Aptamers

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The Chemical Biology of Aptamers

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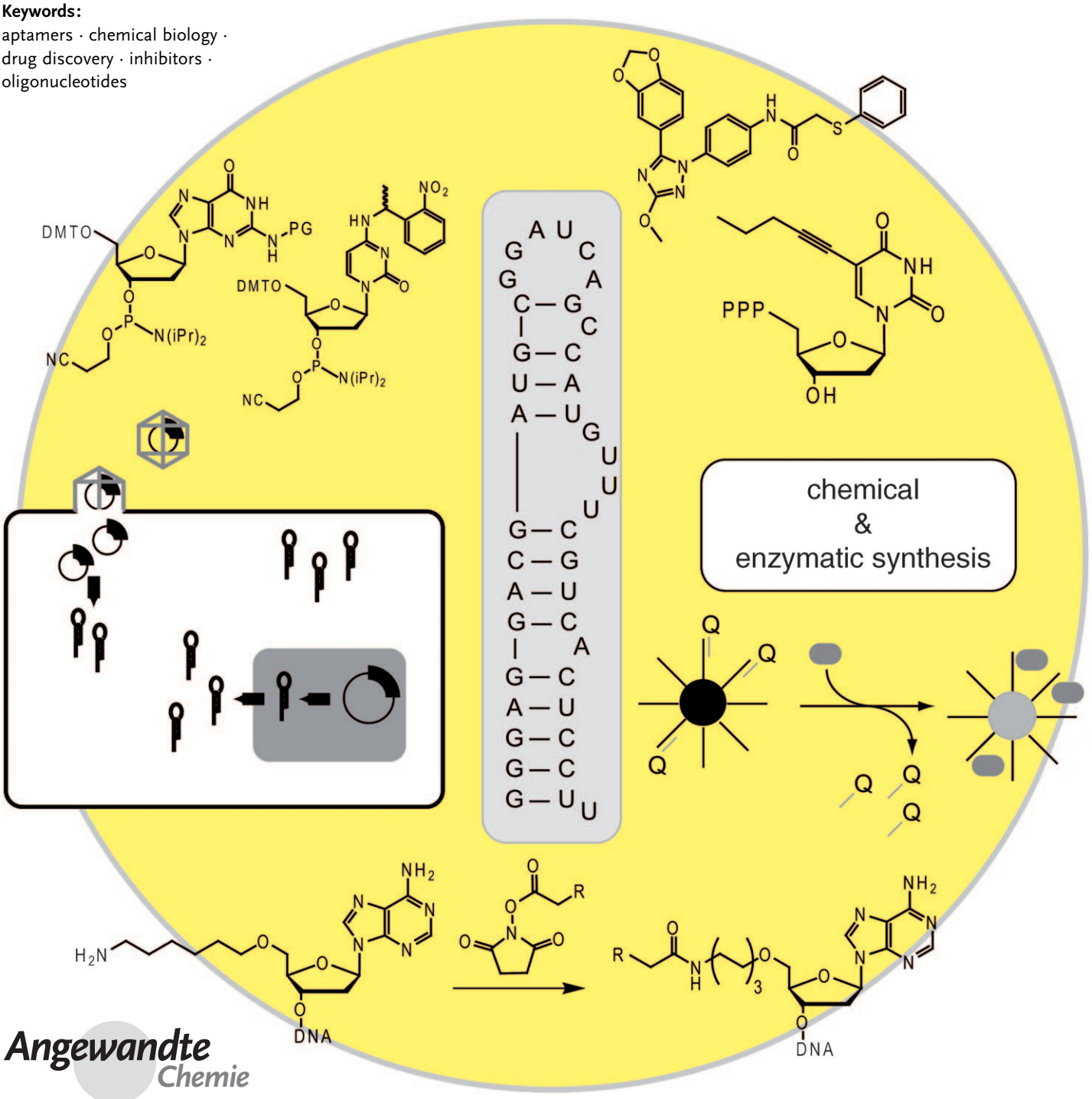

Aptamers are small single-stranded nucleic acids that fold into a well-defined three-dimensional structure. They show a high affinity and specificity for their target molecules and inhibit their biological functions. Aptamers belong to the nucleic acids family and can be synthesized by chemical or enzymatic procedures, or a combination of the two. They can, therefore, be considered as both chemical and biological substances. This Review summarizes the most convenient approaches to their preparation and new developments in the field of aptamers. The application of aptamers in chemical biology is also discussed.

From the Contents

1. Introduction

Spiegelman and co-workers described the first evolutionary experiments with nucleic acids in the 1960s.[1–4] Their work was based on the use of an RNA-dependent RNA replicase for the replication of a given RNA species. The subsequent introduction of the polymerase chain reaction (PCR) in 1986 by Mullis et al. has had a major impact on molecular biology in general and particularly on evolutionary experiments.[5–9] The systematic identification of nucleic acids with defined functions by using in vitro selection techniques, also termed systematic enrichment of ligands by exponential amplification (SELEX), was described in 1990—only four years after PCR. This method enables the specific in vitro replication of any single- or double-stranded DNA template using complementary primer oligodeoxynucleotides and thermostable DNA polymerases.[1,10] Any nucleic acid of virtually any length and any sequence composition can be amplified by using this technique.

In 1990 three research groups independently reported the isolation of small nucleic acids with predefined functions given by their defined experimental set-ups: Ellington and Szostak reported on RNA molecules that bind to a small organic dye, which they denoted as aptamers—a word chimera built up from the Latin expression "aptus" (to fit) and the Greek word "meros" (part).[11] A second study by Tuerk and Gold described the selection of RNA molecules that bind to the bacteriophage T4 DNA polymerase, and they termed this process SELEX.[12] Robertson and Joyce described the application of in vitro selection for the adaptation of the group I ribozyme so that it cleaved DNA rather than single-stranded RNA.[13] These ground-breaking studies already highlighted the power of in vitro selection for obtaining nucleic acid molecules with complex functions.

Thereafter, various studies showed clearly that the aptamer technology fulfilled these expectations and proved that nucleic acid molecules can serve as sophisticated functional moieties, besides acting as blueprints of the genetic code or as rigid materials for nanoarchitectures.[14–20] Further evidence of the versatility of nucleic acids is given by the discovery of small noncoding RNA molecules and riboswitches, which play key roles as regulatory elements to control gene expression in bacteria, eukaryotes, and higher organisms.[21–23] Riboswitches in particular demonstrate that nature already makes use of aptamer domains to ensure high affinity for and specific binding to small metabolites.[24–26] Nucleic acid sequences have both a defined function, as a result of their distinct 3D shape, and carry the blueprint for their own synthesis in their primary sequence. Consequently, chemical synthesis can be applied to equip an aptamer with a variety of additional functionalities, thereby tailoring it for diverse applications. Combinatorial solid-phase synthesis first produces a diverse library of nucleic acids, an in vitro selection process based on enzymatic methods then follows, and the resulting aptamers can in turn be manipulated by chemical synthesis (Figure 1).

The early selection protocols targeting proteins were cumbersome, and it frequently took several months to identify aptamers that specifically targeted the desired protein. Today, the selection process has been reduced to several days by elaborated handling protocols including methods based on single beads,[27] capillary electrophoresis,[28–32] surface plasmon resonance,[33,34] HPLC,[35] and automated processes.[36–38] The development of nucleic acid libraries with superior replication properties, designed to avoid the accumulation of artefacts, such as molecular parasites, led to sophisticated selection procedures. The protocols that make use of robotic workstations are optimized to do without any purification steps of the nucleic acid intermediates through immobilization of a target molecule to magnetic beads.[37,39] By this means, the simultaneous selection against up to eight target molecules is possible. Besides linear nucleic acid libraries (Figure 1B), structurally constrained libraries with predefined secondary structure elements have been successfully applied for in vitro selection

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purposes.[39,40] These libraries have the advantage that truncation and structural characterization of a representative aptamer is straightforward.[41] In some cases, these libraries have been proven to require fewer selection cycles. Recently, Bugaut et al. demonstrated that chemical synthesis can be efficiently combined with SELEX procedures.[42] They synthesised a library containing 2′-amino-2′-deoxyuridine with 14 randomized positions. Prior to incubation with the target molecule, this library was post-transcriptionally modified with a set of three aldehydes (Scheme 1). After incubation and elution of the bound RNA species, the aldehydes were removed to allow enzymatic replication and amplification of the isolated RNA molecules.

The major disadvantage of the SELEX approach is the uncertain prediction of the success of the selection. While the inherent parameters of aptamers, such as affinity and specificity, can be influenced during the selection process, it is not possible to judge the ability of a target molecule to be suitable for the selection process and whether an aptamer can be successfully raised against the target (aptamerogenicity) a priori. In the case of proteins, the isoelectric point (pI) might indicate whether the target is suitable for SELEX or not. In general, proteins which are positively charged under physiological conditions (pH 7.0–7.4) are regarded as excellent targets. However, a pI value below 7 does not necessarily indicate that the target is not suited for the enrichment of aptamers; various studies have shown the successful selection of nucleic acid aptamers that target proteins with pI values below 7. These examples indicate that the pI value alone might not be a reliable criterion to judge the suitability of a target protein for SELEX. A serious problem is the conformational instability of a target protein, especially under the conditions employed for the selection process. Since aptamers recognize a defined 3D shape, conformational changes to the target molecule consequently result in a loss of potential binders during the selection—an effect that can occur from one selection cycle to the other. This difficulty can be avoided, at least to some extent, by the preparation of fresh protein solutions and affinity matrixes for each selection cycle. There is no reliable predicting tool yet available for the a priori assessment of the success of the selection for a target molecule. Thus, the complete selection process must be carried out to completion to obtain aptamers for a target molecule. The success rate of automated in vitro selection procedures for a homogeneous protein target is around 75 %.[244]

Günter Mayer, born in Munich, studied chemistry at the Ludwig-Maximilians University in Munich and completed his PhD with M. Famulok at the University of Bonn on the functional analysis of cytohesin-1 in T cells with RNA intramers. In 2001 he joined the biotech company NascaCell, where he headed the combinatorial biotechnology department. In 2003 he cofounded NascaCell IP GmbH and in 2004 he rejoined the Famulok group at the University of Bonn, and is currently working on his "Habilitation". His research interests are the precise control of aptamer activity, the discovery of new targets based on aptamer selection, and riboswitches.

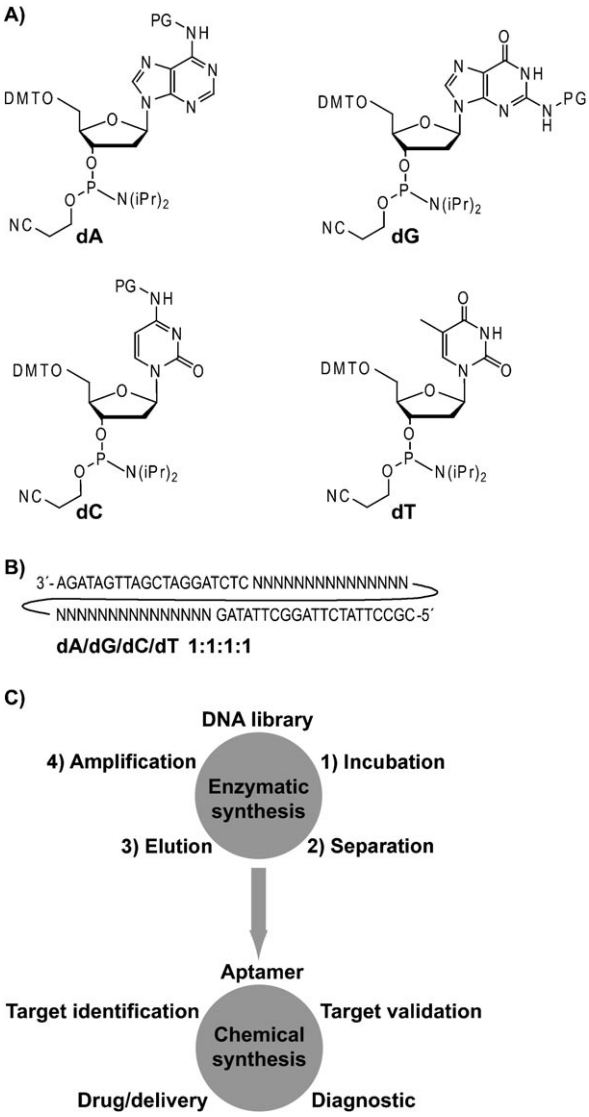
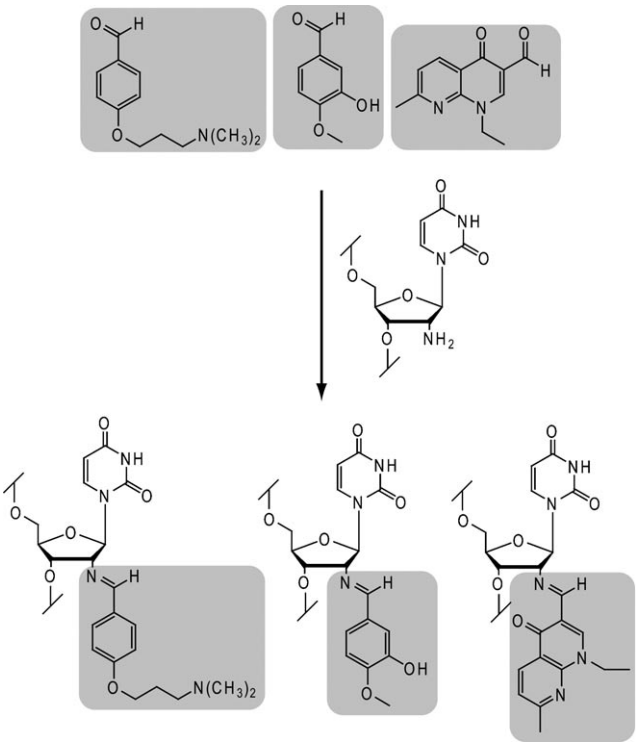


Figure 1. A) Deoxynucleoside phosphoramidites protected with dimethoxytrityl (DMT) groups for the solid-phase synthesis of DNA (PG: protecting group). B) Nucleic acid libraries can be synthesized with defined primer binding sites flanking a random region (N). C) Enzymatic methods are mandatory for the in vitro selection process (top circle), which consists of four key steps: Incubation of the nucleic acid library (DNA library) with a target molecule of choice, separation of bound from nonbound nucleic acid species, elution of bound nucleic acid species, and amplification of eluted nucleic acid species. Once an aptamer has been identified it can be tailored for distinct applications (bottom circle), such as target validation, diagnostics, drug and delivery purposes, and for target identification.

The ever-increasing number of studies describing the use of aptamers, particularly during the last five years, emphasizes the emerging use and acceptance of aptamers as tools for basic research and medical applications.[23,43–45] A search of the keyword "aptamer*" on the Web of Science identified 2417 published items and 44158 citations (status: 21.11.2008). It is noteworthy that the number of published items and



Scheme 1. RNA libraries containing 2′-amino-2′-deoxyuridine can be modified with aldehydes (gray boxes), thereby increasing the chemical diversity of the RNA libraries.[42]

citations per year have increased continuously, with a strong surge from 2002 onwards (Figure 2).

In this Review, the most recent developments in the aptamer field are summarized with an emphasis on the modification of aptamers by enzymatic and chemical synthesis, with the ultimate goal of tailoring aptamers for specific applications. It should become clear how synthetic aptamers can be used for target identification and as validation tools as well as for diagnostic and drug-discovery processes.[46]

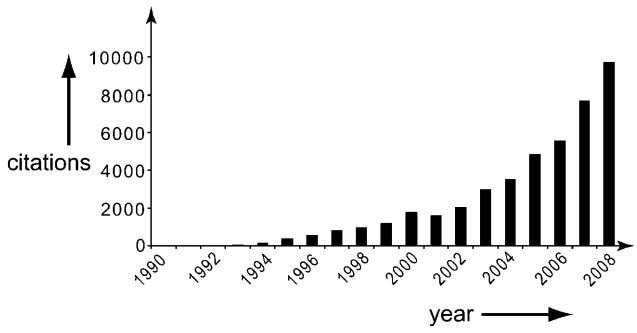


Figure 2. Citations found on the Web of Science when searching the keyword "aptamer*" (status: 21.11.2008).

2. Aptamers—Biological and Chemical Substances

Aptamers are short single-stranded nucleic acids with a defined three-dimensional shape that allows them to interact with high affinity with a target molecule. DNA and RNA polymerases are indispensable for the in vitro selection process since they provide a satisfactory means for the proper replication of selected sequences and for the mutual introduction of point mutations to obtain covariations of selected nucleic acids. Various modifications of nucleotides have been described which are compatible with the enzymatic steps of the in vitro selection procedure and thus enhance the chemical diversity and the biological properties of nucleic acid libraries (Figure 3).[47] These modifications were introduced either at the phosphate/ribose backbone or at the nucleobases.[48] The replacement of the DNA phosphate backbone by a phosphorothioate has enhanced the stability against nucleases and the cellular availability of such molecules. Besides antisense molecules, such as vitravene, aptamers with a phosphorothioate backbone have also been described.[49,50] However, the most prominent modification of aptamers is the derivatization of the 2′-ribose. This position contributes significantly to the stability of RNA aptamers, and 2′-fluoro- and 2′-amino-2′-deoxy pyrimidine triphosphates have frequently been used for the direct selection of nuclease-stabilized RNA aptamers. Chelliserrykattil and Ellington developed a variant of the T7 RNA polymerase that can use 2′-methoxypyrimidine nucleotide triphosphates and 2′-methoxyadenine nucleotide triphosphate as substrates for in vitro transcription.[51,52] However, the utility of the latter modification during in vitro selection procedures is hampered since RNA molecules that bear those modifications can not be considered as templates for the reverse transcriptase. Wengel and co-workers described recently the suitability of locked-nucleic acid (LNA) triphosphates for PCR and in vitro transcription.[53,54] LNAs bear a methylene ether bridge between the 2′-oxygen atom and the 4′-carbon atom. In this way, the 3′-carbon atom is "locked" in the LNA ribose in an *endo* conformation. The adaptation of LNAs for SELEX processes might pave the way to construct nucleic acid libraries with LNA building blocks and the de novo selection of LNA aptamers.

Modifications of the nucleobases that are compatible with enzymatic replication have also been reported, with modifications at the C5-position of uridine and deoxyuridine being the most prevalent.[55–57] In this way distinct chemical moieties have been introduced in nucleic acid libraries and were applied for the in vitro selection of aptamers that require modification for target binding. Moreover, the introduction of alkyne groups opens up a route for the modification of aptamers at the completion of the selection, for example, through reaction with azide-derivatized compounds by copper(I)-catalyzed 1,3-dipolar Huisgen cycloadditions.[58–61] This allows an aptamer to be equipped with various functionalities and further adapted to individual applications. Our research group has developed aptamers whose activity can be regulated by light. This can be accomplished by the post-selective and site-specific incorporation of photolabile protecting groups, such as *o*-nitrophenylpropyl (NPP) and *o*-nitrophe-

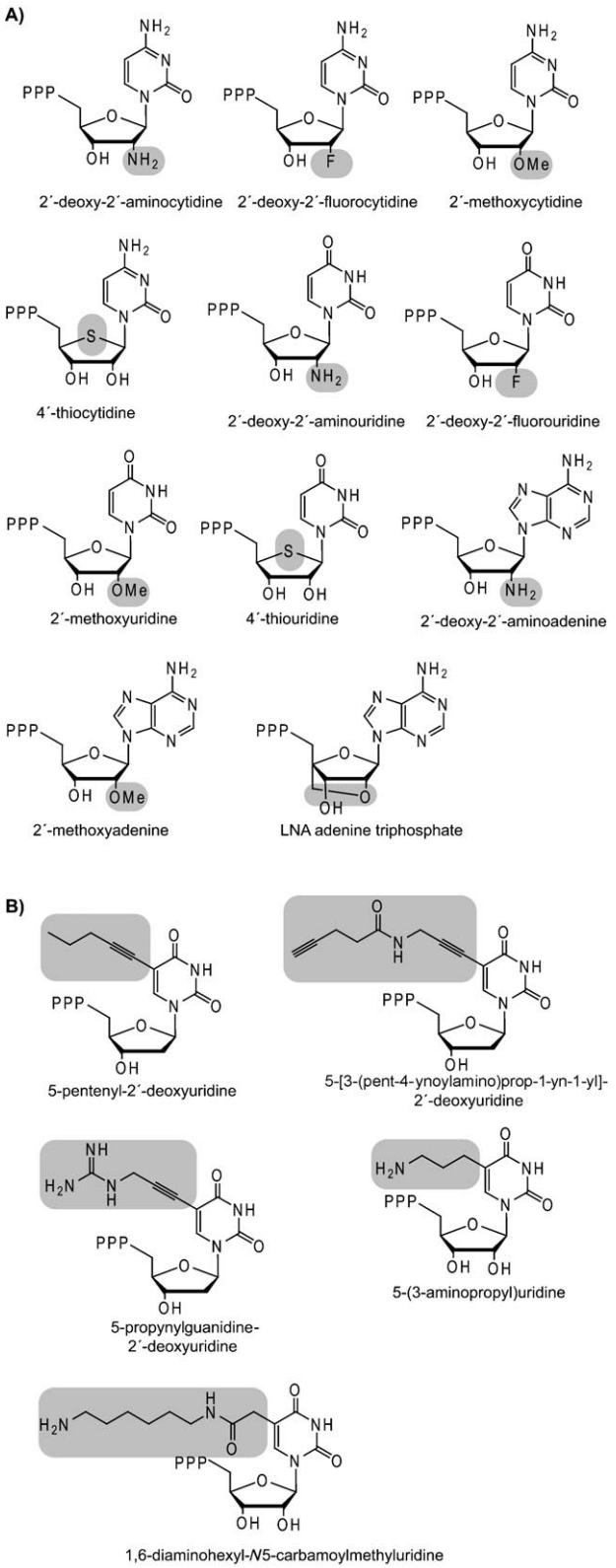
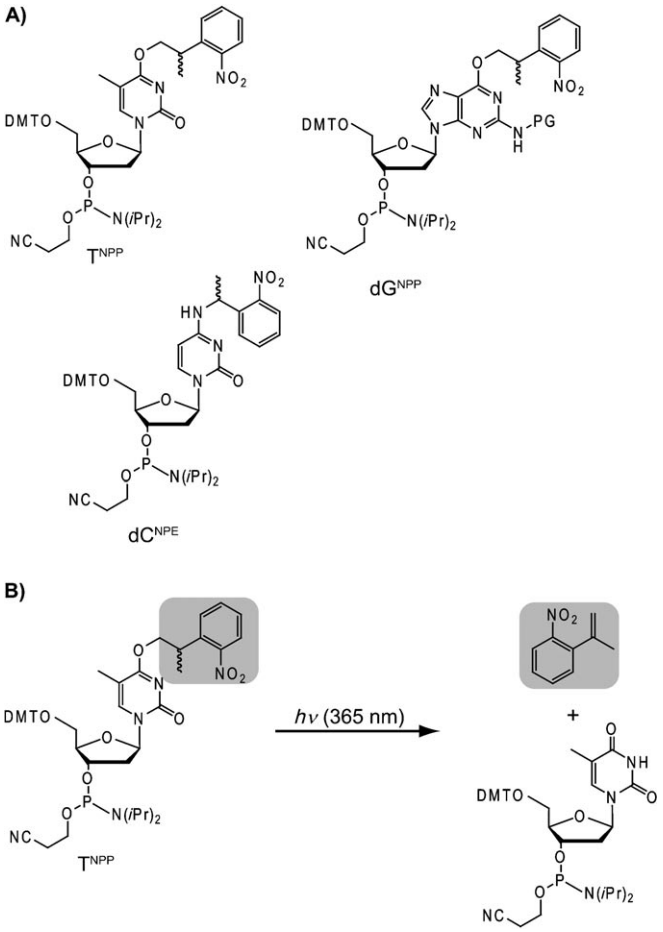


Figure 3. Modifications of nucleotides at either the 2′-position (A) or the C5-position of uridine nucleotides (B) that are compatible with the enzymatic steps of the SELEX procedure (PPP = triphosphate). Modifications are highlighted by gray boxes.

nylethyl (NPE), by solid-phase synthesis by employing modified nucleosides and phosphoramidite approaches (Scheme 2).[62] Aptamers with photolabile protecting groups offer the possibility for the spatiotemporal regulation of aptamer activity and thus control over the activity of the target molecule. Aptamers, like nucleic acids, have the advantage that they can be designed to be either activated or deactivated by light.[63–65] This enables access to numerous light-regulated aptamer-based protein inhibitors.



Scheme 2. A) Phosphoramidites of nucleotides with photolabile protecting groups at the exocyclic positions O^4 (T^{NPP}), O^6 (dG^{NPP}), and N^4 (dC^{NPE}) for the site-specific incorporation of photolabile protecting groups (NPP, NPE) into aptamers by solid-phase synthesis (PG: Protecting group). B) Irradiation of the modified nucleobases, shown for T^{NPP}, with light ($\lambda = 365$ nm) results in the release of the protecting group (gray box) and the native nucleobases.

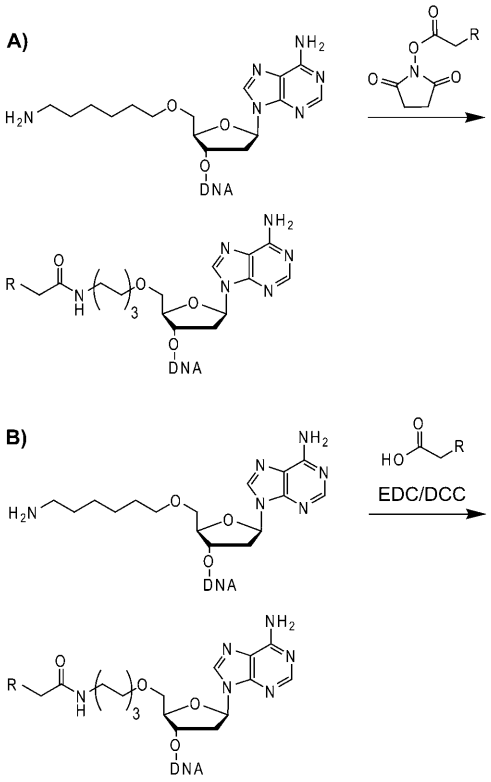
2.1. Aptamers and Their Modifications for Diagnostic Purposes

Forcing aptamers to fluoresce under certain conditions enables their use as fluorescent probes. Several approaches have been described, and excellent reviews dealing with this topic have been published recently.[66–68] Aptamers can be modified at their 5′-position by chemical synthesis, enzymatic synthesis, or by a combination of the two.[69,70] DNA aptamers can be modified by the use of appropriate fluorescently labeled primer molecules and PCR amplification followed by

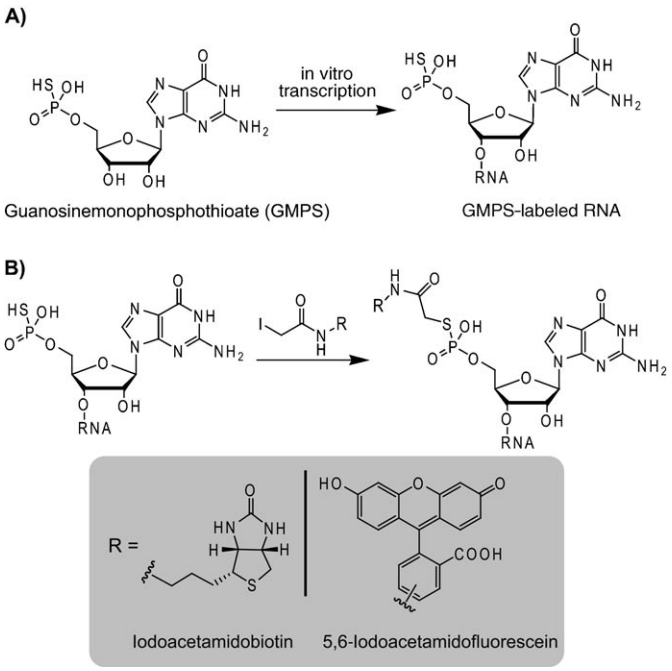
single-strand displacement, or directly by chemical synthesis. In the latter case the introduction of a 5′-amino group represents a versatile anchor that can be further modified by using *N*-hydroxysuccinimide (NHS) or ethylenediamine carbodiimide (EDC). In this way, the desired fluorescent molecule or any other functional moiety can be introduced (Scheme 3).[71,72]

Fluorescing RNA aptamers can be synthesized directly by solid-phase synthesis. However, synthesis is limited since RNA molecules longer than 80 nucleotides are hard to obtain. Alternatively, modifications of RNA molecules at the 5′ ends can be made enzymatically by in vitro transcription with T7 RNA polymerases in the presence of so-called initiator nucleotides, such as guanosine monophosphothioate (GMPS).[69,73,74] The incorporation of GMPS at the 5′ end of RNA molecules enables the modification of RNA molecules with fluorescent moieties or other reporter molecules by iodoacetamido-based approaches (Scheme 4).[69]

Recently, Jäschke and co-workers developed a novel initiator nucleotide that resulted in an aldehyde modification at the 5′ end of an enzymatically synthesized RNA which can then be modified with amino- or hydrazine-functionalized groups (Scheme 5).[75] This approach allows the synthesis of a broader range of RNA molecules through a combination of chemical and enzymatic approaches and might be very useful for the introduction of diverse functional groups. Besides the modification of the 5′ end of RNA molecules, the 3′ ends can also be modified efficiently. One route employs redox reactions and derivatization with hydrazide or semithiocarbazide groups to introduce functional moieties at the 3′ end of RNA molecules (Scheme 6).[76]



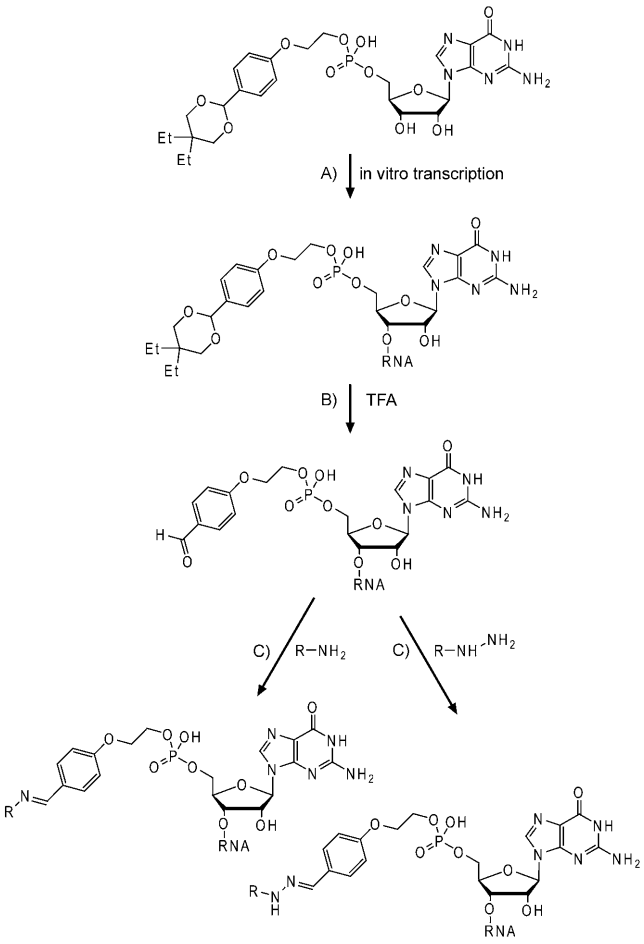
Scheme 3. Derivatization of 5′-amino-modified DNA aptamers by using NHS (A) or EDC/DCC approaches (B). DCC = *N*,*N*′dicyclohexylcarbodiimide.



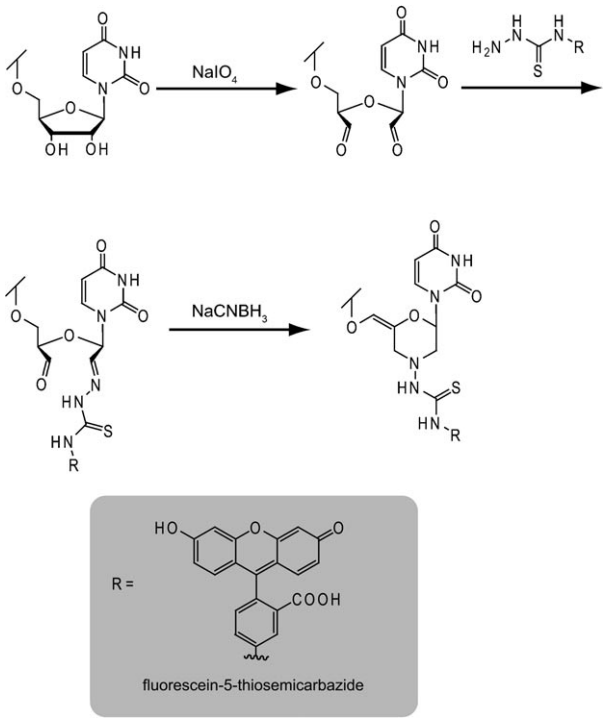
Scheme 4. A) Introduction of GMPS moieties at the 5′-position of RNA molecules by in vitro transcription. B) GMPS-modified RNA can be further derivatized with iodoacetamido groups. In this way, either biotin or fluorescent groups (gray box) can be introduced.

Unfortunately, modification after selection can have a negative effect on the aptamer activities, thereby limiting their utility for diagnostic purposes. Approaches that circumvent this problem make use of aptamers that bind to small molecules, such as malachite green, whose fluorescent properties change in the aptamer-bound state.[77–82] The combination of such reporter aptamers with other functional nucleic acid subunits might pave the way to highly developed diagnostic tools. Excellent reports discussing the use of aptamer-based electronic sensors and microarrays have been published.[83–92] Gold and co-workers introduced the so-called photo-SELEX approach, which enables the photochemical cross-linking of an aptamer to a target protein through incorporated 5-bromodeoxyuridin nucleotides. This allows a significant enhancement of the signal to noise ration in microarrays employing photosensitive aptamers as recognition elements.[93] An elegant approach in which structure-switching aptamers are applied has been described by Li and co-workers.[94,95] These aptamers are designed to induce a change in the fluorescence intensity when complexed to the target molecule, thereby exploiting the adaptive binding nature of aptamers to receptor molecules.[96,97]

Similar to antibodies, modified aptamers have been used in diverse assays such as ELISA-like formats,[98,99] Western Blot analysis,[100] capillary electrophoresis,[30,101] flow cytometry,[102] in vivo imaging,[103] HPLC,[35,104] and microarrays[93,105] that allow the sensitive detection of biomolecules. An ingenious development in the field of aptamer diagnostics is the use of aptamers as both the recognition element and the



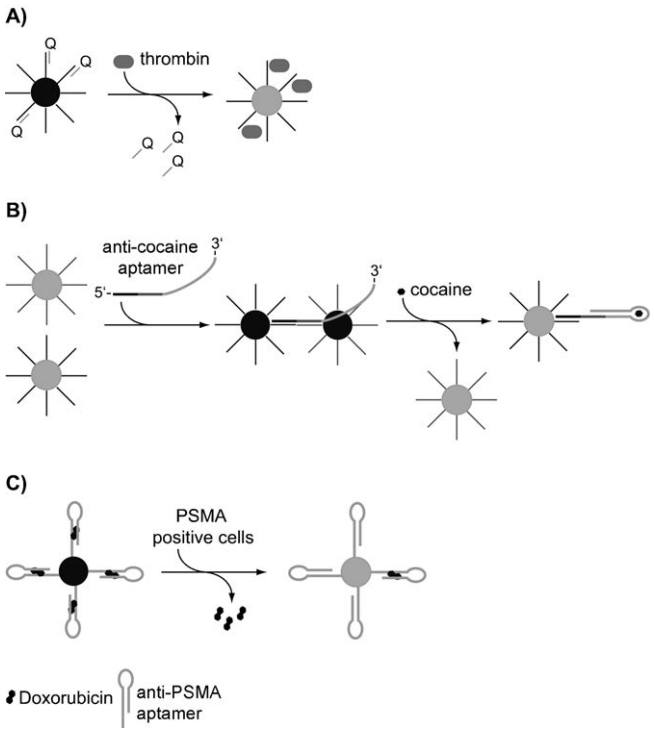
Scheme 5. A) The acetal-protected aldehyde-guanosine phosphate can be introduced at the 5′ end of RNA molecules during in vitro transcription. B) Cleavage of the acetal by trifluoracetic acid (TFA) releases the corresponding aldehyde, which can be subsequently modified with amino- or hydrazide-derivatized functional moieties R (C).



Scheme 6. Modification of 3′ ends of RNA molecules by oxidation with sodium periodate and condensation of the aldehyde with thiosemicarbazide derivatives, such as biotin or fluorescein. Ring closure is achieved by reduction with sodium cyanotrihydridoborate.

template for amplification reactions by either rolling circle amplification[106,107] or aptamer-affinity PCR.[108] Landegren and co-workers adapted aptamers for assay formats based on proximity ligation, thus allowing the very sensitive detection of analytes in complex mixtures.[109,110] Proximity ligation relies on the ligation of DNA molecules located nearby each other because of receptor–ligand interaction, thus resulting in a new template which is sensitive for detection by PCR. However, no easy-to-use, commercially available clinical assay yet exists; this might be due to the different recognition properties of aptamers compared to the antibodies that are used daily in clinical practice: Aptamers require an intact and folded target structure to maintain high affinity and specific binding, while antibodies merely recognize a linear epitope, also in a slightly and totally denatured state of the target protein. Thus, different prerequisites are required in target handling prior to the use of aptamers as detection reagents, and the habits adopted in the use of highly established methods might be hard to overcome.

The detection of small molecules is a field in which aptamers will probably start to make a great impact, since in this case the handling of the target is not a big issue. The combination of aptamers with quantum dot technology has provided novel advanced diagnostic assay formats.[111–117] For example, Levy et al. immobilized a thrombin-recognizing aptamer on quantum dots by employing a streptavidin–biotin approach.[113] An annealed complementary strand of the aptamer equipped with an eclipse quencher was efficiently displaced by the addition of thrombin, thus resulting in enhanced fluorescence of the quantum dots (Scheme 7A).[118] Liu and Lu described a similar approach: They derivatized quantum dots with two different short adapter oligodeoxynucleotides. The addition of an aptamer, which partially acts as a splint, causes the quantum dots to aggregate together, thereby resulting in a quenched fluorescence signal. The addition of the aptamer ligand results in removal of the splint and thus disassociation of the quantum dots. This was quantified by a colorimetric assay (Scheme 7B).[114,119,120] Besides the streptavidin–biotin approach, Lu and co-workers used thiol-modified aptamers to efficiently immobilize the aptamers on the Au surfaces of the quantum dots. They even went a step further and developed a simple dip-stick assay format based on quantum dot/aptamer conjugates for the rapid and easy detection of cocaine.[120,121] Stojanovic and Landry also used the same anti-cocaine aptamer and developed a colorimetric detection assay. This assay is, in principle, based on a competition between cocaine and a cyanine dye prebound to the aptamer.[115] In an elegant approach, the anti-prostate-specific membrane antigen (anti-PSMA) aptamer A10 has been coupled to quantum dots, whose fluorescence intensity was quenched by a doxorubicin (DOX) molecule



Scheme 7. The combination of quantum dots and aptamers. A) Thrombin-recognizing aptamers are immobilized onto quantum dots (black circles). The fluorescence of the quantum dot is quenched by a complementary oligodeoxynucleotide equipped with a quencher molecule (Q), which hybridizes to the aptamer sequence. The addition of thrombin induces release of the quencher and thus the fluorescence of the quantum dots (gray circles) is detectable. B) Two different oligodeoxynucleotide sequences are immobilized on quantum dots (gray circles) and the anti-cocaine aptamer annealed to both sequences. This brings the quantum dots in proximity and the quenching of the fluorescence is induced (black circles). The addition of cocaine leads to the release of the aptamer and the quantum dots separate, thereby resulting in enhanced fluorescence signaling (gray circles). C) The anti-PSMA aptamer, in a complex with DOX, is immobilized onto quantum dots (black circle), whereby DOX quenches the fluorescence of the quantum dots. The addition of PSMA-positive cells leads to a release of DOX and thus to enhanced fluorescence of the quantum dot (gray circles).

that was also noncovalently associated with the aptamer (Scheme 7C). This multifunctional complex has been shown to be useful for both the specific targeting of tumor cells expressing PSMA and the detection of these cells following DOX release.[111] Simultaneously, the cytostatic activities of the anthracycline drug DOX inhibits cell growth of the targeted cells.[122] Chen et al. coupled the 5′-amino-modified tenascin-C binding aptamer GBI-10 to quantum dots by using an EDC/sulfo-NHS approach to selectively detect and visualize glioma cells.[123]

2.2. Modified Aptamers as Delivery Vehicles

Aptamers with well-defined structure and function can be assembled into multifunctional molecules containing various functional groups. For example, aptamers targeting cells provide the means to develop drug-delivery vehicles that specifically address a certain malignant cell subtype. The synthesis of aptamers enables the manufacture of highly stable oligonucleotides with dramatically enhanced stability against degradation by nucleases. The bottom-up construction of functional molecular components with at least one aptamer building block allows access to multifunctional molecules. In this regard, aptamers have been applied as guiding modules in multifunctional complexes to target distinct cell subtypes and tissues. The most established and best characterized aptamer in this regard is the nucleic acid molecule A10, which binds to the prostate-specific membrane antigen (PSMA), a cell-surface molecule associated with the onset and progression of cancer.[124] Several studies have been concerned with the modification of this aptamer with additional molecules, thereby allowing their selective aptamer-triggered delivery to cancer cells (Figure 4). For example, polymer-coated nanoparticles with encapsulated chemotherapeutics (such as docetaxel) have been attached to the 5′-amino end of the aptamer by an NHS/EDC approach.[125–127] The systemic application of these complexes allows the efficient targeting of solid tumors. In a breakthrough study Langer, Farokzhad, and co-workers showed the efficacy of such a chimeric aptamer cargo molecule in an in vivo xenograft rat tumor model system. The aptameric subunit A10 specifically localized the complex in a solid tumor where the adjacent chemotherapeutic induced a reduction in the size of the tumor up to total remission.[126] It is anticipated that such aptamer-based tumor-targeting systems will result in the unwanted side effects of therapeutics being significantly reduced. In a second example, the same research group directly complexed aptamer A10 with DOX, thereby obtaining so-called physical conjugates (noncovalent complexes between aptamers and DOX). Again, treatment of prostate cancer cells resulted in a significant reduction of tumor-cell proliferation.[122] Besides chemotherapeutics, siRNA molecules can also be used to assemble chimeric siRNA–aptamer conjugates, thereby facilitating the cell-specific delivery of siRNA molecules. In this regard, siRNA molecules have been coupled to aptamer A10 either directly by nucleotidic extensions[128] or indirectly through the assembly of tetrameric streptavidin–biotin complexes consisting of two biotinylated aptamers and two biotinylated siRNA molecules per streptavidin moiety.[129] Both approaches were successful in the cell-specific siRNA-mediated reduction of the corresponding mRNA and protein levels. Aptamer–toxin conjugates were also generated and applied to give specificity for the toxin gelonin, a ribosome-inactivating protein.[130]

Recently, Rossi and co-workers added a second aptamer to the list of aptamer-based siRNA-delivering agents. They fused an anti-gp120-binding aptamer with siRNAs that are specific for the HIV transcripts tat and rev through a nucleotide linker.[132] Treatment of HIV-1-infected cells with these chimeras resulted in the specific down-regulation of the appropriate mRNA transcript. Several other aptamers have been described that specifically recognize cell-surface molecules. Of these, aptamers that target the $\alpha_v\beta_3$-integrin,[133] the glutamate receptor channel GluR2,[134] VCAM-1,[135] epidermal growth factor receptor-3,[136] or the DC-SIGN[137] protein

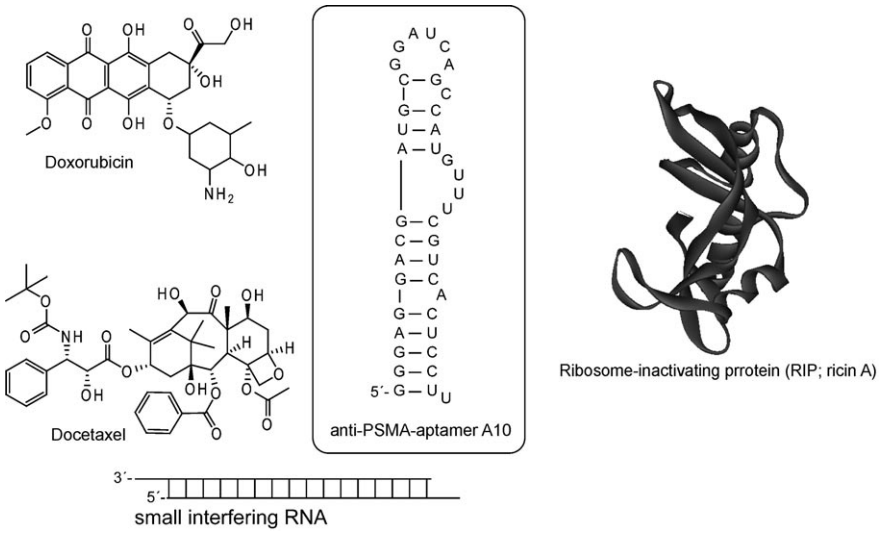


Figure 4. The anti-PSMA aptamer A10 (boxed) has been conjugated to various agents that exert antitumor activity. All the pyrimidine nucleotides are 2′-fluoro-2′-deoxynucleotides. Besides coupling to the small molecules doxorubicin and docetaxel, short interfering RNA (siRNA) molecules and the ribosome-inactivating protein gelonin were also attached. The depicted 3D structure represents ricin A, a protein which is highly homologous with gelonin and whose crystal structure was employed for superimposition of the structure of gelonin.[131]

might be also suitable to generate sophisticated and cell-specific multifunctional tools for the delivery of therapeutically active agents.

3. Aptamers for the Identification of Biomolecules

An emerging direction in the aptamer field is the systematic identification of target molecules that are associated with distinct cellular states, either pathogenic or not. The identification of new therapeutically and diagnostically relevant biomolecules can be facilitated with the ultimate goal of developing an individualized medical approach. In this regard, aptamers that target a specific cell type or subpopulation of malignant cells (for example, tumor cells) are selected and characterized. After generation of the monoclonal aptamers, the cognate target molecules on the cell-surfaces can be subsequently identified by employing aptamer-based pull-down protocols followed by SDS-PAGE, protease digestion, and liquid-chromatography/mass spectrometry (LC-MS) analysis.[138,139] The first example of such an approach was reported by Blank et al.[71] In this study, aptamers with specificity for rat endothelial glioblastoma cells (YPEN) were identified. One aptamer has been shown, after modification with a fluorescent label at its 5′ end, to be efficient for staining tumor cells. LC-MS analysis identified the protein pigpen as the putative target of the aptamer. Pigpen is an endothelial proliferation marker protein that is expressed in the proliferating endothelium but its expression is down-regulated until confluency (full coverage of sample vial) is reached.[140] This finding indicates that pigpen is indeed a marker of the angiogenic state of endothelial cells, as already suggested by the aptamer-based approach introduced by Blank et al.[141] A subsequent study by the Gold research group further emphasizes the feasibility of the cell–SELEX approach for target identification. In this similar study the authors identified a tumor-cell-specific tenascin-C protein by an aptamer that had previously been selected to specifically target a different glioblastoma cell line.[142]

SELEX procedures that target various eukaryotic cells, bacteria, parasites, and viruses have since been described.[143–146] Anthrax spores and trypanosomes have been subjected to in vitro selection protocols aimed at the discovery of pathogen-neutralizing aptamers useful for therapeutic intervention. The aptamers were successfully applied to inhibit the virulence of the targeted microorganisms, although the cognate targets have not yet been identified. This is clearly an advantage of the aptamer technology, where cell-surface molecules and transmembrane receptors can be targeted within their native environment.[147] Cumbersome purification procedures are not required, which in turn lead to suboptimal activities and stabilities of the biomolecules. Taken together, the targeting of cells with SELEX allows a forward-genetic approach to be established based on a phenotype-correlated selection of aptamers (for example, a tumor cell). Thereafter, the identification of the associated targets and antigens is possible (Figure 5).

Recently, Tan and co-workers applied in vitro selection to identify single-stranded DNA (ssDNA) aptamers that target

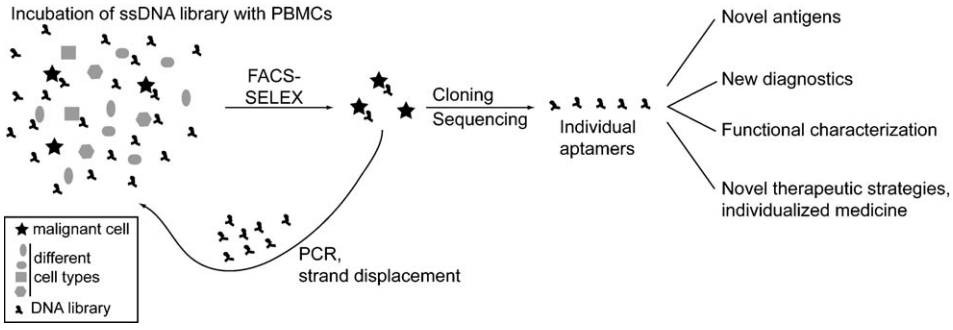


Figure 5. Identification of aptamers that target a distinct cell subpopulation can be accomplished by SELEX procedures that utilize fluorescent-activated cell-sorting devices.[148,149] Individual aptamers serve as the starting point for the identification of novel antigens, the development of new diagnostic tools, the characterization of the biological function of the antigen, and the development of novel therapeutic strategies. PBMC = peripheral blood mononuclear cell.

several immortalized tumor cell lines.[150–153] They employed a discriminating counter-SELEX protocol and enriched aptamers that specifically interact with a distinct tumor cell line and most remarkably also with primary cells from patients.[154] Aptamer-based pull-down analysis identified the membrane-bound heavy μ chain of immunoglobulin as the putative target molecule presented on the Burkitt's lymphoma cell line (Ramos B-cell) used for the in vitro selection process; RTK7 was deconvulated as the target molecule of a second aptamer, selected to specifically interact with T-cell lymphomas.[155,156] Our research group has successfully implemented the fluorescent-activated cell-sorting (FACS) technology in the in vitro selection process (Figure 5), thus allowing the selection of aptamers that not only possess high affinity, but also target a defined cellular phenotype in composite cell mixtures.[148] This approach might enable the direct targeting of subpopulations found in primary blood tumor cells, and thus offers a route for the direct selection of patient-specific aptamers, thereby paving the way for individual diagnostics and therapeutics. Recently, Krylov and co-workers applied a similar whole-cell SELEX approach to mature and immature dendritic cells (DCs). Instead of using monoclonal aptamers, they employed the entire diverse enriched library as an affinity matrix for the simultaneous identification of target molecules by LC-MS analysis.[149] These target molecules might represent novel biomarkers associated with a distinct differentiation level of mature and immature cells. However, none of the identified target molecules of the cell-specific aptamers has been purified and no binding analysis in homogenous assays has been conducted so far.

The limited number of proteins which have been successfully identified by aptamer-based pull-down analysis demonstrates that aptamer selection involving living cells is a difficult task, and is still in its infancy. Moreover, identification of the target molecules requires a large number of bound molecules, hence a large number of cells. Fitter and James showed that a subset of proteins, which are found in whole blood, for example, can also be employed directly as the target mixture for in vitro selection.[157] A similar selection protocol has been used by Layzer and Sullenger. They selected aptamers against the gamma-carboxyglutamic acid domain containing protein proteome from human plasma.[158] In both approaches it was possible to simultaneously enrich aptamers against several targets. The limited number of possible targets means that the identification of the appropriate aptamer–target pairs might be simplified in these approaches compared to whole-cell–SELEX experiments. Wendel and co-workers identified aptamers that target stem cells and applied them for the isolation of the stem cells from composite mixtures, with the ultimate goal of developing functionalized materials with a preference for aggregating a certain cell-subtype.[159–163] This approach could lead to the development of smart materials based on aptamers.

4. Aptamers for Target Validation

Aptamers are potent inhibitors of their target molecules and therefore they are particularly important for target validation and functional characterization. In the last decade several studies have been described in which aptamers were employed to validate the function of a biomolecule in cell-culture experiments and in vivo.[164–166] In the latter case, the stability and pharmacokinetic parameters of aptamers can be manipulated by site-specific chemical modifications, such as with 2′-fluoro, 2′-amino, and 2′-methoxy nucleotides, and the addition of CAP structures, such as 3′-3′-dT, and polyethylene glycol (PEG).[167,168] This enables the aptamers to be subjected to versatile experimental set-ups and ultimately the use of aptamers as drugs. Aptamers offer a wide range of applications for target validation and the direct targeting of endogenous proteins. The synthesis of RNA aptamers by the cell's own transcription machinery is a great advantage and offers an alternative to transfection (Figure 6).[165,169–172] Recently, selection approaches based on the yeast three hybrid system have been described to assess the in vivo properties of in vitro selected RNA aptamers and to improve the activities of aptamers in vivo.[173–177]

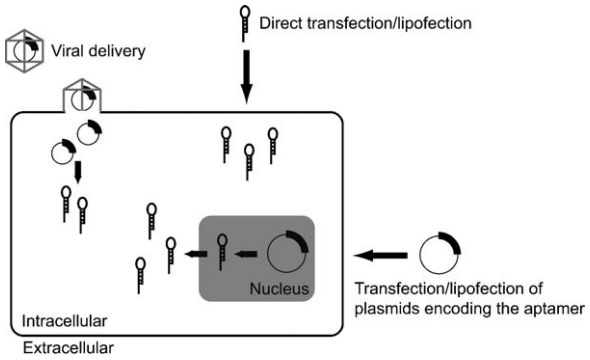


Figure 6. Cellular inclusion of aptamers. Aptamers can be transfected by state-of-the-art lipofection protocols or expressed by the cellular transcription machinery. In the latter case either the transfection of plasmids encoding for the aptamer sequence or the infection with recombinant viruses bearing the genetic information for aptamer production has been described.

Lipid-based transfection experiments, which are used for the transmembrane location of plasmids and siRNA molecules, can also be used to deliver aptamers into the cytoplasm (Figure 6).[178] Other methodologies that might be useful for delivering aptamers inside cells include microinjection, receptor-mediated internalization, and the construction of transgenic animals expressing the aptamer under the control of conditional promoters, as shown in a landmark study by Lis and co-workers.[179] The validation of intracellular target molecules with aptamers is a difficult task since this not only depends on the kinetic and thermodynamic properties of the aptamer, but also challenges the limitations of delivering aptamers into the cytoplasm. Each method has its own inherent limitations and makes optimization necessary with respect to the transfection efficiency (which also depends on the cell-type used) and read-out format. Primary cells, tissues, and organs in living animals are not particularly applicable to state-of-the-art lipid-mediated transfection protocols. However, the application of engineered recombinant viruses might

be useful in these cases, but the labor-intensive procedures means that such examples based on these approaches are rare.[164,165,180] Future examples are required to prove the general utility of viral delivery methods in this regard.

An alternative approach might be an adaption of the siRNA-transfection method. siRNA molecules have been successfully distributed into diverse tissues by equipping them with suitable delivery molecules such as peptides, lipoids, cholesterol, and even cell-specific aptamers.[128,181–185] However, these approaches have not yet been applied for the delivery of aptamers. It would be of great interest to explore whether lipid- or peptide-mediated delivery is also feasible for the cellular delivery of aptamers, even in living organisms. Similarly to off-target effects associated with siRNA approaches,[186,187] the secondary endogenous targets of aptamers need to be addressed extensively in future experiments to allow the precise interpretation of aptamer-induced phenotypes. The comprehensive and reliable comparison of the aptamer technology with other techniques for validation of the target molecule, at least at a cellular level, makes the compilation of these data indispensable.[188] Data on the function of the target molecule at the protein level through the application of aptamers can be regarded as complementary to data derived from siRNA and genetic knock-out studies. The combination of both approaches might give access to a precise analysis of the function of a biomolecule.

In a recent study Clary, Kontos, and co-workers demonstrated that the synergistic effects of an aptamer targeting NF-κB can be exploited to suppress drug-resistant phenotypes of cancer cells that are no longer susceptible to DOX treatment.[174,189,190] DOX resistance has been shown to be caused by the activation of NF-κB, hence the anti-NF-κB aptamer had a beneficial effect. In a different study, Chan et al. established that the expression of the anti-NF-κB aptamer in combination with anti-NF-κB siRNA molecules leads to a quantitative inhibition of NF-κB function in mammalian (Hela) cells. In contrast, the expression of only the aptamer or siRNA molecule leads to nonquantitative inhibition of the target.[191] The aptamer AGRO100, also known as AS1411, targets nucleolin and belongs to a family of G-rich oligodeoxynucleotides that are known to fold into a stable G-quartet structure and inhibit the proliferation of cancer cells.[192,193] The aptamer is undergoing clinical trials as an anticancer agent. A detailed analysis of its mode of action revealed that the aptamer inhibits cancer cell proliferation by forming a competition between the association of nucleolin with distinct co-factors, such as protein arginine methyltransferase 5 and NF-κB essential modulator (NEMO), thereby indicating that nucleolin is necessary for NF-κB regulation.[194,195] Furthermore, the treatment of breast cancer cells with AGRO100 resulted in an increased instability of the BCL-2 mRNA transcript.[196] These data demonstrate that a detailed analysis of the mode-of-action of an aptamer might uncover new insights into the molecule biology of the target molecule and pave the way for potential new targets for the development of novel therapeutics.

5. Drug Discovery with Aptamers

The modification of aptamers with fluorescing reporter molecules enables the establishment of high-throughput screening assays. In these formats the aptamer is applied as a competitive reporter probe, and small-molecule libraries are searched for compounds that are able to bind to the protein target and to compete with the aptamer for binding. The first report on using aptamers as competitors in screening assays was published in 2001. Green et al. employed an anti-PDGF aptamer and screened a small library of compounds which were already known to bind to and inhibit PDGF function, analogous to the parent aptamer.[197,198] Whereas the study by Green et al. used radioactively labeled aptamers, Famulok and co-workers employed so-called aptazymes for high-throughput screening.[199,200] Aptazymes are RNA molecules that contain two domains: one ribozyme domain which can be designed to undergo *cis* or *trans* cleavage and a second aptamer domain that binds to an effector molecule and thereby induces conformational changes within the ribozyme domain.[201,202] This set-up can be employed for the exogenous control of ribozyme activity, and was used to screen a library of diverse antibiotics that compete with the anti-HIV-1 rev aptamer for binding to the rev protein. Remarkably, the identified compound, coumermycin A1, was shown to contain aptamer-inherited properties and was able to inhibit virus replication in cell cultures (Figure 7). HIV-1 reverse transcriptase inhibitors were identified by a very similar approach, but using a commercially available library of diverse small

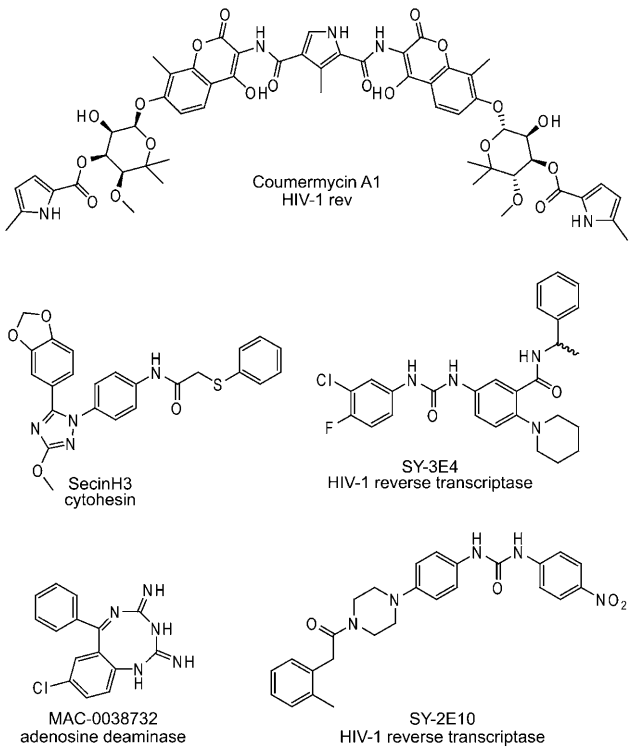


Figure 7. Small organic molecules identified by aptamer-based high-throughput screening experiments. The name of the compound (top) and its primary biological target (below) are given.

molecules (Figure 7).[203] The compound SY-3E4 has been shown to inhibit virus replication in HIV-1 strains that are resistant to known reverse transcriptase inhibitors. Since the design of the aptazyme is rather complex, and the future applications of aptazymes might focus on the regulation of gene expression,[204–207] straightforward protocols are required that allow an aptamer to be used for general screening purposes.

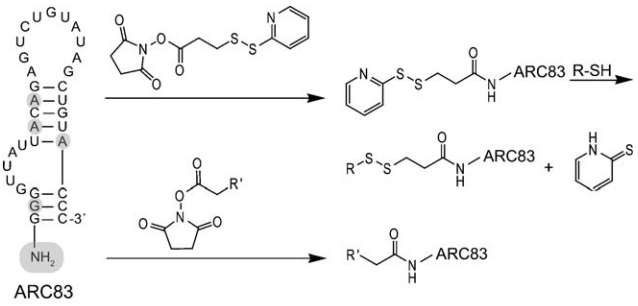
One such approach is based on fluorescence polarization and utilizes fluorescently labeled aptamers. In this method, the read-out signal is directly derived from the aptamer and strictly correlates with it being bound to the target molecule or not.[208,209] The application of such an approach led to the identification of the small molecule SecinH3, which binds to cytohesin and competes with the anti-cytohesin aptamer M69 (Figure 7).[164,210] Moreover, the compound could be used to further investigate the function of cytohesins in animals.[210,211] In a different approach, Li, Brown, and co-workers identified a new adenosine deaminase inhibitor.[212] Their screening assay was based on an aptamer that interacts with adenine, whereas the deaminated adenine (the product of deaminase enzymatic activity) was not recognized. The aptamer was thus used to detect enzymatic activity by differentially sensing the product and the reactant. A similar approach was applied by Srinivasan et al.[213] They used an aptamer that recognizes ADP with a 330-fold greater affinity than adenosine triphosphate. This aptamer is a versatile reagent for detecting adenosine diphosphate, which is formed from adenosine triphosphate by kinase activity.

These examples illustrate that aptamers can be employed for the identification of small organic molecules that have protein-inhibiting properties. The most exciting approach is represented by the direct conversion of the chemical information stored in the structure of an aptamer into a small molecule by direct competition assays and fluorescent detection.[208,214] Compounds identified by these approaches displayed aptamer-similar activities, and represent a direct link from macromolecular to small-molecule chemistry. An overview of novel protein inhibitors identified by aptamer-based approaches is given in Figure 7.

6. Aptamers as Drugs

Besides the application of aptamers for the discovery of small-molecule drug candidates, they can also be applied as drugs themselves.[215] Actually, this is one of the first areas that is envisioned to be ideally suited for aptamer applications. Since aptamers have restricted membrane-penetration capabilities, the vast majority of aptamers for therapeutic purposes were selected to target extracellular proteins. As described in Section 2, aptamers with enhanced stability against nucleases can be selected directly by using nucleotides with modifications that are compatible with the enzymatic steps of the in vitro selection process. Alternatively, aptamers can be modified after the selection. In this way they can be equipped with distinct chemical moieties to enhance their stability and improve their pharmacokinetics.[167,168]

Healy et al. compared the pharmacokinetic and biodistribution of an anti-TGFβ-2 aptamer equipped with different modifications, such as PEG20, PEG40, cholesterol, or cell-permeating peptides (tat/antennapedia; Scheme 8).[168] The



Scheme 8. The anti-TGFβ-2 aptamer ARC83 consists of 2′-fluoropyrimidine nucleotides and 2′-methoxy-substituted purines (unmodified positions are highlighted gray). The 5′-position bears an amino group that allows the introduction of further modifications; for example, the commercially available *N*-succinimidyl 3-(2-pyridyldithio) proprionate reagent (SPDP, Pierce) was used to couple peptides through the C-terminal cysteine residues, while NHS was used to couple polyethylene glycol moieties.

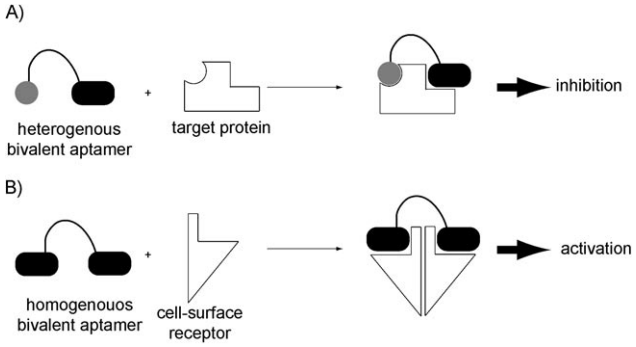
aptamer itself was modified with 2′-fluoro and 2′-methoxy groups, since in its unmodified version it was completely eliminated from the blood stream 48 h following injection. Consistent with previous studies, conjugation with PEG led to an enhanced half-life. The cholesterol-modified aptamer was cleared from plasma, while the addition of cell-permeating peptides did not promote clearance. Conjugation with the antennapedia peptide resulted in a pronounced accumulation of the aptamer in the kidneys. All modifications led to a significant enrichment of the aptamer in the kidney, liver, spleen, heart, and mediastinal lymph nodes. This example illustrates that aptamers can be rapidly modified with diverse functional groups, thereby modulating their pharmacokinetic properties. However, each aptamer–drug candidate has to be individually investigated, since sequence-dependent variations of the biodistribution and pharmacokinetic are possible.

One aptamer (Macugen) has been approved to date by the Food and Drug Administration (FDA) and it is important for the field that others follow. Promisingly, a few aptamers and spiegelmers (RNA aptamers consisting of L-enantiomer building blocks)[216,217] are currently in clinical trials for different indications and disease patterns (Table 1). Besides the treatment of cancer and cardiovascular diseases, the most prominent application of aptamers is as anticoagulants.[209] The first aptamer applied for this purpose is an anti-thrombin G-quadruplex-forming 15 mer oligodeoxynucleotide.[218–220] Clinical trials were initiated for the usage of the aptamer, also termed as ARC183 or HD1, during coronary artery bypass graft surgery (CABG). However, development was stopped after completion of the phase I clinical trials because of a low dosage profile.[221]

Table 1: Aptamers currently under investigation in clinical and preclinical trials.

Aptamer	Target	Disease pattern
NOX-B11	ghrelin	adipositas
E-10030	platelet-derived growth factor (PDGF)	age-related macular degeneration (AMD)
NU172	thrombin	coronary artery bypass graft surgery (CABG)
ARC1779	von Willebrand factor	acute coronary syndrome (ACS)
AS1411	nucleolin	acute myelogenous leukemia (AML)
REG1	factor IXa	coronary artery bypass graft surgery (CABG)

Improved anti-thrombin aptamers have been introduced recently by employing the concept of multivalency. In this regard, exosite I and exosite II binding aptamers have been interconnected by linker moieties, thus generating a bivalent aptamer with superior activities (Scheme 9A).[222,223] We demonstrated that the bivalent anti-thrombin aptamer has outstanding anticoagulant activities and, advantageously, its activity can be inactivated efficiently by designed antisense molecules.[224] The concept of using complementary oligonucleotides as antidotes for aptamers was initially introduced by Sullenger and co-workers in 2002.[225–227] They developed an aptamer that targets the blood coagulation factor IXa. A 5′-cholesterol and 3′-deoxythimidine CAP-modified variant of the anti-FIXa aptamer was later shown in vivo to be effective as an anticoagulant. Moreover, a 2′-methoxy-derivatized antisense strand, complementary to 17 nucleotides of the aptamer, was able to interfere with the aptamer activity, as shown in a murine model as well as in humans.[228–230] Another aptamer that has been developed and already in clinical trials targets the von Willebrand factor (vWF). This aptamer functions as an antithrombotic reagent and is being assessed in the treatment of patients with acute coronary syndrome (ACS). Preliminary trials in humans fulfilled the expectations and showed inhibition of vWF-mediated platelet aggregation in vivo.[231] Furthermore, Oney et al. showed that a designed antisense molecule reverses the inhibitory activities of the anti-vWF aptamer.[232] Thus, the safety profile of aptamer-based drugs can be enhanced and adverse side-effects minimized.



Scheme 9. Bivalent aptamers. A) Heterobivalent aptamers that target a protein through two different domains can have improved inhibitory properties. B) Homobivalent aptamers that target cell-surface receptors can induce receptor dimerization and thus activate the cell-signaling pathway.

As stated above, macugen (or pegaptanib sodium injection) is the sole aptamer which is approved by the FDA for the treatment of the wet-form of age-related macula degeneration (AMD).[233] Macugen targets the 165 amino acid splice isoform of the vascular-endothelial growth factor (VEGF), and its administration requires intravitreal injection of 6 nmoles once every 6 weeks. The efficacy of macugen and its high specificity (also in the context of VEGF isoforms) gives it a good safety profile. This is a factor of eminent importance since VEGF plays major roles in a wide range of physiological events. However, the treatment costs are very high, at around 12500 $.[234] Alternatively, antibody-derived VEGF inhibitors, such as bevacizumab and ranibizumab, can be used for AMD treatment.[235,236] The treatment of AMD patients with ranibizumab was approved by the FDA in 2006 and shown to result in the improvement of visual acuity—a remarkable effect that distinguishes the antibody treatment from other available therapies. An explanation for this effect might be the unspecific binding of the antibody to all VEGF isoforms.

Sullenger and co-workers introduced agonistic aptamers that are able to stimulate T cells, thus resulting in an efficient inhibition of tumor growth in mice. They identified an aptamer that targets 4-1BB, a co-stimulatory receptor which is up-regulated on activated T cells.[237] Agonistic antibodies targeting 4-1BB have proven successful in enhancing tumor immunity and tumor rejection in mice.[238] Similarly, a multivalent aptamer construct consisting of two identical aptamer domains assembled through a central stem structure was also shown to be effective in the stimulation of T cells and in inducing tumor rejection in mice (Scheme 9B). Recently, the same research group expanded the bivalent agonistic aptamer approach to aptamers that target OX40, a member of the tumor necrosis factor receptor family of proteins.[239]

These are interesting examples of how an aptamer that binds to cell-surface receptors can be transformed into an agonistic variant, based on a bivalent aptamer, and induce clustering of the receptors—similar to antibodies. Similarly, a study by Wang et al. describes the identification of an aptamer targeting midkine, a heparin-binding growth factor. Application of the aptamer resulted in an expansion of regulatory T cells, thus inducing reduction of symptoms associated with experimental autoimmune encephalomyelitis (EAE), a model for the severe disease multiple sclerosis.[240]

These examples demonstrate that aptamers and engineered variants thereof possess superior activities and might give access to sophisticated therapeutics. The low immunogenicity and cellular-targeting properties of aptamers leads to the hypothesis that they might represent suitable drugs with low side effects. However, adverse effects of aptamers, such as immunological responses on the B- and T-cell level, mainly mediated by the toll-like receptor family, need to be investigated and long-term studies regarding their safety will not be available until more aptamers have been approved and applied in the clinic.

7. Summary and Outlook

Since the first description of aptamers in 1990, the field has developed into a mature technology, providing sophisticated tools for biological and medical sciences. The synthetic and enzymatic access to aptamers enables their site-specific and strategic modification, and thus their adaptation to various applications such as target validation, as diagnostics, and as drugs. Aptamers have the advantage that they do not induce immunological responses, at least at the level of antibody production. Moreover, aptamers can be used for the identification of cell-surface molecules associated with a pathogenic state of a cell; future studies will prove whether the aptamer technology can make significant contributions in this research field. Aptamer technology thus represents an integrated technology platform. In contrast to other technologies based on nucleic acids, such as siRNA and antisense technologies, the identification of an aptamer is a labor-intensive procedure, consequently leading to the frequent use of these other technologies for target validation. A major challenge that needs to be overcome is the development of easy-to-use SELEX procedures, for example, the provision of ready-to-use "SELEX kits" that enable the application of in vitro selection by any skilled researcher or technician. The characterization of an identified target gene by siRNA molecules needs to be verified by the application of inhibitors acting at the protein level, otherwise the interpretation of phenotypes might be suboptimal, because of off-target effects.[241–243] In this regard aptamers and siRNA molecules represent complementary approaches whose combination might be a powerful tool. Aptamers are excellent tools for chemical biology, and the precise application and control of their activities will allow the accurate targeting of biomolecules and the high-resolution analysis of biomolecular function. Recent studies have used aptamer technology for the discovery of small organic molecules that possess aptamer-inherited properties. This might pave the way for the development of novel inhibitors of protein functions. The next few years will show whether aptamers will succeed as a novel drug class and as a diagnostic tool for daily clinical practice.

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[1] D. R. Mills, R. L. Peterson, S. Spiegelman, *Proc. Natl. Acad. Sci. USA* **1967**, *58*, 217.
[2] I. Haruna, K. Nozu, Y. Ohtaka, S. Spiegelman, *Proc. Natl. Acad. Sci. USA* **1963**, *50*, 905.
[3] I. Haruna, S. Spiegelman, *Science* **1965**, *150*, 884.
[4] I. Haruna, S. Spiegelman, *Proc. Natl. Acad. Sci. USA* **1965**, *54*, 579.
[5] G. F. Joyce, *Angew. Chem.* **2007**, *119*, 6540; *Angew. Chem. Int. Ed.* **2007**, *46*, 6420.
[6] K. Mullis, F. Faloona, S. Scharf, R. Saiki, G. Horn, H. Erlich, *Biotechnology* **1992**, *24*, 17.
[7] R. K. Saiki, D. H. Gelfand, S. Stoffel, S. J. Scharf, R. Higuchi, G. T. Horn, K. B. Mullis, H. A. Erlich, *Science* **1988**, *239*, 487.
[8] R. K. Saiki, S. Scharf, F. Faloona, K. B. Mullis, G. T. Horn, H. A. Erlich, N. Arnheim, *Science* **1985**, *230*, 1350.
[9] K. B. Mullis, *Angew. Chem.* **1994**, *106*, 1271; *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 1209.
[10] D. H. Bishop, N. R. Pace, S. Spiegelman, *Proc. Natl. Acad. Sci. USA* **1967**, *58*, 1790.
[11] A. D. Ellington, J. W. Szostak, *Nature* **1990**, *346*, 818.
[12] C. Tuerk, L. Gold, *Science* **1990**, *249*, 505.
[13] D. L. Robertson, G. F. Joyce, *Nature* **1990**, *344*, 467.
[14] A. Heckel, M. Famulok, *Biochimie* **2008**, *90*, 1096.
[15] G. Mayer, D. Ackermann, N. Kuhn, M. Famulok, *Angew. Chem.* **2008**, *120*, 985; *Angew. Chem. Int. Ed.* **2008**, *47*, 971.
[16] G. Rasched, D. Ackermann, T. L. Schmidt, P. Broekmann, A. Heckel, M. Famulok, *Angew. Chem.* **2008**, *120*, 981; *Angew. Chem. Int. Ed.* **2008**, *47*, 967.
[17] C. Lin, X. Wang, Y. Liu, N. C. Seeman, H. Yan, *J. Am. Chem. Soc.* **2007**, *129*, 14475.
[18] P. W. Rothemund, *Nature* **2006**, *440*, 297.
[19] M. Famulok, A. Huttenhofer, *Biochemistry* **1996**, *35*, 4265.
[20] H. Schürer, K. Stembera, D. Knoll, G. Mayer, M. Blind, H. H. Forster, M. Famulok, P. Welzel, U. Hahn, *Bioorg. Med. Chem.* **2001**, *9*, 2557.
[21] K. K. Farh, A. Grimson, C. Jan, B. P. Lewis, W. K. Johnston, L. P. Lim, C. B. Burge, D. P. Bartel, *Science* **2005**, *310*, 1817.
[22] D. P. Bartel, *Cell* **2004**, *116*, 281.
[23] G. Mayer, M. S. Raddatz, J. D. Grunwald, M. Famulok, *Angew. Chem.* **2007**, *119*, 563; *Angew. Chem. Int. Ed.* **2007**, *46*, 557.
[24] J. E. Barrick, R. R. Breaker, *Sci. Am.* **2007**, *296*, 50.
[25] B. J. Tucker, R. R. Breaker, *Curr. Opin. Struct. Biol.* **2005**, *15*, 342.
[26] A. Rentmeister, G. Mayer, N. Kuhn, M. Famulok, *Nucleic Acids Res.* **2007**, *35*, 3713.
[27] J. B. Tok, N. O. Fischer, *Chem. Commun.* **2008**, 1883.
[28] S. D. Mendonsa, M. T. Bowser, *Anal. Chem.* **2004**, *76*, 5387.
[29] S. D. Mendonsa, M. T. Bowser, *J. Am. Chem. Soc.* **2005**, *127*, 9382.
[30] M. Berezovski, A. Drabovich, S. M. Krylova, M. Musheev, V. Okhonin, A. Petrov, S. N. Krylov, *J. Am. Chem. Soc.* **2005**, *127*, 3165.
[31] A. Drabovich, M. Berezovski, S. N. Krylov, *J. Am. Chem. Soc.* **2005**, *127*, 11224.
[32] D. D. Buchanan, E. E. Jameson, J. Perlette, A. Malik, R. T. Kennedy, *Electrophoresis* **2003**, *24*, 1375.
[33] E. Kraus, W. James, A. N. Barclay, *J. Immunol.* **1998**, *160*, 5209.
[34] T. S. Misono, P. K. Kumar, *Anal. Biochem.* **2005**, *342*, 312.
[35] J. Müller, O. El-Maarri, J. Oldenburg, B. Potzsch, G. Mayer, *Anal. Bioanal. Chem.* **2008**, *390*, 1033.
[36] D. Eulberg, K. Buchner, C. Maasch, S. Klussmann, *Nucleic Acids Res.* **2005**, *33*, e45.
[37] J. C. Cox, A. D. Ellington, *Bioorg. Med. Chem.* **2001**, *9*, 2525.
[38] A. Wochner, B. Cech, M. Menger, V. A. Erdmann, J. Glokler, *Biotechniques* **2007**, *43*, 344.

[39] G. Mayer, B. Wulffen, C. Huber, J. Brockmann, B. Flicke, L. Neumann, D. Hafenbradl, B. M. Klebl, M. J. Lohse, C. Krasel, M. Blind, *RNA* **2008**, *14*, 524.
[40] J. H. Davis, J. W. Szostak, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 11616.
[41] J. Hamm, *Nucleic Acids Res.* **1996**, *24*, 2220.
[42] A. Bugaut, J. J. Toulme, B. Rayner, *Org. Biomol. Chem.* **2006**, *4*, 4082.
[43] M. Famulok, G. Mayer, *ChemBioChem* **2005**, *6*, 19.
[44] M. Rimmele, *ChemBioChem* **2003**, *4*, 963.
[45] D. S. Wilson, J. W. Szostak, *Annu. Rev. Biochem.* **1999**, *68*, 611.
[46] As a consequence of the large body of literature that exists on the aptamer topic, I apologize for having missed individual studies.
[47] A. D. Keefe, S. T. Cload, *Curr. Opin. Chem. Biol.* **2008**, *12*, 448.
[48] R. M. Orr, *Curr. Opin. Mol. Ther.* **2001**, *3*, 288.
[49] X. Yang, S. E. Bassett, X. Li, B. A. Luxon, N. K. Herzog, R. E. Shope, J. Aronson, T. W. Prow, J. F. Leary, R. Kirby, A. D. Ellington, D. G. Gorenstein, *Nucleic Acids Res.* **2002**, *30*, 132e.
[50] J. T. Holmlund, *Ann. N. Y. Acad. Sci.* **2003**, *1002*, 244.
[51] J. Chelliserrykattil, A. D. Ellington, *Nat. Biotechnol.* **2004**, *22*, 1155.
[52] R. Padilla, R. Sousa, *Nucleic Acids Res.* **2002**, *30*, 138e.
[53] R. N. Veedu, B. Vester, J. Wengel, *ChemBioChem* **2007**, *8*, 490.
[54] R. N. Veedu, B. Vester, J. Wengel, *J. Am. Chem. Soc.* **2008**, *130*, 8124.
[55] J. A. Latham, R. Johnson, J. J. Toole, *Nucleic Acids Res.* **1994**, *22*, 2817.
[56] S. Jäger, M. Famulok, *Angew. Chem.* **2004**, *116*, 3399; *Angew. Chem. Int. Ed.* **2004**, *43*, 3337.
[57] S. Jäger, G. Rasched, H. Kornreich-Leshem, M. Engeser, O. Thum, M. Famulok, *J. Am. Chem. Soc.* **2005**, *127*, 15071.
[58] N. Lin, J. Yan, Z. Huang, C. Altier, M. Li, N. Carrasco, M. Suyemoto, L. Johnston, S. Wang, Q. Wang, H. Fang, J. Caton-Williams, B. Wang, *Nucleic Acids Res.* **2007**, *35*, 1222.
[59] J. F. Lutz, Z. Zarafshani, *Adv. Drug Delivery Rev.* **2008**, *60*, 958.
[60] P. M. Gramlich, S. Warncke, J. Gierlich, T. Carell, *Angew. Chem.* **2008**, *120*, 3491; *Angew. Chem. Int. Ed.* **2008**, *47*, 3442.
[61] J. Gierlich, G. A. Burley, P. M. Gramlich, D. M. Hammond, T. Carell, *Org. Lett.* **2006**, *8*, 3639.
[62] G. Mayer, A. Heckel, *Angew. Chem.* **2006**, *118*, 5020; *Angew. Chem. Int. Ed.* **2006**, *45*, 4900.
[63] A. Heckel, M. C. Buff, M. S. Raddatz, J. Muller, B. Potzsch, G. Mayer, *Angew. Chem.* **2006**, *118*, 6900; *Angew. Chem. Int. Ed.* **2006**, *45*, 6748.
[64] A. Heckel, G. Mayer, *J. Am. Chem. Soc.* **2005**, *127*, 822.
[65] G. Mayer, L. Krock, V. Mikat, M. Engeser, A. Heckel, *ChemBioChem* **2005**, *6*, 1966.
[66] J. Hesselberth, M. P. Robertson, S. Jhaveri, A. D. Ellington, *J. Biotechnol.* **2000**, *74*, 15.
[67] J. R. Hesselberth, M. P. Robertson, S. M. Knudsen, A. D. Ellington, *Anal. Biochem.* **2003**, *312*, 106.
[68] M. Famulok, G. Mayer, *Curr. Top. Microbiol. Immunol.* **1999**, *243*, 123.
[69] G. Sengle, A. Jenne, P. S. Arora, B. Seelig, J. S. Nowick, A. Jaschke, M. Famulok, *Bioorg. Med. Chem.* **2000**, *8*, 1317.
[70] B. Seelig, A. Jäschke, *Chem. Biol.* **1999**, *6*, 167.
[71] M. Blank, T. Weinschenk, M. Priemer, H. Schluesener, *J. Biol. Chem.* **2001**, *276*, 16464.
[72] H. Ulrich, A. H. Martins, J. B. Pesquero, *Cytometry* **2004**, *59A*, 220.
[73] A. Eisenführ, P. S. Arora, G. Sengle, L. R. Takaoka, J. S. Nowick, M. Famulok, *Bioorg. Med. Chem.* **2003**, *11*, 235.
[74] B. Seelig, A. Jaschke, *Bioconjugate Chem.* **1999**, *10*, 371.
[75] S. Pfander, R. Fiammengo, S. I. Kirin, N. Metzler-Nolte, A. Jaschke, *Nucleic Acids Res.* **2007**, *35*, e25.
[76] F. Hansske, F. Cramer, *Methods Enzymol.* **1979**, *59*, 172.
[77] C. Baugh, D. Grate, C. Wilson, *J. Mol. Biol.* **2000**, *301*, 117.
[78] D. Grate, C. Wilson, *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 6131.
[79] M. Müller, J. E. Weigand, O. Weichenrieder, B. Suess, *Nucleic Acids Res.* **2006**, *34*, 2607.
[80] S. Hanson, G. Bauer, B. Fink, B. Suess, *RNA* **2005**, *11*, 503.
[81] M. N. Stojanovic, P. de Prada, D. W. Landry, *J. Am. Chem. Soc.* **2001**, *123*, 4928.
[82] S. Sando, A. Narita, Y. Aoyama, *ChemBioChem* **2007**, *8*, 1795.
[83] C. A. Savran, S. M. Knudsen, A. D. Ellington, S. R. Manalis, *Anal. Chem.* **2004**, *76*, 3194.
[84] M. D. Schlensog, T. M. A. Gronewold, M. Tewes, M. Famulok, E. Quandt, *Sens. Actuators B* **2004**, *101*, 308.
[85] H. Petach, R. Ostroff, C. Greef, G. M. Husar, *Methods Mol. Biol.* **2004**, *264*, 101.
[86] H. Petach, L. Gold, *Curr. Opin. Biotechnol.* **2002**, *13*, 309.
[87] B. R. Baker, R. Y. Lai, M. S. Wood, E. H. Doctor, A. J. Heeger, K. W. Plaxco, *J. Am. Chem. Soc.* **2006**, *128*, 3138.
[88] M. Eisenstein, *Nat. Methods* **2006**, *3*, 244.
[89] R. Kirby, E. J. Cho, B. Gehrke, T. Bayer, Y. S. Park, D. P. Neikirk, J. T. McDevitt, A. D. Ellington, *Anal. Chem.* **2004**, *76*, 4066.
[90] R. Y. Lai, K. W. Plaxco, A. J. Heeger, *Anal. Chem.* **2007**, *79*, 229.
[91] Y. Xiao, A. A. Lubin, A. J. Heeger, K. W. Plaxco, *Angew. Chem.* **2005**, *117*, 5592; *Angew. Chem. Int. Ed.* **2005**, *44*, 5456.
[92] I. Willner, M. Zayats, *Angew. Chem.* **2007**, *119*, 6528; *Angew. Chem. Int. Ed.* **2007**, *46*, 6408.
[93] C. Bock, M. Coleman, B. Collins, J. Davis, G. Foulds, L. Gold, C. Greef, J. Heil, J. S. Heilig, B. Hicke, M. N. Hurst, G. M. Husar, D. Miller, R. Ostroff, H. Petach, D. Schneider, B. Vant-Hull, S. Waugh, A. Weiss, S. K. Wilcox, D. Zichi, *Proteomics* **2004**, *4*, 609.
[94] R. Nutiu, J. M. Yu, Y. Li, *ChemBioChem* **2004**, *5*, 1139.
[95] R. Nutiu, Y. Li, *Chem. Eur. J.* **2004**, *10*, 1868.
[96] T. Hermann, D. J. Patel, *Science* **2000**, *287*, 820.
[97] Y. Yang, M. Kochoyan, P. Burgstaller, E. Westhof, M. Famulok, *Science* **1996**, *272*, 1343.
[98] D. W. Drolet, L. Moon-McDermott, T. S. Romig, *Nat. Biotechnol.* **1996**, *14*, 1021.
[99] C. S. Ferreira, K. Papamichael, G. Guilbault, T. Schwarzacher, J. Gariepy, S. Missailidis, *Anal. Bioanal. Chem.* **2008**, *390*, 1039.
[100] M. B. Murphy, S. T. Fuller, P. M. Richardson, S. A. Doyle, *Nucleic Acids Res.* **2003**, *31*, 110e.
[101] H. Zhang, X. F. Li, X. C. Le, *J. Am. Chem. Soc.* **2008**, *130*, 34.
[102] Y. F. Huang, H. T. Chang, W. Tan, *Anal. Chem.* **2008**, *80*, 567.
[103] J. Charlton, J. Sennello, D. Smith, *Chem. Biol.* **1997**, *4*, 809.
[104] M. Michaud, E. Jourdan, A. Villet, A. Ravel, C. Grosset, E. Peyrin, *J. Am. Chem. Soc.* **2003**, *125*, 8672.
[105] E. N. Brody, M. C. Willis, J. D. Smith, S. Jayasena, D. Zichi, L. Gold, *Mol. Diagn.* **1999**, *4*, 381.
[106] B. Schweitzer, S. Wiltshire, J. Lambert, S. O'Malley, K. Kukanskis, Z. Zhu, S. F. Kingsmore, P. M. Lizardi, D. C. Ward, *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 10113.
[107] D. A. Di Giusto, W. A. Wlassoff, J. J. Gooding, B. A. Messerle, G. C. King, *Nucleic Acids Res.* **2005**, *33*, e64.
[108] H. Zhang, Z. Wang, X. F. Li, X. C. Le, *Angew. Chem.* **2006**, *118*, 1606; *Angew. Chem. Int. Ed.* **2006**, *45*, 1576.
[109] S. Fredriksson, M. Gullberg, J. Jarvius, C. Olsson, K. Pietras, S. M. Gustafsdottir, A. Ostman, U. Landegren, *Nat. Biotechnol.* **2002**, *20*, 473.
[110] S. M. Gustafsdottir, S. Wennstrom, S. Fredriksson, E. Schallmeiner, A. D. Hamilton, S. M. Sebti, U. Landegren, *Clin. Chem.* **2008**, *54*, 1218.
[111] V. Bagalkot, L. Zhang, E. Levy-Nissenbaum, S. Jon, P. W. Kantoff, R. Langer, O. C. Farokhzad, *Nano Lett.* **2007**, *7*, 3065.
[112] J. A. Hansen, J. Wang, A. N. Kawde, Y. Xiang, K. V. Gothelf, G. Collins, *J. Am. Chem. Soc.* **2006**, *128*, 2228.

[113] M. Levy, S. F. Cater, A. D. Ellington, *ChemBioChem* **2005**, *6*, 2163.
[114] J. Liu, J. H. Lee, Y. Lu, *Anal. Chem.* **2007**, *79*, 4120.
[115] M. N. Stojanovic, D. W. Landry, *J. Am. Chem. Soc.* **2002**, *124*, 9678.
[116] A. P. Alivisatos, W. Gu, C. Larabell, *Annu. Rev. Biomed. Eng.* **2005**, *7*, 55.
[117] S. Dwarakanath, J. G. Bruno, A. Shastry, T. Phillips, A. A. John, A. Kumar, L. D. Stephenson, *Biochem. Biophys. Res. Commun.* **2004**, *325*, 739.
[118] L. C. Bock, L. C. Griffin, J. A. Latham, E. H. Vermaas, J. J. Toole, *Nature* **1992**, *355*, 564.
[119] J. Liu, Y. Lu, *Nat. Protoc.* **2006**, *1*, 246.
[120] J. Liu, D. Mazumdar, Y. Lu, *Angew. Chem.* **2006**, *118*, 8123; *Angew. Chem. Int. Ed.* **2006**, *45*, 7955.
[121] M. Famulok, G. Mayer, *Nature* **2006**, *439*, 666.
[122] V. Bagalkot, O. C. Farokhzad, R. Langer, S. Jon, *Angew. Chem.* **2006**, *118*, 8329; *Angew. Chem. Int. Ed.* **2006**, *45*, 8149.
[123] X. Chen, Y. Deng, Y. Lin, D. Pang, H. Qing, F. Qu, H. Xie, *Nanotechnology* **2008**, *19*, 235105.
[124] S. E. Lupold, B. J. Hicke, Y. Lin, D. S. Coffey, *Cancer Res.* **2002**, *62*, 4029.
[125] O. C. Farokhzad, S. Jon, A. Khademhosseini, T. N. Tran, D. A. Lavan, R. Langer, *Cancer Res.* **2004**, *64*, 7668.
[126] O. C. Farokhzad, J. Cheng, B. A. Teply, I. Sherifi, S. Jon, P. W. Kantoff, J. P. Richie, R. Langer, *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 6315.
[127] J. Cheng, B. A. Teply, I. Sherifi, J. Sung, G. Luther, F. X. Gu, E. Levy-Nissenbaum, A. F. Radovic-Moreno, R. Langer, O. C. Farokhzad, *Biomaterials* **2007**, *28*, 869.
[128] J. O. McNamara II, E. R. Andrechek, Y. Wang, K. D. Viles, R. E. Rempel, E. Gilboa, B. A. Sullenger, P. H. Giangrande, *Nat. Biotechnol.* **2006**, *24*, 1005.
[129] T. C. Chu, K. Y. Twu, A. D. Ellington, M. Levy, *Nucleic Acids Res.* **2006**, *34*, e73.
[130] T. C. Chu, J. W. Marks III, L. A. Lavery, S. Faulkner, M. G. Rosenblum, A. D. Ellington, M. Levy, *Cancer Res.* **2006**, *66*, 5989.
[131] M. V. Hosur, B. Nair, P. Satyamurthy, S. Misquith, A. Surolia, K. K. Kannan, *J. Mol. Biol.* **1995**, *250*, 368.
[132] J. Zhou, H. Li, S. Li, J. Zaia, J. J. Rossi, *Mol. Ther.* **2008**, *16*, 1481.
[133] J. Mi, X. Zhang, P. H. Giangrande, J. O. McNamara II, S. M. Nimjee, S. Sarraf-Yazdi, B. A. Sullenger, B. M. Clary, *Biochem. Biophys. Res. Commun.* **2005**, *338*, 956.
[134] Z. Huang, W. Pei, S. Jayaseelan, H. Shi, L. Niu, *Biochemistry* **2007**, *46*, 12648.
[135] F. Chauveau, Y. Aissouni, J. Hamm, H. Boutin, D. Libri, F. Duconge, B. Tavitian, *Bioorg. Med. Chem. Lett.* **2007**, *17*, 6119.
[136] C. H. Chen, G. A. Chernis, V. Q. Hoang, R. Landgraf, *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 9226.
[137] Y. Hui, L. Shan, Z. Lin-Fu, Z. Jian-Hua, *Mol. Cell. Biochem.* **2007**, *306*, 71.
[138] J. A. Phillips, D. Lopez-Colon, Z. Zhu, Y. Xu, W. Tan, *Anal. Chim. Acta* **2008**, *621*, 101.
[139] S. M. Shamah, J. M. Healy, S. T. Cload, *Acc. Chem. Res.* **2008**, *41*, 130.
[140] M. C. Alliegro, M. A. Alliegro, *J. Biol. Chem.* **2002**, *277*, 19037.
[141] M. C. Alliegro, *Cell Biol. Int.* **2001**, *25*, 577.
[142] D. A. Daniels, H. Chen, B. J. Hicke, K. M. Swiderek, L. Gold, *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 15416.
[143] J. G. Bruno, J. L. Kiel, *Biosens. Bioelectron.* **1999**, *14*, 457.
[144] F. Chen, J. Zhou, F. Luo, A. B. Mohammed, X. L. Zhang, *Biochem. Biophys. Res. Commun.* **2007**, *357*, 743.
[145] M. Homann, M. Lorger, M. Engstler, M. Zacharias, H. U. Goringer, *Comb. Chem. High Throughput Screening* **2006**, *9*, 491.
[146] M. Homann, H. U. Goringer, *Bioorg. Med. Chem.* **2001**, *9*, 2571.
[147] L. Cerchia, F. Duconge, C. Pestourie, J. Boulay, Y. Aissouni, K. Gombert, B. Tavitian, V. de Franciscis, D. Libri, *PLoS Biol.* **2005**, *3*, e123.
[148] M. S. Raddatz, A. Dolf, E. Endl, P. Knolle, M. Famulok, G. Mayer, *Angew. Chem.* **2008**, *120*, 5268; *Angew. Chem. Int. Ed.* **2008**, *47*, 5190.
[149] M. V. Berezovski, M. Lechmann, M. U. Musheev, T. W. Mak, S. N. Krylov, *J. Am. Chem. Soc.* **2008**, *130*, 9137.
[150] H. W. Chen, C. D. Medley, K. Sefah, D. Shangguan, Z. Tang, L. Meng, J. E. Smith, W. Tan, *ChemMedChem* **2008**, *3*, 991.
[151] D. Shangguan, Y. Li, Z. Tang, Z. C. Cao, H. W. Chen, P. Mallikaratchy, K. Sefah, C. J. Yang, W. Tan, *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 11838.
[152] D. Shangguan, L. Meng, Z. C. Cao, Z. Xiao, X. Fang, Y. Li, D. Cardona, R. P. Witek, C. Liu, W. Tan, *Anal. Chem.* **2008**, *80*, 721.
[153] Z. Tang, D. Shangguan, K. Wang, H. Shi, K. Sefah, P. Mallikratchy, H. W. Chen, Y. Li, W. Tan, *Anal. Chem.* **2007**, *79*, 4900.
[154] D. Shangguan, Z. C. Cao, Y. Li, W. Tan, *Clin. Chem.* **2007**, *53*, 1153.
[155] P. Mallikaratchy, Z. Tang, S. Kwame, L. Meng, D. Shangguan, W. Tan, *Mol. Cell. Proteomics* **2007**, *6*, 2230.
[156] D. Shangguan, Z. Cao, L. Meng, P. Mallikaratchy, K. Sefah, H. Wang, Y. Li, W. Tan, *J. Proteome Res.* **2008**, *7*, 2133.
[157] S. Fitter, R. James, *J. Biol. Chem.* **2005**, *280*, 34193.
[158] J. M. Layzer, B. A. Sullenger, *Oligonucleotides* **2007**, *17*, 1.
[159] K. Guo, H. P. Wendel, L. Scheideler, G. Ziemer, A. M. Scheule, *J. Cell. Mol. Med.* **2005**, *9*, 731.
[160] K. T. Guo, R. Schäfer, A. Paul, A. Gerber, G. Ziemer, H. P. Wendel, *Stem Cells* **2006**, *24*, 2220.
[161] K. T. Guo, D. Scharnweber, B. Schwenzer, G. Ziemer, H. P. Wendel, *Biomaterials* **2007**, *28*, 468.
[162] J. Hoffmann, A. Paul, M. Harwardt, J. Groll, T. Reeswinkel, D. Klee, M. Möller, H. Fischer, T. Walker, T. Greiner, G. Ziemer, H. P. Wendel, *J. Biomed. Mater. Res. Part A* **2008**, *84*, 614.
[163] R. Schäfer, J. Wiskirchen, K. Guo, B. Neumann, R. Kehlbach, J. Pintaske, V. Voth, T. Walker, A. M. Scheule, T. O. Greiner, U. Hermanutz-Klein, C. D. Claussen, H. Northoff, G. Ziemer, H. P. Wendel, *Rofo* **2007**, *179*, 1009.
[164] G. Mayer, M. Blind, W. Nagel, T. Bohm, T. Knorr, C. L. Jackson, W. Kolanus, M. Famulok, *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 4961.
[165] J. Mi, X. Zhang, Y. Liu, S. K. Reddy, Z. N. Rabbani, B. A. Sullenger, B. M. Clary, *Biochem. Biophys. Res. Commun.* **2007**, *359*, 475.
[166] M. Famulok, J. S. Hartig, G. Mayer, *Chem. Rev.* **2007**, *107*, 3715.
[167] T. G. McCauley, J. C. Kurz, P. G. Merlino, S. D. Lewis, M. Gilbert, D. M. Epstein, H. N. Marsh, *Pharm. Res.* **2006**, *23*, 303.
[168] J. M. Healy, S. D. Lewis, M. Kurz, R. M. Boomer, K. M. Thompson, C. Wilson, T. G. McCauley, *Pharm. Res.* **2004**, *21*, 2234.
[169] R. E. Martell, J. R. Nevins, B. A. Sullenger, *Mol. Ther.* **2002**, *6*, 30.
[170] H. K. Lee, H. Y. Kwak, J. Hur, I. A. Kim, J. S. Yang, M. W. Park, J. Yu, S. Jeong, *Cancer Res.* **2007**, *67*, 9315.
[171] K. H. Choi, M. W. Park, S. Y. Lee, M. Y. Jeon, M. Y. Kim, H. K. Lee, J. Yu, H. J. Kim, K. Han, H. Lee, K. Park, W. J. Park, S. Jeong, *Mol. Cancer Ther.* **2006**, *5*, 2428.
[172] H. K. Lee, Y. S. Choi, Y. A. Park, S. Jeong, *Cancer Res.* **2006**, *66*, 10560.
[173] J. Konig, C. Julius, S. Baumann, M. Homann, H. U. Goringer, M. Feldbrugge, *RNA* **2007**, *13*, 614.
[174] L. A. Cassiday, L. J. Maher III, *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 3930.

[175] C. Lorenz, N. Piganeau, R. Schroeder, *Nucleic Acids Res.* **2006**, *34*, 334.
[176] N. Piganeau, U. E. Schauer, R. Schroeder, *RNA* **2006**, *12*, 177.
[177] N. Piganeau, R. Schroeder, *Nat. Protoc.* **2006**, *1*, 689.
[178] M. G. Theis, A. Knorre, B. Kellersch, J. Moelleken, F. Wieland, W. Kolanus, M. Famulok, *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 11221.
[179] H. Shi, B. E. Hoffman, J. T. Lis, *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 10033.
[180] M. Blind, W. Kolanus, M. Famulok, *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 3606.
[181] C. Wolfrum, S. Shi, K. N. Jayaprakash, M. Jayaraman, G. Wang, R. K. Pandey, K. G. Rajeev, T. Nakayama, K. Charrise, E. M. Ndungo, T. Zimmermann, V. Koteliansky, M. Manoharan, M. Stoffel, *Nat. Biotechnol.* **2007**, *25*, 1149.
[182] A. Akinc, A. Zumbuehl, M. Goldberg, E. S. Leshchiner, V. Busini, N. Hossain, S. A. Bacallado, D. N. Nguyen, J. Fuller, R. Alvarez, A. Borodovsky, T. Borland, R. Constien, A. de Fougerolles, J. R. Dorkin, K. Narayanannair Jayaprakash, M. Jayaraman, M. John, V. Koteliansky, M. Manoharan, L. Nechev, J. Qin, T. Racie, D. Raitcheva, K. G. Rajeev, D. W. Sah, J. Soutschek, I. Toudjarska, H. P. Vornlocher, T. S. Zimmermann, R. Langer, D. G. Anderson, *Nat. Biotechnol.* **2008**, *26*, 561.
[183] R. Rennert, I. Neundorf, A. G. Beck-Sickinger, *Adv. Drug Delivery Rev.* **2008**, *60*, 485.
[184] J. Soutschek, A. Akinc, B. Bramlage, K. Charisse, R. Constien, M. Donoghue, S. Elbashir, A. Geick, P. Hadwiger, J. Harborth, M. John, V. Kesavan, G. Lavine, R. K. Pandey, T. Racie, K. G. Rajeev, I. Rohl, I. Toudjarska, G. Wang, S. Wuschko, D. Bumcrot, V. Koteliansky, S. Limmer, M. Manoharan, H. P. Vornlocher, *Nature* **2004**, *432*, 173.
[185] P. Kumar, H. Wu, J. L. McBride, K. E. Jung, M. H. Kim, B. L. Davidson, S. K. Lee, P. Shankar, N. Manjunath, *Nature* **2007**, *448*, 39.
[186] E. Anderson, Q. Boese, A. Khvorova, J. Karpilow, *Methods Mol. Biol.* **2008**, *442*, 45.
[187] Y. Fedorov, E. M. Anderson, A. Birmingham, A. Reynolds, J. Karpilow, K. Robinson, D. Leake, W. S. Marshall, A. Khvorova, *RNA* **2006**, *12*, 1188.
[188] T. Mori, A. Oguro, T. Ohtsu, Y. Nakamura, *Nucleic Acids Res.* **2004**, *32*, 6120.
[189] J. Mi, X. Zhang, Z. N. Rabbani, Y. Liu, S. K. Reddy, Z. Su, F. K. Salahuddin, K. Viles, P. H. Giangrande, M. W. Dewhirst, B. A. Sullenger, C. D. Kontos, B. M. Clary, *Mol. Ther.* **2008**, *16*, 66.
[190] J. Mi, X. Zhang, Z. N. Rabbani, Y. Liu, Z. Su, Z. Vujaskovic, C. D. Kontos, B. A. Sullenger, B. M. Clary, *Nucleic Acids Res.* **2006**, *34*, 3577.
[191] R. Chan, M. Gilbert, K. M. Thompson, H. N. Marsh, D. M. Epstein, P. S. Pendergrast, *Nucleic Acids Res.* **2006**, *34*, e36.
[192] V. Dapic, V. Abdomerovic, R. Marrington, J. Peberdy, A. Rodger, J. O. Trent, P. J. Bates, *Nucleic Acids Res.* **2003**, *31*, 2097.
[193] C. R. Ireson, L. R. Kelland, *Mol. Cancer Ther.* **2006**, *5*, 2957.
[194] Y. Teng, A. C. Girvan, L. K. Casson, W. M. Pierce, Jr., M. Qian, S. D. Thomas, P. J. Bates, *Cancer Res.* **2007**, *67*, 10491.
[195] A. C. Girvan, Y. Teng, L. K. Casson, S. D. Thomas, S. Juliger, M. W. Ball, J. B. Klein, W. M. Pierce, Jr., S. S. Barve, P. J. Bates, *Mol. Cancer Ther.* **2006**, *5*, 1790.
[196] S. Soundararajan, W. Chen, E. K. Spicer, N. Courtenay-Luck, D. J. Fernandes, *Cancer Res.* **2008**, *68*, 2358.
[197] L. S. Green, C. Bell, N. Janjic, *Biotechniques* **2001**, *30*, 1094.
[198] J. Floege, T. Ostendorf, U. Janssen, M. Burg, H. H. Radeke, C. Vargeese, S. C. Gill, L. S. Green, N. Janjic, *Am. J. Pathol.* **1999**, *154*, 169.
[199] J. S. Hartig, S. H. Najafi-Shoushtari, I. Grune, A. Yan, A. D. Ellington, M. Famulok, *Nat. Biotechnol.* **2002**, *20*, 717.
[200] J. S. Hartig, M. Famulok, *Angew. Chem.* **2002**, *114*, 4440; *Angew. Chem. Int. Ed.* **2002**, *41*, 4263.
[201] G. A. Soukup, R. R. Breaker, *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 3584.
[202] A. Jenne, J. S. Hartig, N. Piganeau, A. Tauer, D. A. Samarsky, M. R. Green, J. Davies, M. Famulok, *Nat. Biotechnol.* **2001**, *19*, 56.
[203] S. Yamazaki, L. Tan, G. Mayer, J. S. Hartig, J. N. Song, S. Reuter, T. Restle, S. D. Laufer, D. Grohmann, H. G. Krausslich, J. Bajorath, M. Famulok, *Chem. Biol.* **2007**, *14*, 804.
[204] M. Wieland, J. S. Hartig, *Angew. Chem.* **2008**, *120*, 2643; *Angew. Chem. Int. Ed.* **2008**, *47*, 2604.
[205] M. Wieland, J. S. Hartig, *ChemBioChem* **2008**, *9*, 1873.
[206] B. Suess, J. E. Weigand, *RNA Biol.* **2008**, *5*, 24.
[207] T. S. Bayer, C. D. Smolke, *Nat. Biotechnol.* **2005**, *23*, 337.
[208] M. Hafner, E. Vianini, B. Albertoni, L. Marchetti, I. Grune, C. Gloeckner, M. Famulok, *Nat. Protoc.* **2008**, *3*, 579.
[209] G. Mayer, M. Famulok, *ChemBioChem* **2006**, *7*, 602.
[210] M. Hafner, A. Schmitz, I. Grune, S. G. Srivatsan, B. Paul, W. Kolanus, T. Quast, E. Kremmer, I. Bauer, M. Famulok, *Nature* **2006**, *444*, 941.
[211] B. Fuss, T. Becker, I. Zinke, M. Hoch, *Nature* **2006**, *444*, 945.
[212] N. H. Elowe, R. Nutiu, A. Allali-Hassani, J. D. Cechetto, D. W. Hughes, Y. Li, E. D. Brown, *Angew. Chem.* **2006**, *118*, 5776; *Angew. Chem. Int. Ed.* **2006**, *45*, 5648.
[213] J. Srinivasan, S. T. Cload, N. Hamaguchi, J. Kurz, S. Keene, M. Kurz, R. M. Boomer, J. Blanchard, D. Epstein, C. Wilson, J. L. Diener, *Chem. Biol.* **2004**, *11*, 499.
[214] P. Porschewski, M. A. Grattinger, K. Klenzke, A. Erpenbach, M. R. Blind, F. Schafer, *J. Biomol. Screening* **2006**, *11*, 773.
[215] G. Mayer, A. Jenne, *BioDrugs* **2004**, *18*, 351.
[216] W. G. Purschke, D. Eulberg, K. Buchner, S. Vonhoff, S. Klussmann, *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 5173.
[217] A. Vater, F. Jarosch, K. Buchner, S. Klussmann, *Nucleic Acids Res.* **2003**, *31*, 130e.
[218] S. M. Nimjee, C. P. Rusconi, R. A. Harrington, B. A. Sullenger, *Trends Cardiovasc. Med.* **2005**, *15*, 41.
[219] A. DeAnda, Jr., S. E. Coutre, M. R. Moon, C. M. Vial, L. C. Griffin, V. S. Law, M. Komeda, L. L. Leung, D. C. Miller, *Ann. Thorac. Surg.* **1994**, *58*, 344.
[220] A. Joachimi, G. Mayer, J. S. Hartig, *J. Am. Chem. Soc.* **2007**, *129*, 3036.
[221] Archemix, http://www.archemix.com/website/_popup_press_-release.php?release=17 **2005**, press release.
[222] J. Müller, B. Wulffen, B. Potzsch, G. Mayer, *ChemBioChem* **2007**, *8*, 2223.
[223] Y. Kim, Z. Cao, W. Tan, *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 5664.
[224] J. Müller, D. Freitag, B. Pötzsch, G. Mayer, *J. Thromb. Haemost.* **2008**, *6*, 2105.
[225] C. P. Rusconi, J. D. Roberts, G. A. Pitoc, S. M. Nimjee, R. R. White, G. Quick, Jr., E. Scardino, W. P. Fay, B. A. Sullenger, *Nat. Biotechnol.* **2004**, *22*, 1423.
[226] S. M. Nimjee, J. R. Keys, G. A. Pitoc, G. Quick, C. P. Rusconi, B. A. Sullenger, *Mol. Ther.* **2006**, *14*, 408.
[227] C. P. Rusconi, E. Scardino, J. Layzer, G. A. Pitoc, T. L. Ortel, D. Monroe, B. A. Sullenger, *Nature* **2002**, *419*, 90.
[228] C. K. Dyke, S. R. Steinhubl, N. S. Kleiman, R. O. Cannon, L. G. Aberle, M. Lin, S. K. Myles, C. Melloni, R. A. Harrington, J. H. Alexander, R. C. Becker, C. P. Rusconi, *Circulation* **2006**, *114*, 2490.
[229] M. Y. Chan, M. G. Cohen, C. K. Dyke, S. K. Myles, L. G. Aberle, M. Lin, J. Walder, S. R. Steinhubl, I. C. Gilchrist, N. S. Kleiman, D. A. Vorchheimer, N. Chronos, C. Melloni, J. H. Alexander, R. A. Harrington, R. M. Tonkens, R. C. Becker, C. P. Rusconi, *Circulation* **2008**, *117*, 2865.

[230] M. Y. Chan, C. P. Rusconi, J. H. Alexander, R. M. Tonkens, R. A. Harrington, R. C. Becker, *J. Thromb. Haemostasis* **2008**, *6*, 789.
[231] J. C. Gilbert, T. DeFeo-Fraulini, R. M. Hutabarat, C. J. Horvath, P. G. Merlino, H. N. Marsh, J. M. Healy, S. Boufakhreddine, T. V. Holohan, R. G. Schaub, *Circulation* **2007**, *116*, 2678.
[232] S. Oney, S. M. Nimjee, J. Layzer, N. Que-Gewirth, D. Ginsburg, R. C. Becker, G. Arepally, B. A. Sullenger, *Oligonucleotides* **2007**, *17*, 265.
[233] R. S. Apte, *Expert Opin. Pharmacother.* **2008**, *9*, 499.
[234] W. E. Smiddy, *Ophthalmology* **2007**, *114*, 847.
[235] S. S. Lynch, C. M. Cheng, *Ann. Pharmacother.* **2007**, *41*, 614.
[236] P. J. Rosenfeld, D. M. Brown, J. S. Heier, D. S. Boyer, P. K. Kaiser, C. Y. Chung, R. Y. Kim, *N. Engl. J. Med.* **2006**, *355*, 1419.
[237] J. O. McNamara, D. Kolonias, F. Pastor, R. S. Mittler, L. Chen, P. H. Giangrande, B. Sullenger, E. Gilboa, *J. Clin. Invest.* **2008**, *118*, 376.
[238] I. Melero, W. W. Shuford, S. A. Newby, A. Aruffo, J. A. Ledbetter, K. E. Hellstrom, R. S. Mittler, L. Chen, *Nat. Med.* **1997**, *3*, 682.
[239] C. M. Dollins, S. Nair, D. Boczkowski, J. Lee, J. M. Layzer, E. Gilboa, B. A. Sullenger, *Chem. Biol.* **2008**, *15*, 675.
[240] J. Wang, H. Takeuchi, Y. Sonobe, S. Jin, T. Mizuno, S. Miyakawa, M. Fujiwara, Y. Nakamura, T. Kato, H. Muramatsu, T. Muramatsu, A. Suzumura, *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 3915.
[241] A. C. Gavin, M. Bosche, R. Krause, P. Grandi, M. Marzioch, A. Bauer, J. Schultz, J. M. Rick, A. M. Michon, C. M. Cruciat, M. Remor, C. Hofert, M. Schelder, M. Brajenovic, H. Ruffner, A. Merino, K. Klein, M. Hudak, D. Dickson, T. Rudi, V. Gnau, A. Bauch, S. Bastuck, B. Huhse, C. Leutwein, M. A. Heurtier, R. R. Copley, A. Edelmann, E. Querfurth, V. Rybin, G. Drewes, M. Raida, T. Bouwmeester, P. Bork, B. Seraphin, B. Kuster, G. Neubauer, G. Superti-Furga, *Nature* **2002**, *415*, 141.
[242] A. C. Gavin, G. Superti-Furga, *Curr. Opin. Chem. Biol.* **2003**, *7*, 21.
[243] W. A. Weiss, S. S. Taylor, K. M. Shokat, *Nat. Chem. Biol.* **2007**, *3*, 739.
[244] M. Blind (NascaCell Technologies AG), personal communication.