

## **Aptamers as an Emerging Player in Biology**

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## ***Abstract***

Aptamers are single stranded nucleic acid molecules which are emerged as chemical rival of antibodies. They are generated through an *in vitro* evolution process called SELEX. These single stranded nucleic acid aptamers can acquire various secondary and tertiary structures to bind their cognate targets with high affinity and specificity. In the last decade aptamers are regarded as an emerging player in biology attributed to their various biological applications including but not limited to sensing, diagnostics, biomarker discovery, drug delivery, bio-imaging and molecular therapy. Further ease in synthesis and modification make them an attractive candidate for various biological applications. In this review we have summarized various applications of aptamers in biology.

Key words: aptamers, diagnostic, imaging, biomarker, therapy, drug delivery, sensors

## ***Introduction***

Aptamers are oligonucleotides, such as ribonucleic acid (RNA) and single-strand deoxyribonucleic acid (ssDNA) that can bind to their targets with high affinity and specificity due to specific secondary and tertiary structures. The whole idea of nucleic acids binding with a particular target emerged in the 1980s from the research on human immunodeficiency virus (HIV) and adenovirus. These viruses encode a number of small structured RNAs that bind to viral or cellular proteins with high affinity and specificity [1-4]. Aptamers were first reported independently by two research groups- Gold's group and Szostak's group in 1990. The Gold lab selected RNA ligands against T4 DNA polymerase; and the Szostak lab, generated RNA ligands against various organic dyes [5,6]. Both these groups employed an *in vitro* selection process called Systematic Evolution of Ligands by EXponential enrichment (SELEX) for the generation of aptamers (Figure 1). Since then, SELEX is being used as a basic technique for the generation of aptamers. Continuous efforts are being put towards improving the SELEX

process and development of more sophisticated and faster techniques of aptamer screening such as Capillary Electrophoresis microfluidic chips (CE-Microfluidics), Cell-SELEX etc[7,8]. Also, methods such as AFM, electrophoretic mobility shift assays (EMSA), and surface plasmon resonance (SPR), have been performed in connection with SELEX [9-12].

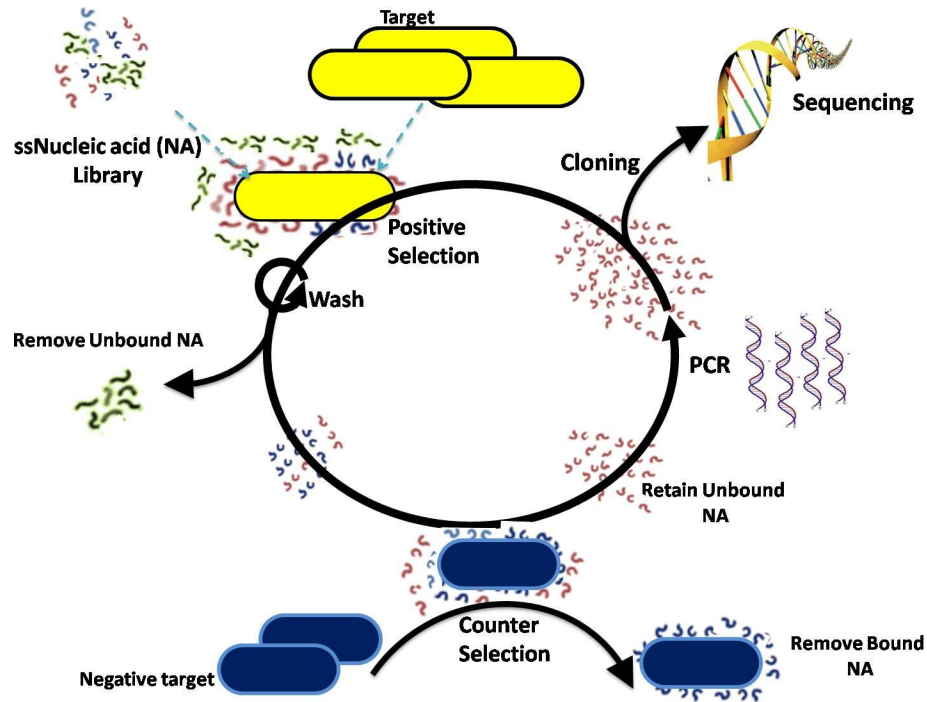


Figure1: Schematic illustration of SELEX process

Aptamers are different from antibodies, yet they mimic properties of antibodies in a

variety of diagnostic formats. As an emerging class of molecules that rivals antibodies in both therapeutic and diagnostic application, aptamers have become the focus of attention for their novel properties, such as highly selective and specific target recognition and binding. Aptamers offer additional advantages with respect to cost, stability, ability to sustain reversible denaturation, lack of toxicity and immunogenicity biocompatibility and ease and speed of synthesis against a variety of targets including proteins, small molecules and whole cells. These physical and chemical properties make aptamers ideal candidates for a wide variety of applications. Various applications of aptamers are portrayed in Figure 2.

Aptamers have been most commonly exploited for the use as biosensors where the aptamer functions as the molecular recognition probe for the detection of a number of target molecules. A transducer that works on different principles detects the signal [13]. In addition, several nanomaterials like such as gold nanoparticles, DNA hydrogels, carbon nanotubes (CNTs), quantum dots (QDs) have also been used in combination with aptamers for the development of tools for biodiagnostic applications [14]. In 2004, the Food and Drug Administration (FDA) approved Macugen, a vascular endothelial growth factor (VEGF) specific aptamer, for the treatment of neovascular (wet) age-related macular degeneration (AMD) [15]. Since then, aptamers have attracted significant attention as therapeutic agents, anti-infective agents, drug delivery vehicles, bio-imaging agents in cancer and infectious disease detection and point of care diagnostics [16-25]. This review focuses on the development of aptamer technology as an eminent player for diagnostic and therapeutic applications in biology.

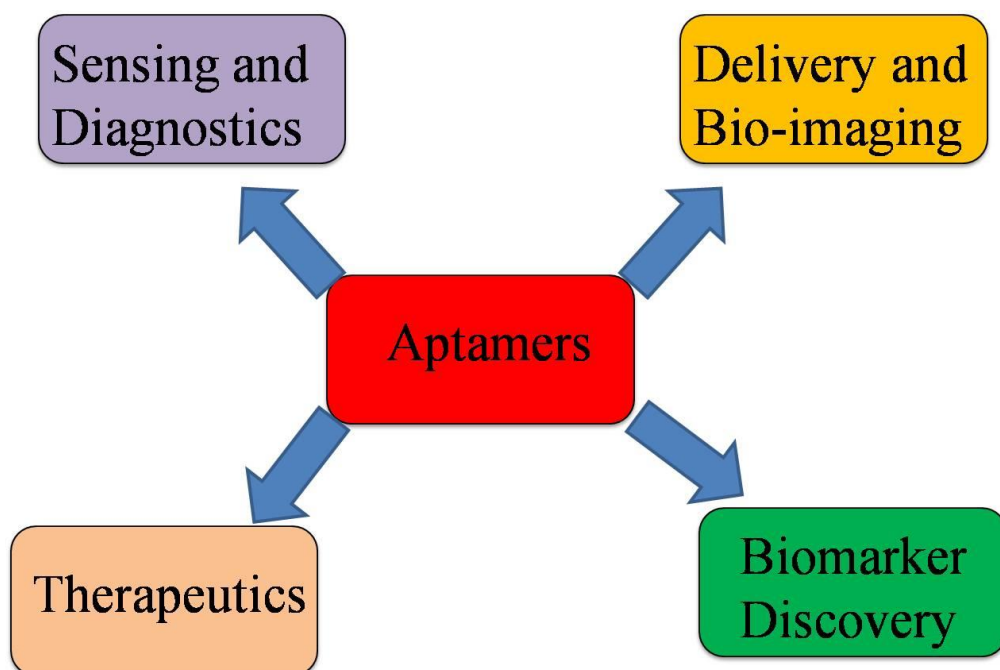


Figure 2: Various application of aptamers

### ***Aptamers as aptasensor***

A biosensor is an analytical device for the recognition/detection of an analyte. It has two main components (i) a ligand that acts as a molecular recognition element (MRE), which produces a primary signal, upon successful binding to the target; and (ii) a transducer that acts as a detector element, which converts the signal obtained from the MRE into a detectable form. A biosensor that is based on aptamers as a recognition element is called

an aptasensor. Different types of aptasensors have been developed based on the principle of detection including optics, electrochemistry, mass based detection [13]. They detect a range of targets including proteins, small molecules, intracellular analytes, bacterial pathogens, and mammalian cells etc. [26-29].

Aptasensors were first used in 1996 as the selective component in an optical sensor application where they were part of a model system consisting of human-neutrophil-elastase-coated beads that interact with fluorescent-tagged aptamers [30].

The optical sensors have gained the most popularity because of their relatively high sensitivity, fast response and simple mode of operation. Different optical aptasensors are based on different working principles-colorimetry, fluorescence, chemiluminescence, Surface Plasmon Resonance (SPR), Surface Enhanced Raman Scattering (SERS), Dynamic light scattering (DLS), ellipsometry. Many optical aptasensors for various small biomolecules have been developed, such as ATP, adenosine, cocaine, dopamine, NADP, ochratoxin A, theophylline, flavin mononucleotide, tyrosinamide, kanamycin, oxytetracycline, glucose, bisphenol A etc [31].

Galarreta et al. reported an aptasensor for ochratoxin A assisted by ellipsometry. They immobilized an aptamer for ochratoxin A on the surface of gold nanostructures. In the presence of ochratoxin A, the aptamer transformed its form from coil to its specific secondary structure that was observed as a change in ellipsometry[32].

Ma et al. developed an electrochemical aptasensor for detection of *Salmonella* using a *Salmonella*-specific recognition aptamer. It was based on the changes in electrochemical properties between the electrode and the electrolyte observed when *Salmonella* was bound to the aptamer linked onto the carbon electrode via gold nanoparticles. The electrochemical impedance spectrum was measured to quantify the *Salmonella*. A detection limit as low as 3 CFU/mL was obtained. This novel method is specific and fast, and it has the potential for real sample detection [33].

Aptamer based quartz crystal microbalance (QCM) biosensors were used for the detection of immunoglobulin E (IgE), human chorionic gonadotropin and urinary protein [34-36]. The detection of the analyte was observed in terms of the frequency shifts of the

QCM. This biosensor was highly specific, sensitive (detection limit up to 50pM) and could be recycled with little loss of sensitivity.

### ***Aptamers as delivery agent***

The active targeting of drugs to a particular cell, tissue or disease-specific manner represents a potentially powerful and important technology with widespread applications in medicine, including the treatment of many infectious diseases and cancers. The properties of high affinity and specificity for their targets make aptamers the molecules of choice to be used as delivery agents. Aptamers can be designed as targeting ligands, particularly when generated by cell-based SELEX, where live cells are used to select aptamers. The aptamers are selected for cell surface molecules which include the mainly cell surface proteins. The aptamers thus generated can differentiate diseased cells from the healthy one, thus enabling the selective delivery of therapeutic compounds to target cells. Zhou and coworkers demonstrated the cell specific delivery of anti-human immunodeficiency virus (anti-HIV) siRNAs through fusion to an anti-gp120 [37]. The envelope glycoprotein is expressed on the surface of HIV-1-infected cells, allowing binding and internalization of the aptamer–siRNA chimeric molecules. Cells expressing HIV-1 gp120 specifically take up the chimera and Dicer processes the siRNA; this releases an anti-tat/rev siRNA, which, in turn, inhibits HIV replication.

Another example of aptamer mediated targeted delivery was shown by Dassie et al. [38]. They bound an anti-PSMA RNA aptamer to a 21-mer siRNA portion, resulting in a chimera that targets polo-like kinase 1 (PLK1) and BCL2, two survival genes overexpressed in most human tumors. The aptamer portion of the chimeras selectively binds to PSMA, whereas the therapeutic siRNA portion interferes and knocks down gene expression and inhibits the growth activity of the cancer cells.

In addition, aptamers were also used as a component of polyvalent therapeutics termed as “Poly-Aptamer-Drug”, which composed of multiple aptamer units and physically intercalated chemotherapy agents. Zhang et.al. used leukemia cell-binding aptamer and doxorubicin as a model system for the polyaptamer drug strategy. They demonstrated that the use of polyvalent aptamer is significantly more effective than its monovalent counterpart in targeting and killing leukemia cells due to enhanced binding affinity and

cell internalization via multivalent effects [39].

Liposomes have been used in order to increase the cell membrane penetration of aptamers. This liposome nanostructure can efficiently increase cell permeability and enhance drug delivery. Another advantage of liposomes is their ability to increase the plasma residence time of aptamers from several minutes to 23 hours [40].

Aptamer conjugated, multifunctional liposomes have been fabricated to encapsulate and deliver cisplatin to breast cancer cells (MCF-7). Here, polymeric nanocarriers in combination with aptamer mediated targeting, can be used to selectively deliver drug payloads to cancerous cells with high efficiency and specificity [41].

Several groups have reported the use of aptamers bound to nanoparticles such as gold and silver for targeted photothermal killing of cancerous cells. [42-46]. Also, other nanomaterials like CNTs, QDs and magnetic nanoparticles have been used extensively [47-50]. The emerging integration of aptamers with nanotechnology and chemical biology is envisioned to produce more versatile target-specific molecules, stimulate further new diagnostic and therapeutic nanotechnologies, and provide significant potential for many research and clinical applications in the near future.

### ***Aptamers as diagnostic tools and in biomarkers discovery***

Biomarkers can be expressed in different forms, including, for example, proteins unique to specific cell types and subtypes [51]. Proteins are the most useful form of biomarkers since they reflect the genotype and phenotype of a particular disease. Alterations of proteins within the cell, as well as overexpression and down regulation of certain proteins, can have profound effects on the cell as a whole. It is quite challenging to develop a biomarker system able to provide accurate evidence that a protein is a reflection of the physiological state of the cell at some defined stage. Traditional methods employed the use of antibodies which was limited for large scale screening of biomarkers [52]. However, detection systems based on aptamers have the ability to overcome such pitfalls, making them extremely valuable as a diagnostic tool and for discovery of biomarkers. Generation of aptamers using Cell-SELEX technology has become an effective tool for molecular medicine and biomarker discovery [8]. Aptamers selected in this manner can target overexpressed proteins on the cell surface and even detect small differences among



cell- surface proteins. This capability allows aptamers to differentiate unique cellular characteristics, particularly those between cancerous and noncancerous cells based on their unique cell-surface homology.

Mi et al. developed a unique approach to isolate RNA aptamers in vivo against tumor cells in living mice. The aptamer selected was used to identify the biomarker for hepatic colon cancer metastases. The aptamer binds to protein p68, an RNA helicase that has been shown to be unregulated in colorectal cancer [53].

Recently, Kim et al. used the cell-SELEX technique to isolate DNA aptamers against the glioblastoma brain tumour initiating cells with high affinity and specificity. Glioblastoma is classified as one of the most lethal cancers. Therefore, the selection of aptamers that can specifically bind the Cancer Stem Cells of Glioblastoma could help discover and characterize new biomarkers for these cells [54].

Aptamers can not only provide a way of discovering biomarkers that help to distinguish between cancer cells and normal cells, but they can also help to differentiate the stage of carcinogenesis within one subtype of cancer. A study conducted by Ostroff et al. using a DNA-based aptamer with very specific affinity for protein binding revealed 44 different biomarkers able to distinguish among Stage I–III lung cancer [55].

The aptamer technology can accelerate the discovery of new specific biomarkers that provide an insight to the cellular mechanisms and pathways; which would facilitate the development of highly specific and effective targeting ligands and platforms for diagnostic and therapeutic purposes.

### ***Aptamers as Therapeutic Agent***

In the 1980s, it was discovered that HIV and adenovirus contain several small RNA sequences or regions that can specifically bind to viral or cellular proteins with high affinity and inhibit viral replication. Functional studies indicated that such viral RNA-protein interactions could be exploited as competitive anti-viral therapeutics [1-4].

With the development of SELEX in the 1990s, several aptamers were quickly developed against important clinical targets such as vonWillebrand Factor (vWF), Platelet-derived growth factor (PDGF), E-selectin, Vascular endothelial growth factor (VEGF), Nuclear factor kB (NFkB), tenascin-C and Prostate Specific Membrane Antigen (PSMA) etc. The

aptamers were generated with a mind set to function as novel anti-coagulants and therapeutics in case of cardiovascular diseases, angiogenesis, diabetes and cancer [15, 56].

Pegaptanib (brand name Macugen), is an anti-vascular endothelial growth factor (VEGF) RNA aptamer and is the first nucleic acid aptamer approved by the US FDA. It was approved in December 2004 as a anti-angiogenic therapeutic agent for neovascular (wet) age-related macular degeneration (AMD), a disease of the eye which does not cause total blindness, but a loss of central vision, leaving only the side vision intact [15]. Other aptamers developed against clinically important targets are currently undergoing clinical trials, and hopefully in future there would be more aptamer based therapeutics.

Thrombin is a serine protease and a key activator of several proteins in the coagulation cascade. Nu172 is a DNA aptamer that selectively binds and inhibits thrombin. It is administered intravenously during acute cardiovascular surgical procedures to prevent formation of blood clots. This aptamer is in Phase II clinical trials for anticoagulation in heart disease treatments [57].

The von Willebrand factor (vWF) plays an important role in the clotting cascade by recruiting platelets to damaged arteries. ARC1779 is a DNA aptamer selected to bind to a region of vWF and blocks binding to platelet membrane glycoprotein Ib receptors, thus inducing antithrombotic effects [58]. It provides a potential therapeutic benefit in acute coronary syndromes and von Willebrand disease. Currently ARC1779 is in Phase II clinical trials for thrombotic microangiopathies and in patients with carotid artery disease undergoing carotid endarterectomy.

ARC1905 is an RNA aptamer selected to bind complement component 5 (C5), an important protein in AMD. The complement system is part of the innate immune system that is based on a number of proteins. When the system is triggered, a cytokine cascade is started which recruits inflammatory cells and activates the membrane attack complex (MAC). Inhibition of C5 by ARC1905 potentially inhibits the inflammation and angiogenic components of AMD [59]. ARC1905 is administered by intravitreal injection and is currently in Phase I clinical trials by Ophthotech.

AS1411 is a 26 nucleotide in length G-rich DNA aptamer considered to be the first

anticancer aptamer. AS1411 targets nucleolin, a nuclear matrix protein which can be found on the surface of cancer cells. It has been demonstrated to be effective in many different preclinical cancers such as human breast cancer, lung cancer, pancreatic cancer, and acute myelogenous leukemia [60-63]. It is currently in Phase II clinical trials for acute myeloid leukemia by Antisoma Research.

### ***Aptamers as bioimaging agent***

The features of aptamers also make them a favourable candidate to be used as bioimaging agents. Aptamers can be synthesized with specific functional groups on 5' or 3' termini for site-specific conjugation to imaging agents without significantly affecting their binding affinity.

The first reports of using aptamers for imaging were in 1997 to identify sites of inflammation. Using a rat model, Charlton and colleagues used radiolabelled NX21909, a DNA aptamer which targets elastase and compared its benefits with a clinically proven, radiolabelled IgG-based imaging agent [64]. Radiolabelled NX21909 was able to specifically accumulate at the site of inflammation and achieve a high target to background ratio.

DNA aptamer A9 and A10 targeted to prostate specific membrane antigen (PSMA) have been used in combination with QDs and super-paramagnetic iron oxide NPs for the recognition of cell receptors as well as optical imaging or MRI of the cancerous cells [65-66].

*In vivo* studies regarding imaging of tumors inside mice have also been made possible with the help of aptamers. TD05, an aptamer that specifically binds to Ramos cells (Human Burkitt's lymphoma cell line) was modified with fluorophores, successfully exhibiting aptamer fluorescence in an area overlapping with the site of the tumor in the mice [67].

According to recent report, an activatable aptamer based on sgc8 DNA aptamer which targets a cell membrane protein on CCRF-CEM cancer cells (Human T cell lymphoblast-like cell line) was used as an imaging probe [68]. This strategy exploits the property where the aptamer is able to adopt a new conformation upon binding of its

target. The optical signal is quenched when it is unbound, but once the aptamer binds its target a conformational change occurs, thereby resulting in fluorescence.

Bioimaging using aptamer technology provides a new direction for imaging of biomolecules. It offers rapid uptake by the target molecules, faster clearance of the residual aptamers and superior tumour imaging. In addition, it reduces the toxicity to normal tissues, which was often observed when radiolabeled antibodies were used.

### ***Conclusion***

Aptamer technology is evolving rapidly and is becoming more accessible by the introduction of new developments including for example improvisation and automation of the SELEX method making the selection process faster and more reproducible. In addition, the inherent properties of aptamer and the ease of modification make it the molecule of interest for a variety of applications. The advancements in the fields of nanotechnology, chemical biology and RNA interference have taken the aptamer technology to a new horizon with a plethora of applications in different spheres of life. In this review, we have tried to give a glimpse of the applications of aptamers in the field of biology. Aptamers have truly emerged as a strong player in form of biology including but not limited to sensing, diagnostics and therapeutics. More recently, the aptamer technology is being used extensively for biomarkers discovery and targeted cancer diagnosis in order to find solutions to some of the major problems faced today.

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