

## APTAMERS ROLE IN BASIC DRUG RESEARCH AND DEVELOPMENT - AN OVERVIEW

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## ABSTRACT

Aptamers, also termed as “decoys” or “chemical antibodies,” represent an emerging class of therapeutics. They are short DNA or RNA oligonucleotides or peptides that assume a specific and stable three-dimensional shape *in vivo*, thereby providing specific tight binding to protein targets. In some cases and as opposed to antisense oligonucleotides, effects can be mediated against extracellular targets, thereby preventing a need for intracellular transportation. In the simplest view, Aptamers can be thought of as nucleic acid analogs to antibodies. Aptamers have wide application in many areas of biomedical sciences such as-Purification processes, Target validation, Drug development diagnostics, and MRI-based cell tracking therapy. Combining Nano biotechnology with Aptamers opens the way for more sophisticated applications in molecular diagnosis. Moreover, Aptamers are used to study regulatory circuits and molecular mechanisms of disease processes. Aptamers can readily be adopted for analytical or diagnostic applications. *In vivo* experiments demonstrate that they generally exhibit low toxicity and immunogenicity characteristics, which make them suitable for therapeutic use. This article presents a view of recent developments in Systematic Evolution of Ligands by Exponential enrichment (SELEX) technologies and new emerging applications of Aptamers.

**Keywords:** Aptamers, Oligonucleotides, SELEX technology.

## INTRODUCTION

Aptamers are short single stranded DNA or RNA molecules that recognize their targets on basis of shape complementarily generated by a method called SELEX (systemic evaluation of Ligands by exponential enrichment) have been widely used in various biomedical applications. These often called chemical antibodies; These properties of aptamers have led to their application in many areas of biomedical sciences such as- Purification processes, Target validation, Drug development, Diagnostics, MRI-based celltracking therapy<sup>1,2,3</sup>. Aptamers are poised to take on the monoclonal antibodies in therapeutics, diagnostics, and drugdevelopment.<sup>4</sup>

Since the introduction of the technology for isolation of nucleic acid Aptamers in 1990, there has been a steady annual increase in the number of publications related to Aptamers. During the last 10 years Aptamers binding to for example cells, proteins, peptides, low molecular weight molecules (like antibiotics, amino acids, and dyes) or nucleic acids have been isolated. Aptamers display a high specificity and affinity for their target molecules, which make them suitable for a variety of applications. A continuously growing number of nucleic acid Aptamers are used as research tools to study specific protein functions and interactions.

The first Aptamers-based therapeutics is now being tested in clinical trials. The most efficient way to find Aptamers is through a method utilizing nucleic acid libraries in conjunction with a selection scheme based on *in vitro* evolution principles. The process has been termed SELEX, standing for Systematic Evolution of Ligands by Exponential enrichment. Since DNA and RNA molecules adopt stable and intricately folded three-dimensional shapes they are capable of providing a scaffold for the interaction with functional side groups of a bound ligand. Randomized sequence pools of oligonucleotides can be synthesized with sequence complexities in the range of 10<sup>15</sup> different molecules. This represents a structural diversity not matched by any other combinatorial technique and it is one of the prime advantages of the SELEX method.<sup>5</sup>

## PROPERTIES OF APTAMERS

- They are RNA/DNA oligonucleotides molecules.
- They have the ability to bind to proteins with high affinity and high specificity.
- They adopt vast no. of 3-D shapes-which enables them to bind tightly to a specific molecular target.
- They are usually very small in size but can easily distinguish between very closely related proteins also.

## THERAPEUTIC USES OF APTAMERS

- **Pathway elucidation:** Aptamers can be used as specific inhibitors to dissect intracellular signalling and transport pathways.
- **Tools for purification:** The affinity and specificity of aptamers is sufficient for use as affinity chromatography media.
- **Target validation:** Aptamers are very helpful in validating and screening no.of drug molecules against certain proteins.
- **Cancer:** Aptamers specifically bind to certain growth factors such as VEGF (vascular endothelial growth factor) which is involved in tumor development, and inactivate them and thus are helpful in the treatment of certain types of cancers.
- **AIDS:** A series of aptamers which interfere with virus multiplication in human cells, have been developed. The aptamers interact with various components involved in virus propagation and prevent the formation of intact virus particles.
- **Anti-infective agents:** Two active areas of interest are the use of aptamers as inhibitors of the chronic viral infections - HIV & Hepatitis C.<sup>6</sup>

## METHODOLOGY

## Aptamers Discovery

## The SELEX Process

Aptamers are generally discovered using the *in vitro* selection process referred to as SELEX (Systematic Evolution of Ligands by Exponential enrichment). SELEX is an interactive process used to identify an aptamer to a chosen molecular target from a large pool of nucleic acids<sup>7</sup>. The process relies on standard molecular biological techniques, which allow a single researcher to conduct multiple selection experiments in parallel.

## Step One: Pool Generation

At the outset of a SELEX experiment, a pool of nucleic acid molecules is generated using standard automated oligonucleotide synthesis methods. Natural RNAs/DNAs have short half-lives in serum due to nuclease degradation. Nuclease activity may be blocked by modifications to the 2'-ribose position (e.g., 2'-O-methyl) on the oligonucleotide backbone. Therefore, in addition to using DNA-based pools, SELEX is also conducted using pools of nucleic acids containing fully 2'-O-methyl nucleotides, or mixtures of 2'-deoxy and 2'-O-methyl nucleotides. At the outset of a selection, each pool contains approximately 10<sup>14</sup> oligonucleotides, each with a unique sequence that

can, in principle, adopt a unique three-dimensional structure. A few of these molecules the aptamers present a contact surface that is complementary to an "apitope" on the target molecule.

**Step Two: Selection**

The selection step is designed to find those molecules with the greatest affinity for the target of interest. The library of nucleotide sequences is exposed to the target protein and allowed to incubate for a period of time. The molecules in the library with weak or no affinity for the target have a tendency to remain free in solution, while those with some capacity to bind will tend to associate with the target. Any one of several methods is used to physically isolate the aptamer-target complexes from the unbound molecules in the mixture, and then the unbound molecules are discarded. The target-bound molecules, among which are the highest affinity aptamers, are purified away from the target and used for the subsequent steps in the SELEX process.

**Step Three: Amplification**

The captured, purified sequences are copied enzymatically, or "amplified", to generate a new library of molecules that is substantially enriched with oligonucleotides that can bind to the target. The enriched library is used to initiate a new cycle of selection, partitioning and amplification. One complete cycle of binding, partitioning and amplification is referred to as a "round" of SELEX.

**Step Four: Aptamer Isolation**

After five to 15 cycles of the complete SELEX process, the library of molecules is reduced from  $10^{14}$  unique sequences to a small number that bind tightly to the target of interest. At this stage, the nucleotide sequences of individual members of the library are determined. The target binding affinity and specificity of selected sequences are then measured and compared.<sup>7</sup>

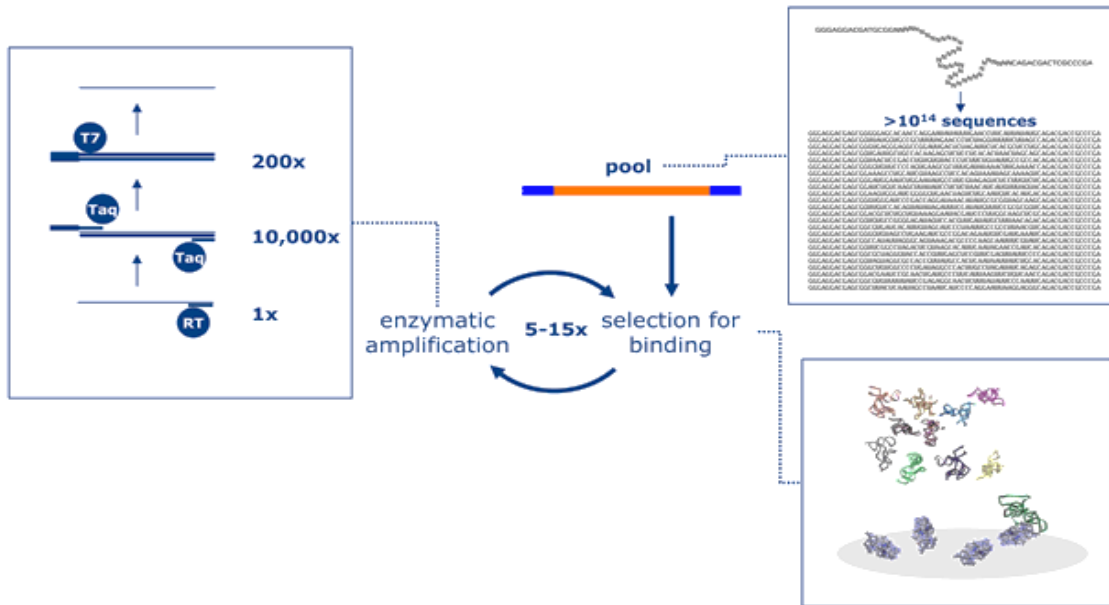


Fig. 1: SELEX Process

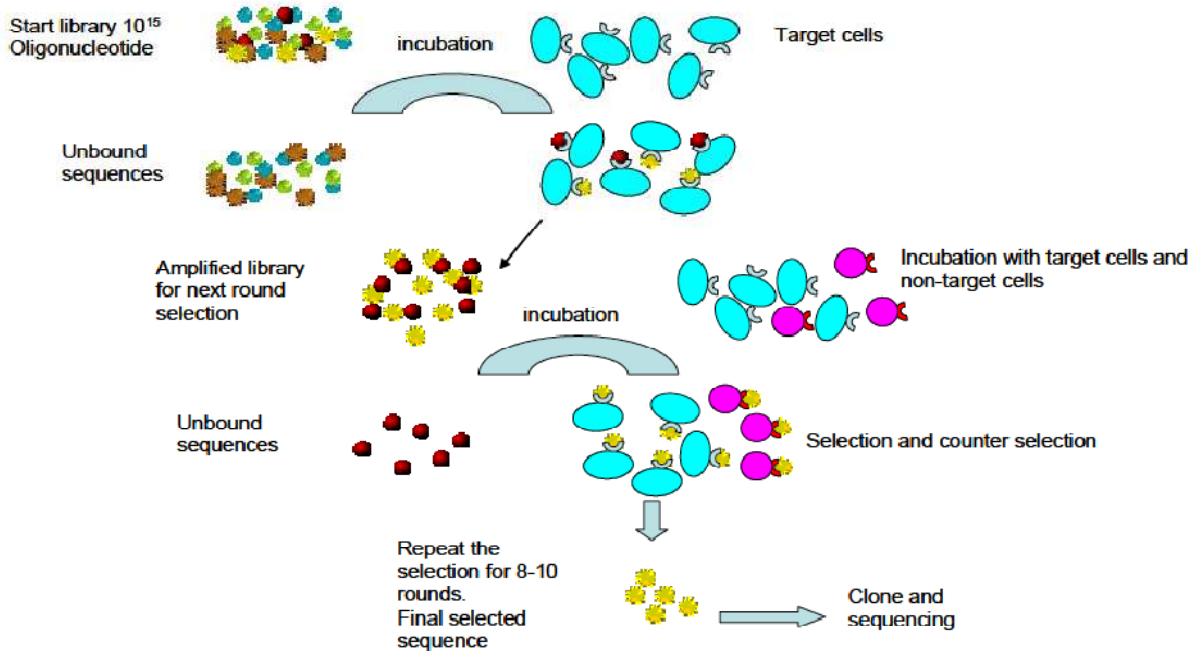


Fig. 2: SELEX Process.

### Post-SELEX Modification Processes

The aptamers isolated by the SELEX process exhibit affinity and specificity for the selected target, but often exhibit chemical characteristics that may limit their potential as therapeutics. Accordingly, following the SELEX process, we use proprietary chemistry techniques, which we call post-SELEX modification, to design, stabilize and optimize the early lead series of aptamers to create aptamer product candidates for clinical development.

The steps involved in post-SELEX modification include:

- **Minimization**

The initial aptamer sequences isolated by SELEX are typically 70 to 80 nucleotides long. Commercializing aptamers of this length would be difficult and expensive using current manufacturing techniques, and production yields would be low. Accordingly, we apply our proprietary methods to identify the active site or core of the aptamer and remove unnecessary nucleotides from the molecule. We are typically able to reduce the aptamer to between 20 and 40 nucleotides in length without compromising the affinity, specificity or functional activity of the aptamer for the target of interest.

- **Optimization**

**Affinity and functional activity improvements:** here sequence and chemical modifications to improve an aptamer's affinity for its target and functional activity using a technique in which sets of variant aptamers are chemically synthesized. Then compare these variant aptamers to each other and to the starting aptamer in order to determine which modifications improve affinity, functional activity or both.

**Nuclease resistance:** If not chemically altered, aptamers composed of unmodified nucleotides may be rapidly degraded, or metabolized, by enzymes which are naturally present in the blood and tissues. These enzymes, known as nucleases, bind to and metabolize the aptamer. While rapid drug clearance and a short duration of action are desirable for some clinical applications, a prolonged duration of action is necessary for other disease categories. Accordingly, proprietary methods are used to identify the specific sites within an aptamer that are most susceptible to nuclease metabolism.

- **PEGylation**

Duration of action is often correlated to how long the aptamer remains in the body. Because aptamers are small in size, they may be

naturally excreted before they have achieved their intended therapeutic effect. To slow the rate of excretion from the body, we increase the size of the aptamer by attaching it to another molecule known as polyethylene glycol, or PEG, to create a larger molecule. This process is known as PEGylation. We can achieve the desired duration of action by using different sizes, structures and attachment locations of PEG molecules. Once the aptamer is PEGylated, it is tested to determine whether it has achieved the desired duration of action.

Through this combination of SELEX and post-SELEX modification processes, we are able to design and confirm the desired properties of an aptamer that we believe will address the proposed therapeutic indication.<sup>8,9</sup>

### Drug Metabolism and Pharmacokinetics

Oligonucleotides composed of unmodified RNA or DNA are rapidly metabolized by serum and tissue nucleases. While having rapid drug clearance and a short duration of action is desirable for some clinical applications, most of the time a prolonged duration of action is preferable. Over the past 20 years a number of chemical modifications have been described that significantly stabilize oligonucleotides to nuclease-mediated metabolism. Implemented several of these well characterized modifications in the process of creating aptamers that are highly resistant to nuclease degradation.<sup>10</sup>

During post-SELEX modification processes, we study metabolic stability of early lead molecules in animal and human serum (a nuclease containing matrix). We assess the rate of disappearance of the full length molecule and then, by liquid chromatography and tandem mass spectroscopy, we identify the specific metabolites and sites susceptible to cleavage. With this information, we can then introduce site specific stabilizing modifications into the parent molecule and retest to confirm that nuclease resistance has been achieved. Once the aptamer core has been sufficiently stabilized, we often conjugate the aptamer with a polyethylene glycol (PEG) moiety to create a larger molecule that will be resistant to renal filtration and clearance via excretion. Large molecular weight PEG has been a commonly used and well validated strategy for increasing the circulation half-life of protein and peptide therapeutics. The PEG conjugated aptamer can then be tested *in vivo* to establish if the desired pharmacokinetics have been achieved.<sup>11</sup>

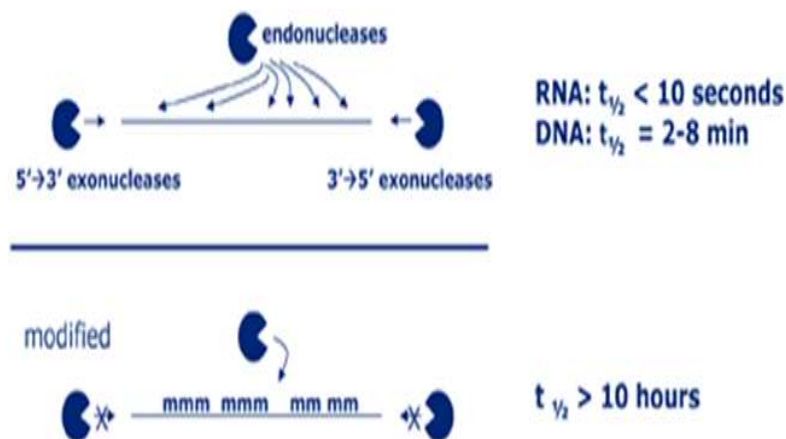


Fig. 3: Tuning metabolic stability through chemical modification

Through this combination of optimization of metabolism by chemical stabilization and elimination by PEGylation, we are able to precisely design and then confirm the desired PK properties of the resultant development candidate.<sup>11</sup>

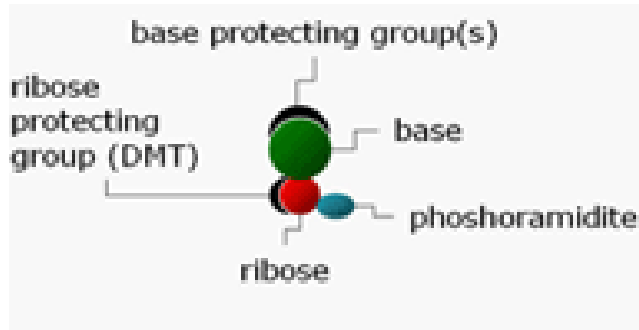
### Chemistry, Manufacturing, and Controls

Although aptamers are a new entry, the broader field of therapeutic oligonucleotides has been around for about 20 years, beginning with

antisense oligonucleotides in the 1980s. As a result, a great deal of technology investment has been made in medicinal chemistry, chemical process development, and analytical chemistry that has enabled the efficient manufacture of therapeutic oligonucleotides of high quality.

Oligonucleotides are made by solid-phase chemical synthesis that involves the progressive addition of the appropriate nucleotide to the growing chain. This process is well defined using commercially

available instrumentation and raw materials. A great strength of aptamer technology is the ability to rapidly scale the synthesis process and move from milligram to gram to kilogram scale very quickly. The discovery and development teams do not have significant delays waiting for drug. This is in contrast to bio therapeutics (such as monoclonal antibodies) where large timeline delays are incurred during cell line development and material scale-up.



**Fig. 4: Oligonucleotides by solid-phase chemical synthesis**

Recent advances in analytical chemistry tools and techniques have enabled an improved ability to characterize both the raw materials and the end product of therapeutic oligonucleotides. The same analytical tools enable us to build a detailed understanding of stability and thereby ensure a quality product throughout the stated shelf life.

Even though oligonucleotide manufacture and characterization is relatively well understood and utilizes established technologies, the know-how is consolidated into the few companies that have specialized in this field.<sup>12</sup>

#### Selection methods

One of the most crucial aspects of the selection experiment is the partition of bound and unbound aptamer species, which requires the immobilization or separation of the target or target-aptamer complex. There is a variety of selection methods, depending on the nature of the target and the possibilities available. Filtration using nitrocellulose filters was one of the first selection methods to trap a target-aptamer complex. This method was successfully used to select ricin-binding aptamers.

Centrifugation has also been used in the selection step. Aptamers binding to intact parasites could easily be collected by centrifugation. A new elegant approach for selection was established, where the target molecule is bound to colloidal gold to confer a higher mass at the target, for further purification by centrifugation. A less common method is separation by molecular size on polyacrylamide gels (gel shift), where the complex is isolated from the gel and the aptamer is eluted and amplified.

However, separation on columns is an often used method, where the columns contain Sepharose or agarose. The target can be modified to contain a tag, GST (glutathione-S-transferase) or His (6-8 residues linked to the N- or C-terminal of the protein), which binds to the column matrix.<sup>13, 14, 15</sup>

For selection of toxin-binding aptamers, conjugation of the target to magnetic beads has been used. This selection method has also been useful in the development of an automated SELEX process.<sup>16, 17</sup>

#### Stabilization of aptamers

Modification of single-stranded RNA may be performed for stabilization since the aptamers are sensitive to degrading enzymes. This is of special importance when the aptamer is used for therapy and the environmental parameters cannot be modified. Degrading enzymes are common in body fluids as serum or urine. The stability is increased by changing the 2'-OH group of ribose to 2'-amino (NH<sub>2</sub>) or 2'-fluoro (F)- groups on pyrimidines. Such modified nucleoside tri phosphates function as substrate for T7 polymerase in *invitro* transcription and may be included in the SELEX protocol.<sup>18, 19</sup>

An aptamer can also be protected from exonuclease degradation by capping its 3'-end after the SELEX process is completed. Other ways of stabilization are by enlargement of the molecule through extended sequence or by conjugating the aptamers to larger macromolecules like polyethyleneglycol (PEG). Embedding into liposomes is another method to protect the aptamer. Stabilized aptamers were coupled to a dialkylglycerol (DAG) moiety and inserted into the lipid bi-layer of liposomes. These aptamers have different orientations; some are directed inwards and others out into the surrounding solution. Liposomes have also been used for delivery of aptamers, to improve the cellular uptake. Moreover, the Spiegelmers technology is useful to overcome the degradation problem. This technology is based on mirror image nucleic acid chemistry. The selection of Spiegelmers is performed by a modified SELEX method where an aptamer is selected against the mirror image of the natural target. After trimming, the equivalent "unnatural form" (L-form) of the aptamer is synthesized. Such a Spiegelmer binds both the natural target and the mirror target with high affinity and displays high resistance to enzymatic degradation compared with the natural D-form of the aptamer.<sup>20, 21</sup>

#### Automated SELEX robotic systems

The expanding information on complete genome sequences and organismal proteomes raises the need for high throughput aptamer selection methods to identify aptamers to new targets, entire proteomes or metabolites. The *in vitro* selection can be extremely time-consuming, from weeks to months. However, the introduction of robotic systems has made it possible to isolate aptamers by automated RNA selection. These automated selection systems can be used in the discovery of new small molecule drugs as well as for selection of nucleic acid enzymes. The automated SELEX process has also been used for isolation of Spiegelmers in the purpose to identify drug candidates.<sup>22, 23</sup>

#### Targets for selected aptamers

Aptamers can be selected for almost any type of substance. A wide variety of molecules have been targeted by *in vitro* selection experiments yielding specific aptamers. Many small organic molecules with molecular weights of 100-1000 Da have been shown to be good targets for selection. A variety of Aptamers are targeted against peptides or proteins, even those that do not normally interact with nucleic acid within their natural context.<sup>24</sup> This includes antibodies, phospholipases, hormones and growth factors. Aptamers can also be selected against targets (such as toxins or prions), which are non-immunogenic or toxic. Furthermore, aptamers binding a variety of clinically important substances as well as micro-organisms and parasites have been isolated. A compilation of more than 1100 sequences of aptamers is found in The Aptamerdatabase@The Ellington Lab.<sup>25</sup> The majority of aptamers, which have been described, are RNA molecules. Some examples of targets for isolated aptamers are shown in Table 1.

#### Binding properties and structure of aptamers

Important characteristics for the success of analytical and diagnostic assays or therapy protocols based on aptamers are the affinity and the specificity of the nucleic acid that provides molecular recognition. Aptamers have shown a very high affinity and selectivity for their targets, with dissociation constants (K<sub>d</sub>) comparable to those of some monoclonal antibodies, sometimes even better. The K<sub>d</sub> values of aptamers differ depending on the nature of the target substance. Normally, aptamers have K<sub>d</sub>s between 10 nM and 10 pM for proteins, which are usually present in cells at concentrations between μM and nM. For small molecules, aptamers have K<sub>d</sub>s between 10 μM and 10 nM (see Table 3). Small molecules are present in cells at concentrations far higher than protein concentrations; perhaps M to M.<sup>26</sup> During the selection process there is an enrichment of aptamer sequences, based on binding of the target. This causes the three-dimensional structure of the aptamer complexes to reflect optimized scaffolds for specific target recognition. Aptamers bind their targets with high selectivity by discriminating closely related molecules from their targets on the basis of small structural changes. An example is a theophylline aptamer with a 10,000-fold lower affinity for caffeine, which differs

in only a methylgroup.<sup>27</sup> The architecture of aptamer-target complexes is valuable for the study of molecular recognition processes. Furthermore, structural data on aptamer-target complexes will be especially helpful for the rational exploration and optimization of new drug targets. Three-dimensional structures

have been determined at high resolution for a number of aptamers in complex with their cognate ligands. Appendix 1 contains aptamer-ligand complexes for which structural data are available in the Protein Data Bank.<sup>28</sup> The affinity constants (Kd) for aptamers are taken from Hermann and Patel.<sup>29</sup>

**Table 1: Examples of targets for nucleic acid aptamers. Unless otherwise indicated, the examples have been taken from The Aptamer Database@The Ellington Lab.**

Target Type	Target	Nucleic acid type	
		DNA	RNA
Inorganic	Zn <sup>2+</sup>	-	X
	Malachite Green	-	X
	Vitamin B12	-	X
Nucleic	Adenine	-	X
	TAR element of HIV-1	X	X
	Yeast Phe-tRNA	-	X
Organic	MDA (4,4'-methylenedianiline) <sup>31</sup>	-	X
	L-Arginine	-	X
	Cocaine	X	-
	Sialyl Lewis X	-	X
	Tetracycline	-	X
Peptides	Rex-peptide of HTLV-1	-	X
	IFN-gamma	X	-
	Substance P	-	X
Proteins	Ricin A-chain	-	X
	RNase H, HIV-1 virus	X	-
	Tax protein, HTLV virus	-	X
	NF-kappa B	-	X
	Coagulation factor Factor VIIa	-	X
	G-protein coupled receptor of neurotensin, NTS-1	-	X
	Human acetylcholinesterase	-	X
	Myasthenic autoantibody mAB198	-	X
Others	Anthrax spores	X	-
	African trypanosomes	-	X
	Tumour microvessels (rat brain)	X	-

**Table 2: Examples of aptamers for which structural data are available in the Protein Data Bank**

Target	Aptamer	Affinity, Kd ( $\mu$ M)	Pdb ID
ATP	DNA	~6 (AMP)	1AW4
Neomycin B	RNA	~0,115	1NEM
Thrombin	DNA	~0,025	148D
Coat protein, MS2	RNA	~0,002	5MSF
	RNA	~0,082	6MSF

## CLINICAL APPLICATIONS

### Aptamers and gene silencing

When conjugated to aptamers, the mediate targeted delivery of small interfering RNAs (siRNAs) can be greatly improved resulting in higher therapeutic efficacy and safety<sup>30, 31</sup>. This approach has the advantage that therapeutics can be composed entirely of RNA. The aptamer-siRNA chimeric RNAs are capable of cell type-specific binding and delivery of functional siRNAs into cells<sup>32</sup>. For example, the aptamer portion of the chimer as mediates binding to PSMA (prostate-specific membrane antigen), a cell-surface receptor over expressed in prostate cancer cells and tumor vascular endothelium, whereas the siRNA portion targets the expression of survival genes. When applied to cells expressing PSMA, these RNAs are internalized and processed by Dicer, resulting in depletion of the siRNA target proteins and cell death. In contrast, the chimeras do not bind to or function in cells that do not express PSMA. These reagents also specifically inhibit tumor growth and mediate tumor regression in a xenograft model of prostate cancer<sup>33</sup>. These studies demonstrate a novel approach for targeted delivery of siRNAs with numerous potential applications, including cancer therapeutics. Short aptamers (25–35bp) have been generated that bind to a wide variety of

targets with a high affinity. It should be possible to design siRNA-aptamer chimera as with longer strands (45–55bp) that are within the range of technical and commercial feasibility for synthesis, however, molecules longer than that may be difficult to synthesize on a commercial scale.

### APTAMERS FOR TARGET VALIDATION

Target validation is the determination that a drug target is involved in disease pathology and is potentially a point of intervention for new drugs. The conventional approach for determining the relevance of a given protein to a particular disease is to inhibit the expression of the gene and observe the effects on an appropriate disease model. Target validation methods therefore have been devised that attack gene expression at the DNA, RNA, and protein levels. (One method that attacks gene expression at the DNA or genetic level is to construct a knock-out mouse by homologous recombination. The advantages of the gene knockout approach are that gene expression is completely abolished and that the function of a given gene can be examined in every system of the organism simultaneously. Two major disadvantages of gene knockouts are that the approach is extremely laborious and that results observed in mice may not translate to humans. Also, it is disquieting that

knockout mice often show a phenotype that is inconsistent with extensive *in vitro* research. These conflicting phenotypes often manifest as either a very mild phenotype, possibly as a result of an

additional redundant gene, or as an animal that has developmental defects resulting in lethality. Validating a target that falls into either the mild or lethal categories is extremely difficult.<sup>34</sup>

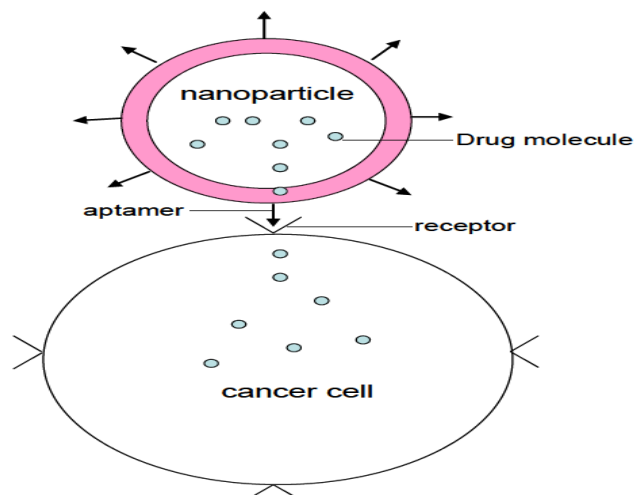


Fig. 5: Aptamer conjugated Nano particle for delivery of drugs to treat cancer

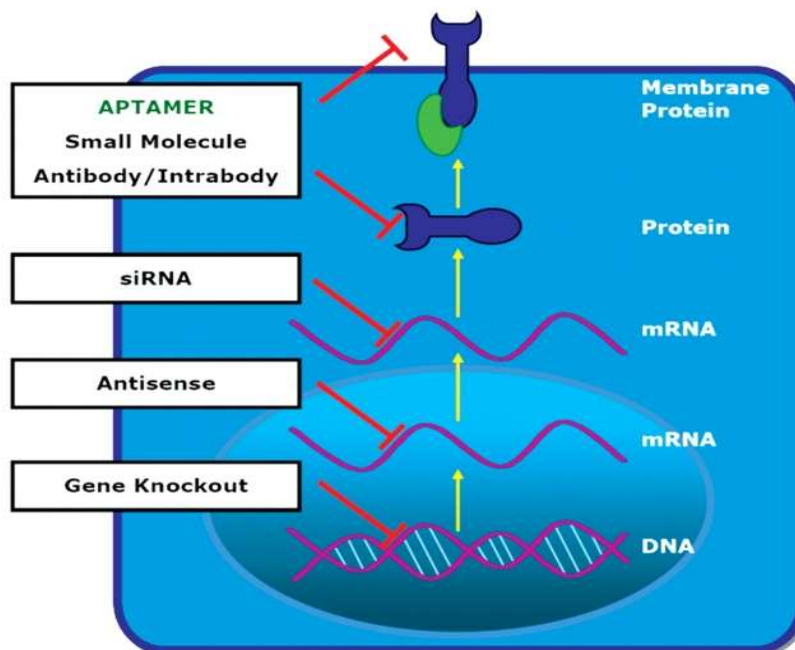


Fig. 6: Levels of gene expression and corresponding target validation techniques

#### Aptamers and antibiotics

One way of studying the mode of action of RNA-binding antibiotics is to determine the structure of RNA-antibiotic complexes. The natural target sites of RNA-binding antibiotics are often too large for structural characterization. Two methods of reducing the RNA length are dissection of the natural target RNA to that of the antibiotic RNA binding domain or isolation (via *in vitro* selection) of small RNAs that bind specifically to antibiotics. Aptamers are perfect tools for studying the interaction of small ligands with RNA. RNA aptamers that bind to antibiotics include binders to tetracycline, tobramycin, lividomycin, kanamycin B,<sup>35</sup> moenomycin A,<sup>36</sup> neomycin B,<sup>37</sup> streptomycin,<sup>38</sup> spectinomycin,<sup>39</sup>

#### Tools for purification

Affinity chromatography is a powerful method for rapid selection and purification of compounds from complex mixtures. The affinity

and specificity of aptamers is now sufficient for them to be used as affinity chromatography media. Two DNA aptamers were covalently linked to fused silica capillary columns to serve as stationary-phase reagents in capillary electro chromatography. Separations of binary mixtures of amino acids (D-trp and D-tyr), enantiomers (D-trp and L-trp), and polycyclic aromatic hydrocarbons (naphthalene and benzo [a] pyrene) were achieved.<sup>40</sup> Moreover, aptamers immobilized in fused silica columns have been used to affinity purify the protein L-selectin. Aptamers have also been used for separation of adenosine and analogues. A biotinylated-DNA aptamer that binds adenosine and related compounds in solution was immobilized by reaction with Streptavidin. The Streptavidin was covalently attached to porous chromatographic supports. The aptamer medium was packed into fused silica capillaries to form affinity chromatography columns. It was demonstrated that the column could selectively retain and separate cyclic-AMP, NAD<sup>+</sup>, AMP, ADP, ATP, and adenosine, even in complex mixtures such as tissue extracts.

## Diagnosis and Analysis

### Diagnosis of infectious diseases

Aptamers are new alternatives to antibodies in the diagnostics of pathogenic organisms. Aptamers directed against spores of *Bacillus anthracis* vaccine strains Sterne and A 16R have been isolated and characterized. Furthermore, a patent has been published concerning an aptamer chip, containing spore-binding aptamers. At binding of spores to the aptamer chip an electric and/or photochemical change occurs, giving a specific signature. A lot of attention has been focused on developing new technology for diagnosis and treatment of infections caused by the Hepatitis C virus. A number of aptamers directed to various targets of this virus have been reported. One of the targets is then on-structural protein 3, which has two distinct activities, as a protease and a helicase. Binding of the aptamer inhibited the protease activity. The level of inhibition in cells was increased four-fold by construction of a tandem chimeric aptamer. Hepatitis C virus RNA replicase has been identified in patient's blood using an RNA aptamer immobilized on magnetic bead and analyzed by MALDI-TOF mass spectrometry. Aptamers directed to toxins recently, DNA aptamers directed to the Cholera toxin and the Staphylococcal Enterotoxin B (SEB) have been isolated. The main purpose was to use these aptamers as tools for detection.

SEB is involved in several severe disease patterns and it has been used as a target for the generation of biologically stable mirror-image aptamers, Spiegelmers.<sup>71</sup> Since the full-length protein is not accessible to chemical peptide synthesis, a stable domain of 25 amino acids was identified as a suitable selection target. The isolated Spiegelmer bound the whole protein target with only slightly reduced affinity and had a dissociation constant of 420 nM. These data also demonstrate the possibility to identify Spiegelmers against large protein targets by a domain approach. Verotoxins are produced by *Enterohemorrhagic Escherichia coli*, and are classified into two types based on their different amino acid sequences. Verotoxin-1 is the same toxin as the Shiga toxin produced by *Shigella dysenteriae* type 1, and is called the Shiga-like toxin. Recently, a DNA aptamer directed against the toxin was isolated. A high affinity ligand capable of selectively recognizing Verotoxin-1 can be advantageously used for detection and quantitative determination of the toxin. Microcystin is a toxin generated from the *Cyano* bacteria causing water blooms. A DNA aptamer showing specific binding to this toxin has been isolated. The direct detection range using an immobilized aptamer and a surface Plasmon resonance biosensor was 50-1000 µg/ml.<sup>41</sup>

### Diagnosis of brain diseases

Alzheimer's disease is correlated with the deposition of amyloid peptides in the brain of the patients. The amyloid is thus a major target in the search for novel diagnostic and therapeutic approaches. High-affinity RNA aptamers against the amyloid peptide have been isolated from a combinatorial library and the binding of the RNA to the amyloid fibrils was confirmed by electron microscopy.<sup>42</sup> The chemical synthesis of these nucleic acids enables tailor-made modifications. By introduction of specific reporter groups these RNAs can become suitable tools for analytical and diagnostic purposes.

### Diagnosis of cardiovascular diseases

Elevated total homocysteine in plasma or serum is used as a diagnostic marker of certain forms of cardiovascular disease. S-adenosyl homocysteine (SAH) is a key intermediate in the metabolism of the amino acid methionine and also the direct and only source of homocysteine. An RNA aptamer binding SAH with an affinity and selectivity comparable to that of a monoclonal antibody and with a high diagnostic potential has been isolated.<sup>43</sup>

### Biosensors based on aptamers

Aptamers have been used extensively in biosensing applications. Aptamers are well suited to application in biosensors to specifically detect a large variety of molecules like, for example, proteins, metabolites, amino acids, and nucleotides. Aptamers can also be utilized when the targets, such as toxic agents or non-immunogenic

substances, are hard to address by using antibody sensors. The first biosensor using an aptamer was designed for measurements of L-adenosine.<sup>44</sup> An RNA aptamer directed against L-adenosine was attached through an avidin-biotin bridge to the core of a multimode fiber. The sensor indicates binding of FITC-labeled L-adenosine and real-time fluorescence measurements are performed by the competition with the L-adenosine (unlabeled) of the sample in the micro mol arrange.

### Aptamers specific for various signalling targets and cancer therapy

The vascular endothelial growth factor (VEGF) is an example of a factor involved in tumor growth. It has been shown to stimulate the growth of vessels into the tumors, which are growing more rapidly. Another example is the VEGF stimulation of growth of new vessels in the eye which cause age-related sight defects. RNA aptamers, which specifically bind and inactivate VEGF *in vitro*, have been isolated. The effect of an anti-VEGF aptamer was initially tested in monkeys with introduced eye defects. The test showed that the aptamer retained activity after 28 days in the vitreous body, but that the half-time was 94 hours. No toxicological effect was noted in the eye and no antibody formation occurred. Based on the results the authors recommended a dose of 1-2 mg aptamer per eye per month. A clinical study (presented in 2002) on humans with injection of anti-VEGF-aptamers in the eye showed that 80 % of the patients (15) retained or improved the eye sight and they had no side-effects.<sup>44</sup>

## CONCLUSION

The encouraging results obtained by aptamers based on their intrinsic properties and the versatility of the SELEX procedure generates new applications in diagnostic as well as therapeutic fields. The ability to discriminate between two closely related targets, for example a cancerous cell and an untransformed cell of the same tissue type, makes aptamers suitable as imaging reagents for noninvasive diagnostic procedures. Even though the combined use of aptamers with Nano particles is still a novel concept, a growing number of valuable results have been reported contributing to cancer medicine and molecular medicine and opening up a huge potential for numerous clinical applications. Unlike many other drug molecules, use of oligonucleotides like aptamers as therapeutic aids gives rise to specific action against the drug target. The chances of side effects are minimized. Using aptamers untouched diseases like cancer, AIDS, diabetes etc can also be targeted. Proofs that aptamers can specifically inhibit bio medically relevant proteins and modify cellular metabolism augur well for future drug development.

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