicon oxide structure cross section and top-view (inset) [4]. (C) Silicon nanowires top view and cross section (inset) [27]. (D) Au-hexagon structures [29]. (E) CPPy nanotube-networks on electrodes [32]. (F) Molybdenum selenide flower-like nanostructures [56]. Adapted with permission from the respective references.

light interferences and are commonly used for biosensing applications in a reflective interferometer Fourier transform spectroscopy (RIFTS) mode [57–59], see Figure 1B. Additionally, cell capture on porous nanostructures has been reported [28, 60, 61]. The technique enables rapid capture and detection of cells with minimal instrumentation and without the need of labeling. One example where the advantages of aptamers have been combined with optical porous silicon structures was recently demonstrated for distinct capture of live probiotic bacteria [28]. Herein, the importance of spacing between aptamer and biosensor surface as well as immobilization density was highlighted as it directly affected optical signals and the ability of the structure to capture bacteria cells. The porous silicon matrix in concert with the tethered highly specific aptamers, showed fast and robust optical signals upon bacteria capture on the nanostructured surface and importantly, could distinguish between live and dead bacteria populations based on the specificity of the utilized aptamer [28].

Due to the availability of aptamers against many other bacteria species, several systems for their capture have recently been reported (e.g. against *Escherichia coli* O157:H7 [62], *Salmonella typhimurium* [63] or *Staphylococcus* aureus [64]), however, to the best of our knowledge, none of the so far published examples, harnesses the advantages of nanostructured surfaces, but are rather designed utilizing different types of nanoparticles.

Biosensing

A classification of biosensors is usually firstly made by separating label-free and label-based approaches. Secondly, biosensors of different signal transductions (optical, electrical etc.) can be divided. In the following, we are presenting several examples of biosensors, covering all types, and outlining their beneficial combination of nanomaterials and aptamer capture probes.

Contrary to the previously described cell capture methods, in biosensors, most schemes involving the use of nanomaterials, take advantage of them for the purpose of signal transduction and/or amplification. The most popular material, chosen not only for its intrinsic properties but also due to facile and well-characterized immobilization of oligonucleotides, is probably gold. While it is being widely utilized as planar material for functional-coating or as electrode material [65], researchers have also increased efforts in studying gold nanostructures due to their plasmonic properties as well as absorbance, coupling and scattering properties which are depending on their geometry [29, 30, 37].

Chemisorption of thiols to elemental gold is a well-known mechanism resulting in stable self-assembled monolayers (SAM) [66, 67]. With the facile possibility of post-synthesis functionalization of oligonucleotides with thiols, the immobilization of thiolated DNA to gold in any form, has proven to be a viable strategy and remains widely utilized [68] as can be seen from the examples presented in the following.

Surface-enhanced Raman spectroscopy (SERS) is a promising technique for the sensitive detection of chemical or biological species. Herein, characteristic molecular vibrations are observed as the inelastic scattering of monochromatic light by surface-tethered species. The use of noble metal nanostructures (i.e. gold) enhances the signals obtained significantly due to (i) the localized surface plasmons on the gold surface that get excited and (ii) the formation of a target-analyte complex enabling charge transfers [22, 29, 69]. It is crucial, that distinct Raman signals are observed only upon binding of the target molecule. One example by Peters et al. [29], presents the detection of dopamine, also investigating the influence of different gold nano-geometries on the signal output. Herein, hexagonal Au is used as substrate for the immobilization of the capturing aptamer, see Figure 1D. Raman signals are observed only when the target is present and bound.

Unlike the previously mentioned assay, in an example by Yang et al. [30], an additional TAMRA-tag (5-carboxytetramethylrhodamine) on the targeted vasopressin protein hormone is necessary to achieve distinct and enhanced Raman signals upon target capture. The authors of this study on a SERS biosensor pay specific attention to optimization of the aptamer immobilization density, aptamer orientation and surface effects on the Au coated silicon nanopillars. One interesting aspect they emphasize, is the treatment of the gold layer with mercaptohexanol (MCH), which on the one hand blocks access of analyte and buffer components to the gold surface (causing unspecific adsorption), and on the other hand, supports the vertical orientation of the aptamers when conjugated to the surface (by preventing interaction of aptamer and gold surface, thereby enhancing functional structures). However, the authors do not report if they observed a ligand exchange induced by prolonged exposure to MCH. This is highly relevant when choosing utilized MCH concentrations and incubation times [30, 70]. Their hypothesis is confirmed by significantly higher Raman peak intensities for samples treated with MCH. With their optimized setup, highly reliable and quantitative detection of picomolar concentrations was achieved. In achieving sensitive signals, the utilized nanostructure, namely silicon nanopillars, was of the essence: trapping target molecules between the nanopillars resulted in intense Raman scattering enhanced by the localized plasmon resonance induced by the gold-capped pillars leaning towards each other, see Figure 1A.

Among the label-free methods, field effect transistors (FET) have recently gained a lot of attention due to the advantages of nanostructured materials that can be integrated to the gate of the transistor [71]. In one example by So et al., the well-studied and in this case amine-modified thrombin-aptamer was conjugated to single walled carbon nanotubes (CNTs) within a FET via carbodiimidazole chemistry. Thanked to the small size of the aptamers and the conducting properties of the nanotubes, the FET enabled robust thrombin detection at low nanomolar concentrations with the additional possibility of biosensor regeneration for consecutive sensing cycles. It should be noted that in label-free FETs where the use of CNTs provides significant advantages, their nanostructure is not means of signal attainment, but a suitable and highly beneficial material enabling the observation of changes in its conductance upon target binding. Other nanomaterials such as graphene or SiNWs are also widely used as FET-gate materials due to their tunable material properties [71]. Another novel nanomaterial that has been demonstrated for the use as FET-gate material are carboxylic-acid-functionalized polypyrrole (CPPy) nanotubes. In a study by Yoon et al. [32], similarly as previously described for CNTs, the conducting polymer nanotubes, possessing a carboxy-functionality, were conjugated to the electrodes and gate surface and subsequently modified with amine-terminated aptamers targeting thrombin. Besides the facile synthesis of the material, its stable attachment to the FET basis and a reliable biosensing performance at thrombin concentrations between 50 and 500 nM, the authors show the beneficial effects of the formation of interconnected CPPy networks on the gate surface between source and drain electrode, evoking higher signal amplification as well as the improved sensor sensitivity at high aptamer-densities [32], see Figure 1E.

Oxidized porous silicon ($PSiO_2$) nanostructures serving as optical transducers are an example where the nanomaterial not only provides increased surface area but also facilitates signal transduction: when an aptamer is immobilized onto a $PSiO_2$ structure, it can serve for the capture of target proteins while the induced changes in optical properties of the functionalized scaffold can be recorded with a spectrometer. This simple experimental setup was proven successfully with an aptamer directed against his-tagged proteins, demonstrating rapid protein detection in a reversible manner and with outstanding

simplicity [4]. Herein, the signal upon target capture, arises through the changes in refractive index of the nanostructured matrix induced by the formation of the aptamer-target complex. Noteworthy is the reversibility of the target binding and complete regeneration of the sensor for multiple consecutive biosensing cycles resulting in highly reproducible signals.

Likewise utilizing multifunctional porous silicon substrates, Yoo et al. demonstrated a biosensor for the detection of adenosine [33]. Herein, the authors take advantage of the target induced dissociation of a TAMRA-labeled complementary strand from the aptamer-functionalized porous silicon surface. Thereby the fluorescence of the label, which was previously effectively quenched by the silicon surface properties, is restored and a fluorescence signal can be observed. The authors demonstrate this simple one-step assay with submicromolar concentrations of adenosine and propose the application of this scheme for the detection of other biomolecules. Despite the simple design, the assay relies on a labeled component impeding the reuse of the biosensor and additionally requires fluorescence detection which implies the necessity of sophisticated laboratory instruments.

Electrochemical aptasensors for thrombin utilizing amperometry or impedance spectroscopy (EIS) as well as electrochemical quartz crystal microbalance (EQCM) have been studied extensively by the group of Hianik [34, 35, 72–75]. Pre-treatment of their multiwalled CNTs (MWCNTs) with methylene blue (MG) has shown significant improvements in sensitivity for most of their biosensing schemes [34, 35]. This is not attributed to a direct effect on the target affinity of the biosensor, but rather improved immobilization of aptamers due to the MB's positive charge counterbalancing the negative charges of the carboxy-terminated MWCNTs and aptamers respectively. Thus, MWCNT-MB composites possess a higher aptamer-density after functionalization and subsequently display lower detection limits. Furthermore, MB in its role as a phenothiazine dye provides means of signal detection by its change in redox-status upon interaction of immobilized aptamers and its target analyte. The group has demonstrated a wide range of detection schemes and consequently improved the performance of their biosensors. Noteworthy is also their investigation of so called aptabodies [73]. Heterodimers of two anti-thrombin aptamers modified each with a poly-A or poly-T tag respectively form Y-shaped aptabodies due to the complementarity of the tags, subsequently each possessing two binding sites for the target protein. Contrary to improved sensitivity in EQCM, investigations for EIS emphasize the fragile balance of aptamer density and steric hindrance phenomena as well as charge-related affinity losses: the authors were not able to further improve biosensor sensivity from their reported 0.3 nM for the EQCM biosensing scheme and assume a negative effect of the high density negative charges on the coordination of the binding motifs [34, 35].

Going beyond the commonly demonstrated thrombin model-systems, a highly relevant application of an electrochemical aptasensor has been demonstrated by Miodek et al. [36]. For the sensitive detection of human cellular prions, the authors combined several elements: a MWCNT-coated gold surface served as electrode, while a layer of fourth generation polyamidoamine dendrimers coupled to the MWCNTs further increased available surface functionalities for the following conjugation of modified ferrocene markers [76]. Finally, traditional biotin-streptavidin was used to immobilize prion specific aptamers. Binding of human cellular prions significantly impacted the electron transfer in the system, enabling specific detection of prions at concentrations as low as 0.5 pM and notably, applicability directly in blood plasma [36].

The range of the presented examples in this review makes no pretence to be complete, however, in the authors' opinion it reflects the variety of nanomaterials, aptamer-immobilization strategies and different target analytes well and shall give the reader a good idea of the capabilities and limitations of currently studied aptasensors employing nanostructured materials.

Combination of nanostructured surfaces and nanoparticles

In search of mechanisms to amplify attained signals and enhance specificity, some assays rely on sandwich formats where aptamer-conjugated nanoparticles can serve as secondary capture probes and labels [77, 78]. While the advantages and different applications for aptamer-functionalized nanoparticles will be discussed in the next chapter, herein, we would like to give one example where both, a nanostructured material and nanoparticles are implemented with aptamer-assisted target capture.

In the study by Huang et al. [37], molybdenum selenide nanoflowers were prepared by a simple hydrothermal method on the surface of an electrode. This material was chosen due to its high surface area, the exceptional intrinsic electrical conductivity and finally its electrocatalytical activity induced by the selenite component, see Figure 1F. They constructed a highly sensitive electrochemical sensor for the detection of ochratoxin A (OTA) by integration of oligonucleotide-functionalized gold nanoparticles (AuNPs). Therein, the aptamers were

also hybridized with a second complementary sequence. Methylene blue (MB) was utilized as the electrochemical probe due to its specific interaction with single stranded DNA (ssDNA). Upon target binding, complementary DNA was released and thus became available (as ssDNA) for interaction with MB. This can be observed by the change in redox currents. With this construction of a combination between nanostructured surfaces and additional nanoparticles, the authors achieved a highly sensitive assay with detection limits as low as 0.08 pM OTA.

Aptamer-modified nanoparticles

Special considerations for aptamer immobilization on nanoparticles

For aptamer conjugation to nanoparticles, the surface charge of the nanoparticles has to be considered. Direct immobilization of aptamers on cationic surfaces, such as polyethylenimine (PEI), may lead to an aptamer-PEI-complex which interferes with correct aptamer folding and thereby renders the aptamer useless as targeting molecule. Thus, nanoparticles composed of neutral material [e.g. polymers such as polylactic acid (PLA) or polylactic-co-glycolic acid (PGLA)] may be most convenient for conjugation with aptamers [79].

An important goal during aptamer-immobilization is maintaining the binding affinity and selectivity the aptamer displays in solution [14]. This is usually accomplished by covalent binding of the aptamer to a surface bound linker and, in some cases, non-covalent attachment by physisorption [14]. In the last few years, many advantages in synthesis and characterization of different nanoparticles such as metallic, silica, magnetic, hydrogel or polymeric nanoparticles and CNTs have been revealed [80]. These nanomaterials generally possess a large surface area in combination with a unique size and shape. Due to their small sizes, nanoparticles can potentially move through cell and tissue barriers and their cellular uptake can be compared much easier as for larger drug delivery systems [81, 82]. The large surface-area-to-volume ratio leads to a greater drug delivery efficiency [81]. Additionally, the high surface area allows for high loading of targeting or drug molecules [83]. Furthermore, nanoparticles display composition and size dependent physical properties such as SPR, fluorescence and/or magnetism [80].

One advantage of aptamer immobilization on nanoparticle surfaces, for example, is the influence of aptamer on the nanoparticle stability. Wang et al. showed that gold nanoparticles were more stable in high salt concentrations when modified with aptamers [84]. High salt concentrations shield the electric field and un-modified nanoparticles form aggregates more frequently due to dipole interactions [85]. To stabilize gold nanoparticles against aggregation, negatively charged aptamers can be coupled to nanoparticle surfaces and prevent the aggregation due to the electrostatic repulsion forces between similarly charged surfaces [85].

Application of aptamer-modified nanoparticles

The modification of nanoparticles with specific aptamers has proven advantageous in different areas of applications. The properties of aptamer-modified nanoparticles can among others be used for biosensing. If cell-specific aptamers are used, resulting conjugates can be used for cell targeting and targeted drug delivery [80].

There are four different types of nanoparticles conjugated with aptamers which are commonly used for biological imaging applications: Gold nanoparticles (AuNP), quantum dots (QD), silica nanoparticles (SiNP) and magnetic nanoparticles (MNP).

In the following, we present examples for applications of aptamer-modified nanoparticles in cell and intracellular targeting, drug delivery and biosensing.

Cell targeting

One prominent application of aptamer-modified nanoparticles is cell targeting. To date, countless aptamers for various cellular targets are available. Targets can be cell-surface bound proteins, viruses, and so on [86]. Table 3 summarizes several applications of aptamer-modified nanoparticles along with the used aptamer, its target, the mode of detection and the type of NP.

Most applications aim to target cancer cells. Gao et al. developed biodegradable nanoparticles consisting of polyethylene glycol (PEG) and polycaprolactone (PCL), which are also functionalized with AS1411 aptamer and loaded with doxorubicin (DOX). They were able to target and enhance cellular uptake by glioma cells in vitro [89].

Another example for cell targeting is found in literature: Farokhzad et al. developed a nanoparticle-aptamer bioconjugate for targeting prostate cancer cells. They synthesized nanoparticles consisting of poly(lactic acid)-block-polyethylene glycol (PLA-PEG) and coupled an aptamer to their surface which selectively binds to

Table 3: Overview about aptamers, their targets and types of nanoparticles on which they could be conjugated (continued).

Aptamer	Target	Detection	Type of NP	References
A9	Prostate-specific membrane antigen (PSMA)		QD	[87]
A10	PSMA	Colorimetric	SPION[a]	[88]
AS1411	Nucleolin	Fluorescent	Polymer-NP, MSN	[89–91]
MUC1	Mucin-1	Fluorescent	QD, SiNP	[90]
TTA1	Tenascin-C	Fluorescent	MNP	[92]
A30	HER-2	Fluorescent	AuNP[b]	[93]
Sgc8c	CCRP-CEM cells	Fluorescent	AgNP[c]	[1]
TD05	Ramos (B-cell lymphoma)	Fluorescent	QD	[90]
S6	A549	Fluorescent	Polymer-NP, QD	[90]
SA17, SA61	*S. aureus*	Light scattering	AuNPs	[94]

[a]SPION, Superparamagnetic iron oxide nanoparticle; [b]AuNP, gold nanoparticle; [c]AgNP, silver nanoparticle.

prostate-specific membrane antigen (PSMA) [80, 93, 95]. Farokhzad and coworkers successfully demonstrated that their nanoparticle-aptamer conjugates targeted prostate cancer epithelial cells and were internalized by them [93].

The imaging and targeting of PSMA has also been realized by using superparamagnetic iron oxide nanoparticles (SPION) functionalized with the A10 aptamer [88]. The A10 aptamer is an RNA aptamer specifically binding an extracellular domain of the PSMA. SPIONs are characterized by low toxicity and detection limits, for example. An important application of SPIONs is to serve as a magnetic resonance imaging (MRI) contrast agent for cancer diagnosis [96]. As mentioned, Wang and coworkers developed A10 aptamer-modified thermally cross-linked SPIONs enabling the detection (by MRI) and treatment of PSMA [88].

Jalalian and coworkers developed epirubicin loaded SPIONs functionalized with 5TR1 aptamer, which binds specifically to mucin-1, a glycoprotein which is overexpressed on many epithelial tumors and adenocarcinomas [97]. They investigated the internalization of the aptamer-modified particles and cell viability after incubation with drug loaded and modified particles. Detection of the internalization was performed by using flow cytometry analysis. Cell viability was assessed by MTT assay [97].

Aptamer-modified QDs are used particularly for cancer cell imaging [98]. MUC-1 aptamer-conjugated QDs for the detection of mucin-1 positive cells serve as an example for fluorescent cell imaging [90]. QDs are also widely used for targeting breast cancer cells (MCF-7). Gedi and Kim successfully targeted such cells with aptamer-modified QDs, resulting in a strong red fluorescence signal [99].

Ulusoy and coworkers developed aptamer-modified QDs for the detection and imaging of lung cancer cells [100]. They used the S15 aptamer directed against the lung cancer cell line A549 [101]. Fluorescence microscopy showed that aptamer-modified QDs were successfully internalized by lung cancer cells while unmodified QDs were not taken up [100].

Silica is a biocompatible but inorganic material often used for biological applications such as artificial implants [83]. It was found that silica is an appropriate compound for the development of drug releasing systems. Mesoporous silica nanoparticles (MSN) are responsive to external (e.g. light or magnetic field) and internal stimuli (e.g. enzymes or pH). They are also used for imaging, controlled release of therapeutics and cell targeting. When MSNs are conjugated with aptamers, they can be used for targeting cancer cells [102]. Li and coworkers developed MSNs and conjugated them to AS1411 aptamer which is specific to nucleolin, a protein overexpressed on several types of cancer cells [89, 102]. The conjugated MSNs have successfully targeted MCF-7 cells [102, 103]. Investigating the success of cell targeting, Li and coworkers prepared fluorescein-modified MSNs, conjugated to AS1411 aptamer and then incubated them with MCF-7 cells. Utilizing confocal microscopy, the targeting and internalization of the particles were observed [102].

Su and coworkers coated MSNs with carbon quantum dots and conjugated them with aptamers for the electroluminescent detection of MCF-7 cells. The aptamer used was directed against mucin1. Specifically, Su et al. used a surface which was cast with a three dimensional graphene (3D-GR). Additionally, AuNPs were attached to the 3D-GR to improve the electronic transmission. Subsequently, MCF-7 cells were seeded on the modified electrode and were incubated with aptamer-modified MSNs. Detection was carried out with electrochemical impedance spectroscopy [103].

MNP are widely used for cell targeting as biological samples mostly exhibit no magnetic properties. Thus, MNPs may yield ultrasensitive detection with no interfering background signals [92]. MNPs conjugated with aptamers have been applied for cell targeting especially in cancer cell targeting. An example is the detection of Tenascin-C in glioma cells. Iliuk and coworkers used the GB-10 aptamer specifically binding to the Tenascin-C receptor on glioma cells and conjugated it to MNPs. The interaction between aptamer-modified MNPs and cancer cells was determined by scanning electron microscopy. Only aptamer-modified particles interacted with the glioma cells [104].

Not only cancer cells or their receptors can be targeted, but also bacteria like *Staphylococcus aureus* [94]. Chang and coworkers selected two aptamers against *S. aureus* and conjugated them to AuNPs. They modified AuNPs with one of the selected aptamers (SA17 and SA61) and detected the interaction between aptamer-modified particles and *S. aureus* cells by direct detection (resonance light-scattering signals).

Aptamer-modified AuNPs are also used for the detection of *Escherichia coli* (*E. coli*) O157:H7 and *Salmonella typhimurium* (*S. typhimurium*). Aptamers can stabilize AuNPs against aggregation in presence of high salt concentrations. Furthermore, AuNPs change color when they aggregate. Wu and coworkers took advantage of these properties and modified AuNPs with aptamers. Subsequently, they incubated *E. coli* and *S. typhimurium* with the conjugates. The conformation of the aptamers changed upon binding to the bacteria. Thus, the particles could not be stabilized by aptamers anymore and after applying high salt-concentrations, AuNPs aggregated and the dispersion changed its color. Color change was detected by UV/Vis spectroscopy [105].

As illustrated by the presented applications, aptamer-modified nanoparticles are very promising candidates for cell targeting and diagnostic detection. Especially in the context of in vivo imaging, there are still some problems to be solved. The main issue is the investigation of long term in vivo cytotoxicity.

Drug delivery

Drug delivery systems aim for specific transportation of pharmaceuticals to the desired site of action. By targeted delivery of drugs, solely to diseased cells, systemic side effects should be avoided. To enable specific delivery, a targeting ligand and its specific binding of the target cells as well as efficient intracellularization is necessary. The key advantage of such drug delivery systems is the ability to change pharmacokinetics. Additionally, the targeted distribution of drugs results in reduced effects on non-targeted tissues [106]. Aptamer-conjugated nanoparticles serving as targeting delivery systems commonly consist of iron oxid nanoparticles, gold nanoparticles, CNTs, dendrimers, quantum dots, liposomes or polymeric nanoparticles [106]. The drug can either be encapsulated within the nanoparticle or attached to the nanoparticle surface [81, 107]. Zhang and coworkers developed an aptamer-nanoparticle conjugate for co-delivery of both, an entrapped and a surface-attached drug. The surface-attached drug release was approximately 80%, while the entrapped drug release was 45% in the same time interval. These properties may find application for time-controlled drug delivery [108]. Different research groups showed the effect of aptamer-functionalized and drug-loaded nanoparticles on cancer cells [89, 91]. For example, Aravind et al. used polymeric nanoparticles consisting of poly(lactic-co-glycolic acid) (PGLA), loaded with paclitaxel (PTX) and immobilized with AS1411. They successfully targeted cancer cells in vitro. Additionally, they showed that cell viability of cancer cells decreased after incubation with aptamer-modified and drug-loaded particles. Thus, after targeting the cancer cells, drug release was induced [91].

Today there are many targeted drug delivery systems which are able to specifically enhance cellular uptake and increase cytotoxicity in vitro. Some groups have already investigated the applicability of aptamer-modified nanoparticles for targeted drug delivery in vivo. For example, Liu et al. showed that aptamer-modified nanoparticles could accumulate at tumor sites in mice. They used ApS6 and ApS10 directed against breast cancer cells and demonstrated that the systemic toxicity to other organs was decreased, compared to systematic strategies of administration [109]. A problem which has to be solved, is the multicancer drug resistance. Multidrug resistance (MDR) hampers the efficacy of chemotherapy [110]. Utilization of aptamer-modified nanoparticles is one approach to solve this problem by enhancement of intracellular drug concentration in cancer cells can be achieved with nanoparticles. Simultaneously, the toxicity to healthy cells is minimal. Due to their small size, nanoparticles are able to cross the leaky and hyperpermeable tumor vascular [111]. It is also possible to incorporate anticancer drugs and an additional chemosensitizer. Such delivery systems, consisting of two different drugs to overcome multi drug resistance have been reported. Sengupta and coworkers, for example, developed a nanoparticle delivery system loaded with combretastatin and doxorubicin

[112]. The drug attached to the nanoparticle surface was released first (combretastatin) and caused the destruction of tumors vasculature. Doxorubicin, which was entrapped within the nanoparticles, was released secondly and subsequently caused cytotoxicity [112]. This may be a suitable approach to overcome MDR.

Intracellular sensing

While the aforementioned examples deal with the specific targeting of cell surface bound receptors, aptamer-modified nanoparticles can also be exploited for the detection of targets within cells. Different types of nanoparticles were already applied for intracellular imaging, such as quantum dots, silica nanoparticles or graphene oxide nanoparticles [113]. Furthermore, AgNPs, AuNPs and QDs functionalized with aptamers were used for intracellular protein imaging [114–116]. It is possible to target specific proteins and trace their endocytic pathway [114]. AuNPs are the most commonly used nanoparticles in intracellular sensing. Zheng et al. developed an assay with aptamer-modified AuNPs which could detect intracellular adenosine triphosphate (ATP) concentrations [117]. Wang and coworkers used aptamer-modified silica nanoparticles to detect ATP [118]. They immobilized a Cy-5 labeled aptamer on nanoparticle surfaces and upon exposure to ATP, the aptamer changed its structure. Formation of an aptamer-target-complex induced the release of the immobilized aptamer and finally a strong fluorescence signal is observed in the presence of ATP. If ATP is absent, no fluorescence is detectable. The detection limit was reported to be ~34 μM [118].

Biosensing

Recently, aptamer-based biosensors (aptasensors) have attracted particular attention. The best known aptamer-modified nanoparticles for biosensing are metallic nanoparticles like gold and silver nanoparticles [119]. Besides, aptamer-functionalized MNP are used for small-molecule and protein detection (e.g. for detection of human R-thrombin protein, SPIONs were functionalized with aptamers) [80]. Aptamer-modified magnetic particles bind the target protein and MRI is used for detection. In presence of the target protein, a MRI contrast change is detectable [120].

There are three main categories of aptasensors: electrochemical, optical and mass sensitive sensor systems [121]. Table 4 quotes different aptasensors, their detection limit, corresponding analyte targeted by the aptamer and application.

Mass sensitive aptasensors are a category of label-free bioassays which include wave-based sensors like surface-plasmon resonance (SPR), acoustic wave-based sensors [quartz crystal microbalance (QCM)] and surface acoustic wave (SAW) sensors [121]. Mass sensitive aptasensors are capable of displaying changes on the sensor surface without any additional labeling. Furthermore, they can operate in real-time. Applications of mass sensitive aptasensors have been reported for the detection of large molecules like proteins or cells. Small molecules are hard to detect due to the minor change in mass induced by binding of small molecules to the sensor [104].

Optical sensors can be divided into fluorescent and colorimetric sensors [121]. Many colorimetric sensors are based on size dependent optical properties. An example

Table 4: Different aptasensors for targeting biomolecules (continued).

Sensor	Detection limit	Target	Application	References
Colorimetric	–	Cocaine	AuNP	[122]
Colorimetric	20 nM	Thrombin	AuNP	[86]
Colorimetric	5 μM	Ibuprofen	AuNP	[123]
Colorimetric	17 nM	Glutathione	AuNP	[86, 124]
Fluorescence	5 nM	OTA	AuNP	[86]
Fluorescence	0.5 μM (signal-off mode)	Cocaine	QD	[125]
Fluorescence	10 μM	ATP	Graphene	[86]
Electrochemical	0.1 nM	ATP	AuNP	[86]
Electrochemical	0.5 μM	Cocaine	AuNP	[86]
Electrochemical	9.4 nM	Kanamycin	AuNP; self-assembled nano-composite	[126]
Electrochemical	0.5 nM	Heavy metals	AuNP	[126]
Electrochemical	5 nM	BPA	AuNP	[126]
Fluorescent flow	5 mM	ATP	QD	[127]
FRET		OTA	AuNP	[128]

for colorimetric sensing are aptamer-conjugated AuNPs [126]. Colloidal gold nanoparticles exhibit a red color and when they aggregate, their color changes to blue due to SPR effects [116, 129]. SPR is dependent on shape and particle size, as well as the distance between AuNPs; consequently their absorption wavelength changes upon formation of agglomerates [126].

Another example for a colorimetric aptasensor was demonstrated for the detection of digoxin. Herein, Emrani and coworkers used AuNPs and coupled aptamers on the surface of the particles. When digoxin was absent, the aptamers were attached to the surface of the AuNPs by electrostatic interaction between aptamer and particles. Thus, the particles were stabilized against high salt-concentrations and consequently aggregation. In presence of digoxin, the aptamer changed its structure and an aptamer-digoxin-complex was formed. AuNPs aggregated after adding NaCl and the red colored colloidal AuNPs change their color into blue upon aggregation [130].

Fluorescence-based biosensors could be designed with fluorescent labeled aptamers or with label-free aptamers [131]. Many fluorescence biosensors apply the competitive binding principle. This is based on the competition of binding of the analyte or hybridization of a complementary strand to the aptamer. One example for competitive binding is a QD-based aptasensor for the detection of cocaine or the detection of human neutrophil elstase (HNE) [126, 132].

The fluorescence of the sensors could either be achieved by (i) direct modification of the aptamer with fluorophores, (ii) structural changes could cause fluorescence of a dye or (iii) the fluorescence resonance energy transfer (FRET) between two dyes could be affected [131]. As mentioned above, an example for a fluorescence-based aptasensor was reported by Sharma et al. for the detection of cocaine with aptamer-conjugated QDs [126]. Herein, distinction is made between two different types of sensors: signal-on and signal-off sensors. In case of the signal-on sensor, an oligonucleotide is labeled with the fluorescent dye Cy-5. FRET (between Cy-5 and QDs) quenches the signal and no fluorescence is detectable. If cocaine is present, an aptamer-cocaine-complex is formed resulting in the restoration of fluorescence exhibited by the labeled oligonucleotide [125, 126, 131]. In case of the signal-off sensor, the aptamer is hybridized to a complementary (Cy-5 labeled) strand and coupled to the QD surface. If cocaine is absent, the fluorescence of Cy-5 is detectable due to FRET between QD and Cy-5. In case of cocaine presence, the aptamer-target-complex is formed and the Cy-5 labeled oligonucleotide is released from the QD surface. Thus, decreased Cy-5 fluorescence signal indicates the presence of cocaine [125, 126, 131].

Aptasensors can also be of electrochemical nature and implement aptamer-functionalized gold nanoparticles. Li and coworkers self-assembled AuNPs on a gold electrode. Subsequently, the aptamer was immobilized on the nanoparticle surface and the electrical potential was measured. Upon detection of cocaine, a higher electrical potential was measured [133].

Conclusions

Potential applications of aptamer-modified nanoparticles or nanostructured materials are almost as multifarious as aptamer-sequences and their corresponding targets themselves. We believe that they will contribute to the solution of many analytical or other problems based on target recognition in the future. Even though the need of optimization for every aptamer-target pair and every utilized nanostructure persists, we are convinced that the set of considerations presented in this review can be a helpful resource when conjugating aptamers to nanoparticles or nanostructured surfaces. The applications presented here are demonstrating the success of such optimization processes for a wide range of cell-capture and biosensor applications based on aptamer-modified nanomaterials.

Author's statement

Conflict of interest: Authors state no conflict of interest.

Materials and methods

Informed consent: Informed consent has been obtained from all individuals included in this study.

Ethical approval: The research related to human use has been complied with all the relevant national regulations, institutional policies and in accordance the tenets of the Helsinki Declaration, and has been approved by the authors' institutional review board or equivalent committee.

References

1. Tuerk C, Gold L. Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. Science. 1990;249:505–10.
2. Jayasena SD. Aptamers: an emerging class of molecules that rival antibodies in diagnostics. Clin Chem. 1999;45:1628–50.
3. Heilkenbrinker A, Reinemann C, Stoltenburg R, Walter JG, Jochums A, Stahl F, et al. Identification of the target binding site of ethanolamine-binding aptamers and its exploitation for ethanolamine detection. Anal Chem. 2015;87:677–85.

4. Urmann K, Walter J-G, Scheper T, Segal E. Label-free optical biosensors based on aptamer-functionalized porous silicon scaffolds. Anal Chem. 2015;87:1999–2006.
5. Kokpinar O, Walter JG, Shoham Y, Stahl F, Scheper T. Aptamer-based downstream processing of his-tagged proteins utilizing magnetic beads. Biotechnol Bioeng. 2011;108:2371–9.
6. Lönne M, Bolten S, Lavrentieva A, Stahl F, Scheper T, Walter JG. Development of an aptamer-based affinity purification method for vascular endothelial growth factor. Biotechnol Rep. 2015;8:16–23.
7. Walter JG, Stahl F, Scheper T. Aptamers as affinity ligands for downstream processing. Eng Life Sci. 2012;12:496–506.
8. Schax E, Lönne M, Scheper T, Belkin S, Walter JG. Aptamer-based depletion of small molecular contaminants: A case study using ochratoxin A. Biotechnol Bioproc E. 2015;20:1016–25.
9. Meyer M, Scheper T, Walter JG. Aptamers: versatile probes for flow cytometry. Appl Microbiol Biotechnol. 2013;97:7097–109.
10. Modrejewski J, Walter J-G, Kretschmer I, Kemal E, Green M, Belhadj H, et al. Aptamer-modified polymer nanoparticles for targeted drug delivery. BioNanoMaterials. 2015;17:43–51.
11. Walter JG, Petersen S, Stahl F, Scheper T, Barcikowski S. Laser ablation-based one-step generation and bio-functionalization of gold nanoparticles conjugated with aptamers. J Nanobiotechnol. 2010;8:21.
12. Patel DJ, Suri AK, Jiang F, Jiang LC, Fan P, Kumar RA, et al. Structure, recognition and adaptive binding in RNA aptamer complexes. J Mol Biol. 1997;272:645–64.
13. Walter JG, Kökpinar O, Friehs K, Stahl F, Scheper T. Systematic investigation of optimal aptamer immobilization for protein-microarray applications. Anal Chem. 2008;80:7372–8.
14. Balamurugan S, Obubuafo A, Soper SA, Spivak DA. Surface immobilization methods for aptamer diagnostic applications. Anal Bioanal Chem. 2008;390:1009–21.
15. Ocana C, del Valle M. A comparison of four protocols for the immobilization of an aptamer on graphite composite electrodes. Microchim Acta 2014;181:355–63.
16. Zhu GH, Lubbecke M, Walter JG, Stahl F, Scheper T. Characterization of optimal aptamer-microarray binding chemistry and spacer design. Chem Eng Technol. 2011;34:2022–8.
17. Lin PH, Chen RH, Lee CH, Chang Y, Chen CS, Chen WY. Studies of the binding mechanism between aptamers and thrombin by circular dichroism, surface plasmon resonance and isothermal titration calorimetry. Colloids Surf B Biointerfaces. 2011;88:552–8.
18. Witt M, Walter J-G, Stahl F. Aptamer microarrays – current status and future prospect. Microarrays. 2015;4:115–32.
19. Justino CIL, Freitas AC, Pereira R, Duarte AC, Santos TAPR. Recent developments in recognition elements for chemical sensors and biosensors. TrAC Trends Anal Chem. 2015;68:2–17.
20. Liang H, Zhang XB, Lv YF, Gong L, Wang RW, Zhu XY, et al. Functional DNA-containing nanomaterials: cellular applications in biosensing, imaging, and targeted therapy. Acc Chem Res. 2014;47:1891–901.
21. Wang ZH, Yu JB, Gui RJ, Jin H, Xia YZ. Carbon nanomaterials-based electrochemical aptasensors. Biosens Bioelectron. 2016;79:136–49.
22. Wang G, Wang Y, Chen L, Choo J. Nanomaterial-assisted aptamers for optical sensing. Biosens Bioelectron. 2010;25:1859–68.
23. Bunka DHJ, Stockley PG. Aptamers come of age – at last. Nat Rev Microbiol. 2006;4:588–96.
24. Wang L, Zhu C, Zheng Q, He X. Preparation of homogeneous nanostructures in 5 minutes for cancer cells capture. J Nanomater. 2015;2015:6.
25. Wan Y, Mahmood MAI, Li N, Allen PB, Kim Y-t, Bachoo R, et al. Nanotextured substrates with immobilized aptamers for cancer cell isolation and cytology. Cancer. 2012;118:1145–54.
26. Chen L, Liu X, Su B, Li J, Jiang L, Han D, et al. Aptamer-mediated efficient capture and release of T lymphocytes on nanostructured surfaces. Adv Mater. 2011;23:4376–80.
27. Xue L, Lyu Z, Luan Y, Xiong X, Pan J, Chen G, et al. Efficient cancer cell capturing SiNWAs prepared via surface-initiated SET-LRP and click chemistry. Polym Chem. 2015;6:3708–15.
28. Urmann K, Arshavsky-Graham S, Walter JG, Scheper T, Segal E. Whole-cell detection of live lactobacillus acidophilus on aptamer-decorated porous silicon biosensors. Analyst. 2016, doi: 10.1039/C6AN00810K.
29. Peters RF, Gutierrez-Rivera L, Dew SK, Stepanova M. Surface enhanced Raman spectroscopy detection of biomolecules using EBL fabricated nanostructured substrates. J Vis Exp. 2015, doi:10.3791/52712:e52712.
30. Yang J, Palla M, Bosco FG, Rindzevicius T, Alstrøm TS, Schmidt MS, et al. Surface-enhanced Raman spectroscopy based quantitative bioassay on aptamer-functionalized nanopillars using large-area Raman mapping. ACS Nano. 2013;7:5350–9.
31. So H-M, Won K, Kim YH, Kim B-K, Ryu BH, Na PS, et al. Single-walled carbon nanotube biosensors using aptamers as molecular recognition elements. J Am Chem Soc. 2005;127:11906–7.
32. Yoon H, Kim J-H, Lee N, Kim B-G, Jang J. A novel sensor platform based on aptamer-conjugated polypyrrole nanotubes for label-free electrochemical protein detection. ChemBioChem. 2008;9:634–41.
33. Yoo L, Ahn K-Y, Ahn J-Y, Laurell T, Lee YM, Yoo PJ, et al. A simple one-step assay platform based on fluorescence quenching of macroporous silicon. Biosens Bioelectron. 2013;41:477–83.
34. Evtugyn G, Porfireva A, Ryabova M, Hianik T. Aptasensor for Thrombin based on carbon nanotubes-methylene blue composites. Electroanalysis. 2008;20:2310–6.
35. Porfireva AV, Evtugyn GA, Ivanov AN, Hianik T. Impedimetric aptasensors based on carbon nanotubes – poly(methylene blue) composite. Electroanalysis. 2010;22:2187–95.
36. Miodek A, Castillo G, Hianik T, Korri-Youssoufi H. Electrochemical aptasensor of human cellular prion based on multiwalled carbon nanotubes modified with dendrimers: a platform for connecting redox markers and aptamers. Anal Chem. 2013;85:7704–12.
37. Huang K-J, Shuai H-L, Chen Y-X. Layered molybdenum selenide stacking flower-like nanostructure coupled with guanine-rich DNA sequence for ultrasensitive ochratoxin A aptasensor application. Sens Actuators B Chem. 2016;225:391–7.
38. White RJ, Phares N, Lubin AA, Xiao Y, Plaxco KW. Optimization of electrochemical aptamer-based sensors via optimization of probe packing density and surface chemistry. Langmuir. 2008;24:10513–8.
39. Daniel C, Roupioz Y, Gasparutto D, Livache T, Buhot A. Solution-phase vs surface-phase aptamer-protein affinity from a label-free kinetic biosensor. PLoS One. 2013;8:e75419.
40. Hasegawa H, Savory N, Abe K, Ikebukuro K. Methods for improving aptamer binding affinity. Molecules (Basel, Switzerland). 2016;21:421.
41. Balamurugan S, Obubuafo A, McCarley RL, Soper SA, Spivak DA. Effect of linker structure on surface density of aptamer

monolayers and their corresponding protein binding efficiency. Anal Chem. 2008;80:9630–4.
42. Lim JY, Donahue HJ. Cell sensing and response to micro- and nanostructured surfaces produced by chemical and topographic patterning. Tissue Eng. 2007;13:1879–1891.
43. Teixeira AI, Abrams GA, Bertics PJ, Murphy CJ, Nealey PF. Epithelial contact guidance on well-defined micro- and nanostructured substrates. J Cell Sci. 2003;116:1881–92.
44. Plaks V, Koopman CD, Werb Z. Circulating tumor cells. Science (New York, N.Y.). 2013;341:1186–8.
45. Glaves D, Mayhew E. Selective therapy of metastasis. I. Quantitation of tumorigenic circulating and covert cancer cells disseminated from metastatic and nonmetastatic tumors. Canc Drug Del. 1984;1:293–302.
46. Butler TP, Gullino PM. Quantitation of cell shedding into efferent blood of mammary adenocarcinoma. Cancer Res. 1975;35:512–6.
47. Alix-Panabières C, Pantel K. Circulating tumor cells: liquid biopsy of cancer. Clin Chem. 2013;59:110–8.
48. Liotta LA, Kleinerman J, Saidel GM. Quantitative relationships of intravascular tumor cells, tumor vessels, and pulmonary metastases following tumor implantation. Cancer Res. 1974;34: 997–1004.
49. Bettinger CJ, Langer R, Borenstein JT. Engineering substrate topography at the micro- and nanoscale to control cell function. Angew Chem Int Ed. 2009;48:5406–15.
50. Wang L, Asghar W, Demirci U, Wan Y. Nanostructured substrates for isolation of circulating tumor cells. Nano Today. 2013;8:374–87.
51. Garipcan B, Odabas S, Demirel G, Burger J, Nonnenmann SS, Coster MT, et al. In vitro biocompatibility of n-type and undoped silicon nanowires. Adv Eng Mater. 2011;13:B3–9.
52. Fischer KE, Alemán BJ, Tao SL, Daniels RH, Li EM, Bünger MD, et al. Biomimetic nanowire coatings for next generation adhesive drug delivery systems. Nano Lett. 2009;9:716–20.
53. Wang S, Wang H, Jiao J, Chen K-J, Owens GE, Kamei K-i, et al. Three-dimensional nanostructured substrates toward efficient capture of circulating tumor cells. Angew Chem Int Ed. 2009;48:8970–3.
54. Wang S, Liu K, Liu J, Yu ZTF, Xu X, Zhao L, et al. Highly efficient capture of circulating tumor cells by using nanostructured silicon substrates with integrated chaotic micromixers. Angew Chem Int Ed. 2011;50:3084–8.
55. Dasgupta NP, Sun J, Liu C, Brittman S, Andrews SC, Lim J, et al. 25th anniversary article: semiconductor nanowires–synthesis, characterization, and applications. Adv Mater. 2014;26:2137–84.
56. Tang H, Dou K, Kaun C-C, Kuang Q, Yang S. $MoSe_2$ nanosheets and their graphene hybrids: synthesis, characterization and hydrogen evolution reaction studies. J Mater Chem A. 2014;2:360–4.
57. Pacholski C, Yu C, Miskelly GM, Godin D, Sailor MJ. Reflective interferometric fourier transform spectroscopy: a self-compensating label-free immunosensor using double-layers of porous SiO_2. J Am Chem Soc. 2006;128:4250–2.
58. Guinan T, Godefroy C, Lautrédou N, Pace S, Milhiet P-E, Voelcker N, et al. Interaction of antibiotics with lipid vesicles on thin film porous silicon using reflectance interferometric Fourier transform spectroscopy. Langmuir. 2013;29:10279–86.
59. Shtenberg G, Segal E. Porous silicon optical biosensors. In: Canham L, editor. Handbook of Porous Silicon. Cham, Switzerland: Springer International Publishing; 2014:857–68.
60. Tenenbaum E, Segal E. Optical biosensors for bacteria detection by a peptidomimetic antimicrobial compound. Analyst. 2015;140:7726–33.
61. Massad-Ivanir N, Shtenberg G, Tzur A, Krepker MA, Segal E. Engineering nanostructured porous SiO_2 surfaces for bacteria detection via “direct cell capture”. Anal Chem. 2011;83:3282–9.
62. Khang J, Kim D, Chung KW, Lee JH. Chemiluminescent aptasensor capable of rapidly quantifying Escherichia Coli O157:H7. Talanta. 2016;147:177–83.
63. Duan N, Chang BY, Zhang H, Wang ZP, Wu SJ. Salmonella typhimurium detection using a surface-enhanced Raman scattering-based aptasensor. Int J Food Microbiol. 2016;218: 38–43.
64. Cheng D, Yu MQ, Fu F, Han WY, Li G, Xie JP, et al. Dual recognition strategy for specific and sensitive detection of bacteria using aptamer-coated magnetic beads and antibiotic-capped gold nanoclusters. Anal Chem. 2016;88:820–5.
65. Lucarelli F, Marrazza G, Turner APF, Mascini M. Carbon and gold electrodes as electrochemical transducers for DNA hybridisation sensors. Biosens Bioelectron. 2004;19:515–30.
66. Palegrosdemange C, Simon ES, Prime KL, Whitesides GM. Formation of self-assembled monolayers by chemisorption of derivatives of oligo(ethylene glycol) of structure HS(CH2)11(OCH2CH2) mOH on gold. J Am Chem Soc. 1991;113:12–20.
67. Walczak MM, Popenoe DD, Deinhammer RS, Lamp BD, Chung CK, Porter MD. Reductive desorption of alkanethiolate monolayers at gold: a measure of surface coverage. Langmuir. 1991;7:2687–93.
68. Hegner M, Wagner P, Semenza G. Immobilizing DNA on gold via thiol modification for atomic force microscopy imaging in buffer solutions. FEBS Lett. 1993;336:452–6.
69. Hu M, Chen J, Li Z-Y, Au L, Hartland GV, Li X, et al. Gold nanostructures: engineering their plasmonic properties for biomedical applications. Chem Soc Rev. 2006;35: 1084–94.
70. Wijaya A, Hamad-Schifferli K. Ligand customization and DNA functionalization of gold nanorods via round-trip phase transfer ligand exchange. Langmuir. 2008;24:9966–9.
71. Adzhri R, Arshad KM, Gopinath SCB, Ruslinda AR, Fathil MFM, Ayub RM, et al. High-performance integrated field-effect transistor-based sensors. Anal Chim Acta. 2016;917:1–18.
72. Evtugyn G, Porfireva A, Ivanov A, Konovalova O, Hianik T. Molecularly Imprinted polymerized methylene green as a platform for electrochemical sensing of aptamer–thrombin interactions. Electroanalysis. 2009;21:1272–7.
73. Hianik T, Porfireva A, Grman I, Evtugyn G. Aptabodies – new type of artificial receptors for detection proteins. Protein Pept Lett. 2008;15:799–805.
74. Hianik T, Wang J. Electrochemical Aptasensors – Recent achievements and perspectives. Electroanalysis. 2009;21:1223–35.
75. Porfirieva A, Evtugyn G, Hianik T. Polyphenothiazine modified electrochemical aptasensor for detection of human α-thrombin. Electroanalysis. 2007;19:1915–20.
76. Miodek A, Castillo G, Hianik T, Korri-Youssoufi H. Electrochemical aptasensor of cellular prion protein based on modified polypyrrole with redox dendrimers. Biosens Bioelectron. 2014;56:104–11.
77. Ocana C, del Valle M. Three different signal amplification strategies for the impedimetric sandwich detection of thrombin. Anal Chim Acta. 2016;912:117–24.

78. Kwon MJ, Lee J, Wark AW, Lee HJ. Nanoparticle-enhanced surface plasmon resonance detection of proteins at attomolar concentrations: comparing different nanoparticle shapes and sizes. Anal Chem. 2012;84:1702–7.
79. Amiji MM. Nanotechnology for Cancer Therapy. Boca Raton, FL, USA: CRC Press; 2006.
80. Yang L, Zhang XB, Ye M, Jiang JH, Yang RH, Fu T, et al. Aptamer-conjugated nanomaterials and their applications. Adv Drug Deliver Rev. 2011;63:1361–70.
81. Singh R, Lillard JW. Nanoparticle-based targeted drug delivery. Exp Mol Pathol. 2009;86:215–23.
82. Farokhzad OC, Langer R. Impact of nanotechnology on drug delivery. ACS Nano. 2009;3:16–20.
83. Slowing II, Vivero-Escoto JL, Wu CW, Lin VSY. Mesoporous silica nanoparticles as controlled release drug delivery and gene transfection carriers. Adv Drug Deliver Rev. 2008;60:1278–88.
84. Wang LH, Liu XF, Hu XF, Song SP, Fan CH. Unmodified gold nanoparticles as a colorimetric probe for potassium DNA aptamers. Chem Commun. 2006;36:3780–2.
85. Sperling RA, Parak WJ. Surface modification, functionalization and bioconjugation of colloidal inorganic nanoparticles. Phil Trans R Soc A. 2010;368:1333–83.
86. Kim YS, Raston NHA, Gu MB. Aptamer-based nanobiosensors. Biosens Bioelectron. 2016;76:2–19.
87. Chu TC, Shieh F, Lavery LA, Levy M, Richards-Kortum R, Korgel BA, et al. Labeling tumor cells with fluorescent nanocrystal-aptamer bioconjugates. Biosens Bioelectron. 2006;21:1859–66.
88. Wang AZ, Bagalkot V, Vasilliou CC, Gu F, Alexis F, Zhang L, et al. Superparamagnetic iron oxide nanoparticle-aptamer bioconjugates for combined prostate cancer imaging and therapy. ChemMedChem. 2008;3:1311–5.
89. Gao HL, Qian J, Cao SJ, Yang Z, Pang ZQ, Pan SQ, et al. Precise glioma targeting of and penetration by aptamer and peptide dual-functioned nanoparticles. Biomaterials. 2012;33:5115–23.
90. Dougherty CA, Cai WB, Hong H. Applications of aptamers in targeted imaging: state of the art. Curr Top Med Chem. 2015;15:1138–52.
91. Aravind A, Varghese SH, Veeranarayanan S, Mathew A, Nagaoka Y, Iwai S, et al. Aptamer-labeled PLGA nanoparticles for targeting cancer cells. Cancer Nanotechnol. 2012;3:1–12.
92. Liu QL, Jin C, Wang YY, Fang XH, Zhang XB, Chen Z, et al. Aptamer-conjugated nanomaterials for specific cancer cell recognition and targeted cancer therapy. NPG Asia Mater. 2014;6:e95.
93. Farokhzad OC, Jon S, Khademhosseini A, Tran T-NT, LaVan DA, Langer R. Nanoparticle-aptamer bioconjugates: a new approach for targeting prostate cancer cells. Cancer Res. 2004;64:7668–72.
94. Chang YC, Yang CY, Sun RL, Cheng YF, Kao WC, Yang PC. Rapid single cell detection of Staphylococcus aureus by aptamer-conjugated gold nanoparticles. Sci Rep. 2013;3:1863.
95. Levy-Nissenbaum E, Radovic-Moreno AF, Wang AZ, Langer R, Farokhzad OC. Nanotechnology and aptamers: applications in drug delivery. Trends Biotechnol. 2008;26:442–9.
96. Rosen JE, Chan L, Shieh DB, Gu FX. Iron oxide nanoparticles for targeted cancer imaging and diagnostics. Nanomedicine. 2012;8:275–90.
97. Jalalian SH, Taghdisi SM, Hamedani NS, Kalat SAM, Lavaee P, ZandKarimi M, et al. Epirubicin loaded super paramagnetic iron oxide nanoparticle-aptamer bioconjugate for combined colon cancer therapy and imaging in vivo. Eur J Pharm Sci. 2013;50:191–7.
98. Li ZM, Huang P, He R, Lin J, Yang S, Zhang XJ, et al. Aptamer-conjugated dendrimer-modified quantum dots for cancer cell targeting and imaging. Mater Lett. 2010;64:375–8.
99. Gedi V, Kim YP. Detection and characterization of cancer cells and pathogenic bacteria using aptamer-based nano-conjugates. Sensors. 2014;14:18302–27.
100. Ulusoy M, Walter JG, Lavrentieva A, Kretschmer I, Sandiford L, Le Marois A, et al. One-pot aqueous synthesis of highly strained CdTe/CdS/ZnS nanocrystals and their interactions with cells. RSC Adv. 2015;5:7485–94.
101. Zhao ZL, Xu L, Shi XL, Tan WH, Fang XH, Shangguan DH. Recognition of subtype non-small cell lung cancer by DNA aptamers selected from living cells. Analyst. 2009;134:1808–14.
102. Li LL, Yin Q, Cheng JJ, Lu Y. Polyvalent mesoporous silica nanoparticle-aptamer bioconjugates target breast cancer cells. Adv Healthc Mater. 2012;1:567–72.
103. Su M, Liu H, Ge L, Wang YH, Ge SG, Yu JH, et al. Aptamer-based electrochemiluminescent detection of MCF-7 cancer cells based on carbon quantum dots coated mesoporous silica nanoparticles. Electrochim Acta. 2014;146:262–9.
104. Iliuk AB, Hu LH, Tao WA. Aptamer in bioanalytical applications. Anal Chem. 2011;83:4440–52.
105. Wu WH, Li M, Wang Y, Ouyang HX, Wang L, Li CX, et al. Aptasensors for rapid detection of Escherichia coli O157:H7 and Salmonella typhimurium. Nanoscale Res Lett. 2012;7:658.
106. de Aguiar Ferreira C, Branco de Barros AL. Aptamer functionalized nanoparticles for cancer targeting. J Mol Pharm Org Process Res. 2013;1:105.
107. Wu X, Chen J, Wu M, Zhao JXJ. Aptamers: active targeting ligands for cancer diagnosis and therapy. Theranostics. 2015;5:322–44.
108. Zhang LF, Radovic-Moreno AF, Alexis F, Gu FX, Basto PA, Bagalkot V, et al. Co-delivery of hydrophobic and hydrophilic drugs from nanoparticle–aptamer bioconjugates. ChemMedChem. 2007;2:1268–71.
109. Liu J, Wei T, Zhao J, Huang YY, Deng H, Kumar A, et al. Multifunctional aptamer-based nanoparticles for targeted drug delivery to circumvent cancer resistance. Biomaterials. 2016;91:44–56.
110. Kapse-Mistry S, Govender T, Srivastava R, Yergeri M. Nanodrug delivery in reversing multidrug resistance in cancer cells. Front Pharmacol. 2014;5:159.
111. Dong XW, Mumper RJ. Nanomedicinal strategies to treat multidrug-resistant tumors: current progress. Nanomedicine. 2010;5:597–615.
112. Sengupta S, Eavarone D, Capila I, Zhao GL, Watson N, Kiziltepe T, et al. Temporal targeting of tumour cells and neovasculature with a nanoscale delivery system. Nature. 2005;436:568–72.
113. Xing H, Wong NY, Xiang Y, Lu Y. DNA aptamer functionalized nanomaterials for intracellular analysis, cancer cell imaging and drug delivery. Curr Opin Chem Biol. 2012;16:429–35.
114. Chen LQ, Xiao SJ, Hu PP, Peng L, Ma J, Luo LF, et al. Aptamer-mediated nanoparticle-based protein labeling platform for intracellular imaging and tracking endocytosis dynamics. Anal Chem. 2012;84:3099–110.
115. Chen LQ, Xiao SJ, Peng L, Wu T, Ling J, Li YF, et al. Aptamer-based silver nanoparticles used for intracellular protein imaging and single nanoparticle spectral analysis. J Phys Chem B. 2010;114:3655–9.

116. Lonne M, Zhu GH, Stahl F, Walter JG. Biosensors based on aptamers and enzymes. Adv Biochem Eng Biotechnol. 2014;140:121–54.
117. Zheng D, Seferos DS, Giljohann DA, Patel PC, Mirkin CA. Aptamer nano-flares for molecular detection in living cells. Nano Lett. 2009;9:3258–61.
118. Wang, YY, Wang, YS, Liu, B. Fluorescent detection of ATP based on signaling DNA aptamer attached silica nanoparticles, Nanotechnology. 2008;19:415605.
119. Chiu TC, Huang CC. Aptamer-functionalized nano-biosensors. Sensors. 2009;9:10356–88.
120. Yigit MV, Mazumdar D, Kim HK, Lee JH, Dintsov B, Lu Y. Smart "turn-on" magnetic resonance contrast agents based on aptamer-functionalized superparamagnetic iron oxide nanoparticles. ChemBioChem. 2007;8:1675–8.
121. Song SP, Wang LH, Li J, Zhao JL, Fan CH. Aptamer-based biosensors. Trends Anal Chem; TrAC. 2008;27:108–17.
122. Stojanovic MN, Landry DW. Aptamer-based colorimetric probe for cocaine. J Am Chem Soc. 2002;124:9678–9.
123. Kim YS, Kim JH, Kim IA, Lee SJ, Gu MB. A novel colorimetric aptasensor using gold nanoparticle for a highly sensitive and specific detection of oxytetracycline. Biosens Bioelectron. 2011;26:4058–63.
124. Xu H, Wang YW, Huang XM, Li Y, Zhang H, Zhong XH. Hg^{2+}-mediated aggregation of gold nanoparticles for colorimetric screening of biothiols. Analyst. 2012;137:924–31.
125. Zhang CY, Johnson LW. Single quantum-dot-based aptameric nanosensor for cocaine. Anal Chem. 2009;81:3051–5.
126. Sharma R, Ragavan KV, Thakur MS, Raghavarao KSMS. Recent advances in nanoparticle based aptasensors for food contaminants. Biosens Bioelectron. 2015;74:612–27.
127. Bogomolova A, Aldissi M. Real-time aptamer quantum dot fluorescent flow sensor. Biosens Bioelectron. 2011;26:4099–103.
128. Duan N, Wu SJ, Zhu CQ, Ma XY, Wang ZP, Yu Y, et al. Dual-color upconversion fluorescence and aptamer-functionalized magnetic nanoparticles-based bioassay for the simultaneous detection of Salmonella typhimurium and Staphylococcus aureus. Anal Chim Acta. 2012;723:1–6.
129. Lee JH, Yigit MV, Mazumdar D, Lu Y. Molecular diagnostic and drug delivery agents based on aptamer-nanomaterial conjugates. Adv Drug Deliv Rev. 2010;62:592–605.
130. Emrani AS, Danesh NM, Lavaee P, Jalalian SH, Ramezani M, Abnous K, et al. Sensitive and selective detection of digoxin based on fluorescence quenching and colorimetric aptasensors. Anal Methods. 2015;7:3419–24.
131. Wang RE, Zhang Y, Cai J, Cai W, Gao T. Aptamer-based fluorescent biosensors. Curr Med Chem. 2011;18:4175–84.
132. He JL, Wu ZS, Zhang SB, Shen GL, Yu RQ. Fluorescence aptasensor based on competitive-binding for human neutrophil elastase detection. Talanta. 2010;80:1264–8.
133. Li XX, Qi HL, Shen LH, Gao Q, Zhang CX. Electrochemical aptasensor for the determination of cocaine incorporating gold nanoparticles modification. Electroanalysis. 2008;20:1475–82.