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Aptamer-Modified Nanoparticles in Medical Applications

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Contents

Abstract Since aptamers have been selected against a broad range of target structures of medical interest and nanoparticles are available with diverse properties, aptamer-modified nanoparticles can be used in various diagnostic and therapeutic applications. While the aptamer is responsible for specificity and affinity of the conjugate, the nanoparticles' function varies from signal generation in diagnostic approaches to drug loading in drug delivery systems. Within this chapter different medical applications of aptamer-modified nanoparticles will be summarized and underlying principles will be described.

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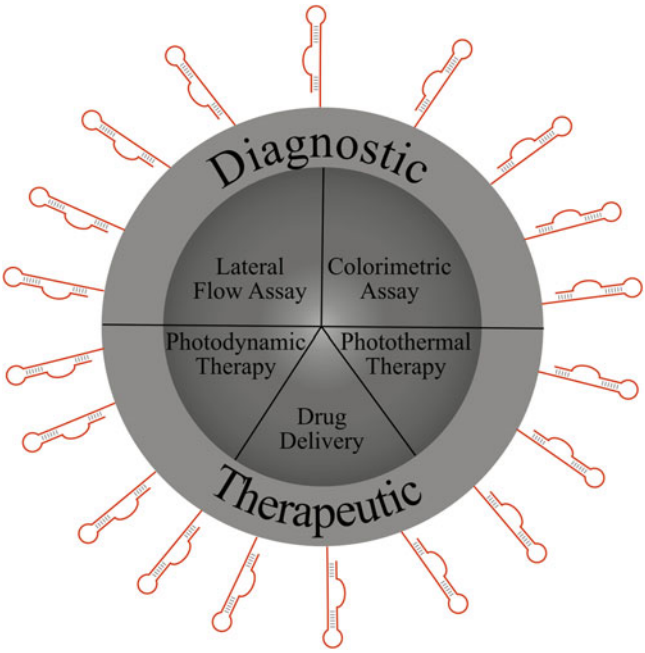
Graphical Abstract



Keywords Aptamer, Biosensor, Diagnostics, Drug delivery, Nanoparticle, Therapy

1 Introduction

Aptamers were first described in 1990 [1]. Since then many aptamers against a wide variety of target molecules have been selected [2]. At the same time, nanotechnology has advanced, thus providing the use of aptamer-coupled nanoparticles for many applications in therapeutic applications, for biosensing or cellular imaging [2, 3]. In contrast to free drug molecules, it was shown that targeted nanoparticles can accumulate in cancer tissue. So systemic side effects of drugs can be prevented in therapeutic applications, and in vivo imaging of target tissue or biomarkers can be enabled in diagnostics [3]. Also ex vivo applications for biomarker detection like lateral flow assays can be performed with aptamer-modified nanoparticles, where the nanoparticles are used for imaging and the aptamers to achieve specificity [4].

Nanoparticles are structures with a size smaller than 100 nm. They can be composed of different materials, resulting in many different types of nanoparticles such as gold nanoparticles, quantum dots, iron oxide nanoparticles, polymer nanoparticles, dendrimers, liposomes, and carbon nanotubes. Although derived from different materials, nanoparticles commonly display a high surface area to

volume ratio and offer the opportunity to modify this surface with different molecules. For example, targeting ligands such as aptamers can be used for nanoparticle modification [5]. Important aspects of aptamer coupling to nanoparticles are highlighted in the section below.

This chapter deals with the application of aptamer-modified nanoparticles with a focus on both therapeutic (Sect. 3) and diagnostic (Sect. 2) applications.

1.1 Coupling of Aptamers to Nanoparticles

Aptamers can either be immobilized to nanoparticles by covalent coupling or in a non-covalent way [6–9]. Most immobilization strategies require a site-specific modification of the aptamer; these modifications are commonly introduced during the aptamer synthesis [10]. Covalent coupling can, e.g., be performed via carbodiimide coupling chemistry [6]. Non-covalent coupling can be performed by utilizing affinity ligands (e.g., streptavidin and biotin) [9]; physisorption, e.g., between thiol-modified aptamers and gold nanoparticles [7]; or π-stacking between nucleotide bases of the aptamer and the side walls of single-walled carbon nanotubes (SWNTs) [8].

To ensure the functionality of nanoparticle-coupled aptamers, some parameters have to be considered. Aptamers depend on their three-dimensional structure for target recognition and binding; disrupting the native structure of an aptamer may lead to a loss of affinity [11]. Therefore the density of immobilized aptamers must be optimized carefully. Too high densities can result in non-correct folding of aptamers [12], while too low aptamer density can reduce the binding capacity of the functionalized nanoparticle. Furthermore the surface charge of the nanoparticle can affect aptamer folding, e.g., a positive-charged surface can interact electrostatically with the negative-charged aptamers and lead to aptamer unfolding. In some cases aptamers that are bound directly to a surface cannot adapt their correct folding because of their proximity to the surface; in these cases spacers (e.g., poly T spacers) can be used to increase the distance of the aptamers to the surface and therefore allow correct folding. The orientation of aptamer immobilization can also affect aptamer functionality; in this case both 3′ and 5′ terminal modifications should be investigated to optimize functionality of the aptamer orientation [13].

If these aspects are taken into account, aptamer-modified nanoparticles can be a promising tool for medical applications including diagnostics and therapy.

1.2 Aptamer-Modified Nanoparticles and Multivalent Binding

Nanoparticles hold the potential to carry more than one aptamer, thereby facilitating multivalent binding with dramatically increased affinity. Here either several copies

of one aptamer or different aptamers directed against the same target can be immobilized on a nanoparticle. This can dramatically increase the affinity of the system due to avidity effects [14]. While the term affinity describes the interaction between one ligand and its target structure, avidity describes the overall affinity of multiple binding events between the multimeric ligand construct and the target [15]. Avidity effects are possible when either different aptamers binding to distinct sites of a target molecule are available or in case of multivalent target structures, such as multimeric proteins or targets expressed in high density on a cell surface [15].

Multivalent aptamer-modified nanoparticles have already been proposed for therapeutic applications. They were used to develop theragnostic agents for cancer cell therapy [16] and drug delivery [17]. Moreover aptamer-modified nanoparticles were used to develop a system to control thrombin activity [18, 19]; one of these systems is described in Sect. 3.3 of this chapter in detail.

2 Assays with Aptamer-Modified Nanoparticles in Diagnostics

Common types of diagnostic applications are immunoassays using antibodies or analytical methods like liquid chromatography (LC) [20–23]. Because of their high costs and some other disadvantages, efforts are made to replace antibodies by aptamers [4, 6, 12, 24]. Due to their low cost production and the possibility to target even small and toxic analytes, aptamers provide the possibility for new diagnostic assays and applications. For generation of a signal, the aptamers often get conjugated to nanoparticles [12, 23]. The nanoparticles can produce a visible signal, either based on their color like gold nanoparticles or, e.g., an electrochemical signal [25]. Different assays exploiting aptamer-modified nanoparticles for diagnostic applications are discussed in the following sections.

2.1 Assays Using Aptamer-Modified Gold Nanoparticles

In assays with gold nanoparticles (AuNPs), the optical properties of AuNPs are utilized. Color shifts due to the size and distance-dependent light absorption can be analyzed by the naked eye. Colloidal gold nanoparticles are red; agglomerated particles show a color shift over purple to blue and black eventually. The AuNPs are mostly either produced by reduction of tetrachloroauric acid ($HAuCl_4$) by sodium citrate or by laser ablation of gold [26].

AuNPs can easily be modified with ligands, such as aptamers but also proteins like antibodies or other molecules [27, 28].

Often the high affinity of thiol toward gold is exploited, e.g., to immobilize thiol-modified aptamers on the AuNP surface, resulting in a strong semi-covalent

physisorption [29, 30]. Coating gold nanoparticles with a silica layer leads to free hydroxyl groups on the surface, which can further be functionalized with aptamers [31, 32]. Additionally "click" reactions of terminal alkynes and azides can be performed. These "click" reactions utilize the Au-thiol bond, which can further be used for modifying AuNPs with Polyethylene glycol (PEG) ligands [33]. Non-modified aptamers get adsorbed as well, just with a weaker, electrostatic interactions [34].

The assays use the ability of aptamers to specifically bind their target. Some assays need an additional interaction partner, to generate signals. In case of small molecule detection, oligonucleotides complementary to the aptamers are frequently used. The assay setup can be a sandwich or a competitive assay; properties like size and binding mechanism of the aptamer, target, and oligonucleotide are crucial for the selected setup.

In this chapter, the most common assays using aptamer-modified AuNPs are presented.

2.1.1 Lateral Flow Assays

Lateral flow assays (LFAs) are paper-based platforms for detection and sometimes even quantification of an analyte. Many LFAs work with complex media, such as blood, urine, saliva, or serum [35]. The most popular LFA is the pregnancy test, invented in the 1970s [36], using urine or blood as sample probe. But there are numerous LFAs for different analytes, some of them shown in Table 1. They all have in common that the visible signal determining between positive or negative is derived by ligand-modified AuNPs. In the case of the pregnancy test the ligands are antibodies [37], but more recently also aptamers have been used. The aptamers are immobilized to the nanoparticles and bind their target based on their three-dimensional structure.

In case of aptamer-based LFAs, two different setups are most frequently used, competitive LFAs with a complementary oligonucleotide (cOligo) or sandwich format with two different aptamers binding the analyte simultaneously. Depending on the setup, the presence of the targeted analyte results in a colored or uncolored test zone.

Table 1 Assays with aptamer-modified gold nanoparticles

Setup	Target	Limit of detection	Sample	Reference
Lateral flow assay	Cholera toxin	2–10 ng mL^{-1}	Spiked buffer	[45]
Lateral flow assay	Kanamycin	35 nM	Food samples	[46]
Lateral flow assay	Zearalenone	5–200 ng mL^{-1}	Spiked corn samples	[47]
Colorimetric assay	Cholic acid	1 μM	Spiked buffer	[48]
Colorimetric assay	17 β-estradiol	0.1 ng mL^{-1}	Spiked buffer	[49]
Colorimetric assay	Human insulin	0.0156 ng mL^{-1}	Serum	[50]
Colorimetric assay	Serotonin	52 ng mL^{-1}	Spiked buffer	[51]

Figure 1 shows the scheme of a LFA; it consists of three to four different parts: sample pad, where the liquid sample is applied; conjugate pad (CP) which possesses the dried AuNP conjugates; membrane (mostly nitrocellulose) which has one test and one control zone; and absorbent pad (AP), which soaks up the test liquid. The LFA uses the capillary force; therefore the sample flows through all the pads while collecting the AuNPs of the CP, interacting on the membrane and finally reaching the AP [35, 38].

The analyte concentration in the sample can affect the assay and result in a concentration-dependent intensity of the test zone, thereby allowing quantification. For reliable results LFAs have a readout window after running the test; afterward the signals can change due to detachment of the particles or analytes. In general they are point-of-care devices that deliver a fast result and are easy to use, even for untrained personnel [38] and exist for various medical targets, as shown in Table 1. The listed LFAs are currently still restricted to research applications, but the usage in complex samples for diagnostic analysis is assumed to allow clinical use.

2.1.2 Colorimetric Assays

In contrast to LFAs, which use a membrane as a solid phase, AuNPs can also be used in liquid-phase assays. Colorimetric assays with gold nanoparticles use the color shifting effect from red to purple/blue, when AuNPs aggregate or assemble [39]. There are two different setups, one with aptamers adsorbed on the particles and the other with stable aptamer-AuNP conjugates.

Colloidal AuNPs have a special localized surface plasmon resonance (LSPR), resulting in their optical properties. The LSPR is sensitive to the size of the AuNPs and the local refractive index near their surface. Changes in the LSPR are visible due to the color change of the AuNPs [40]. AuNPs synthesized with citrate are stable in solution because the citrate ions form an adsorbed protective layer around the AuNPs, and the electrostatic repulsion from these anions keeps the AuNPs separated [41]. When salt (e.g., NaCl) is added, the AuNPs aggregate, because the salt ions shield the negative charge on the AuNPs and the interparticle distance decreases, thereby the LSPR changes and the AuNPs solution turns purple [42, 43].

Ligands like aptamers can be bound to the AuNPs and stabilize the AuNPs at higher salt concentrations [44]. Aptamers can form electrostatic interactions with AuNPs and adsorb reversibly on their surface or they can be conjugated covalently or covalently like, as described in Sect. 1.1. The negatively charged aptamers provide stabilization of AuNPs by electrostatic repulsion and steric stabilization, so that the AuNP solution retains it red color. If an analyte is added and two aptamers bind in a sandwich setup simultaneously, the AuNPs assemble and the interparticle distance gets reduced; therefore the LSPR shifts and the visible color changes to purple [39].

The different colorimetric assay setups are shown in Fig. 2.

Salt-induced aggregation assays with adsorbed aptamers are shown in Fig. 2a, and directed assembly of conjugates is shown in Fig. 2b.

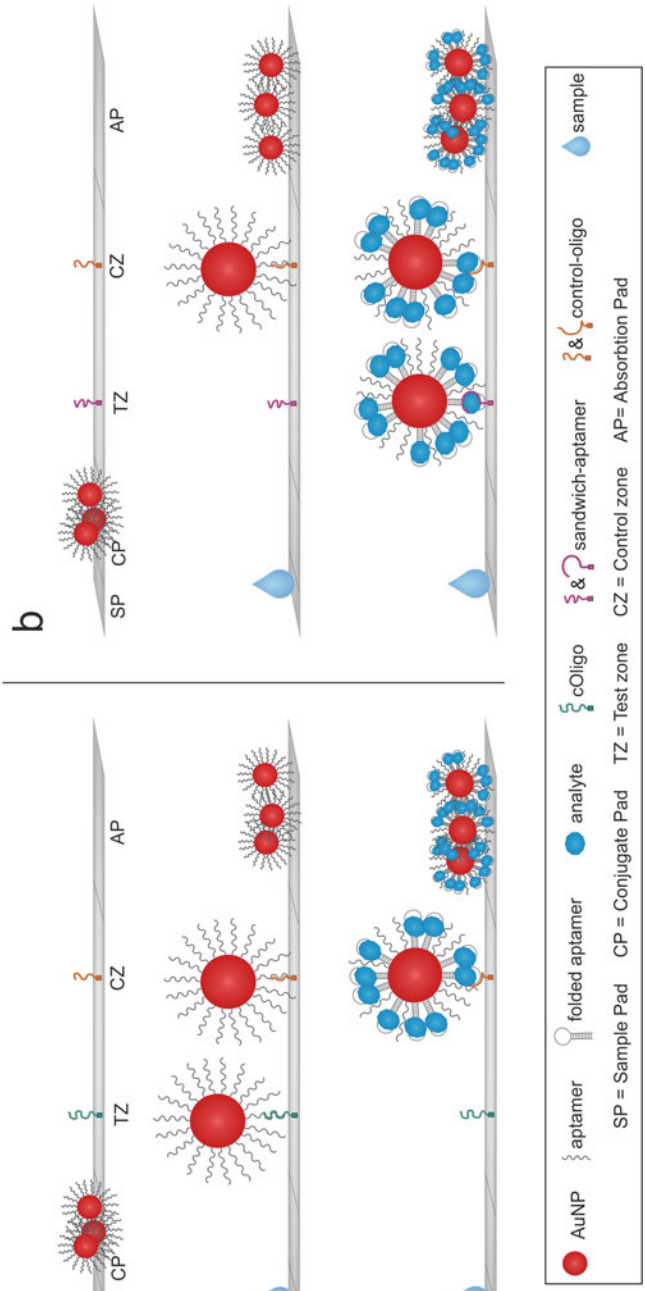


Fig. 1 Lateral flow assay with aptamer-modified gold nanoparticles. (**a**) Competitive assay format, cOligo on test zone, control oligo on control zone (binds, e.g., part of aptamer on spacer sequence, which doesn't interact with target/is free to bind even when target binds). (**b**) Sandwich format, aptamer on test zone which binds target, conjugated AuNPs bind target as well. AuNP sizes from CP/AP and NZ differ for better visualization

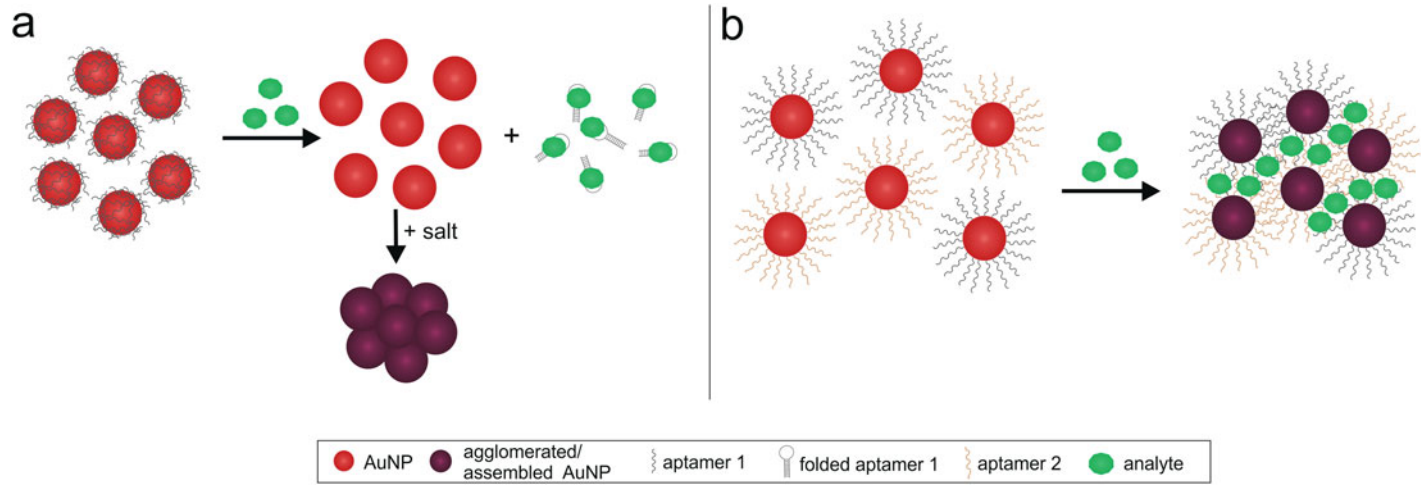


Fig. 2 Colorimetric assay with aptamer-modified gold nanoparticles, (**a**) adsorbed aptamers detach from AuNP to bind their target, AuNPs aggregate due to salt addition. (**b**) AuNPs are modified with aptamer 1 or 2, which can bind in a sandwich format to the added analyte and AuNPs build an assembly

By comparing different samples, the samples' color shift gives information about the analyte concentration, and therefore the assay can be quantitative.

An overview of various assays with aptamer-modified gold nanoparticles is given in Table 1.

2.2 *Biosensors Using Aptamer-Modified Nanoparticles*

Besides colorimetric applications, aptamer-modified nanoparticles can be used in various types of biosensors. Depending on the nanoparticle composition, fluorescence signals, UV-Vis analysis, or electrochemical signals can be used for biosensors.

2.2.1 Fluorescence Biosensors

Fluorescence sensors benefit from the property of metal nanoparticles to alter the fluorescence of fluorophores. Dependent on the nanoparticles size and the distance of the fluorophore to the particle, the fluorescence can be enhanced or more likely be reduced; hence most particles quench the fluorescence signal. Crucial for the fluorescence change is the plasmon field around the particle, which is generated by incident light and the dipole energy around the particle [27, 52].

Many sensors are FRET (Förster resonance energy transfer)-based sensors [53]. For FRET sensors the aptamer or cOligo is modified with a fluorophore. In one setup fluorescence-modified aptamers get adsorbed onto nanoparticles and due to quenching generate a low fluorescence signal. When the analyte is present, aptamers detach from the nanoparticles to bind their target; thus the fluorescence increases. This principle is called "signal-on." Depending on the nanoparticles and fluorophore properties, the opposite "signal-off" can be used as the sensor setup. For the "signal-off" sensor, the aptamer is covalently or semi-covalently bound to the nanoparticle, with the fluorophore having the furthest distance from the nanoparticle as possible. Once the target is present, the aptamers' structure changes, and therefore the distance of the fluorophore to the nanoparticle decreases, leading to a higher quenching efficiency and lower signal intensity [52, 54].

Another setup has aptamers immobilized to the nanoparticle. When the cOligo is fluorescence-modified and is competing with the target to bind the aptamer conjugated to the AuNP, the cOligo fluorophore quenching evaluates the assay. A sandwich assay would work similarly.

Another fluorescence sensor setup uses the aptamers' structural changes, operating in a fluorophore distance change [53]. Fluorescent-modified aptamers that bound to nanoparticles generate different signals depending whether the target is bound or not bound to the aptamer. Crucial for this signal change is the fluorophore distance to the particle, which has to change distinctly with the aptamer structural change upon

Table 2 Fluorescence biosensors using aptamer-modified nanoparticles

Setup	Target	Nanoparticle	Reference
FRET	Cocaine	Quantum dots	[55]
FRET	Human cardiac troponin I	Gold NPs, CdSeS/ZnS quantum dots	[56]
FRET	Tumor marker Mucin 1	CdSe quantum dots	[57]
FRET	$VEGF_{165}$	Silver NPs, Mn-ZnS quantum dots	[58]
Fluorescence detection	Theophylline	Gold NPs	[59]
Fluorescent enhancement	Protein of H5N1 influenza virus	Silica-coated silver NPs	[60]

target binding. Thus this setup only works for aptamers which exhibit a large conformational change upon target binding [52].

The methods for fluorescence biosensors listed above consist of labeled aptamers, but there are some label-free methods as well. A competitive assay setup uses aptamer-modified nanoparticles with free fluorophores intercalated to the aptamers. Upon addition of the target, the fluorophores detach from the aptamer, and the fluorescence signal increases, resulting in a signal-on sensor [53].

An overview of different fluorescence biosensors is listed in Table 2.

2.2.2 Other Biosensors

Besides the fluorescence sensors, there are various other applications using aptamer-modified nanoparticles. For once there are UV-Vis sensors using absorption properties of nanoparticles. For example, most AuNPs have an absorption peak around 520 nm, which can be influenced by different factors [61]. It shifts to higher wavelength with the particles' aggregation, or the absorption intensity can change upon binding of analytes. The aggregation shift can easily be monitored by a colorimetric assay due to the sample color shift. When aptamer-modified AuNPs bind the aptamer target, such an intensity shift can occur; the intensity changes proportional to the analyte concentration. The intensity can be enhanced or decreased, depending on assay components [62].

Some electrochemical sensors measure the change in proton relaxation times (ΔT_2). Magnetic nanoparticles (MNPs) can enhance the magnetic resonance signal of protons from the surrounding water molecules [63]. Whether aptamer-modified MNPs aggregate or disassemble their cluster due to target binding results in a signal change, measurable by NMR (nuclear magnetic resonance), MRI (magnetic resonance imaging), or relaxometry [64].

Measuring voltammetry for aptamer-based sensors is a further application. These sensors use various materials as the sensors base, like graphene nanocomposites or carbon electrodes or other nanohybrids, but often use a layer of AuNPs on top for aptamer conjugation. Due to target binding to the aptamer, the electrodes measure changes in cyclic voltammetry, differential pulse voltammetry, or square wave voltammetry [65–67].

2.3 Cell Detection with Aptamer-Modified Nanoparticles

The assays and biosensors described in the previous sections allow the detection of small molecules or proteins. But detection of whole cells is also possible.

Specific ligands on the cell membrane can be targeted by the aptamers [68–76]. Mainly assays targeting bacterial cells or human cancer cells have been described so far.

Table 3 gives an overview of different methods for cell detection with aptamer-modified nanoparticles.

3 Aptamer-Modified Nanoparticles for Therapeutic Applications

Besides analytical and diagnostic applications, aptamer-modified nanoparticles are also a promising approach for treatment of different diseases like cancer, Alzheimer's disease, or blood-clotting disorders [19, 79, 80]. Due to their size, nanoparticles cannot penetrate the vasculature of healthy tissues. Thus nanoparticles

Table 3 Cell detection methods with aptamer-modified nanoparticles

Setup	Target	Nanoparticle	Reference
Electrochemical, H_2O_2 reduction	A549 lung cancer cells	Gold NPs	[68]
Fluorescence and magnetic separation	HER2 and MUC1 breast cancer cells	Silica NPs	[77]
Light scattering with plasma mass spectrometry	Hs578T breast cancer cells	Gold NPs	[70]
Paper-based electrochemiluminescence electrode	MCF-7 breast cancer cells	Gold NPs	[71]
Electroluminescence	HL-60 (cancer) cells	Gold NPs	[78]
Fluorescence – flow cytometry	Leukemia cells	Fluorescent silica NPs	[73]
Dual-recognition units based on FRET	*Staphylococcus aureus*	Gold NPs	[74]
Magnetically assisted surface-enhanced Raman scattering	*Staphylococcus aureus*	Superparamagnetic ferrite NPs, gold NPs	[75]
Electrochemical impedance spectroscopy	*Salmonella typhimurium*	Gold NPs	[76]

accumulate in tissue with leaky vasculature such as inflamed tissue or tumors [79]. This effect, called enhanced permeability and retention effect (EPR effect), alters the biodistribution and pharmacokinetics of a drug, so it can reduce the systemic side effects of nanoparticle-bound drugs and increase drug concentrations in target tissues [5, 79].

The addition of a targeting ligand such as an aptamer further increases the specificity of the system and can promote target binding and cellular uptake of nanoparticles (e.g., via receptor-mediated endocytosis) [5, 79]. This results in higher cytotoxicity of aptamer-modified nanoparticle systems and therefore can reduce the amount of the drug that is needed for therapy [81, 82].

There are several possibilities to use aptamer-modified nanoparticles for therapeutic applications. A frequently used approach is the use of nanoparticles for drug delivery (see Sect. 3.1). Another therapeutic application using aptamer-modified nanoparticles is the photothermal and photodynamic therapy, which uses the characteristics of the nanoparticle material to create damaging effects to cells upon irradiation with certain wavelengths (see Sect. 3.2). In Sect. 3.3 some less frequently used applications of aptamer-modified nanoparticles for therapeutic applications are shown.

3.1 Drug Delivery with Aptamer-Modified Nanoparticles

The most common approach of using aptamer-modified nanoparticles for therapeutic applications is the delivery of drugs to the target tissue with nanoparticles as carrier materials. By the modification with aptamers, these drug-nanoparticle conjugates obtain their specificity for the target cells. Several different materials can be used as carrier materials. Their specific advantages and disadvantages will be discussed below. In Table 4 a summary of different aptamer-based targeted drug delivery systems (TDDS) is shown.

3.1.1 Gold Nanoparticles

Gold nanoparticles (AuNPs) can be produced with different methods; commonly citrate-based methods resulting in citrate-functionalized AuNPs or the Brust-Schiffrin method resulting in alkanethiolate-modified AuNPs are used. Preparation methods usually offer high yields and result in nanoparticles with characteristic sizes and shapes (e.g., spherical or nonspherical). The sizes of AuNPs can be between a few nanometer and over 200 nm. The cytotoxicity of AuNPs is dependent on their size and surface modification and has to be investigated for each system individually. The surface area to volume ratio of AuNPs is usually high, and the surface can be modified in many different ways (e.g., with thiols or amines) [83]. Targeting ligands can easily be added to AuNPs, e.g., by physisorption of thiol-modified aptamers or by coupling to functional groups on the nanoparticle surface [7, 83]. For targeted

Table 4 Examples of aptamer-based targeted drug delivery systems

Nanomaterial	Aptamer	Target	Drug	Possible application	Reference
Au	sgc8	Protein tyrosine kinase 7 (PTK 7)	Doxorubicin	Leukemia treatment	[7]
Au	sgc8 and AS1411	PTK 7 and nucleolin	Daunorubicin	Leukemia treatment	[86]
Liposome	sgc8	Protein tyrosine kinase 7 (PTK 7)	FITC-dextran (model drug)	Leukemia treatment	[91]
Liposome	AS1411	Nucleolin	siRNA (anti BRAF)	Treatment of malignant melanoma	[92]
PEG-PCL	S15 and S6	A549 cells	Paclitaxel	Lung cancer treatment	[6]
PEG-PCL	AS1411	Nucleolin	Docetaxel	Brain glioma treatment	[95]
PLGA-b-PEG	A10	Prostate-specific membrane antigen (PSMA)	Docetaxel	Prostate cancer treatment	[81]
PLGA-b-PEG	A10	Prostate-specific membrane antigen (PSMA)	Cisplatin	Prostate cancer treatment	[82]
PLGA-b-PEG	A10	Prostate-specific membrane antigen (PSMA)	Doxorubicin + docetaxel	Prostate cancer treatment	[84]
PLGA-PEG	A10	Prostate-specific membrane antigen (PSMA)	Cisplatin + docetaxel	Prostate cancer treatment	[97]
CA-PLGA-b-TPGS	AS1411	Nucleolin	Docetaxel	Breast cancer treatment	[98]
PLA-PEG-COOH	A10	Prostate-specific membrane antigen (PSMA)	Rhodamine-dextran (model)	Prostate cancer treatment	[108]
PEG-PLA	FB4	bEND5 cells	Flurbiprofen	Alzheimer's disease treatment	[80]
PLGA	AS1411	Nucleolin	Paclitaxel	Glial cancer treatment	[100]
PLGA	S2.2	MUC1	Paclitaxel	Breast cancer treatment	[101]
PEI-citrate	CD 30 aptamer	CD 30	siRNA	Anaplastic large-cell lymphoma (ALCL) treatment	[102]
Mesoporous silica nanoparticle	AS1411	Nucleolin	Doxorubicin	Breast cancer treatment	[104]

(continued)

Table 4 (continued)

Nanomaterial	Aptamer	Target	Drug	Possible application	Reference
Mesoporous silica nanoparticle	EpCAM aptamer	EpCAM	Doxorubicin	Colon cancer treatment	[109]
Mesoporous silica nanoparticle	ATP aptamer	ATP	Dye (model)	ATP-responsive controlled release of drugs	[103]
Mesoporous silica nanoparticle	ATP aptamer	ATP	Fluorescein (model)	ATP-responsive controlled release of drugs	[105]
Dendrigraft poly-L-lysine (DGL)	AS1411; cytochrome c aptamer; ATP aptamer	Nucleolin; cytochrome C and ATP	Doxorubicin	Treatment of multidrug-resistant cancer cells	[106]
MS2 bacteriophage capsid	G-quadruplex targeting aptamer	MCF-7 cells	Porphyrins for PDT	Breast cancer treatment	[107]

drug delivery based on AuNPs, drugs can be integrated into the system by three commonly used ways that are described below (see Fig. 3).

The first way is the intercalation of drugs into hairpin regions of DNA (see Fig. 3a). For example, the drug doxorubicin is able to intercalate into DNA hairpins. As specificity-mediating ligands, aptamers can be bound to the surface in addition to the hairpin DNA [7].

The second way is the direct intercalation of drugs into aptamers (see Fig. 3b). For example, the aptamer A10 has a GC-rich stem region, where the drug doxorubicin can intercalate [84]. This intercalation often takes place at GC-rich parts of oligonucleotides, e.g., in G-quadruplex structures [85].

Thirdly the drug can be loaded to the surface of AuNPs (see Fig. 3c). For example, Taghdisi et al. loaded gold nanoparticles with the drug daunorubicin [86]. Figure 3 shows the components of different AuNP-based targeted drug delivery systems.

Additionally AuNPs offer the opportunity of photodynamic therapy which is discussed in Sect. 3.2.1. One disadvantage of AuNPs in drug delivery is that only the surface of the AuNPs or the surface-bound ligands can be used as a drug carrier. Some other nanoparticle types do also allow the utilization of the nanoparticle volume to enhance loading capacity, as discussed in the next paragraphs.

3.1.2 Liposomes and Micelles

Liposomes usually consist of a bilayer of natural or synthetic phospholipids (e.g., phosphatidylcholine or phosphatidylethanolamine (see Fig. 4) [85, 87]. The bilayer structure enables encapsulation of hydrophilic drugs inside of the core and hydrophobic drugs inside of the lipid bilayer [85]. At body temperature the bilayers are in a fluid state, making them leaky for encapsulated drugs. If cholesterol is added, the structure of the liposomes gets more stable, and unintended drug release can be prevented [87]. Liposomes can be prepared in different ways; the first described method was the rehydration of a thin film of lipids with aqueous solvents, which was generated by organic solvent evaporation in a round bottom flask. The size can further be reduced by sonication or extrusions through a polycarbonate membrane. One limitation of this method is the rather low encapsulation efficiency [87, 88]. Another method is the addition of lipids dissolved in an organic solvent into an aqueous drug solution (solvent injection technique) and subsequent removal of the organic solvent [87, 89]. However, removal of the organic solvent can be difficult. By using microfluidics, liposomes with a defined size can be produced continuously, thereby offering the opportunity for an industrial preparation method in a large scale [87, 90]. One drawback of liposomes is their short half-life in vivo. However, this can be improved by surface modification with polyethylene glycol (PEG). PEG can prevent plasma protein binding and therefore increase the half-life of liposomes ("stealth liposome") [87].

Targeting ligands like aptamers can be attached, e.g., via coupling to maleimide or *N*-hydroxysuccinimide-activated PEG-modified phospholipids [91, 92]. A

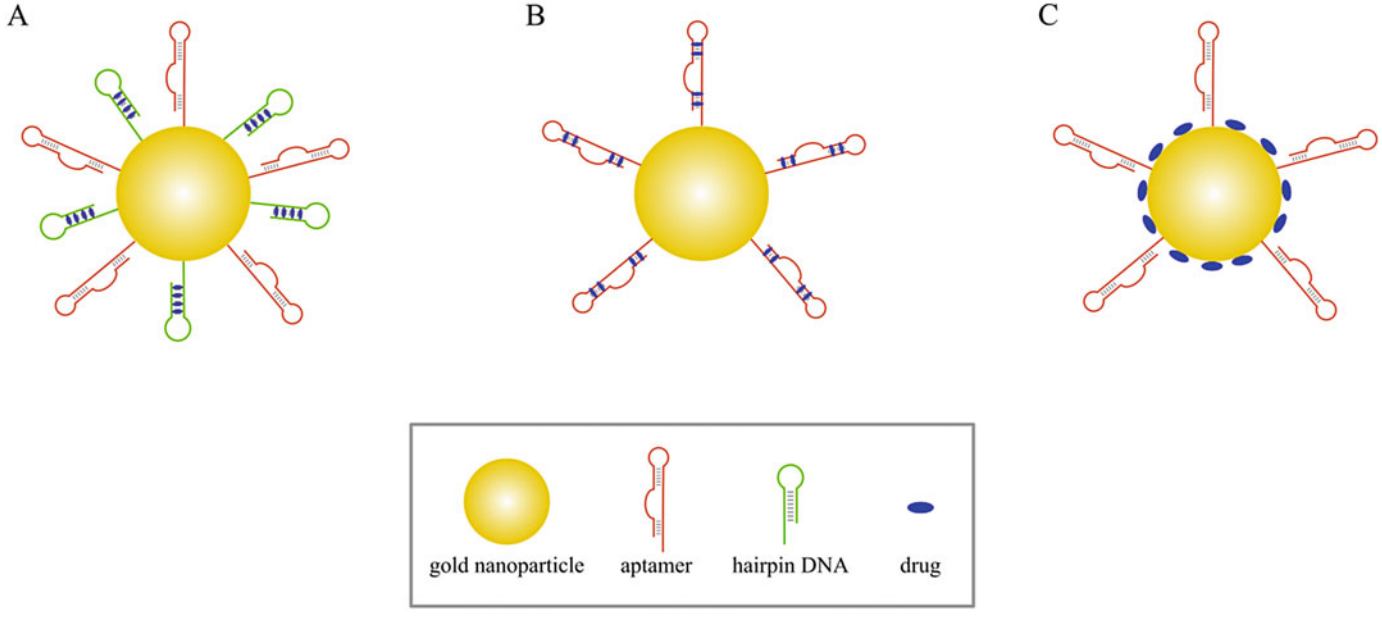


Fig. 3 Different designs of targeted drug delivery systems containing aptamer-modified gold nanoparticles. Drugs can either be intercalated into hairpin DNA (**a**), directly into some aptamers (**b**), or loaded to the surface of the particles (**c**) (according to [85])

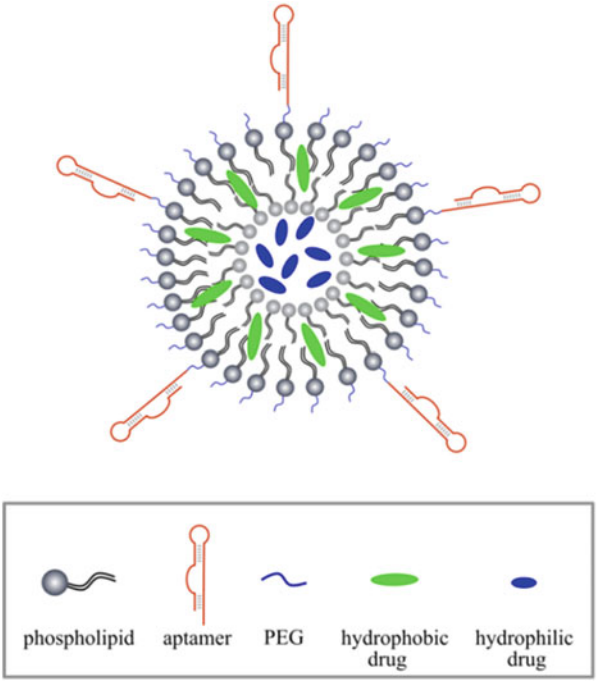


Fig. 4 A possible composition of a liposome for targeted drug delivery (according to [85])

PEG-modified liposome with encapsulated doxorubicin was the first FDA-approved nanodrug (Doxil®), but this system was still without a targeting ligand [93]. The results of Kang et al. showed that aptamers increase the binding of the system to target cells, which could be a promising advancement for future applications [91]. Figure 4 shows the components of different liposome-based targeted drug delivery systems.

3.1.3 Polymer Nanoparticles

Polymer nanoparticles are commonly used nanoparticles for drug delivery. They can be prepared out of preformed polymers or directly out of monomers by different methods. Polymer nanoparticles can be organized in a complete solid structure (nanospheres), or they can contain a fluid core (nanocapsules) [94]. In the literature many examples are described, in which block copolymers are used, composed of a hydrophilic and a hydrophobic part [6, 80, 82, 84, 95–98]. These block copolymers usually build nanoparticles through spontaneous self-assembly [99]. The resulting polymer nanoparticles usually have a hydrophobic core and hydrophilic shell (see Fig. 5a). This hydrophobic core enables to encapsulate hydrophobic drugs, whereas targeting ligands, such as aptamers, can conveniently be coupled to the hydrophilic shell compounds [85]. PEG is often used as a shell compound, which decelerates

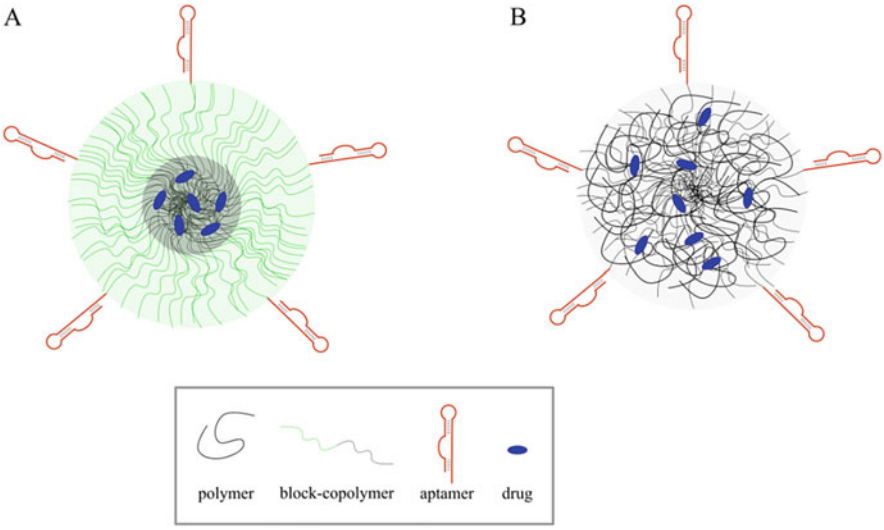


Fig. 5 Design of aptamer-modified polymer nanoparticles for drug delivery. Nanoparticles can be produced out of block copolymers that have a hydrophobic and a hydrophilic part and therefore form a hydrophobic core in aqueous solution. In this hydrophobic core, hydrophobic drugs can be encapsulated (**a**). If nanoparticles are produced only out of a hydrophobic polymer, there is no core-shell structure, but also hydrophobic drugs can be encapsulated (**b**). Aptamers are usually coupled covalently (according to [85])

systemic clearance by preventing the binding of plasma proteins and thereby preventing recognition by the mononuclear phagocyte system [84, 87].

Despite the popularity of block copolymers, other nanoparticles are composed of only a single type of polymer, e.g., poly(lactic-*co*-glycolic acid) (PLGA), and therefore do not form a core-shell structure but a nanosphere (see Fig. 5b) [100, 101].

The aptamers serve as affinity ligands for target cell binding and thereby enable specificity, and in some cases they can also promote the internalization of nanoparticles into target cells, e.g., via receptor-mediated endocytosis [80, 81, 96, 98, 100–102]. Figure 5 shows the components of different polymer nanoparticle-based targeted drug delivery systems.

3.1.4 Mesoporous Silica

Mesoporous silica nanoparticles (MSNs) are used for drug delivery because of their good biocompatibility, their high surface area that allows high drug loading, and the possibility of easy functionalization [103]. Furthermore there are several applications to control the drug release from the MSN, including pH-dependent drug release or target-controlled drug release (see Fig. 6) [103–105].

One possibility for controlled drug release is locking the drug-containing pores with DNA hybrids formed by aptamers and oligonucleotides complementary to

portions of the aptamer sequence. Here drugs cannot escape the pores until the target molecule binds to the aptamer resulting in dissociation of the aptamer from the oligonucleotide (see Fig. 6a). Upon target binding, the aptamer dissociates from the MSN, and due to the flexibility of the complementary DNAs the MSNs pores are open and facilitate the release of the drug [103]. Alternatively, pores can be blocked by aptamer-modified gold nanoparticles (see Fig. 6b). Therefore molecules have to be coupled to the MSN, to which the used aptamer also binds, but the binding strength has to be weaker than to the target molecule (e.g., adenosine also binds to ATP-targeting aptamers, but binding strength of the aptamer is stronger for ATP, so adenosine is released from the ATP aptamer in the presence of ATP). If there is no target, the aptamer-modified gold nanoparticles bind to the surface of the MSN and prevent drug release. In the presence of the target, it will bind to the aptamers, thereby releasing the aptamer-modified AuNPs from the MSN, the pores aren't blocked anymore, and the drug is released [105]. For example, Zhu et al. and He et al. developed this controlled drug release system using ATP as target molecule to trigger drug release [103, 105].

The surface of MSN can easily be functionalized with different molecules. If drugs are bound to MSN by electrostatic interactions with surface molecules, drugs can be released due to pH shift. For example, Li et al. developed a MSN system with phosphate-modified inner channels that can bind positively charged doxorubicin via electrostatic interactions at physiological pH. If the pH decreases, like in tumor tissue, the charge of the phosphate gets positive, and doxorubicin is released from the pores. Aptamers were bound to the surface of the MSN for targeted cell binding and receptor-mediated endocytosis [104]. Figure 6 shows the components of different MSN-based targeted drug delivery systems.

3.1.5 Others

There are some nanomaterials that are less frequently used for drug delivery but also worth mentioning. These are explained in more detail in the following sections; a short summary of the systems can be found in Table 4.

Dendrimers

Dendrimers are branched polymers that contain a central inner core. To this core several repeating groups of the polymer are bound, which themselves can bind several repeating groups, so they build a branched outer layer [85]. The surface can be modified with several functional groups, thus providing a wide range of modifications, e.g., with targeting ligands such as aptamers [106]. Drugs can be bound inside the dendrimers. Chen et al. developed a sophisticated dendrimer-based drug delivery system containing three different aptamers for mitochondrial targeting and to circumvent multidrug resistance (MDR) of tumor cells. The aptamer AS1411 was used for targeting and internalization into nucleolin presenting cells. Furthermore, a cytochrome c targeting aptamer was used for internalization of the dendrimers into the mitochondria, and an ATP aptamer was used for selective

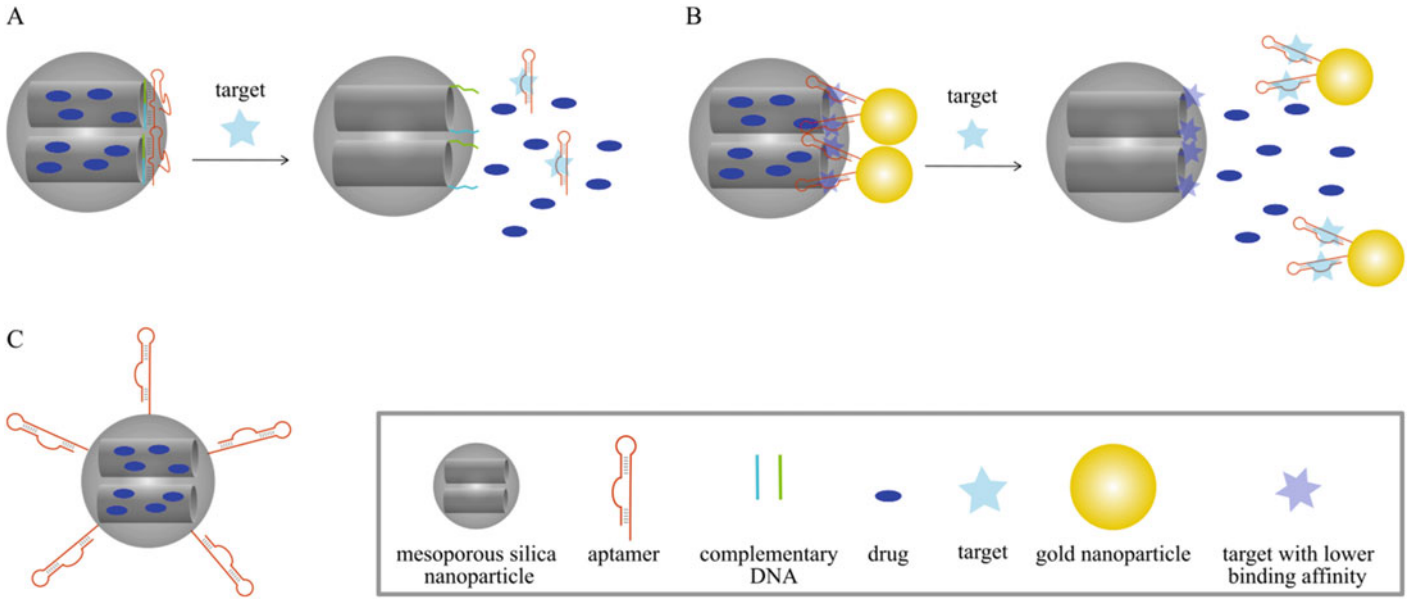


Fig. 6 Different designs of aptamer-modified mesoporous silica nanoparticles. Drug release can be prevented by blocking of drug-containing pores with complementary DNA and aptamer upon target binding (**a**), or with aptamer-modified gold nanoparticles, that bind on targets with lower binding strength next to the drug-containing pores upon competitive displacement by the targets (**b**). By using electrostatic interactions for drug binding inside the pores, drug can be released by pH shift (**c**) (according to [103–105])

drug release. The ATP aptamer was hybridized with a complementary DNA, and the drug doxorubicin was intercalated in this DNA duplex. At high concentrations of ATP, the ATP aptamer binds ATP and therefore dissociates from the duplex structure resulting in drug release. This system ensures that drugs are only released within the mitochondria, where the ATP level is high. Using these multifunctional nanoparticles, Chen et al. could show that this drug delivery system is even effective in multidrug-resistant cancer cells (MCF-7/ADR cells) [106].

Virus Capsid

Virus capsids are protein-based nanoparticles that can be used to encapsulate different molecules; the surface can also be modified with different molecules, such as aptamers [85]. Cohen et al. developed a virus capsid based on the MS2 bacteriophage and loaded it with cationic porphyrins, which can be used for photodynamic therapy. The surface was modified with an aptamer that binds to MCF-7 cancer cells. This system showed cytotoxicity to MCF-7 cells, but no cytotoxicity to non-targeted cells; furthermore no cytotoxicity was observed when a non MCF-7 binding aptamer was used [107].

3.2 Photothermal and Photodynamic Therapy with Aptamer-Modified Nanoparticles

Apart from nanoparticles as delivery vehicles for drugs, nanoparticles themselves can be the therapeutic part of an aptamer-modified nanoparticle system. Some materials have the properties to cause photothermal or photodynamic effects [110].

In photodynamic therapy (PDT) usually a nontoxic photosensitizer is activated by irradiation of the targeted tissue. Thereby, singlet oxygen ($^1O_2$) is generated by the photosensitizer using the energy from the radiation. Singlet oxygen is highly reactive and causes severe reactions with cellular molecules which finally lead to cell death. In PDT it is possible that the nanoparticle itself is the photosensitizer or the photosensitizers are conjugated to nanoparticles via aptamers. In the latter case, the nanoparticles quench the cytotoxic effects if the photosensitizers are close to the nanoparticles in the absence of the target. By conformational changes upon target binding of the aptamer, the photosensitizer is not quenched any longer, and cytotoxic effects can occur after illumination (see Fig. 7) [8].

In photothermal therapy (PTT) nanoparticles prepared of a suitable material (e.g., gold) are irradiated. The resulting photon energy is transformed into heat which then causes cell damage and finally cell death (see Fig. 8a) [7]. Table 5 shows the components of aptamer-modified nanoparticles for PTT and PDT.

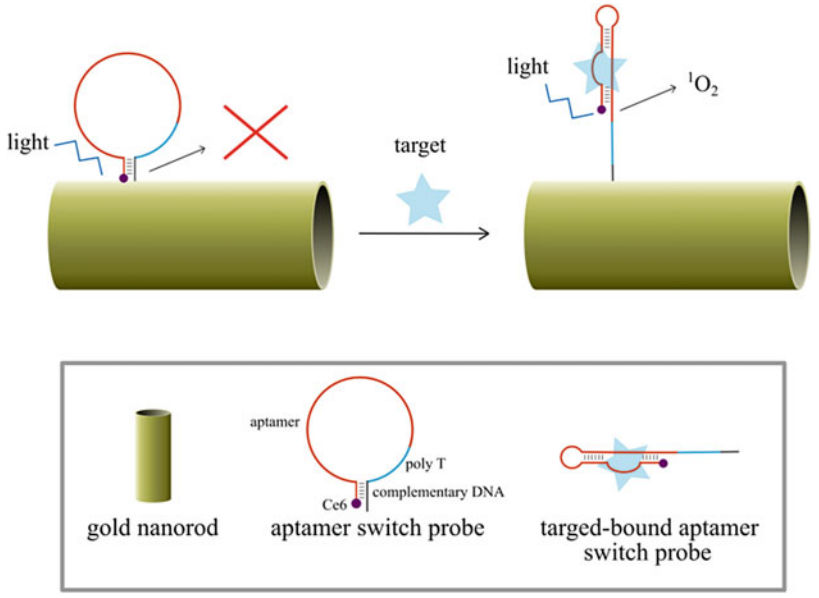


Fig. 7 Design of aptamer-modified gold nanorods for PDT. The aptamer is elongated with a poly T spacer and a DNA sequence that is complementary to the terminus of the aptamer that is conjugated with the photosensitizer Ce6. Without target binding the photosensitizer is close to the gold nanorod, which quenches the Ce6 activity. Upon target binding the Ce6 isn't close to the gold nanorod anymore and light irradiation leads to formation of singlet oxygen ($^1O_2$) that causes cell damage (according to [8])

3.2.1 Gold Nanoparticles for Photodynamic and Photothermal Therapy

Gold nanoparticles have a high plasmon resonance and low quantum yield; therefore photon energy which is absorbed by AuNPs after irradiation is nearly completely transformed into heat. This offers the opportunity for their usage for photothermal therapy.

Additionally the generated heat can lead to a controlled release of drugs if the AuNPs are used for drug delivery (see Fig. 8b) [7]. Nevertheless, not all tissues can be reached by direct laser irradiation. For example, a laser with a wavelength of 532 nm, which was used by Luo et al. for controlled release of drugs from AuNPs, can only penetrate the skin to a depth of 0.3 to 0.5 mm [7, 111]. However, by the use of fiber optics, irradiation can reach nearly all targeted tissues in a minimal invasive way [112].

3.2.2 Single-Walled Carbon Nanotubes (SWNT)

Besides gold nanoparticles also single-walled carbon nanotubes (SWNTs) can be used for photodynamic therapy [8]. They consist of a cylinder out of a single

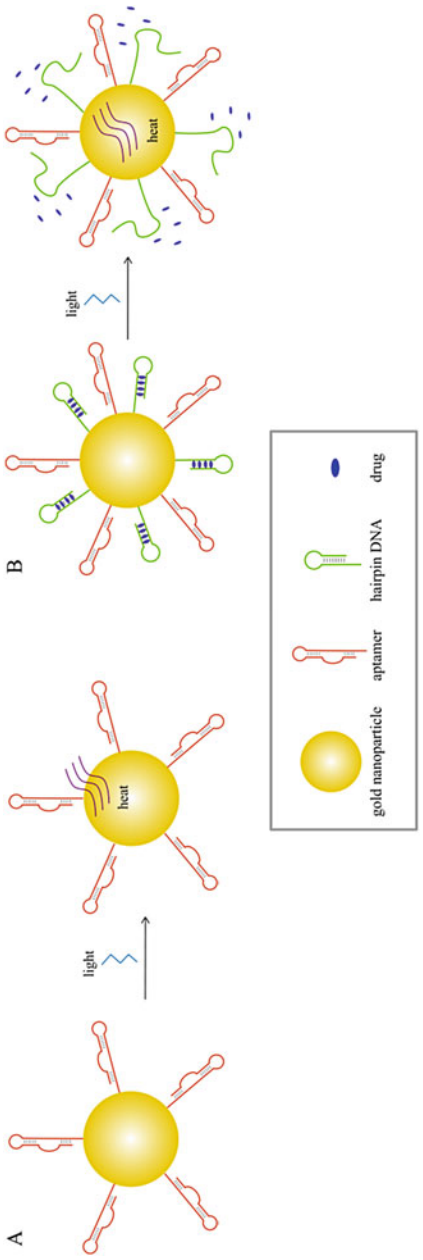


Fig. 8 Different designs of aptamer-modified gold nanoparticles for PTT. Illumination of the AuNPs leads to generation of heat, which causes cell damage (**a**), or drugs that are intercalated into hairpin DNA are released after irradiation due to light-induced heat development (**b**)

Table 5 Examples of aptamer-modified nanoparticles for PTT and PDT

Nanomaterial	Aptamer	Target	Drug	Possible application	Reference
Gold nanorods (AuNR)	sgc8	Protein tyrosine kinase 7 (PTK 7)	–	Leukemia treatment	[110]
Au	sgc8	Protein tyrosine kinase 7 (PTK 7)	Doxorubicin	Leukemia treatment	[7]
SWNT	Tmb aptamer	Human α-thrombin	–	Treatment of diseases related to blood-clotting disorders	[8]

graphene layer with a diameter of around 1 nm and a length from 1 to 100 μm. There are three common ways to prepare SWNT, the carbon arc-discharge technique, the laser-ablation technique, and the chemical vapor deposition technique [113].

SWNTs are known to be good quenchers of fluorescence. Furthermore they can quench the activity of photosensitizers. This characteristic enables SWNTs for the following example application. Aptamer-coupled photosensitizers can be bound to SWNTs non-covalently by π-stacking interactions between the aptamer and the surface of the SWNTs when no target is bound by the aptamer (see Fig. 9). The close proximity of the SWNT quenches the cytotoxic effects of the photosensitizer. Upon target binding, the structure of the aptamer changes and the aptamer is released from the SWNT. Therefore the photosensitizer is no longer quenched by the SWNT, and singlet oxygen is produced upon irradiation [8].

3.3 Other Therapeutic Applications of Aptamer-Modified Nanoparticles

The following section presents selected special applications of aptamer-modified nanoparticles that can be used for therapeutic applications.

Aptamer-Modified Superparamagnetic Nanoparticles as Nanosurgeons
Superparamagnetic nanoparticles are based on magnetite (Fe_3O_4) and therefore can be controlled by external magnetic fields [114, 115]. They have sizes between a few nanometers and 180 nm. Their half-life in the blood circulation is dependent on size and modification [115]. Some formulations of superparamagnetic nanoparticles are approved for magnetic resonance imaging, but they are moreover a promising tool for usage in nanosurgery [114, 115]. They can be transported to the target tissue by usage of a three-dimensional magnetic field generator [114, 116]. The surface of these magnetic nanoparticles can easily be modified and aptamers can be coupled. If aptamers are chosen which bind, e.g., to cancer cells, they selectively can be separated from healthy cells (see Fig. 10). For example, Nair et al. were able to separate targeted cells from non-targeted cells; furthermore a large extend of the

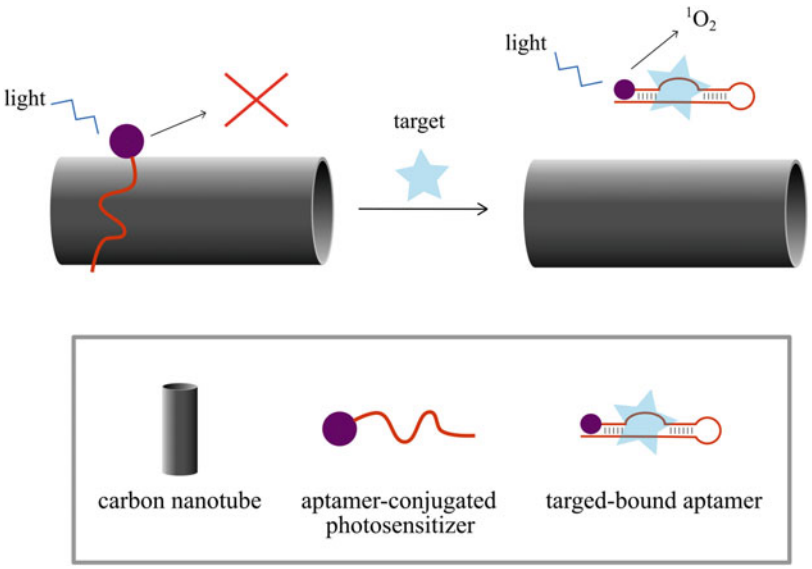


Fig. 9 Design of a single-walled carbon nanotube-aptamer system for photodynamic therapy. In absence of the target of the used aptamer, the activity of the photosensitizer is quenched by the SWNT. Upon target binding the aptamer dissociates from the SWNT and singlet oxygen can be produced by the photosensitizer upon irradiation (according to [8])

targeted cells could be detached from a cell culture surface by rapid changes of the direction of the magnetic field. This method might also be useable in the treatment of solid tumors. Additionally the target cells can be destroyed by the nanosurgeons presumably caused by mechanical strain-induced apoptosis or necrosis. Therefore aptamer-modified superparamagnetic nanoparticles are a promising tool for surgical actions on cellular level [114]. Table 6 shows the compounds of the nanosurgeon system developed by Nair et al.

Aptamer-Modified Gold Nanoparticles for Reversible Treatment of Blood-Clotting Disorders

Another extraordinary method that uses an aptamer-modified nanoparticle system was developed by Huang et al. They used modified aptamers as drugs in combination with gold nanoparticles. These triblock aptamers contained a poly A sequence for self-assembly on the surface of the gold nanoparticles, a short sequence that can be used for hybridization and a thrombin binding block. Two different triblock aptamers were used which contain two thrombin binding aptamers that bind to different parts of thrombin. For drug preparation both triblock aptamers were hybridized and then immobilized to gold nanoparticles (they build a self-assembled monolayer on the nanoparticle surface). Due to the spatial proximity of the two aptamers, they can bind thrombin effectively and thus prevent the conversion of fibrin into fibrinogen and further prevent blood clotting. In emergencies, where blood clotting is required, the aptamers can be detached from the gold nanoparticles

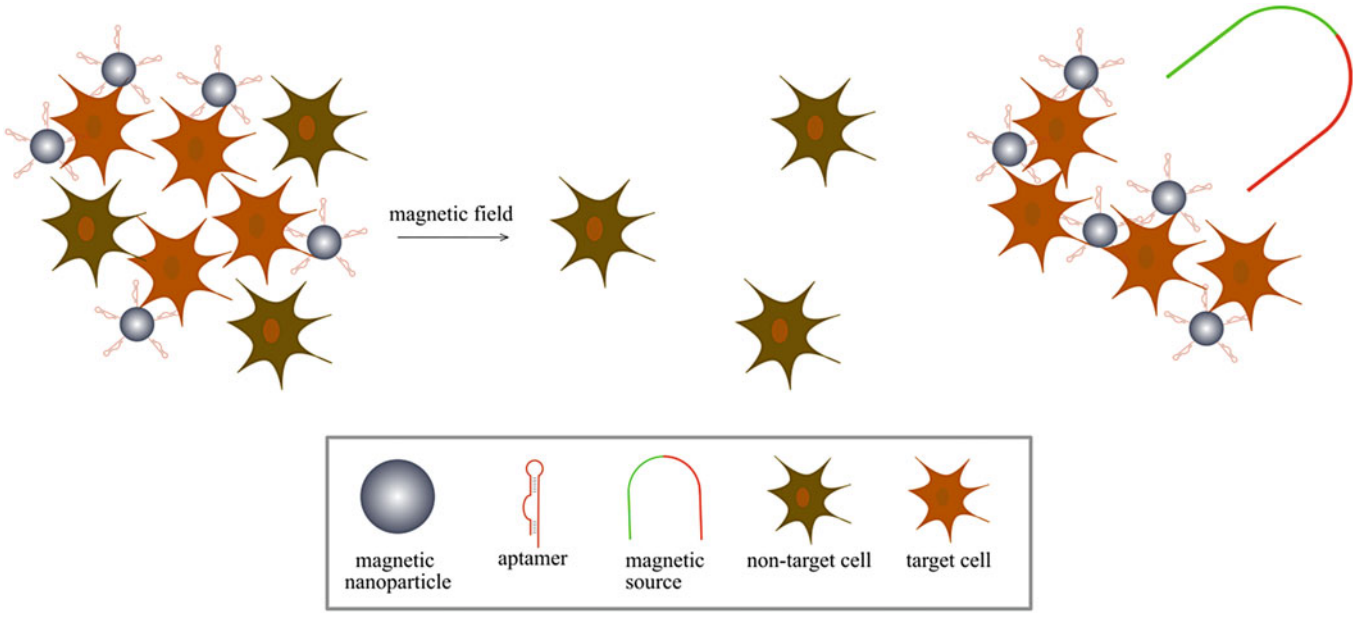


Fig. 10 A possible application of aptamer-modified superparamagnetic nanoparticles for nanosurgeon. Aptamer-modified nanoparticles can be transported to the target tissue by an external magnetic field, where they could bind to their target cells. Subsequently the position of the external magnetic field could be changed and nanoparticle-bound cells can be separated from non-target cells

Table 6 Compounds of unusual aptamer-modified nanoparticle systems

Nanomaterial	Aptamer	Target	Drug	Possible application	Reference
Superparamagnetic NP	GB-10	Tenascin C receptor	–	Glioblastoma treatment	[114]
Au	Triblock aptamer for thrombin binding	Thrombin	–	Treatment of diseases related to blood-clotting disorders	[19]

by irradiating with 532 nm light. The spatial proximity of the two aptamers is lost, and thrombin cannot be effectively bound any longer, so the blood can clot again [19]. However, light with a wavelength of 532 nm can only penetrate the skin to a depth of 0.3 to 0.5 mm [111]. Table 6 shows the compounds of the above described system.

Aptamer-modified nanoparticles can be used for various targets in therapeutic applications. Thereby the nanomaterial plays an important role for the possible applications. As of now there are some nanodrugs or aptamers as drugs in clinical use or trials, but the combination of both is still not in clinical stages [10, 117]. Nonetheless, this combination might be an advantageous tool for targeted treatment of diseases in future.

4 Summary and Conclusions

Aptamer-modified nanoparticles for medical applications are in the focus of current research efforts. Currently there are many diagnostic applications for small molecule detection but also for whole cancer cell detection described in the literature. Their common usage in, e.g., hospitals or for consumers at home is often not yet established, but the setup principle and components are well described. For therapeutic applications in vivo, different clinical stages have to be finished, before their deployment in routinely usage can be proven.

For some cancer types, the combination of diagnostics and therapeutics has already been efficiently shown. For imaging purposes in computed tomography (CT) and magnetic resonance imaging (MRI) with combination of drug delivery for therapy, different aptamer-modified nanoparticles have been utilized [117–121]. Therefore the deployment of aptamer-modified nanoparticles in diagnostics and therapeutics, or the combination of both, provides various application possibilities and research areas in future.

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