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REVIEW ARTICLE

Aptamers against pathogenic microorganisms

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Abstract

An important current issue of modern molecular medicine and biotechnology is the search for new approaches to early diagnostic assays and adequate therapy of infectious diseases. One of the promising solutions to this problem might be a development of nucleic acid aptamers capable of interacting specifically with bacteria, protozoa, and viruses. Such aptamers can be used for the specific recognition of infectious agents as well as for blocking of their functions. The present review summarizes various modern SELEX techniques used in this field, and of several currently identified aptamers against viral particles and unicellular organisms, and their applications. The prospects of applying nucleic acid aptamers for the development of novel detection systems and antibacterial and antiviral drugs are discussed.

Abbreviations: CFU, colony forming units; DPBS, Dulbecco's phosphate-buffered saline; ELISA, enzyme-linked immunosorbent assay; ELONA, enzyme-linked oligonucleotide assay; FRET, fluorescent resonance energy transfer; HAU, hemagglutinin units; HIV, human immunodeficiency virus; HNA, hexitol nucleic acid; LNA, locked nucleic acid; LOD, limit of detection; NMR, nuclear magnetic resonance; PBMC, peripheral blood mononuclear cells; PCR, polymerase chain reaction; PFU, plague forming units; pRNA, packaging RNA; RISC, RNA-induced silencing complex; RT-PCR, real-time PCR; SARS, severe acute respiratory syndrome; SELEX, systematic evolution of ligands by exponential enrichment; SERS, surface-enhanced Raman spectroscopy; siRNA, small interfering RNA; SPR, surface plasmon resonance; TAR, *trans*-activation response element.

Keywords

Antiviral and antimicrobial agents, aptasensors, nucleic acid aptamers, pathogen detection, SELEX

History

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Introduction

Infectious diseases that result from the invasion of viral, bacterial or protozoan agents into a human organism are the major cause of human pathogenesis and mortality, surpassing cardiovascular diseases and cancer in both industrialized and developing countries. Infectious diseases are highly contagious and can also be easily transmitted through food and water. Taken together with an issue of hospital-acquired infections that can hardly be cured using standard therapeutics, this fact dictates a necessity to search for new approaches of fast and reliable diagnostics and treatment. Nowadays, the following methods of diagnostics of infectious diseases are broadly used (Kaittanis et al., 2010; Shinde et al., 2012).

Microbiological method (*isolation, growth, and microscopy of pathogens from clinical samples*). This method, although highly specific, has several limitations. First, the method requires at least 24 h incubation of the pathogen culture. Second, some microorganisms cannot easily grow in culture (for example, *Mycobacterium tuberculosis*). The application of this method for the identification of viruses is even more restricted due to the small size of viral particles which cannot be studied by conventional optical microscopy and requires the use of electron microscopy for visualization.

PCR. PCR detection is based on the identification of DNA/RNA fragments corresponding to a pathogen's genome. This diagnostic method is the most sensitive and specific and can be applied for the detection of a wide spectrum of pathogens. Despite these advantages, PCR detection requires undamaged microbial DNA/RNA and is unable to discriminate between alive and dead organisms. In general, PCR diagnostics is a highly complex technique that requires appropriate instruments, specialized reagents, and experienced personnel.

Immunological methods. These are widely used methods based on an application of specific antibodies targeting proteins or carbohydrate moieties unique to the pathogen. Such methods include agglutination test, ELISA, and Western blot analysis. Immunoassays are very sensitive but require special conditions for the storage and handling of antibodies to prevent their denaturation.

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Thus, the development of fast, cost-effective, and reliable pathogen detection methods remains a necessity. Another pressing challenge is a design of therapeutic agents for the treatment of infectious diseases. Despite the existence of a large variety of antibacterial and antiviral drugs, the problems of side effects and drug resistance are still unsolved (see, for example, rev. (Guidry et al., 2014; Llor & Bjerrum, 2014). During the last decade, RNA and DNA aptamers targeting microorganisms and viruses have attracted increasing attention. The aptamers are DNA or RNA oligonucleotides capable of specific interaction with microbial targets due to the formation of a unique 3D-structure. The specificity and affinity of aptamers is close to that of monoclonal antibodies; however, the aptamers possess a number of advantages. An aptamer could be created against almost any target (even toxic or non-immunogenic), it is a relatively stable compound that can be synthesized via the standard oligonucleotide chemical synthesis procedures, with different chemical modifications if necessary. RNA and DNA aptamers that are able to bind specifically to the surface determinants of microorganisms and viruses could be used as recognizing elements for new diagnostic systems. Such characteristics of aptamers as thermal and chemical stability, lower cost, and less batch-to-batch variation make aptamer-based approaches a promising alternative to immunological methods. Moreover, high affinity and selectivity allow aptamers to discriminate very close-related targets, for example, different protein isoforms (Conrad & Ellington, 1996). In some cases, aptamers can also block the functions of target proteins and thereby could serve as a basis for the development of novel therapeutics for the treatment of infections.

Whereas several problem-oriented reviews discuss antibacterial or antiviral aptamers and their applications (Gedi & Kim, 2014; Ozalp et al., 2013; Shum et al., 2013; Zimbres et al., 2013), this review tends to systemize recent data concerning aptamers selected against viruses, bacteria, and unicellular parasites and their potential therapeutic and diagnostic applications.

General SELEX scheme

Aptamers are obtained from combinatorial libraries of nucleic acids by selection *in vitro*. As a rule, the library is represented by a pool of oligonucleotides containing the randomized region (20–60 nt) flanked by two constant regions necessary for primer binding and PCR amplification. Single-stranded DNA libraries are obtained by standard methods of oligonucleotide synthesis, using a mixture of all four monomers for the synthesis of randomized region. To synthesize a single-stranded RNA library, a promoter sequence for T7 RNA polymerase is introduced into the 5′-end region of ssDNA library, followed by its conversion into dsDNA and subsequent transcription *in vitro*.

A general SELEX scheme includes three major stages: incubation of the library with the target, separation of aptamer-target complexes from unbound oligonucleotides, and the amplification of bound molecules (Figure 1A). RNA SELEX includes additional steps for *in vitro* transcription to obtain a RNA library, and the reverse transcription of bound RNA molecules to obtain cDNA and its subsequent amplification (Figure 1B).

During the selection, the library is enriched by sequences possessing higher target affinity. Most of the time 5–15 SELEX rounds are needed to obtain aptamers, although in some cases much faster enrichment can be achieved. After the selection, the library is cloned and sequenced to determine the sequences of individual aptamers. The analysis of the structures of aptamers followed by a search for consensus regions allows the determination of the minimal size of the aptamers necessary for target binding, and the affinity of aptamers to their targets.

At present, both DNA and RNA libraries are widely used for aptamer SELEX. RNA aptamers could potentially form much larger variety of secondary structures then DNA aptamers. However, RNA aptamers are more sensitive to cellular nucleases; thus, the introduction of protective

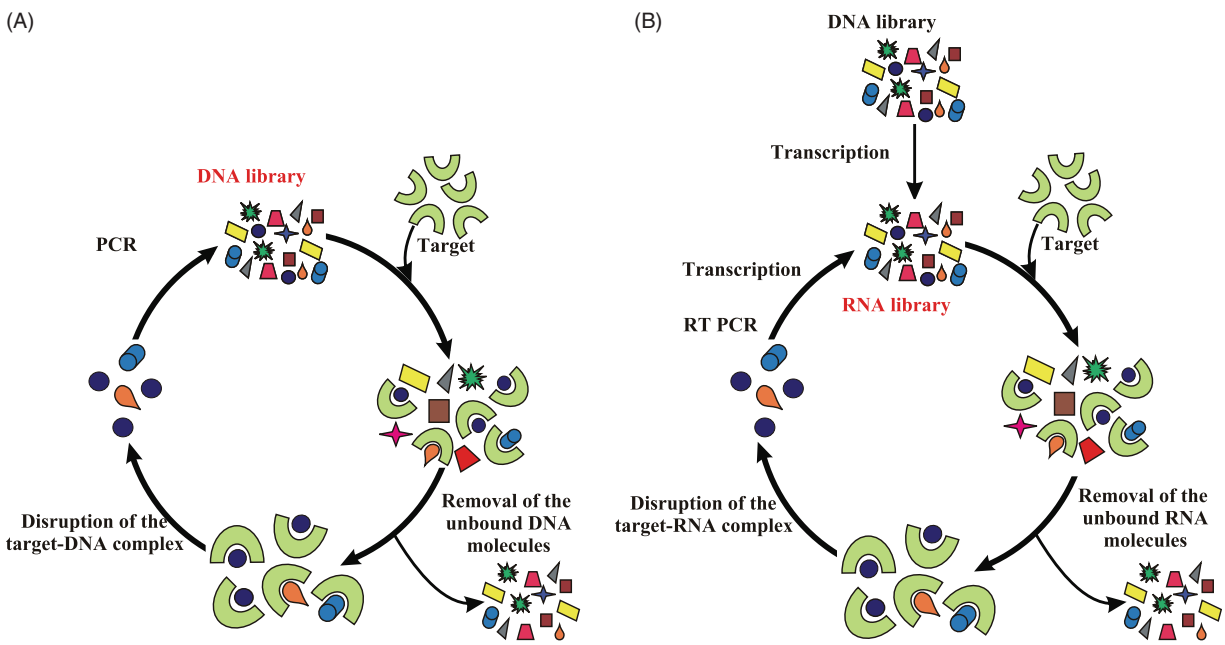

Figure 1. A general schemes of SELEX method for DNA (A) and RNA libraries (B).

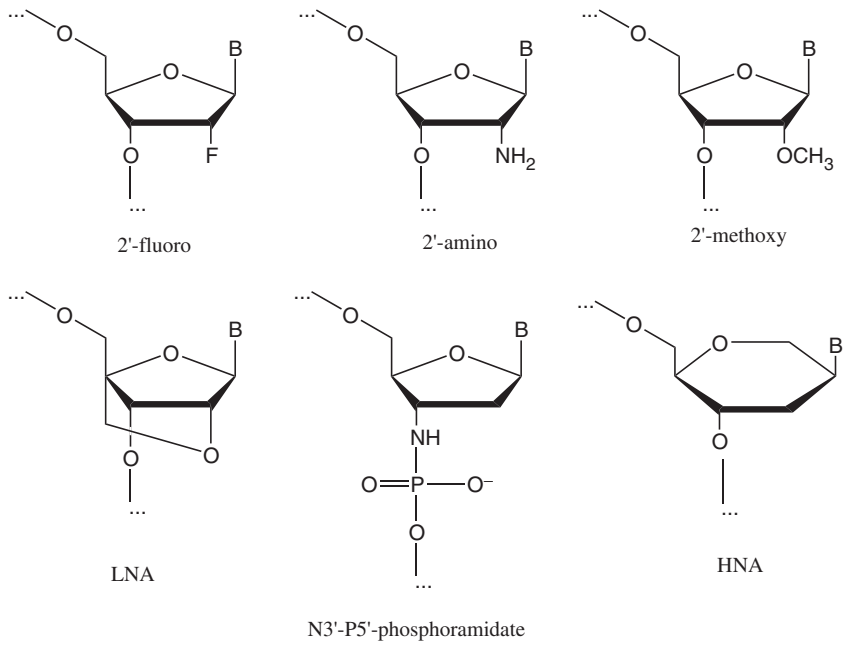

Figure 2. Chemical modifications commonly used for antiviral and antibacterial aptamers.

chemical modifications becomes necessary (see Keefe & Cload, 2008). DNA aptamers are more stable under a large range of conditions (Breaker, 1997), but their biological stability can also be improved. The introduction of different substituents at the 2′ position of ribose and modifications of phosphate/ribose backbone are the most commonly used chemical modifications of aptamers (Figure 2). 2′-Fluoro- and 2′-amino ribonucleoside substitutions could be introduced into initial libraries due to their compatibility with enzymatic reactions during SELEX protocol.

Antiviral aptamers

Human immunodeficiency virus

Human immunodeficiency virus is the causative agent of acquired immunodeficiency syndrome, which is responsible for 3 million deaths every year. Despite the development of the inhibitors of major virus enzymes and combined therapy, obtaining new antiretroviral drugs remains an urgent task because of high HIV variability. Reverse transcriptase of HIV-1 virus plays one of the key roles in viral replication, the synthesis of the DNA copy of the viral RNA genome before its integration into the DNA of the host cell. At present, a wide spectrum of aptamers has been obtained for effective reverse transcriptase binding and/or the inhibition of its activity *in vitro* (Burke et al., 1996; DeStefano & Cristofaro, 2006; Ditzler et al., 2011; Green et al., 1995; Held et al., 2006, 2007; Kensch et al., 2000; Kissel et al., 2007a, b; Lai & DeStefano, 2012; Li et al., 2008; Michalowski et al., 2008; Mosing et al., 2005; Tuerk et al., 1992; Whatley et al., 2013). Furthermore, several aptamers were able to inhibit the viral replication in cells (Chaloin et al., 2002; Joshi & Prasad, 2002; Joshi et al., 2005; Lange, 2012). The selected antiviral aptamers possess a large variety of secondary structures, including a subgroup of pseudoknot-forming aptamers first obtained by Gold and co-authors (Green et al., 1995; Tuerk et al., 1992). Such structural variability could probably be determined by the large amount of potential binding sites located at different functional domains of the enzyme. Aptamers binding to HIV-1 reverse transcriptase are also characterized by different selectivities. For example, RNA aptamers obtained by Li et al. (2008) were able to discriminate wild-type RT from its mutant form. On the contrary, broad-spectrum of DNA aptamers (Kissel et al., 2007b; Michalowski et al., 2008) can bind to the phylogenetically diverse HIV-1 reverse transcriptases.

HIV-1 entry process includes several stages: the surface glycoprotein gp120 binds to CD4 receptor on the host cell surface, and due to conformational changes enables an interaction with the alternative coreceptors, CCR5 or CXCR4. The viral and cellular membranes then fuse and the viral RNA enters the cell. Therefore, gp120 is a promising target for construction of virus-inhibiting aptamers. James and co-authors selected 2′-F-modified RNA aptamer B40 binding to the BaL viral strain (Khati et al., 2003), which were shown to inhibit different types of HIV-1 infection in the culture PBMC. A more detailed study of the aptamer B40 demonstrated that the viral infection is neutralized due to the blocking of conservative amino acids in the region of binding of gp120 with the N-terminus of the CCR5 co-receptor (Cohen et al., 2008; Dey et al., 2005a, b; Joubert et al., 2010). It was also shown that B40 aptamer at maximum concentration of 2 μM had no cytotoxic effect on cardiomyocytes and PBMC (Lopes de Campos et al., 2009). In order to enhance biological stability of the aptamer B40, a series of minimized B40 analogs containing different chemical modifications were generated (Cohen et al., 2008; Moore et al., 2011a; Mufhandu et al., 2012). The authors suggest that the combination of such modified aptamers with nuclease-inhibiting Zn^{2+} ions that are able to enhance antiviral effect of chemically modified aptamers could be applied as a basis for the antiviral drug for topical application. Besides the direct therapeutic application, the B40 aptamer was also

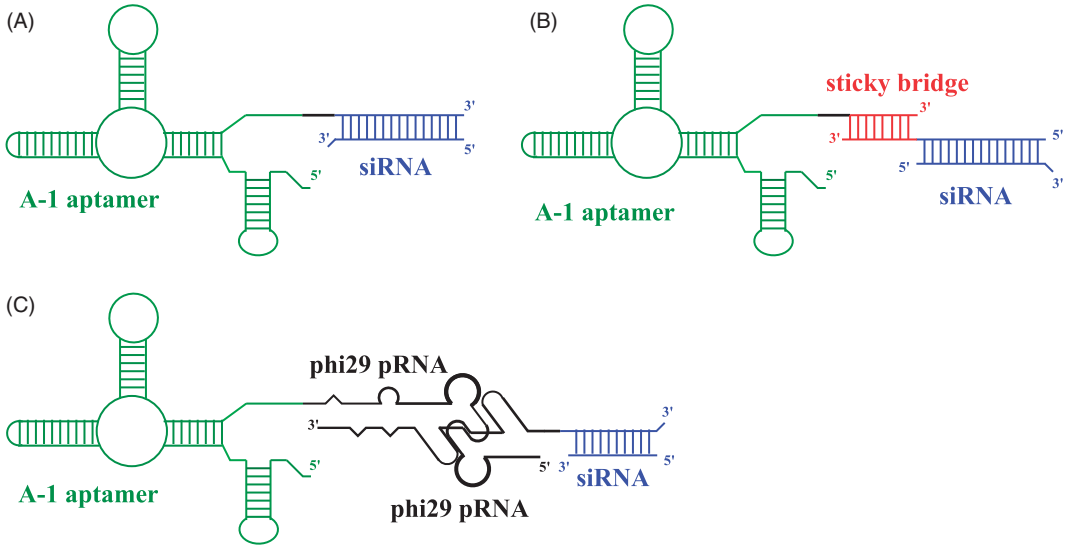

Figure 3. Schematic representation of aptamer-based siRNA delivery system (Zhou et al., 2009, 2011a). (A) Aptamer-siRNA chimera. (B) Aptamer-sticky bridge-siRNA chimera. (C) Aptamer-phi29 pRNA-siRNA chimera.

used as a delivery vehicle for siRNA targeting *tat/rev* HIV-1 exon into HIV-1-infected cells as a part of a chimeric molecule (Zhou et al., 2008).

An alternative selection of 2′-F-modified RNA aptamers against gp120 was made by Rossi and co-authors (Zhou et al., 2009). The obtained aptamers, which were different in the structure and binding sites from those described above, were applied in design of chimeric molecules containing siRNA targeting *tat/rev* exon of HIV-1 (Figure 3, panels A and B) (Zhou & Rossi, 2011; Zhou et al., 2009, 2011a). Aptamer A-1 provided the binding of chimeras with the surface of target cells and the subsequent cell internalization. When inside the cell, the siRNA part of the chimera was processed in the RISC complex and then inhibited HIV-1 replication and infectivity in cell cultures. The intravenous injections of the aptamer A-1 itself or A-1-containing chimeras to model humanized mice after 1-week led to significant inhibition of the viral infection (Neff et al., 2011; Zhou et al., 2013). Chimeras containing ‘‘sticky bridge’’ between aptamer and siRNA (Figure 3B) allow combining different aptamers and siRNA, modified or not modified. This strategy is more universal compared with the direct conjugation of aptamers with siRNA. The A-1 aptamer was also applied to design of a chimeric dimer made of the aptamers, siRNA and pRNA of the bacteriophage phi29 (Figure 3C) (Zhou et al., 2011). These constructs were shown to bind gp120-expressing cells and to inhibit the infection.

The viral integrase, an enzyme providing an integration of viral genome to the host cell genome, is another promising target for the selection of HIV-1 inhibiting aptamers. The DNA aptamer 93del, targeting viral reverse transcriptase and integrase *in vitro*, was obtained (Andreola et al., 2001; De Soultrait et al., 2002), and its ability to inhibit HIV-1 replication (50% of inhibition at 20 nM concentration) was demonstrated (De Soultrait et al., 2002). NMR studies revealed that this 16 nt aptamer forms a dimeric DNA quadruplex in the presence of K^+ ions (Phan et al., 2005). The aptamer was shown to internalize into human cells, and the efficiency of internalization was increased in the presence of the virus (Métifiot et al., 2007). The inhibiting activity of 93del is stipulated by its interaction with viral particles as well as by blocking of the early stages of intracellular viral replication (Faure-Perraud et al., 2011). Aptamer beacons based on 93del were designed for the visualization of viral proteins in living cells (Liang et al., 2011).

Another viral protein Tat (*trans*-activator protein) was also used as a ligand for RNA aptamers’ selection (Matsugami et al., 2003; Yamamoto et al., 2000b). These aptamers had higher affinity to the Tat protein than TAR (the natural target of Tat) and were utilized as the basis of fluorescent beacons (Yamamoto et al., 2000a) and biosensors of several types (Minunni et al., 2004; Ruslinda et al., 2013; Tombelli et al., 2005). RNA aptamers targeting the polyprotein gag (Ramalingam et al., 2011) and the nucleocapsid protein were also successfully acquired (Kim & Jeong, 2004; Kim et al., 2002).

The majority of anti-HIV aptamers were selected against viral proteins; however, viral RNA fragments could also be used as selection targets. Toulmé and co-authors obtained DNA and RNA aptamers against TAR RNA which is responsible for viral transcription (Boiziau et al., 1999; Collin et al., 2000; Ducongé & Toulmé, 1999; Ducongé et al., 2000; Sekkai et al., 2002). RNA aptamers bind tightly to the RNA target due to the formation of the so-called kissing complexes between the loops of the aptamer and the target RNA (Figure 4) (Collin et al., 2000; Ducongé et al., 2000; Lebars et al., 2007b); their specific spatial structure was confirmed by NMR and X-ray analysis and molecular dynamics simulation (Kolb et al., 2005; Lebars et al., 2007b, 2008; Van Melckebeke et al., 2008) (Figure 4).

A cassette coding $R06_{24}$ aptamer was constructed with a goal of inhibition of HIV infection in the cell culture (Kolb et al., 2006). This cassette showed nucleus penetration and consistent expression, resulting in the complete inhibition of HIV replication. Synthetic analogs of the $R06_{24}$ aptamer were obtained as well, bearing a number of chemical modifications to improve biological stability (Darfeuille et al., 2001, 2002a, 2004; Kolb et al., 2005; Lebars et al., 2007a). The analogs containing 2′-OMe nucleotides and N3′-P5′-phosphoramidate

internucleotide linkages possessed high affinity to the TAR fragment ($K_d = 1.5$ and 6.9 nM, correspondingly) and were able to inhibit the viral transcription *in vitro* (Darfeuille et al., 2002a, b). A modified analog of the $R06_{24}$ aptamer in which all the ribose residues replaced by hexitol residues (Figure 2) formed an unstable complex with TAR RNA, while partially modified hexitol-containing aptamer retained high affinity to RNA target. Partially hexitol-modified $R06_{24}$ did not compete with the Tat protein for TAR region binding, in contrast to N3′-P5′-phosphoramidate analogs (Kolb et al., 2005). The uniformly LNA-modified analog of the TAR binding aptamer had a low affinity to the TAR target, while the modified aptamers containing ribo-, deoxyribo-, and LNA-nucleotides possessed high binding affinity and inhibited the formation of a complex between TAR and the Tat protein *in vitro* (Darfeuille et al., 2004, 2006). Analysis of 64 variants of LNA/2′-O-Me modified aptamers had shown that the combination of these modifications results in higher binding affinity as compared with the unmodified RNA aptamer (Di Primo et al., 2007). To obtain genomic RNA aptamers that might reflect natural interactions of the TAR fragment with human RNA transcripts, a genomic SELEX was conducted, taking *in vitro* transcripts of full human genome as a starting library (Watrin et al., 2009). The hairpin-forming aptamers obtained during this process possessed high affinity to their targets (K_d from 4 to 297 nM). A more detailed study of one of the genomic aptamers, a1, showed that it has a stem-loop structure with a 6-nt loop (which is different from $R06_{24}$ aptamer having 8-nt loop) and binding to TAR RNA target is also provided by the formation of kissing complex. Analysis of the localization of the a1 sequence revealed that while its sequence correspond to a small area of the chromosome 16, this aptamer does not correspond to any known human RNA transcripts.

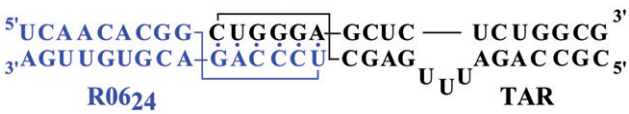

Figure 4. Schematic representation of kissing complex between loops of aptamer and TAR RNA (Kolb et al., 2005).

HIV-1 can also be inhibited by aptamers directed to non-viral targets. For example, human cyclin T1 (CycT1) together with cyclin-dependent kinase 9 (Cdk9) are the components of positive transcription elongation factor b (P-TEFb), which activates viral transcription after interaction with Tat protein. The RNA aptamer Apt4 against human cyclin T1 was shown to block the binding site of CycT1 with Cdk9 thus inhibiting HIV-1 transcription (Um et al., 2012).

The 2′-F-modified RNA aptamer binding to CD4 receptor on the cell surface (Davis et al., 1998) was applied for the inhibition of HIV infection as a part of a chimeric molecule containing siRNA targeting *gag*, *vif* or CCR5 genes (Wheeler et al., 2011). It was shown that these chimeras can directly inhibit the viral infection due to binding with CD4 and blocking cell penetration of viral particles. Moreover, aptamer-siRNA chimeras (Figure 5A) can specifically internalize into CD4+ cells and inhibit the expression of target genes in cultures of primary T-cells and macrophages, artificial tissue cultures, and model humanized mice. Local administration of chimeric constructs in a hydroxyethylcellulose gel resulted in the effective inhibition of infection together with the longer duration of the effect due to promotion of the retention of therapeutic agents at the administration area (Wheeler et al., 2013). It was supposed that such siRNA–aptamer chimeras could be applied for the design of locally acting drugs for the protection of HIV infection transmission.

Zhu et al. (2012) also used CD4 receptor-recognizing aptamers for the delivery of siRNAs targeting the HIV protease gene. It is worth noting that the DNA analog of the original RNA aptamer was applied in this case (Figure 5B). Aptamer-siRNA chimeras were shown to penetrate effectively only into CD4-positive cells and to inhibit target gene expression; in this case the DNA aptamer appeared to be a better delivery vehicle than the analogous RNA aptamer.

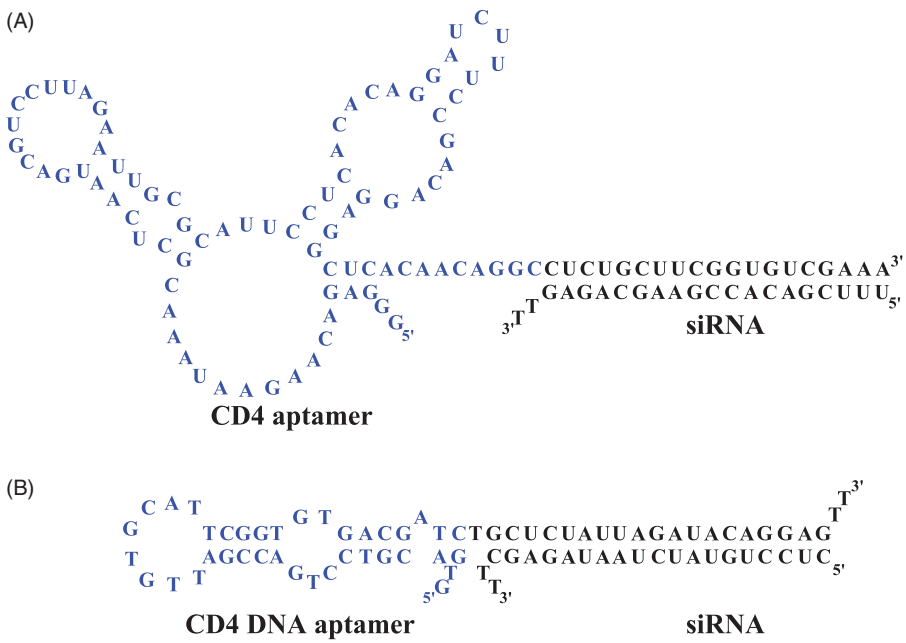

Figure 5. Aptamer-siRNA chimeras. (A) CD4-targeted RNA aptamer directly attached to siRNA (Wheeler et al., 2011). (B) DNA analog of CD4 aptamer attached to siRNA (Zhu et al., 2012).

Influenza viruses

Influenza viruses are RNA viruses of the Orthomyxoviridae family that can be divided into three genera (A, B, and C); the subsequent virus subtype classification is based on the types of surface proteins: hemagglutinin (HA) and neuraminidase (NA). HA plays the key role in initiating the viral infection by binding to the sialic acid-containing receptors and thus mediating viral entry into the cell. Thereby, HA represents a promising target for the design of anti-influenza drugs. DNA aptamers obtained by Jeon et al. (2004) were able to bind to a peptide corresponding with the highly conservative region of HA, common to all influenza A viruses of H3 strain. These aptamers successfully inhibited the HA invasion into the cells and blocked the viral infection in both tissue cultures and animal models. It was shown that the antiviral effect of the aptamers is caused by their interaction with the site of HA responsible for cell receptor binding.

Entire HA molecule of influenza B virus was used as a ligand (Gopinath et al., 2006b) for the selection of RNA aptamers. The obtained aptamers blocked the invasion of the virus into the cells and were able to discriminate between several different types of HA specific for influenza A and influenza B viruses. The same group also obtained an RNA aptamer that could distinguish between different strains of the same H3N2 subtype of influenza A virus. Based on such promising results, this aptamer was suggested as a prospective tool for the genotyping of influenza viruses (Gopinath et al., 2006a).

Avian influenza virus is now of a special interest as an aptamer SELEX ligand. Such aptamers could be applied for both the diagnostic and the therapy of the viral infection. To create potential antiviral drugs, DNA aptamers against the globular H9 HA domain (subtype H9N2) (Choi et al., 2011) and DNA and RNA aptamers against the receptor binding region HA1 of HA (subtype H5N1) (Cheng et al., 2008; Kwon et al., 2014; Park et al., 2011) were selected. All these aptamers possessed antiviral properties and significantly inhibited the viral infection in cell cultures. The development of methods for the rapid detection and identification of avian influenza virus is essential for blocking the expansion of viral infection and the prevention of future outbreaks. A novel detection system, based on Raman spectroscopy, was developed using a DNA aptamer specific to nucleoprotein components of the commercially available vaccine FluarixtInflusplit SSWs 2009/2010 as a recognizing element (Negri et al., 2011, 2012). This system allowed the detection of different influenza strains in biological samples. An alternative detection approach based on the SPR detection was proposed (Bai et al., 2012). In this case, a recognizing element was represented by the DNA aptamer obtained by SELEX against HA1 protein and the entire viral particle (subtype H5N1) (Wang et al., 2013). The resulting aptasensor provided a reliable detection of the avian influenza virus in pure cultures and biological samples in the range of 0.128–1.28 HAU. The specificity of the aptasensor was confirmed by lack of detection of other virus subtypes (H1N1, H2N2, H5N2, H7N2, and H9N2). It is worth noting that the total detection time was only 1.5 h, which is comparable with direct antigen detection by a chromatographic immunoassay. This aptasensor could be considered a prospective convenient tool for avian influenza detection in field.

Hepatitis viruses

Hepatitis C virus belongs to Flaviviridae family; about 3% of the world population carries this disease. Around 80% of infected patients develop hepatic cirrhosis, and in some cases, hepatocarcinoma. Nowadays, the use of interferon itself or in combination with ribavirin is the most widely used therapy; however, it is expensive and has a high probability of adverse effects. To overcome this problem, a new type of drug was recently developed: direct-acting antiviral agents that target different stages of virus development and replication, such as polymerase and protease inhibitors (Ansaldi et al., 2014). However, mutant viruses resistant to these drugs have already emerged; therefore, the search for new therapeutic agents still remains an essential task. RNA aptamers targeting the viral RNA-dependent RNA polymerase were obtained by Biroccio et al. (2002) and their ability to inhibit the enzyme activity was demonstrated, however, without further development. The non-structural NS2 protein, which participates in the initiation of viral RNA replication, was used as a target for DNA SELEX (Gao et al., 2014). It was shown that in virus-infected cell culture, aptamer NS2-3 binds to the NS2 protein and blocks the binding of viral proteins with host cell proteins necessary for viral RNA replication, thus inhibiting the formation of viral particles.

Chen et al. (2009) have selected DNA aptamers against the viral envelope E2 protein expressed on the cell surface, using the same cell line with non-expressing E2 protein as a counter-selection target. As a result, a series of aptamers was obtained; among them, the ZE2 aptamer demonstrated the highest target binding affinity ($K_d = 1$ nM). At the concentration of 100 nM, this aptamer was able to bind viral particles and inhibit their fusion with cells. These results prompted the authors to consider the ZE2 aptamer as a prospective diagnostic and therapeutic agent, as well as a tool for the investigation of virus-cell interactions.

Other viral proteins can also be used as SELEX targets. For example, Nishikawa's group obtained bifunctional RNA aptamers that can bind both the helicase and the protease of hepatitis C virus and inhibit their activity (Fukuda et al., 2004; Kumar et al., 1997; Nishikawa et al., 2003, 2004; Sekiya et al., 2003). Two aptamers recognizing these viral enzymes were obtained separately and then connected by a polynucleotide linker (Umehara et al., 2004, 2005) into a single construct. These two bifunctional aptamers NEO-35-s41 and G925-s50 (Figure 6) inhibited the protease activity of the NS3 viral protein in HeLa cell culture; *in vitro* inhibition of the viral genome replication system by these aptamers was also demonstrated.

Aptamers selected against different proteins of hepatitis C virus were used as recognizing elements for the design of new systems for virus detection. Viral helicase binding aptamer was applied as a receptor molecule for oscillating microcantilever in the detection of ultra-low protein target concentrations (0.1–100 ng/ml) (Hwang et al., 2007); another aptamer targeting the core antigen was applied for the construction of

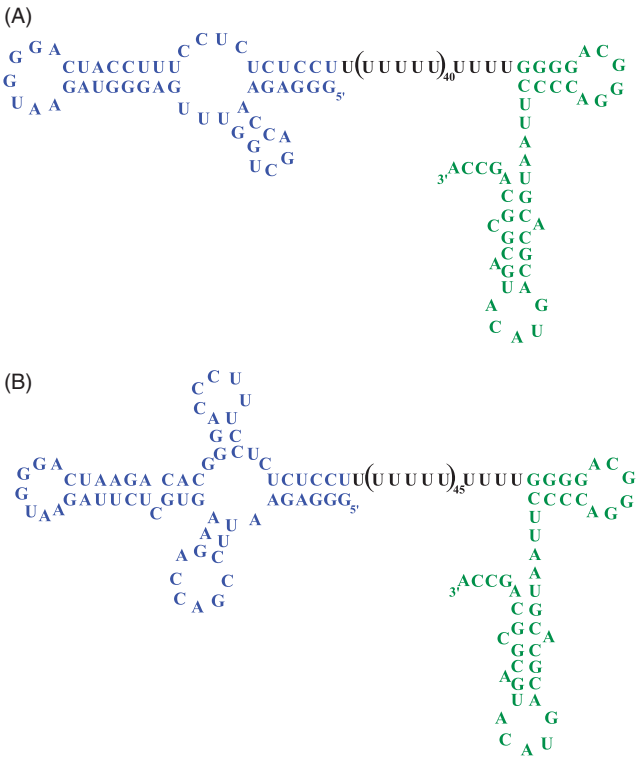

Figure 6. Bifunctional aptamers against helicase and protease of hepatitis C virus (Umehara et al., 2005). (A) NEO-35-s41 aptamer. (B) G925-s50 aptamer. Bifunctional aptamers consisted of anti-protease aptamer at the 5′-end and anti-helicase aptamer at the 3′-end connected by oligoU spacer.

sol–gel-based chips for the core antigen detection in infected patients' serum (Lee et al., 2007).

Hepatitis B virus belongs to the Hepadnaviridae family, and also represents a prospective target for antiviral aptamers. To date, RNA aptamers targeting the P protein of human hepatitis B virus (Feng et al., 2011) and the related duck hepatitis B virus have been obtained (Hu et al., 2004).

Aptamers capable of binding virus-infected eukaryotic cells are of special interest. For example, Liang et al. (2012, 2013) selected DNA aptamers against baby hamster kidney (BHK) cells infected by the rabies virus. These aptamers were able to inhibit the infection in the early stages, and intracranial inoculation of the aptamer FO24 to mice 24 h prior to the inoculation of the lethal dose of the virus provided a survival rate of almost 90%. Laboratory mice intramuscularly infected by the field isolate of rabies virus (more pathogenic than laboratory strains) demonstrated a 30% survival rate when the aptamer was intramuscularly inoculated 48 h prior to infection or simultaneously with virus inoculation. Viability of 50% was observed in the case of intracranial inoculation of the aptamer before or after inoculation with the virus (Liang et al., 2014a). The same group of authors also obtained DNA aptamers that are capable of recognizing BHK cells expressing G protein of the rabies virus on the cell surface, and demonstrated the ability of aptamers to inhibit the viral infection in cell cultures (Liang et al., 2014b). A preventive inoculation of the aptamer GE54 48 h prior to the lethal challenge of the virus increased the survival rate up to 33%, but inoculation of the aptamer after the virus inoculation resulted in only 16% survival. Therefore, aptamer FO24 could protect mice from a lethal dose of rabies virus. The best group had an 87% survival rate, which was much better than for the GE54 aptamer. The authors suggested that aptamer FO24 selected against infected cells could have more common cell surface targets compared with the GE54 aptamer G protein target.

DNA aptamers recognizing A549 cells (human lung cancer cells) infected by vaccinia virus were obtained by Tang et al. (2009). These aptamers were able to bind not only SELEX target cells but also other cell lines infected by the same virus, and demonstrated no affinity to non-infected cells. The same group of authors obtained DNA aptamers against vaccinia virus-infected HeLa cells and discovered their molecular target, which turned out to be hemagglutinin (Parekh et al., 2010). DNA aptamers selected against vaccinia viral particles were able to inhibit the infection *in vitro* (Nitsche et al., 2007). Unfortunately, these aptamers could not recognize vaccinia virus-infected cells. Aptamers selected against the vaccinia virus were applied for the development of an impedimetric aptasensor (Labib et al., 2012c). This biosensor can successfully detect viable vaccinia virus particles (down to 60 PFU in a microliter of DPBS) and distinguish them from nonviable viruses in a label-free electrochemical assay format.

Aptamers as prospective therapeutic agents were obtained for treatment of human cytomegalovirus (Wang et al., 2000), herpes simplex virus (Gopinath et al., 2012; Moore et al., 2011b), SARS coronavirus (Jang et al., 2008), hemorrhagic septicemia virus (Punnarak et al., 2012), and viral protein 35 of Ebola virus (Binning et al., 2013). A possibility for the development of a new aptamer-based diagnostic systems was demonstrated by aptamers targeting SARS coronavirus (Ahn et al., 2009), Rift Valley fever virus (Ellenbecker et al., 2012), and foot-and-mouth disease virus (Bruno et al., 2008a).

A summary of a wide range of antiviral aptamers that could be used either as alternative therapeutic agents or as recognizing elements of biosensors is presented in Table 1. It is worth noting that some of the anti-HIV-1 aptamer-based therapeutics have already been tested on animals and could soon be used for the treatment of this wide-spread disease.

Antibacterial aptamers

Salmonella

Salmonella enterica is a dangerous pathogen that can be transmitted through contaminated food and water. Certain components of the bacteria as well as whole bacterial cells can be used as ligands for aptamer selection. The RNA aptamer S-PS$_{8.4}$, which specifically binds type IVB pili of *S. enterica* serovar Typhi, was acquired using a pilin structural protein as a selection target (Pan et al., 2005). It was shown that the S-PS$_{8.4}$ aptamer forms a tight complex with the target protein ($K_d = 8.56$ nM). Just 2.0 µg of S-PS$_{8.4}$ provided 71% inhibition of the invasion of pil + *S.* Typhi into human monocytes (THP-1 suspended cell line) due to specific aptamer binding. It was proposed that this aptamer can potentially be applied as an alternative to antibiotic-based therapy. The aptamer S-PS$_{8.4}$ covalently immobilized on the surface of carboxylated single-walled carbon nanotubes was used as a recognizing element of a potentiometric biosensor

Table 1. Summary of antiviral and antimicrobial aptamers and their applications.

Target	Aptamer type	Application	Ref.
Human immunodeficiency virus			
Glycoprotein gp120	2′-F-RNA	Neutralization of HIV-1 infection in the culture of PBMC siRNA delivery into HIV-1 infected cells	Khati et al. (2003), Dey et al. (2005a, b), Cohen et al. (2008), Lopes de Campos et al. (2009), Joubert et al. (2010); Moore et al. (2011a), Mufhandu et al. (2012), and Zhou et al. (2008)
Glycoprotein gp120	2′-F-RNA	Inhibition of HIV-1 infection in cell cultures and model humanized mice by chimeric molecules containing siRNA targeting *tat/rev* exon of HIV-1	Zhou et al. (2009, 2011a, b, 2013), Zhou & Rossi (2011) and Neff et al. (2011)
Integrase and reverse transcriptase	DNA	Inhibition of HIV-1 in cell cultures	Andreola et al. (2001), de Soultrait et al. (2002), Phan et al. (2005), Métifiot et al. (2007), and Faure-Perraud et al. (2011)
Tat protein	RNA	Quartz crystal microbalance-based aptasensor LOD is 0.65 ppm in protein solution	Minunni et al. (2004)
Tat protein	RNA	SPR detection LOD is 0.25 ppm in protein solution	Tombelli et al. (2005)
TAR RNA	RNA	Inhibition of HIV replication by a cassette coding aptamer	Kolb et al. (2006)
CD4 receptor	2′-F-RNA	Inhibition of HIV infection by a chimeric molecules containing aptamer and siRNA targeting *gag*, *vif* or CCR5 genes	Wheeler et al. (2011, 2013)
CD4 receptor	DNA analog of RNA aptamer	Effective penetration into CD4-positive cells and inhibition of target gene expression by siRNA-aptamer chimeras	Zhu et al. (2012)
Influenza viruses			
Peptide corresponding to a HA region common to all influenza A viruses of H3 strain	DNA	Inhibition of HA invasion into the cells and blocking viral infection both in tissue cultures and in animal models	Jeon et al. (2004)
HA of influenza B virus	RNA	Blocking the invasion of virus into the cells	Gopinath et al. (2006a)
Globular HA domain (subtype H9N2)	DNA	Inhibition of viral infection in cell cultures	Choi et al. (2011)
Receptor binding region HA1 (subtype H5N1)	DNA	Decreasing of viral titer after 72 h of infection in a dose-dependent manner	Cheng et al. (2008)
Receptor binding region HA1 (subtype H5N1)	RNA	High suppression level of agglutination on chicken red blood cells	Park et al. (2011)
Receptor binding region (subtype H5N1)	RNA	Over 50% of the Madin–Darby canine kidney cells survived in the presence of the aptamers	Kwon et al. (2014)
Inactivated influenza vaccine FluarixtInflusplit SSWs 2009/2010	DNA	SERS detection LOD is 1 mg/mL HA in biological samples	Negri et al. (2011, 2012)
HA1 protein and the entire viral particles (subtype H5N1)	DNA	SPR detection LOD is 0.128–1.28 HAU in pure cultures and biological samples	Bai et al. (2012) and Wang et al. (2013)
Hepatitis viruses			
NS2 protein of hepatitis C virus	DNA	Blocking of the binding of viral protein with host cell proteins necessary for viral RNA replication, thus inhibiting the formation of viral particles	Gao et al. (2014)
Protein E2 expressed on the surface of cells infected by hepatitis C virus	DNA	Binding of viral particles and inhibition of their fusion with cells	Chen et al. (2009)
Helicase and protease of hepatitis C virus	RNA (bifunctional)	Inhibition of the protease activity of NS3 viral protein in HeLa cell culture	Umehara et al. (2004, 2005)
Helicase of hepatitis C virus	RNA	Oscillating microcantilever LOD is 0.1–100 ng/ml in protein solution	Hwang et al. (2007)
Core antigen of hepatitis C virus	2′-F-RNA	Sol–gel-based chips Detection in infected patients' serum	Lee et al. (2007)

Other viruses			
BHK cells infected by rabies virus	DNA	Inhibition of viral infection on model infected animals	Liang et al. (2012, 2013, 2014a)
Vaccinia viral particles	DNA	Inhibition of viral infection *in vitro*. Could not recognize vaccinia virus-infected cells.	Nitsche et al. (2007)
Live vaccinia virus	DNA	Impedimetric aptasensor. LOD is 60 PFU/μL in PBS.	Labib et al. (2012c)
N protein of SARS coronavirus	RNA	Nanoarray aptamer chip. LOD is 42 fM in protein solution.	Ahn et al. (2009)
Salmonella			
Pilin structural protein of *S. typhi*	RNA	Inhibition of the invasion of pil+ *S.* Typhi into human monocytes.	Pan et al. (2005)
		Potentiometric aptasensor based on single-walled carbon nanotubes. LOD is 0.2 CFU/ml in bacterial suspension.	Zelada-Guillén et al. (2009)
Mixture of OMPs of *S. typhimurium*	DNA	Aptamers, immobilized on magnetic beads, were applied for the capturing of the pathogen in biological samples with subsequent PCR detection. LOD is 10 CFU/g in biological samples.	Book et al. (2011), Joshi et al. (2009); Jyoti et al. (2011), and Singh et al. (2012)
		Electrochemical aptasensor. LOD is 3 CFU/ml in bacterial suspension.	Ma et al. (2014)
S. typhimurium whole live cells	DNA	Sandwich-type system with fluorescent detection. LOD is 25 CFU/ml in bacterial suspension.	Duan et al. (2013a)
S. typhimurium whole live cells	DNA	Impedimetric aptasensor. LOD is 600 CFU/ml in bacterial suspension.	Labib et al. (2012b) and Kolovskaya et al. (2013)
S. enteritidis whole live cells	DNA	Impedimetric biosensor. LOD is 600 CFU/ml in bacterial suspension.	Labib et al. (2012a)
		Lateral flow aptasensor. Detection in bacterial suspension and biological samples.	Fang et al. (2014)
S. typhimurium whole live cells	Biotin labeled DNA	Detection by quantitative PCR. LOD is 350–3500 CFU/ml in bacterial suspension	Dwivedi et al. (2013)
S. paratyphi A whole live cells	DNA	Optical detection system LOD is 10^4 CFU/ml in city water samples spiked with *S.* Paratyphi A	Yang et al. (2013)
Staphylococcus aureus			
Whole live cells	DNA	Light-scattering detection. Single cell detection in bacterial suspension.	Chang et al. (2013)
Whole live cells	DNA	Flow cytofluorometry in biological samples.	Cao et al. (2009)
		Potentiometric aptasensor based on carbon single-walled nanotubes. LOD is 800 CFU/ml in bacterial suspension and skin samples	Zelada-Guillén et al. (2012)
		Simultaneous dual-color fluorescent detection of *S. aureus* and *S. typhimurium* using aptamer-functionalized magnetic nanoparticles LOD is 8 CFU/ml in bacterial suspension	Duan et al. (2012a)
α-Toxin	DNA	Inhibition of α-toxin-mediated cell death in Jurkat T cells	Vivekananda et al. (2014)
Enterotoxin B	DNA	Specific enterotoxin B detection in toxin-reach culture medium after cultivation of four different strains of *S. aureus*	DeGrasse et al. (2012)

(*continued*)

Table 1. Continued

Target	Aptamer type	Application	Ref.
Escherichia coli			
OMPs of Crooks strain	DNA	FRET detection in bacterial suspension	Bruno et al. (2010)
Fimbriae protein of K88 strain	DNA	Sandwich-type fluorescent assay LOD is 1.1×10^3 CFU/ml in pure culture and 2.1×10^3 CFU/ml in biological samples	Peng et al. (2014)
DH5a strain whole live cells	2′-F-RNA	Field transistor based on single-walled carbon nanotubes LOD is 310 CFU/ml in bacterial suspension	So et al. (2008)
		Sandwich-type detection system LOD is 10 CFU/ml in suspension of two bacterial species	Lee et al. (2009)
		Potentiometric aptasensor LOD is 6 CFU/ml in milk and 26 CFU/ml in apple juice samples	Zelada-Guillén et al. (2010)
Mycobacterium tuberculosis			
Whole live cells	DNA	Decrease of the amount of mycobacteria in MTB-infected mice, alleviation of disease manifestations and prolongation of the survival rate	Chen et al. (2007, 2012, 2013)
MPT64 protein	DNA	Sandwich-type system Detection in clinical samples	Qin et al. (2009)
CFP-10.ESAT-6 protein heterodimer	DNA	ELONA assay in clinical sputum samples	Rotherham et al. (2012)
Unicellular parasites			
Tr. Brucei live parasites	RNA	Delivery of different cargo compounds into trypanosomes	Homann et al. (1999, 2001, 2006), Adler et al. (2008), and Göringer et al. (2006)
VSG surface proteins	2′-F-RNA	Potentiometric aptasensor based on single-walled carbon nanotubes LOD is 10 pM in blood samples containing VSG protein	Lorger et al. (2003) and Zelada-Guillén et al. (2013)
Tr. cruzi at trypomastigote stage	2′-F-RNA	Inhibition of parasite invasion into cells	Ulrich et al. (2002)
DBL1α protein corresponding to the semi-conservative N-terminal domain of PfEMP1 protein of *P. falciparum*	2′-F-RNA	Destruction of rosettes formed by *P. falciparum*	Barfod et al. (2009)
P. vivax PvLDH and PfLDH	DNA	Impedimetric aptasensor LOD is 110–120 fM in blood samples	Lee et al. (2012a, b)
		Colorimetric aptasensor LOD is 75–100 parasites/μl in blood samples	Jeon et al. (2013)

for bacteria detection in solution (Zelada-Guillén et al., 2009). This aptasensor allowed the detection of a single CFU of target *S. enterica* in an assay that was close to real-time. The authors suggested that this aptasensor could be used for a very rapid and reliable detection of pathogens in samples without any kind of pretreatment. The aptasensor was also shown to be specific, as no signal was detected from *Escherichia coli* or *Lactobacillus casei* containing samples.

To select DNA aptamers against *S. enterica* serovar Typhimurium, a mixture of outer membrane proteins (OMP) was used as the positive selection target (Joshi et al., 2009). The SELEX process included four rounds of positive selection preceded by the counter selection against *E. coli* OMP and lipopolysaccharides and three additional rounds of negative selection using a mixture of *E. coli* and *Salmonella* lipopolysaccharides. The obtained aptamers, 33 and 45, were able to bind not only the target proteins but also the outer membrane proteins isolated from seven (7) other serovars as well as the whole bacteria. These aptamers, immobilized on magnetic beads, were applied to capture the pathogen from biological samples with subsequent PCR detection; the detection limit of the system was 10 CFU/g of biological sample. Fluorescence correlation spectroscopy analysis of the binding affinity of aptamers 33 and 45 revealed that both aptamers form tight complexes with bacterial targets with sub-nanomolar K_d (0.1285 nM and 0.3772 nM, respectively), and the receptor density for these aptamers is about 40 receptors per μm^2 (Book et al., 2011). Therefore, both aptamers were considered to be suitable for the development of detection systems. Aptamer 33 was successfully applied for the concentration and detection of pathogenic bacteria in samples obtained from natural reservoirs (such as Ganges and Gomati rivers) (Jyoti et al., 2011; Singh et al., 2012) and also for the development of an electrochemical biosensor (Ma et al., 2014).

Several modified 2′-F-RNA aptamers against the outer membrane protein, OmpC, of *S.* Typhimurium were described in (Han & Lee, 2013). One aptamer, I-2, possessed high affinity to the target protein ($K_d = 20.27$ nM) and, in contrast to the other obtained aptamers, was able to bind intact *S. typhimurium* cells. Its binding selectivity was demonstrated in experiments with Gram-positive bacteria non-expressing OmpC and Gram-negative bacteria expressing this protein.

Whole living *S. typhimurium* cells were used as targets for DNA aptamer selection (Duan et al., 2013b). To achieve the maximal binding specificity for the third and fifth SELEX rounds (nine rounds overall), an additional negative selection stage was included, using a mixture of pathogenic bacteria *Listeria monocytogenes*, *E. coli*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Vibrio parahemolyticus*, and *Cronobacter sakazakii* as negative targets. The obtained ST2P aptamer was applied as a component of sandwich-type system for the detection of *S. typhimurium* in solution. For this purpose, ST2P aptamer was immobilized on the surface of magnetic nanobeads which were used for the concentration of targets from the solution, and then fluorescently labelled ST2P was added to the system with the subsequent detection of the fluorescent signal.

Whole bacterium cells were also used by Berezovski and co-workers for the selection of DNA aptamers against *S. enterica* serotype Typhimurium and *S. enterica* serotype Enteritidis (Kolovskaya et al., 2013; Labib et al., 2012b). A mixture of aptamers selected against *S. typhimurium* inhibited the growth of the bacteria on Petri dishes, and the STYP-3 aptamer with the highest binding affinity ($K_d = 25$ nM) was applied for the development of an impedimetric biosensor. The obtained aptasensor was able to detect DPBS down to 600 CFU/ml and was able to discriminate between different serovars of *S. enterica* as well as between alive and thermoinactivated bacteria. The shortened version of aptamer SE-3 binding to *S. enteritidis* was applied for the design of a lateral flow biosensor for pathogen detection in bacterial suspensions and biological samples (Fang et al., 2014).

For the use in detection systems, aptamers are generally conjugated with reporter groups, nanoparticles, or nanotubes; therefore, the introduction of additional functional groups into an aptamer is necessary. To obtain biotin-containing DNA aptamers against whole cells of *S. typhimurium*, a library of DNA molecules bearing the biotin residue as 5′-terminal modification was applied (Dwivedi et al., 2013). The binding affinity of biotin-containing aptamers S8-46 and S8-7 ($K_d = 0.74$ and $1.73\ \mu M$, respectively) was sufficient for use in bacterial detection by quantitative PCR, and the detection limit of the assay was found to be 3.5×10^2–3.5×10^3 CFU/ml of bacterial suspension.

Salmonella O8 (Liu et al., 2012), *S. enteritidis* (Hyeon et al., 2012; Labib et al., 2012a) and *S. enterica* serovar Paratyphi A (Yang et al., 2013) were also used as targets for aptamer selection. A DNA aptamer selected against the cells of *S. paratyphi* A was utilized as the basis for an optical detection system (Yang et al., 2013). The scheme of the detection is rather complex and includes several steps. First, a chimeric molecule made of DNA aptamer and peroxidase DNAzyme is non-covalently conjugated to the single-walled carbon nanotubes. After the addition of a bacterial culture to be analyzed, the aptamer binds to the target and dissociates from the surface of the nanotubes. Next, the hemin is added to the analysis mixture, which binds the DNAzyme molecule and induces the formation of the active structure of the DNAzyme. At the final stage, after the addition of the luminol, the latter reacts with hydrogen peroxide generating a chemiluminescent signal which is detected by a fluorescent spectrophotometry. The feasibility of this detection system was proven by the example of *Salmonella* detection in city water samples spiked with *S. paratyphi* A.

Staphylococcus aureus

Staphylococcus aureus is a pathogenic Gram-positive bacterium which can cause a wide spectrum of diseases from mild infections to life-threatening diseases such as pneumonia, endocarditis, septicemia, and toxic shock syndrome. To date, two studies have been published relating to the selection of DNA aptamers against *S. aureus*. Chang et al. (2013) used whole bacterial cells as SELEX targets, where every selection round included a negative selection stage against *S. epidermidis*. The 62 nt aptamers SA17 and SA61 obtained possessed high binding affinity and specificity as compared with a number of other bacteria such as *Bacillus subtilis*, *Citrobacter freundii*, *E. coli*, *Klebsiella pneumoniae*, *Listeria*

monocytogenes, *Moraxella catarrhalis*, *S. enterica*, *Shigella boydii*, *Shigella flexneri*, *Streptococcus bovis*, *S. pneumoniae*, *Staphylococcus saprophyticus*, and *Staphylococcus haemolyticus*; nevertheless, the binding, although at a much lower extent, was observed with *S. epidermidis* and *P. aeruginosa*. Interestingly, the immobilization of aptamers SA17 and SA61 on the surface of gold nanoparticles led to the increase in target binding affinity ($K_d = 35$ and 129 nM for free and 3.03 and 9.9 nM for surface-immobilized aptamers). It was assumed that such a change in affinity could be explained by avidity effect reflecting multiple aptamer-target interactions. Conjugates of the aptamers with gold nanoparticles were applied for the development of a detection system that would be sensitive enough to detect a single bacterial cell.

Five different 88 nt DNA aptamers were obtained by Cao et al. (2009) using whole cell SELEX procedure with counter-selection against *Streptococcus* (A5005) or *S. epidermidis* to reduce the amount of non-specific sequences. Each of the selected aptamers demonstrated high affinity and selectivity of binding of *S. aureus*; however, using a mixture of all five aptamers for the detection of bacterial targets in a number of biological samples was found to be even more effective. One of the aptamers obtained by Cao et al. was further used in the construction of a potentiometric aptasensor for *S. aureus* detection in skin (Zelada-Guillén et al., 2012), and in the design of a system for the simultaneous fluorescent detection of two pathogenic bacteria *S. aureus* and *S. typhimurium* (Duan et al., 2012b). Aptamers immobilized on the surface of magnetic beads were used for the pathogens capture from solution; aptamers conjugated with up-conversion nanoparticles $NaY_{0.28}F4$:Yb0.70, Er0.02 and NaY0.78F4:Yb0.2, Tm0.02 were applied for a specific detection of *S. aureus* and *S. typhimurium*.

Aptamers selected against bacterial toxins are of a particular interest. To develop potential drugs against staphylococcal infections, DNA aptamers capable of recognizing the α-toxin of *S. aureus* were selected (Vivekananda et al., 2014). Aptamers AT-27, AT-33, AT-36, and AT-49 significantly inhibited α-toxin-mediated cell death in Jurkat T cells. For example, in the presence of one of the aptamers, the cell survival rate increased from 50–60% to 85–90%. Semi-quantitative RT-PCR analysis revealed an inhibition of α-toxin-mediated transcription of the cytokine genes *NTF-α* and *IL-17*. Enterotoxin B of *S. aureus* was also used as a ligand for the selection (DeGrasse, 2012). The resulting aptamer APT^{SEB1} possessed a selectivity sufficient for a specific detection of enterotoxin B in the mixture of several homologous staphylococcal enterotoxins as well as in toxin-reach culture medium after the cultivation of four different strains of *S. aureus*. It was suggested that this aptamer can be applied in the development of pathogen detection systems in biological samples of different nature.

Escherichia coli

Escherichia coli is one of the most common and normally harmless bacteria found in the intestinal tract of warm blooded animals. However, some virulent strains of *E. coli* are dangerous food-borne pathogens. For instance, *E. coli* O157:H7 strain can cause a wide range of diseases such as hemorrhagic and non-hemorrhagic diarrhea, occasional kidney failure or hemolytic uremic syndrome. In order to obtain new aptamers against *E. coli*, both whole bacteria and bacterial components could be used as SELEX targets.

A DNA aptamer against lipopolysaccharide of *E. coli* O111:B4 was selected (Bruno et al., 2008b) and applied as a basis for a conjugate with human C1qrs protein which activates the complement system responsible for non-specific resistance to bacteria. The resultant conjugate demonstrated an antibacterial activity in the presence of complement proteins, and could be considered as a prospective antibacterial drug. Unfortunately, this approach has not been further developed so far. The same group of authors selected another DNA aptamer targeting outer membrane proteins of an *E. coli* Crooks strain for the specific FRET detection of bacteria (Bruno et al., 2010).

The DNA aptamer binding the fimbriae protein of enteropathogenic *E. coli* strain K88 was obtained by Li et al. (2011). Since that strain is the only one with fimbriae, the acquired aptamer could be applied for the specific pathogen detection. Enteropathogenic *E. coli* strain K88 was also used for cell-SELEX of a DNA aptamer (Peng, 2014). A sandwich-type fluorescent assay was developed for the detection of *E. coli* K88 in pure cultures as well as in spiked biological samples. An individual aptamer or the whole enriched library after 13 SELEX rounds were used as one of the components of this sandwich detection system; a higher signal intensity was demonstrated in the case of the aptamer library. The pathogen detection limit was 1.1×10^3 CFU/ml in pure culture and 2.1×10^3 CFU/ml in biological samples.

The 2′-F-RNA aptamer I-1 against *E. coli* O157:H7 strain was obtained using whole bacterial cells as a target (Lee et al., 2012a, b). It was found that aptamer I-1 can be truncated from 99 to 64 nt and retained the same properties, but further sequence minimization led to a decrease in binding affinity. The study of aptamers binding with different components of the bacterial membrane revealed that the particular target of the aptamer I-1 is a lipopolysaccharide that is unique for the pathogenic *E. coli* strain. Further experiments for using the aptamer for direct pathogen detection on different samples are planned.

So et al. (2008) obtained a 2′-F-RNA aptamer against DH5α *E. coli* cells, which was further applied as a bacteria-recognizing element. For this purpose, the aptamer was non-covalently immobilized on the surface of single-walled carbon nanotubes as part of the field transistor. The limit of detection was 310 CFU/ml; however, a significant difference in bacterial titer determination was observed between the newly proposed method and classic microbiological approach. This aptamer was also applied for the development of a sandwich-type detection system (Lee et al., 2009): first, bacterial cells were immobilized on magnetic beads coated by specific antibodies, then the aptamer was added, and after thermal denaturation of the target–aptamer complexes, the latter was detected by an RT-PCR method. The development of potentiometric biosensor based on aptamer covalently immobilized on single-walled carbon nanotubes was also described (Zelada-Guillén et al., 2010).

Mycobacterium tuberculosis

Tuberculosis caused by *M. tuberculosis* (MTB) still remains a widespread dangerous infection, in particular because of the existence of a large number of drug-resistant MTB strains. The development of conceptually new therapeutic agents would enable improvements in this field. Chen et al. (2007) selected DNA aptamers using whole living bacteria as SELEX target. A single injection of 0.8 μg of NK2 aptamer decreased the amount of mycobacteria in MTB-infected mice, alleviated disease manifestations and prolonged the survival rate. Further studies revealed that all the aptamers obtained (Chen et al., 2007) possess high binding affinity, with the NK2 aptamer being found to be the most effective binder ($K_d = 31$ nM) and being able to inhibit the invasion of MTB into macrophages (Chen et al., 2012, 2013). These aptamers are considered promising therapeutic agents and are also of interest for the development of molecular probes to study the mechanisms of bacterial invasion into cells.

Another approach for obtaining anti-tuberculosis aptamers was developed by Shum et al. (2011). The bacterial enzyme polyphosphate kinase 2 (PPK2) was used as a SELEX target. PPK2 is a polyphosphate-dependent nucleotide diphosphate kinase that plays an important role in the synthesis of mycolic acid and other surface polysaccharides critical for bacterial survival during active growth. The highest target binding affinity ($K_d = 870$ nM) was demonstrated for the quadruplex-forming aptamer G9 and its shortened versions. The aptamer G9 inhibited the activity of the enzyme to 50% at concentration of 39.3 nM. This aptamer could be a potential therapeutic agent as soon as an efficient system for its cell delivery can be developed.

Aptamers can also be applied as a basis for a new MTB detection system. For example, a sandwich-type system was developed for the detection of MPT64 antigen which is expressed during an active bacterial cell division (Qin et al., 2009). The aptamer M64CA selected in this work was immobilized on the surface of a polystyrol microplate for the capture of analyte molecules, and another aptamer, M64RA, was used as a reporter component. The system was tested on 77 samples of culture filtrates including *M. tuberculosis* and other Mycobacterium species and demonstrated high sensitivity and specificity of the assay. The authors noted that the constructed detection system needs to be improved due to the emergence of false-positive and false-negative results. DNA aptamers were also selected against another biomarker of tuberculosis, protein heterodimer CFP-10.ESAT-6. A detection assay *in vitro* using the obtained aptamers showed high sensitivity and specificity, but the detection in bacterial lysates revealed that aptamers also bind to *S. aureus*, *M. smegmatis*, *M. bovis* BCG and, in some cases, *P. aeruginosa*. Moreover, the aptamer-based detection of CFP-10.ESAT-6 in clinical samples demonstrated lower sensitivity and specificity when compared with the antibody-based assay (Rotherham et al., 2012).

Aptamers recognizing MTB proteins could also be applied to study their functions. For example, RNA aptamers were selected against the bacterial protein EsxG, the particular function of which is not currently clear (Ngubane et al., 2014). The obtained aptamers G47 and G48 possessed high target binding affinity ($K_d = 8.04$ nM and 78.85 nM, correspondingly); therefore, the authors suggested that they could be used as prospective molecular probes in the functional studies of EsxG as potential tuberculosis marker.

One of the problems of public health is the growing drug resistance of several microbial pathogens. Selection of antimicrobial aptamers against *S. enterica*, *S. aureus*, *M. tuberculosis*, etc. (Table 1) represents a new way to overcome this problem. Aptamer-based detection systems provide a possibility to detect pathogenic proteins or whole bacteria directly in a real complex matrix without preliminary concentration or purification of the microbial target. This direct detection allows assay time and cost to be decreased.

During the last several years, aptamers against different bacterial species have become the focus of growing interest. DNA aptamers that specifically bind to the surface of spores of anthrax *Bacillus anthracis* (Bruno & Kiel, 1999; Bruno & Carrillo, 2012) and crystal-forming bacteria *B. thuringiensis* (Ikanovic et al., 2007) have been developed. DNA aptamers against *Campylobacter jejuni* (Bruno et al., 2009; Dwivedi et al., 2010), *L. monocytogenes* (Ohk et al., 2010; Suh & Jaykus, 2013), *V. parahemolyticus* (Duan et al., 2012a), *Shigella dysenteriae* (Duan et al., 2013a), *Streptococcus pyogenes* (Hamula et al., 2011), *Francisella tularensis* (Vivekananda & Kiel, 2006), *Pseudomonas aeruginosa* (Wang et al., 2011), *Leishmania infantum* (Ramos et al., 2007), and *Lactobacillus acidophilus* (Hamula et al., 2008) have also been described. All these aptamers can potentially be used as a prospective therapeutic agents and diagnostic tools.

Unicellular parasites

Various diseases caused by unicellular parasites remain a major concern worldwide. Malaria causes the death of approximately 2.7 million people per year (Andreopoulos, 2003); Chagas disease and schistosomiasis affect millions of people in Asia, Africa and South America (Capron et al., 2002; Dias et al., 2002). Most of the known drugs for the parasites' infection treatment are not very effective and have some serious side effects.

Homann & Göringer (1999) obtained RNA aptamers targeting live *Trypanosoma brucei*, protozoan parasites which are causative agents of sleepy sickness. Two *Tr. brucei strains* were used as SELEX targets. An RNA library was incubated with parasites in the blood-stream stage; non-bound molecules were withdrawn by centrifugation. The resulting aptamer 2–16 was efficiently bound to the parasites of both strains in blood-stream stage ($K_d = 60$ nM) and had no affinity to *Tr. brucei* on the other developmental stages. Using photoaffinity modification and fluorescent spectroscopy with the fluorescently labeled aptamer 2–16, it was discovered that its target is the 24 kDa protein located in the flagellar pocket of trypanosome. After binding to this protein, the aptamer invades the trypanosome by receptor-mediated endocytosis, and becomes localized in the endosomes. With the example of the aptamer 2–16 biotin conjugate, the possibility of using this aptamer for cargo delivery into trypanosomes was demonstrated (Homann & Göringer, 2001). To increase the stability of aptamer 2–16 in biological media, pyrimidine nucleotides

were replaced by their 2′-amino or 2′-fluoro analogs. 2′-Aminomodification resulted in the complete loss of binding affinity, while 2′-fluoro-modified aptamers retained the ability to bind trypanosomes ($K_d = 70$ nM) and possessed high resistance to serum nucleases (Göringer et al., 2006). For the further improvement of biological stability, conjugates of aptamer 2–16 with polyethylene glycol of different molecular weight were synthesized. It was shown that conjugation with low-molecular polyethylene glycol had no significant effect on target binding, and the use of high molecular weight polyethylene glycol completely inactivated the aptamer (Adler et al., 2008). It turned out that a more successful strategy was the use of modified RNA libraries containing 2′-fluoro or 2′-amino pyrimidine nucleotides during SELEX. For the selection of 2′-amino RNA aptamers, living trypanosomes were used as targets. The obtained aptamer possessed nearly the same binding affinity as aptamer 2–16 ($K_d = 70$ nM) and bound trypanosomes in the constrained region around the flagellum (Homann et al., 2006). To obtain 2′-fluoro-containing RNA aptamers capable of binding the surface of trypanosomes of different species, the purified VSG surface proteins were used as the SELEX target (Göringer et al., 2003; Lorger et al., 2003). The aptamer cl57 (Lorger et al., 2003) was covalently immobilized onto the surface of single-walled carbon nanotubes and applied for the development of potentiometric sensor for the detection of clinically significant proteins in blood (Zelada-Guillén et al., 2013).

Modified 2′-fluoro RNA aptamers were also obtained against *Trypanosoma cruzi*, the causative agent of Shagas disease (Ulrich et al., 2002). At the stage of trypomastigote, *Tr. cruzi* binds to the host cells and enters them due to interactions with extracellular matrix proteins. The obtained 2′-fluoro RNA aptamers at 1 μM concentration demonstrated a 50–80% inhibition of parasite invasion into cells. There were also 2′-fluoro RNA aptamers selected for the detection of *Tr. cruzi* antigens in biological fluids (Nagarkatti et al., 2014). These aptamers were applied for the detection of antigens in the serum of infected mice, but the particular target of aptamers was not determined.

Aptamers capable of binding parasite proteins present on the host cell surface deserve a special attention. Barfod et al. (2009) obtained 2′-fluoro RNA aptamers against PfEMP1 protein which is expressed on the surface of erythrocytes infected by the malaria plasmodium *Plasmodium falciparum*. This protein promotes the conglutination of erythrocytes (rosettes forming) and the adhesion of infected erythrocytes to the walls of small blood vessels. The SELEX target was represented by the recombinant DBL1α protein corresponding to the semi-conservative N-terminal domain of PfEMP1 protein responsible for the formation of rosettes. The obtained aptamer at the concentration of 387 nM (12 μg/ml) caused almost complete destruction of rosettes, thus making it a potential anti-malaria drug. The production of aptamers capable of recognizing the malaria plasmodium is also interesting from the point of view of malaria diagnostics, in which it could be applied for direct detection of the pathogen in blood samples. For example, two independent research groups selected DNA aptamers binding the plasmodium lactate dehydrogenase (pLDH), which is a biomarker of malaria. The obtained DNA aptamers possessed different nucleotide sequences but similar elements of secondary structure (Cheung et al., 2013; Lee et al., 2012a,b). The binding affinities with protein targets were also within the same range ($K_d = 20$–50 nM). The minimized aptamer obtained by Lee et al. (2012a,b) was applied for the development of electrochemical and colorimetric biosensors for the detection of plasmodia in clinical samples (Jeon et al., 2013). In particular, the electrochemical biosensor on the basis of gold nanoparticles had the detection limit as low as 1 pM and was able to detect both *P. vivax* and *P. falciparum* at concentrations below 100 cells/ml. The authors suggest that a constructed aptasensor can be used for rapid and sensitive diagnostics of malaria.

Conclusions

Despite the remarkable progress in the diagnostics and treatment of infectious diseases during the last half-century, there are still a multitude of problems concerning the development of rapid cost-effective detection methods and that overcoming the side effects of therapeutic agents and pathogen drug resistance. The aptamers could provide a powerful tool for the development of both novel diagnostic methods and therapeutic agents capable of blocking the functions of pathogenic microorganisms. Nowadays, the SELEX technology includes a variety of techniques and is flexible and tunable enough to target any particular protein of interest or a whole bacterial cell or a viral particle. There are also a number of ways to increase the biological stability of aptamers by means of various chemical modifications. The possibilities of a successful SELEX methodology have been demonstrated in particular for the development of antiviral aptamers. Aptamers selected against viral surface proteins can be applied for the inhibition of virus–cell fusion as well as for the design of diagnostic systems. There are also examples of virus-inhibiting aptamers targeted to the key viral enzymes or to the host proteins necessary for the viral life cycle. It is worth noting that although the overwhelming majority of aptamers are selected against protein targets, aptamers were also obtained to bind HIV-1 TAR RNA, which is responsible for viral transcription. From the diagnostic point of view, it is interesting to mention the aptamers recognizing virus-infected eukaryotic cells. As for bacterial targets, most aptamers obtained against different human pathogens were applied for diagnostic purposes as a basis for specific and sensitive biosensors. To illustrate the potential of aptamers as candidate antibacterial agents, we should mention the DNA aptamer selected against whole, living *Mycobacterium tuberculosis* bacteria which was shown to decrease the amount of mycobacteria in MTB-infected mice and alleviate disease manifestations. Unicellular parasites can also be targeted by aptamers, although only a few examples are described up-to-date, including 2′-modified RNA aptamers against trypanosomes and against PfEMP1 protein which is expressed on the surface of erythrocytes infected by the malaria plasmodium. The latter aptamer was shown to prevent the agglutination of erythrocytes and has been also successfully used as a recognizing element for a very sensitive biosensor.

To summarize, the development of aptamer-based diagnostic tools resulted in the whole array of specific and sensitive biosensors for pathogen detection. There is also a number of encouraging examples of aptamers that can inhibit the infections (Table 1). All these data indicate that in the nearest future aptamer-based technology could become a real alternative to the traditional approaches for the diagnostics and therapy of infectious diseases.

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References

Adler A, Forster N, Homann M, Göringer HU. (2008). Post-SELEX chemical optimization of a trypanosome-specific RNA aptamer. Comb Chem High Throughput Screen 11:6–23.

Ahn DG, Jeon IJ, Kim JD, et al. (2009). RNA aptamer-based sensitive detection of SARS coronavirus nucleocapsid protein. Analyst 134: 1896–901.

Andreola ML, Pileur F, Calmels C, et al. (2001). DNA aptamers selected against the HIV-1 RNase H display *in vitro* antiviral activity. Biochemistry 40:10087–94.

Andreopoulos S. (2003). Developing drugs for parasitic diseases. Science 300:430–1.

Ansaldi F, Orsi A, Sticchi L, et al. (2014). Hepatitis C virus in the new era: perspectives in epidemiology, prevention, diagnostics and predictors of response to therapy. World J Gastroenterol 20:9633–52.

Bai H, Wang R, Hargis B, et al. (2012). A SPR aptasensor for detection of avian influenza virus H5N1. Sensors 12:12506–18.

Barfod A, Persson T, Lindh J. (2009). *In vitro* selection of RNA aptamers against a conserved region of the *Plasmodium falciparum* erythrocyte membrane protein 1. Parasitol Res 105:1557–66.

Binning JM, Wang T, Luthra P, et al. (2013). Development of RNA aptamers targeting Ebola virus VP35. Biochemistry 52:8406–19.

Biroccio A, Hamm J, Incitti I, et al. (2002). Selection of RNA aptamers that are specific and high-affinity ligands of the hepatitis C virus RNA-dependent RNA polymerase. J Virol 76:3688–96.

Boiziau C, Dausse E, Yurchenko L, Toulmé JJ. (1999). DNA aptamers selected against the HIV-1 *trans*-activation-responsive RNA element form RNA-DNA kissing complexes. J Biol Chem 274:12730–7.

Book B, Chen J, Irudayaraj J. (2011). Quantification of receptor targeting aptamer binding characteristics using single-molecule spectroscopy. Biotechnol Bioeng 108:1222–7.

Breaker RR. (1997). DNA aptamers and DNA enzymes. Curr Opin Chem Biol 1:26–31.

Bruno JG, Carrillo MP, Phillips T. (2008a). Development of DNA aptamers to a foot-and-mouth disease peptide for competitive FRET-based detection. J Biomol Tech 19:109–15.

Bruno JG, Carrillo MP, Phillips T. (2008b). *In vitro* antibacterial effects of antilipopolysaccharide DNA aptamer-C1qrs complexes. Folia Microbiol 53:295–302.

Bruno JG, Carrillo MP, Phillips T, Andrews CJ. (2010). A novel screening method for competitive FRET-aptamers applied to *E. coli* assay development. J Fluoresc 20:1211–23.

Bruno JG, Kiel JL. (1999). *In vitro* selection of DNA aptamers to anthrax spores with electrochemiluminescence detection. Biosens Bioelectron 14:457–64.

Bruno JG, Phillips T, Carrillo MP, Crowell R. (2009). Plastic-adherent DNA aptamer-magnetic bead and quantum dot sandwich assay for *Campylobacter* detection. J Fluoresc 19:427–35.

Bruno JG, Carrillo MP. (2012). Development of aptamer beacons for rapid presumptive detection of *Bacillus* spores. J Fluoresc 22: 915–24.

Burke DH, Scates L, Andrews K, Gold L. (1996). Bent pseudoknots and novel RNA inhibitors of type 1 human immunodeficiency virus (HIV-1) reverse transcriptase. J Mol Biol 264:650–66.

Cao X, Li S, Chen L, et al. (2009). Combining use of a panel of ssDNA aptamers in the detection of *Staphylococcus aureus*. Nucleic Acids Res 37:4621–8.

Capron A, Capron M, Riveau G. (2002). Vaccine development against schistosomiasis from concepts to clinical trials. Br Med Bull 62: 139–48.

Chaloin L, Lehmann MJ, Sczakiel G, Restle T. (2002). Endogenous expression of a high-affinity pseudoknot RNA aptamer suppresses replication of HIV-1. Nucleic Acids Res 30:4001–8.

Chang YC, Yang CY, Sun RL, et al. (2013). Rapid single cell detection of *Staphylococcus aureus* by aptamer-conjugated gold nanoparticles. Sci Rep 3:1863. doi: 10.1038/srep01863.

Chen F, Hu Y, Li D, et al. (2009). CS-SELEX generates high-affinity ssDNA aptamers as molecular probes for hepatitis C virus envelope glycoprotein E2. PloS One 4:e8142.

Chen F, Zhang X, Zhou J, et al. (2012). Aptamer inhibits *Mycobacterium tuberculosis* (H37Rv) invasion of macrophage. Mol Biol Rep 39: 2157–62.

Chen F, Zhou J, Huang YH, et al. (2013). Function of ssDNA aptamer and aptamer pool against *Mycobacterium tuberculosis* in a mouse model. Mol Med Rep 7:669–73.

Chen F, Zhou J, Luo F, et al. (2007). Aptamer from whole-bacterium SELEX as new therapeutic reagent against virulent *Mycobacterium tuberculosis*. Biochem Biophys Res Commun 357:743–8.

Cheng C, Dong J, Yao L, et al. (2008). Potent inhibition of human influenza H5N1 virus by oligonucleotides derived by SELEX. Biochem Biophys Res Commun 366:670–4.

Cheung YW, Kwok J, Law AW, et al. (2013). Structural basis for discriminatory recognition of *Plasmodium* lactate dehydrogenase by a DNA aptamer. Proc Natl Acad Sci USA 110:15967–72.

Choi SK, Lee C, Lee KS, et al. (2011). DNA aptamers against the receptor binding region of hemagglutinin prevent avian influenza viral infection. Mol Cells 32:527–33.

Cohen C, Forzan M, Sproat B, et al. (2008). An aptamer that neutralizes R5 strains of HIV-1 binds to core residues of gp120 in the CCR5 binding site. Virology 381:46–54.

Collin D, van Heijenoort C, Boiziau C, et al. (2000). NMR characterization of a kissing complex formed between the TAR RNA element of HIV-1 and a DNA aptamer. Nucleic Acids Res 28:3386–91.

Conrad R, Ellington AD. (1996). Detecting immobilized protein kinase C isozymes with RNA aptamers. Anal Biochem 242:261–5.

Darfeuille F, Arzumanov A, Gait MJ, et al. (2002a). 2′-O-Methyl-RNA hairpins generate loop–loop complexes and selectively inhibit HIV-1 Tat-mediated transcription. Biochemistry 41:12186–92.

Darfeuille F, Arzumanov A, Gryaznov S, et al. (2002b). Loop-loop interaction of HIV-1 TAR RNA with N3'–>P5′ deoxyphosphoramidate aptamers inhibits *in vitro* Tat-mediated transcription. Proc Natl Acad Sci USA 99:9709–14.

Darfeuille F, Cazenave C, Gryaznov S, et al. (2001). RNA and N3′–>P5′ kissing aptamers targeted to the trans-activation responsive (TAR) RNA of the human immunodeficiency virus-1. Nucleosides Nucleotides Nucleic Acids 20:441–9.

Darfeuille F, Hansen JB, Orum H, et al. (2004). LNA/DNA chimeric oligomers mimic RNA aptamers targeted to the TAR RNA element of HIV-1. Nucleic Acids Res 32:3101–7.

Darfeuille F, Reigadas S, Hansen JB, et al. (2006). Aptamers targeted to an RNA hairpin show improved specificity compared to that of complementary oligonucleotides. Biochemistry 45:12076–82.

Davis KA, Lin Y, Abrams B, Jayasena SD. (1998). Staining of cell surface human CD4 with 2′-F-pyrimidine-containing RNA aptamers for flow cytometry. Nucleic Acids Res 26:3915–24.

DeGrasse JA. (2012). A single-stranded DNA aptamer that selectively binds to *Staphylococcus aureus* enterotoxin B. PloS One 7:e33410.

DeStefano JJ, Cristofaro JV. (2006). Selection of primer-template sequences that bind human immunodeficiency virus reverse transcriptase with high affinity. Nucleic Acids Res 34:130–9.

Dey AK, Griffiths C, Lea SM, James W. (2005a). Structural characterization of an anti-gp120 RNA aptamer that neutralizes R5 strains of HIV-1. RNA 11:873–84.

Dey AK, Khati M, Tang M, et al. (2005b). An aptamer that neutralizes R5 strains of human immunodeficiency virus type 1 blocks gp120-CCR5 interaction. J Virol 79:13806–10.

Dias JC, Silveira AC, Schofield CJ. (2002). The impact of Chagas disease control in Latin America: a review. Mem Inst Oswaldo Cruz 97:603–12.

Ditzler MA, Bose D, Shkriabai N, et al. (2011). Broad-spectrum aptamer inhibitors of HIV reverse transcriptase closely mimic natural substrates. Nucleic Acids Res 39:8237–47.

Duan N, Wu S, Chen X, et al. (2012a). Selection and identification of a DNA aptamer targeted to *Vibrio parahemolyticus*. J Agric Food Chem 60:4034–8.

Duan N, Wu S, Zhu C, et al. (2012b). Dual-color upconversion fluorescence and aptamer-functionalized magnetic nanoparticles-based bioassay for the simultaneous detection of *Salmonella Typhimurium* and *Staphylococcus aureus*. Anal Chim Acta 723:1–6.

Duan N, Ding X, Wu S, et al. (2013b). *In vitro* selection of a DNA aptamer targeted against *Shigella dysenteriae*. J Microbiol Methods 94:170–4.

Duan N, Wu S, Chen X, et al. (2013a). Selection and characterization of aptamers against *Salmonella typhimurium* using whole-bacterium Systemic Evolution of Ligands by Exponential Enrichment (SELEX). J Agric Food Chem 61:3229–34.

Ducongé F, Di Primo C, Toulmé JJ. (2000). Is a closing ''GA pair'' a rule for stable loop-loop RNA complexes? J Biol Chem 275: 21287–94.

Ducongé F, Toulmé JJ. (1999). *In vitro* selection identifies key determinants for loop–loop interactions: RNA aptamers selective for the TAR RNA element of HIV-1. RNA 5:1605–14.

Dwivedi HP, Smiley RD, Jaykus LA. (2010). Selection and characterization of DNA aptamers with binding selectivity to *Campylobacter jejuni* using whole-cell SELEX. Appl Microbiol Biotechnol 87: 2323–34.

Dwivedi HP, Smiley RD, Jaykus LA. (2013). Selection of DNA aptamers for capture and detection of *Salmonella typhimurium* using a whole-cell SELEX approach in conjunction with cell sorting. Appl Microbiol Biotechnol 97:3677–86.

Ellenbecker M, Sears L, Li P, et al. (2012). Characterization of RNA aptamers directed against the nucleocapsid protein of Rift Valley fever virus. Antiviral Res 93:330–9.

Fang Z, Wu W, Lu X, Zeng L. (2014). Lateral flow biosensor for DNA extraction-free detection of salmonella based on aptamer mediated strand displacement amplification. Biosens Bioelectron 56:192–7.

Faure-Perraud A, Métifiot M, Reigadas S, et al. (2011). The guanine-quadruplex aptamer 93del inhibits HIV-1 replication *ex vivo* by interfering with viral entry, reverse transcription and integration. Antivir Ther 16:383–94.

Feng H, Beck J, Nassal M, Hu KH. (2011). A SELEX-screened aptamer of human hepatitis B virus RNA encapsidation signal suppresses viral replication. PloS One 6:e27862.

Fukuda K, Umehara T, Sekiya S, et al. (2004). An RNA ligand inhibits hepatitis C virus NS3 protease and helicase activities. Biochem Biophys Res Commun 325:670–5.

Gao Y, Yu X, Xue B, et al. (2014). Inhibition of hepatitis C virus infection by DNA aptamer against NS2 protein. PloS One 9:e90333.

Gedi V, Kim YP. (2014). Detection and characterization of cancer cells and pathogenic bacteria using aptamer-based nano-conjugates. Sensors 14:18302–27.

Gopinath SC, Hayashi K, Kumar PK. (2012). Aptamer that binds to the gD protein of herpes simplex virus 1 and efficiently inhibits viral entry. J Virol 86:6732–44.

Gopinath SC, Misono TS, Kawasaki K, et al. (2006a). An RNA aptamer that distinguishes between closely related human influenza viruses and inhibits haemagglutinin-mediated membrane fusion. J Gen Virol 87:479–87.

Gopinath SC, Sakamaki Y, Kawasaki K, Kumar PK. (2006b). An efficient RNA aptamer against human influenza B virus hemagglutinin. J Biochem 139:837–46.

Göringer HU, Homann M, Lorger M. (2003). *In vitro* selection of high-affinity nucleic acid ligands to parasite target molecules. Int J Parasitol 33:1309–17.

Göringer HU, Homann M, Zacharias M, Adler A. (2006). RNA aptamers as potential pharmaceuticals against infections with African trypanosomes. Handb Exp Pharmacol 1:375–93.

Green L, Waugh S, Binkley JP, et al. (1995). Comprehensive chemical modification interference and nucleotide substitution analysis of an RNA pseudoknot inhibitor to HIV-1 reverse transcriptase. J Mol Biol 247:60–8.

Guidry CA, Mansfield SA, Sawyer RG, Cook CH. (2014). Resistant pathogens, fungi, and viruses. Surg Clin N Am 94:1195–218.

Hamula CL, Le XC, Li X. (2011). DNA aptamers binding to multiple prevalent M-types of *Streptococcus pyogenes*. Anal Chem 83:3640–7.

Hamula CL, Zhang H, Guan LL, et al. (2008). Selection of aptamers against live bacterial cells. Anal Chem 80:7812–19.

Han SR, Lee SW. (2013). *In vitro* selection of RNA aptamer specific to *Salmonella typhimurium*. J Microbiol Biotechnol 23:878–84.

Held DM, Kissel JD, Saran D, et al. (2006). Differential susceptibility of HIV-1 reverse transcriptase to inhibition by RNA aptamers in enzymatic reactions monitoring specific steps during genome replication. J Biol Chem 281:25712–22.

Held DM, Kissel JD, Thacker SJ, et al. (2007). Cross-clade inhibition of recombinant human immunodeficiency virus type 1 (HIV-1), HIV-2, and simian immunodeficiency virus SIVcpz reverse transcriptases by RNA pseudoknot aptamers. J Virol 81:5375–84.

Homann M, Göringer HU. (1999). Combinatorial selection of high affinity RNA ligands to live African trypanosomes. Nucleic Acids Res 27:2006–14.

Homann M, Göringer HU. (2001). Uptake and intracellular transport of RNA aptamers in African trypanosomes suggest therapeutic ''piggy-back'' approach. Bioorg Med Chem 9:2571–80.

Homann M, Lorger M, Engstler M, et al. (2006). Serum-stable RNA aptamers to an invariant surface domain of live African trypanosomes. Comb Chem High Throughput Screen 9:491–9.

Hu K, Beck J, Nassal M. (2004). SELEX-derived aptamers of the duck hepatitis B virus RNA encapsidation signal distinguish critical and non-critical residues for productive initiation of reverse transcription. Nucleic Acids Res 32:4377–89.

Hwang KS, Lee SM, Eom K, et al. (2007). Nanomechanical microcantilever operated in vibration modes with use of RNA aptamer as receptor molecules for label-free detection of HCV helicase. Biosens Bioelectron 23:459–65.

Hyeon JY, Chon JW, Choi IS, et al. (2012). Development of RNA aptamers for detection of *Salmonella* Enteritidis. J Microbiol Methods 89:79–82.

Ikanovic M, Rudzinski WE, Bruno JG, et al. (2007). Fluorescence assay based on aptamer-quantum dot binding to *Bacillus thuringiensis* spores. J Fluoresc 17:193–9.

Jang KJ, Lee NR, Yeo WS, et al. (2008). Isolation of inhibitory RNA aptamers against severe acute respiratory syndrome (SARS) coronavirus NTPase/Helicase. Biochem Biophys Res Commun 366:738–44.

Jeon SH, Kayhan B, Ben-Yedidia T, Arnon R. (2004). A DNA aptamer prevents influenza infection by blocking the receptor binding region of the viral hemagglutinin. J Biol Chem 279:48410–19.

Jeon W, Lee S, Manjunatha DH, Ban C. (2013). A colorimetric aptasensor for the diagnosis of malaria based on cationic polymers and gold nanoparticles. Anal Biochem 439:11–16.

Joshi P, Prasad VR. (2002). Potent inhibition of human immunodeficiency virus type 1 replication by template analog reverse transcriptase inhibitors derived by SELEX (Systematic Evolution of Ligands by Exponential Enrichment). J Virol 76:6545–57.

Joshi PJ, North TW, Prasad VR. (2005). Aptamers directed to HIV-1 reverse transcriptase display greater efficacy over small hairpin RNAs targeted to viral RNA in blocking HIV-1 replication. Mol Ther 11: 677–86.

Joshi R, Janagama H, Dwivedi HP, et al. (2009). Selection, characterization, and application of DNA aptamers for the capture and detection of *Salmonella enterica* serovars. Mol Cell Probes 23: 20–8.

Joubert MK, Kinsley N, Capovilla A, et al. (2010). A modeled structure of an aptamer-gp120 complex provides insight into the mechanism of HIV-1 neutralization. Biochemistry 49:5880–90.

Jyoti A, Vajpayee P, Singh G, et al. (2011). Identification of environmental reservoirs of nontyphoidal salmonellosis: aptamer-assisted bioconcentration and subsequent detection of *Salmonella typhimurium* by quantitative polymerase chain reaction. Environ Sci Technol 45: 8996–9002.

Kaittanis C, Santra S, Perez JM. (2010). Emerging nanotechnology-based strategies for the identification of microbial pathogenesis. Adv Drug Deliv Rev 62:408–23.

Keefe AD, Cload ST. (2008). SELEX with modified nucleotides. Curr Opin Chem Biol 12:448–56.
Kensch O, Connolly BA, Steinhoff HJ, et al. (2000). HIV-1 reverse transcriptase-pseudoknot RNA aptamer interaction has a binding affinity in the low picomolar range coupled with high specificity. J Biol Chem 275:18271–8.
Khati M, Schüman M, Ibrahim J, et al. (2003). Neutralization of infectivity of diverse R5 clinical isolates of human immunodeficiency virus type 1 by gp120-binding 2′-F-RNA aptamers. J Virol 77:12692–8.
Kim MY, Jeong S. (2004). Inhibition of the functions of the nucleocapsid protein of human immunodeficiency virus-1 by an RNA aptamer. Biochem Biophys Res Commun 320:1181–6.
Kim SJ, Kim MY, Lee JH, et al. (2002). Selection and stabilization of the RNA aptamers against the human immunodeficiency virus type-1 nucleocapsid protein. Biochem Biophys Res Commun 291:925–31.
Kissel JD, Held DM, Hardy RW, Burke DH. (2007a). Active site binding and sequence requirements for inhibition of HIV-1 reverse transcriptase by the RT1 family of single-stranded DNA aptamers. Nucleic Acids Res 35:5039–50.
Kissel JD, Held DM, Hardy RW, Burke DH. (2007b). Single-stranded DNA aptamer RT1t49 inhibits RT polymerase and RNase H functions of HIV type 1, HIV type 2, and SIVCPZ RTs. AIDS Res Hum Retroviruses 23:699–708.
Kolb G, Reigadas S, Boiziau C, et al. (2005). Hexitol nucleic acid-containing aptamers are efficient ligands of HIV-1 TAR RNA. Biochemistry 44:2926–33.
Kolb G, Reigadas S, Castanotto D, et al. (2006). Endogenous expression of an anti-TAR aptamer reduces HIV-1 replication. RNA Biol 3: 150–6.
Kolovskaya OS, Savitskaya AG, Zamay TN, et al. (2013). Development of bacteriostatic DNA aptamers for *Salmonella*. J Med Chem 56: 1564–72.
Kumar PK, Machida K, Urvil PT, et al. (1997). Isolation of RNA aptamers specific to the NS3 protein of hepatitis C virus from a pool of completely random RNA. Virology 237:270–82.
Kwon HM, Lee KH, Han BW, et al. (2014). An RNA aptamer that specifically binds to the glycosylated hemagglutinin of avian influenza virus and suppresses viral infection in cells. PloS One 9:e97574.
Labib M, Zamay AS, Kolovskaya OS, et al. (2012a). Aptamer-based impedimetric sensor for bacterial typing. Anal Chem 84:8114–17.
Labib M, Zamay AS, Kolovskaya OS, et al. (2012b). Aptamer-based viability impedimetric sensor for bacteria. Anal Chem 84:8966–9.
Labib M, Zamay AS, Muharemagic D, et al. (2012c). Aptamer-based viability impedimetric sensor for viruses. Anal Chem 84:1813–16.
Lai YT, DeStefano JJ. (2012). DNA aptamers to human immunodeficiency virus reverse transcriptase selected by a primer-free SELEX method: characterization and comparison with other aptamers. Nucleic Acid Ther 22:162–76.
Lange MJ, Sharma TK, Whatley AS, et al. (2012). Robust suppression of HIV replication by intracellularly expressed reverse transcriptase aptamers is independent of ribozyme processing. Mol Ther 20: 2304–14.
Lebars I, Legrand P, Aimé A, et al. (2008). Exploring TAR-RNA aptamer loop-loop interaction by X-ray crystallography, UV spectroscopy and surface plasmon resonance. Nucleic Acids Res 36:7146–56.
Lebars I, Richard T, Di Primo C, Toulmé JJ. (2007a). LNA derivatives of a kissing aptamer targeted to the trans-activating responsive RNA element of HIV-1. Blood Cells Mol Dis 38:204–9.
Lebars I, Richard T, Di Primo C, Toulmé JJ. (2007b). NMR structure of a kissing complex formed between the TAR RNA element of HIV-1 and a LNA-modified aptamer. Nucleic Acids Res 35:6103–14.
Lee HJ, Kim BC, Kim KW, et al. (2009). A sensitive method to detect *Escherichia coli* based on immunomagnetic separation and real-time PCR amplification of aptamers. Biosens Bioelectron 24:3550–5.
Lee S, Kim YS, Jo M, et al. (2007). Chip-based detection of hepatitis C virus using RNA aptamers that specifically bind to HCV core antigen. Biochem Biophys Res Commun 358:47–52.
Lee S, Song KM, Jeon W, et al. (2012a). A highly sensitive aptasensor towards *Plasmodium* lactate dehydrogenase for the diagnosis of malaria. Biosens Bioelectron 35:291–6.
Lee YJ, Han SR, Maeng JS, et al. (2012b). *In vitro* selection of *Escherichia coli* O157:H7-specific RNA aptamer. Biochem Biophys Res Commun 417:414–20.
Li H, Ding X, Peng Z, et al. (2011). Aptamer selection for the detection of *Escherichia coli* K88. Can J Microbiol 57:453–9.
Li N, Wang Y, Pothukuchy A, et al. (2008). Aptamers that recognize drug-resistant HIV-1 reverse transcriptase. Nucleic Acids Res 36: 6739–51.
Liang HR, Hu GQ, Li L, et al. (2014a). Aptamers targeting rabies virus-infected cells inhibit street rabies virus *in vivo*. Int Immunopharmacol 21:432–8.
Liang HR, Hu GQ, Xue XH, et al. (2014b). Selection of an aptamer against rabies virus: a new class of molecules with antiviral activity. Virus Res 184:7–13.
Liang HR, Hu GQ, Zhang T, et al. (2012). Isolation of ssDNA aptamers that inhibit rabies virus. Int Immunopharmacol 14:341–7.
Liang HR, Liu Q, Zheng XX, et al. (2013). Aptamers targeting rabies virus-infected cells inhibit viral replication both *in vitro* and *in vivo*. Virus Res 173:398–403.
Liang Y, Zhang Z, Wei H, et al. (2011). Aptamer beacons for visualization of endogenous protein HIV-1 reverse transcriptase in living cells. Biosens Bioelectron 28:270–6.
Liu G, Yu X, Xue F, et al. (2012). Screening and preliminary application of a DNA aptamer for rapid detection of Salmonella O8. Microchim Acta 178:237–44.
Llor C, Bjerrum L. (2014). Antimicrobial resistance: risk associated with antibiotic overuse and initiatives to reduce the problem. Ther Adv Drug Saf 5:229–41.
Lopes de Campos WR, Coopusamy D, Morris L, et al. (2009). Cytotoxicological analysis of a gp120 binding aptamer with cross-clade human immunodeficiency virus type 1 entry inhibition properties: comparison to conventional antiretrovirals. Antimicrob Agents Chemother 53:3056–64.
Lorger M, Engstler M, Homann M, Göringer HU. (2003). Targeting the variable surface of African trypanosomes with variant surface glycoprotein-specific, serum-stable RNA aptamers. Eukaryot Cell 2: 84–94.
Ma X, Jiang Y, Jia F, et al. (2014). An aptamer-based electrochemical biosensor for the detection of *Salmonella*. J Microbiol Methods 98: 94–8.
Matsugami A, Kobayashi S, Ouhashi K, et al. (2003). Structural basis of the highly efficient trapping of the HIV Tat protein by an RNA aptamer. Structure 11:533–45.
Van Melckebeke H, Devany M, Di Primo C, et al. (2008). Liquid-crystal NMR structure of HIV TAR RNA bound to its SELEX RNA aptamer reveals the origins of the high stability of the complex. Proc Natl Acad Sci USA 105:9210–15.
Métifiot M, Faure A, Guyonnet-Duperat V, et al. (2007). Cellular uptake of ODNs in HIV-1 human-infected cells: a role for viral particles in DNA delivery? Oligonucleotides 17:151–65.
Michalowski D, Chitima-Matsiga R, Held DM, Burke DH. (2008). Novel bimodular DNA aptamers with guanosine quadruplexes inhibit phylogenetically diverse HIV-1 reverse transcriptases. Nucleic Acids Res 36:7124–35.
Minunni M, Tombelli S, Gullotto A, et al. (2004). Development of biosensors with aptamers as bio-recognition element: the case of HIV-1 Tat protein. Biosens Bioelectron 20:1149–56.
Moore MD, Bunka DH, Forzan M, et al. (2011b). Generation of neutralizing aptamers against herpes simplex virus type 2: potential components of multivalent microbicides. J Gen Virol 92:1493–9.
Moore MD, Cookson J, Coventry VK, et al. (2011a). Protection of HIV neutralizing aptamers against rectal and vaginal nucleases: implications for RNA-based therapeutics. J Biol Chem 286:2526–35.
Mosing RK, Mendonsa SD, Bowser MT. (2005). Capillary electrophoresis-SELEX selection of aptamers with affinity for HIV-1 reverse transcriptase. Anal Chem 77:6107–12.
Mufhandu HT, Gray ES, Madiga MC, et al. (2012). UCLA1, a synthetic derivative of a gp120 RNA aptamer, inhibits entry of human immunodeficiency virus type 1 subtype C. J Virol 86: 4989–99.
Nagarkatti R, de Araujo FF, Gupta C, Debrabant A. (2014). Aptamer based, non-PCR, non-serological detection of Chagas disease biomarkers in *Trypanosoma cruzi* infected mice. PLoS Negl Trop Dis 8: e2650.
Neff CP, Zhou J, Remling L, et al. (2011). An aptamer-siRNA chimera suppresses HIV-1 viral loads and protects from helper CD4(+) T cell decline in humanized mice. Sci Transl Med 3:66ra6.
Negri P, Chen G, Kage A, et al. (2012). Direct optical detection of viral nucleoprotein binding to an anti-influenza aptamer. Anal Chem 84: 5501–8.

Negri P, Kage A, Nitsche A, et al. (2011). Detection of viral nucleoprotein binding to anti-influenza aptamers via SERS. Chem Commun (Camb) 47:8635–7.

Ngubane NA, Gresh L, Pym A, et al. (2014). Selection of RNA aptamers against the *M. tuberculosis* EsxG protein using surface plasmon resonance-based SELEX. Biochem Biophys Res Commun 449: 114–19.

Nishikawa F, Funaji K, Fukuda K, Nishikawa S. (2004). *In vitro* selection of RNA aptamers against the HCV NS3 helicase domain. Oligonucleotides 14:114–29.

Nishikawa F, Kakiuchi N, Funaji K, et al. (2003). Inhibition of HCV NS3 protease by RNA aptamers in cells. Nucleic Acids Res 31:1935–43.

Nitsche A, Kurth A, Dunkhorst A, et al. (2007). One-step selection of Vaccinia virus-binding DNA aptamers by MonoLEX. BMC Biotechnol 7:48.

Ohk SH, Koo OK, Sen T, et al. (2010). Antibody-aptamer functionalized fibre-optic biosensor for specific detection of *Listeria monocytogenes* from food. J Appl Microbiol 109:808–17.

Ozalp VC, Bilecen K, Kavruk M, Oktem HA. (2013). Antimicrobial aptamers for detection and inhibition of microbial pathogen growth. Future Microbiol 8:387–401.

Pan Q, Zhang XL, Wu HY, et al. (2005). Aptamers that preferentially bind type IVB pili and inhibit human monocytic-cell invasion by *Salmonella enterica* serovar Typhi. Antimicrob Agents Chemother 49: 4052–60.

Parekh P, Tang Z, Turner PC, et al. (2010). Aptamers recognizing glycosylated hemagglutinin expressed on the surface of vaccinia virus-infected cells. Anal Chem 82:8790–7.

Park SY, Kim S, Yoon H, et al. (2011). Selection of an antiviral RNA aptamer against hemagglutinin of the subtype H5 avian influenza virus. Nucleic Acid Ther 21:395–402.

Peng Z, Ling M, Ning Y, Deng L. (2014). Rapid fluorescent detection of *Escherichia coli* K88 based on DNA aptamer library as direct and specific reporter combined with immuno-magnetic separation. J Fluoresc 24:1159–68.

Phan AT, Kuryavyi V, Ma JB, et al. (2005). An interlocked dimeric parallel-stranded DNA quadruplex: a potent inhibitor of HIV-1 integrase. Proc Natl Acad Sci USA 102:634–9.

Di Primo C, Rudloff I, Reigadas S, et al. (2007). Systematic screening of LNA/2'-O-methyl chimeric derivatives of a TAR RNA aptamer. FEBS Lett 581:771–4.

Punnarak P, Santos MD, Hwang SD, et al. (2012). RNA aptamers inhibit the growth of the fish pathogen viral hemorrhagic septicemia virus (VHSV). Mar Biotechnol (NY) 14:752–61.

Qin L, Zheng R, Ma Z, et al. (2009). The selection and application of ssDNA aptamers against MPT64 protein in *Mycobacterium tuberculosis*. Clin Chem Lab Med 47:405–11.

Ruslinda RA, Tanabe K, Ibori S, et al. (2013). Effects of diamond-FET-based RNA aptamer sensing for detection of real sample of HIV-1 Tat protein. Biosens Bioelectron 40:277–82.

Ramalingam D, Duclair S, Datta SA, et al. (2011). RNA aptamers directed to human immunodeficiency virus type 1 Gag polyprotein bind to the matrix and nucleocapsid domains and inhibit virus production. J Virol 85:305–14.

Ramos E, Piñeiro D, Soto M, et al. (2007). A DNA aptamer population specifically detects *Leishmania infantum* H2A antigen. Lab Invest 87: 409–16.

Rotherham LS, Maserumule C, Dheda K, et al. (2012). Selection and application of ssDNA aptamers to detect active TB from sputum samples. PloS One 7:e46862.

Sekiya S, Nishikawa F, Fukuda K, Nishikawa S. (2003). Structure/function analysis of an RNA aptamer for hepatitis C virus NS3 protease. J Biochem 133:351–9.

Sekkai D, Dausse E, Di Primo C, et al. (2002). *In vitro* selection of DNA aptamers against the HIV-1 TAR RNA hairpin. Antisense Nucleic Acid Drug Dev 12:265–74.

Shinde SB, Fernandes CB, Patravale VB. (2012). Recent trends in *in-vitro* nanodiagnostics for detection of pathogens. J Control Release 159:164–80.

Shum KT, Lui EL, Wong SC, et al. (2011). Aptamer-mediated inhibition of *Mycobacterium tuberculosis* polyphosphate kinase 2. Biochemistry 50:3261–71.

Shum KT, Zhou J, Rossi JJ. (2013). Aptamer-based therapeutics: new approaches to combat human viral diseases. Pharmaceuticals (Basel) 6:1507–42.

Singh G, Vajpayee P, Rani N, et al. (2012). Bio-capture of S. Typhimurium from surface water by aptamer for culture-free quantification. Ecotoxicol Environ Saf 78:320–6.

So HM, Park DW, Jeon EK, et al. (2008). Detection and titer estimation of *Escherichia coli* using aptamer-functionalized single-walled carbon-nanotube field-effect transistors. Small 4:197–201.

De Soultrait V, Lozach PY, Altmeyer R, et al. (2002). DNA aptamers derived from HIV-1 RNase H inhibitors are strong anti-integrase agents. J Mol Biol 324:195–203.

Suh SH, Jaykus LA. (2013). Nucleic acid aptamers for capture and detection of *Listeria* spp. J Biotechnol 167:454–61.

Tang Z, Parekh P, Turner P, et al. (2009). Generating aptamers for recognition of virus-infected cells. Clin Chem 55:813–22.

Tombelli S, Minunni M, Luzi E, Mascini M. (2005). Aptamer-based biosensors for the detection of HIV-1 Tat protein. Bioelectrochemistry 67:135–41.

Tuerk C, MacDougal S, Gold L. (1992). RNA pseudoknots that inhibit human immunodeficiency virus type 1 reverse transcriptase. Proc Natl Acad Sci USA 89:6988–92.

Ulrich H, Magdesian MH, Alves MJ, Colli W. (2002). *In vitro* selection of RNA aptamers that bind to cell adhesion receptors of *Trypanosoma cruzi* and inhibit cell invasion. J Biol Chem 277:20756–62.

Um HJ, Kim M, Lee SH, Kim YH. (2012). Preventing the formation of positive transcription elongation factor b by human cyclin T1-binding RNA aptamer for anti-HIV transcription. AIDS 26:1599–15605.

Umehara T, Fukuda K, Nishikawa F, et al. (2004). Designing and analysis of a potent bi-functional aptamers that inhibit protease and helicase activities of HCV NS3. Nucleic Acids Symp Ser (Oxf.) 48: 195–6.

Umehara T, Fukuda K, Nishikawa F, et al. (2005). Rational design of dual-functional aptamers that inhibit the protease and helicase activities of HCV NS3. J Biochem 137:339–47.

Vivekananda J, Kiel JL. (2006). Anti-*Francisella tularensis* DNA aptamers detect tularemia antigen from different subspecies by Aptamer-Linked Immobilized Sorbent Assay. Lab Invest 86: 610–18.

Vivekananda J, Salgado C, Millenbaugh NJ. (2014). DNA aptamers as a novel approach to neutralize *Staphylococcus aureus* α-toxin. Biochem Biophys Res Commun 444:433–8.

Wang J, Jiang H, Liu F. (2000). *In vitro* selection of novel RNA ligands that bind human cytomegalovirus and block viral infection. RNA (NY) 6:571–83.

Wang KY, Zeng YL, Yang XY, et al. (2011). Utility of aptamer-fluorescence in situ hybridization for rapid detection of *Pseudomonas aeruginosa*. Eur J Clin Microbiol Infect Dis 30:273–8.

Wang R, Zhao J, Jiang T, et al. (2013). Selection and characterization of DNA aptamers for use in detection of avian influenza virus H5N1. J Virol Methods 189:362–9.

Watrin M, Von Pelchrzim F, Dausse E, et al. (2009). *In vitro* selection of RNA aptamers derived from a genomic human library against the TAR RNA element of HIV-1. Biochemistry 48:6278–84.

Whatley AS, Ditzler MA, Lange MJ, et al. (2013). Potent inhibition of HIV-1 reverse transcriptase and replication by nonpseudoknot, ''UCAA-motif'' RNA aptamers. Mol Ther Nucleic Acids 2:e71.

Wheeler LA, Trifonova R, Vrbanac V, et al. (2011). Inhibition of HIV transmission in human cervicovaginal explants and humanized mice using CD4 aptamer-siRNA chimeras. J Clin Invest 121:2401–12.

Wheeler LA, Vrbanac V, Trifonova R, et al. (2013). Durable knockdown and protection from HIV transmission in humanized mice treated with gel-formulated CD4 aptamer-siRNA chimeras. Mol Ther 21:1378–89.

Yamamoto R, Baba T, Kumar PK. (2000a). Molecular beacon aptamer fluoresces in the presence of Tat protein of HIV-1. Genes Cells 5: 389–96.

Yamamoto R, Katahira M, Nishikawa S, et al. (2000b). A novel RNA motif that binds efficiently and specifically to the Tat protein of HIV and inhibits the *trans*-activation by Tat of transcription *in vitro* and *in vivo*. Genes Cells 5:371–88.

Yang M, Peng Z, Ning Y, et al. (2013). Highly specific and cost-efficient detection of *Salmonella* Paratyphi A combining aptamers with single-walled carbon nanotubes. Sensors (Basel) 13:6865–81.

Zelada-Guillén G, Riu J, Düzgün A, Rius FX. (2009). Immediate detection of living bacteria at ultralow concentrations using a carbon

nanotube based potentiometric aptasensor. Angew Chem, Int Ed Engl 48:7334–7.

Zelada-Guillén GA, Bhosale SV, Riu J, Rius FX. (2010). Real-time potentiometric detection of bacteria in complex samples. Anal Chem 82:9254–60.

Zelada-Guillén GA, Sebastián-Avila JL, Blondeau P, et al. (2012). Label-free detection of *Staphylococcus aureus* in skin using real-time potentiometric biosensors based on carbon nanotubes and aptamers. Biosens Bioelectron 31:226–32.

Zelada-Guillén GA, Tweed-Kent A, Niemann M, et al. (2013). Ultrasensitive and real-time detection of proteins in blood using a potentiometric carbon-nanotube aptasensor. Biosens Bioelectron 41: 366–71.

Zhou J, Li H, Li S, et al. (2008). Novel dual inhibitory function aptamer–siRNA delivery system for HIV-1 therapy. Mol Ther 16: 1481–9.

Zhou J, Li H, Zhang J, et al. (2011a). Development of cell-type specific anti-HIV gp120 aptamers for siRNA delivery. J Vis Exp 52:1–9.

Zhou J, Rossi JJ. (2011). Aptamer-targeted RNAi for HIV-1 therapy. Methods Mol Biol 721:355–71.

Zhou J, Shu Y, Guo P, et al. (2011b). Dual functional RNA nanoparticles containing phi29 motor pRNA and anti-gp120 aptamer for cell-type specific delivery and HIV-1 inhibition. Methods 54:284–94.

Zhou J, Neff CP, Swiderski P, et al. (2013). Functional *in vivo* delivery of multiplexed anti-HIV-1 siRNAs via a chemically synthesized aptamer with a sticky bridge. Mol Ther 21:192–200.

Zhou J, Swiderski P, Li H, et al. (2009). Selection, characterization and application of new RNA HIV gp 120 aptamers for facile delivery of Dicer substrate siRNAs into HIV infected cells. Nucleic Acids Res 37: 3094–109.

Zhu Q, Shibata T, Kabashima T, Kai M. (2012). Inhibition of HIV-1 protease expression in T cells owing to DNA aptamer-mediated specific delivery of siRNA. Eur J Med Chem 56:396–9.

Zimbres FM, Tárnok A, Ulrich H, Wrenger C. (2013). Aptamers: novel molecules as diagnostic markers in bacterial and viral infections? Biomed Res Int 2013:731516. doi: 10.1155/2013/731516.