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Research Article

APTAMERS: A NOVEL APPROACH FOR BIO-IMAGING, BIO-SENSING AND TARGETED DRUG DELIVERY SYSTEMS

NISHA UPADHYAY*[1], MAULESH VYAS[1], ATANU BEHERA[1], MEGHA SHAH[2], DHANANJAY MESHRAM[2]

Department of Pharmaceutics[1], Pioneer Pharmacy Degree College[1], Department of quality assuarance [2], Pioneer Pharmacy Degree College, Email: nishau188@gmail.com

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ABSTRACT

This review describes recent progress made in the aptamer and application of biomedically relevant aptamers and relates them to their future clinical prospects.

Aptamers are single-stranded nucleic acid or amino acid polymers that recognize and bind to targets with high affinity and selectivity. In nature they exist as a nucleic acid based genetic regulatory element called a riboswitch. Aptamers, simply described as chemical antibodies, are synthetic oligonucleotide ligands or peptides that can be isolated in vitro against diverse targets including toxins, bacterial and viral proteins, virus-infected cells, cancer cells and whole pathogenic microorganisms. They are isolated by the technique called SELEX- systematic evolution of ligands by exponential enrichment. The applications of aptamers range from diagnostics and biosensing, target validation, targeted drug delivery, therapeutics, templates for rational drug design to biochemical screening of small molecule leads compounds, in virology,as novel radio pharmaceuticals.

Keywords: aptamers, systematic evolution of ligands by exponential enrichment, biosensing, oligonucleotide ligands

INTRODUCTION

Aptamers are single stranded short oligonucleotide sequence which is basically made up of nucleic acid with high affinity to specific targets, like ions, whole cells, peptides, proteins, etc. Aptamers have been generated without difficulty that bind to organic dyes, drugs, amino acids, nucleotides such as ATP, vitamins, pharmacologically important proteins such as substance P16, the anticoagulant thrombin17, growth factors, proteases, and several other small and large proteins and enzymes [1,2] Some of the distinguished properties possessed by aptamers are that they can remain stable at the high temperature [3,4]and high pH.[5]. Aptamer mainly comes from the latin language “aptus” means to fit, are artificial specific antibody.[6].

An affinity of the aptamers depends upon its target type. They had also shown their affinities in nano and pico molar concentrations. [7] . Often aptamers are called chemical antibodies, that are poised to take on the monoclonal antibodies in therapeutics, diagnostics, treatment of cancer[8], Alzheimer disease [9], isoforms of prion proteins ,. One reason for the tremendous interest generated by aptamers is the practical advantages of aptamers over antibodies, as they neither exhibit toxicity nor immunogenicity[10].

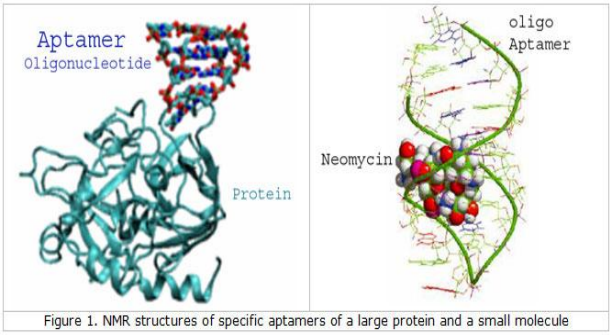

Figure-1: NMR structure of the aptamer.[96]

TYPES OF APTAMERS

There are basically three types of the aptamers:

DNA Aptamers

The DNA aptamers that bind specifically to the human protein fractalkine (FKN) are better suited for targeted drug delivery than other technologies such as antibodies and are non-immunogenic. Fractalkine is involved in different types of cancer such as lymphoma, prostate, lung, and colorectal cancer. [11] DNA aptamers Useful for increasing drug efficacy by targeting correct cells and decreasing drug side effects caused by non-specific delivery to the healthy cells Production of DNA aptamers is not easy as RNA aptamers as they form less 3D structure than RNA aptamers and they bind to the target with the entire sequence. [12]

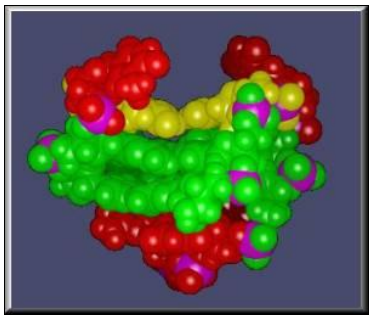

Figure-2:DNA aptamers.[97]

RNA Aptamers

RNA aptamers can fold into complex structures and bind with high affinity and selectivity to various macromolecules, viruses, and cells. They are isolated from a large pool of nucleic acids by a conceptually straightforward iterative selection process called SELEX. Diverse range of structures can be obtained with RNA as compared to DNA, and they are more beneficial for screening process. [13] FDA approved in 2005 and a number of novel RNA aptamer-based therapeutics are currently undergoing clinical trials for treating diseases such as macular degeneration, choroidal neovascularization, intravascular thrombus, acute coronary syndrome, von Willebrand factor related disorders, von Hippel–Lindau syndrome (VHL), angiomas, acute myeloid leukemia, renal

cell carcinoma, non-small cell lung cancer, thrombotic thrombocytopenic purpura, and several others. [14]

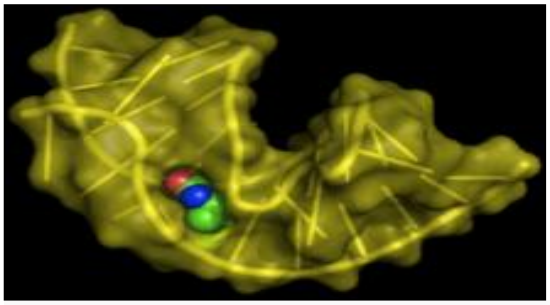

Figure3.Structure of an RNA aptamer specific for biotin[98]

Peptide-Based Aptamers

Peptide aptamers are proteins that are designed to interfere with other protein interactions inside cells. [3] They consist of a variable peptide loop attached at both ends to a protamersein scaffold. This double structural constraint greatly increases the binding affinity of the peptide aptamer to levels comparable to an antibody's.[15] Peptide aptamer selection can be made using different systems, but the most used is currently the yeast two-hybrid system. Peptide aptamer can also be selected from combinatorial peptide libraries constructed by phage display and other surface display technologies such as mRNA display, ribosome display, bacterial display and yeast display. [16]

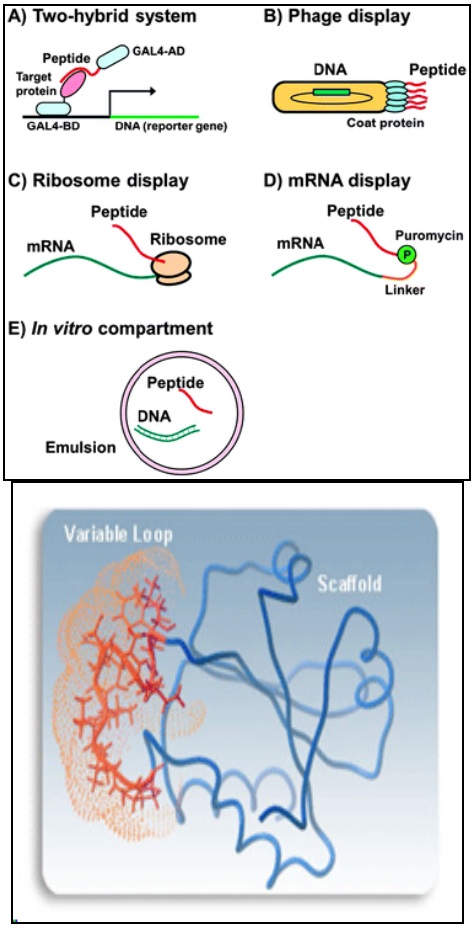

Figure4 A: Combinatorial peptide libraries using surface technologies. [99]B: RNA Aptamer [100]

SCREENING METHOD FOR APTAMERS: SELEX

SELEX (systematic evolution of ligands by exponential enrichment) is a technique used for the screening of the single stranded DNA or RNA ligands from a random library of nucleotide sequences. [17] . The ligands which are selected via SELEX are called as aptamers. SELEX research was first reported in the 1990s by Gold and Ellington [17-20], and a typical process is as follows:

First of all single stranded DNA or RNA is synthesized. The sequence of oligonucleotides in the library is composed of random sequences in the middle and flanked by fixed sequences as primer binding sites. The length of the random region is normally between 20 to 40 base-pairs, which create a library with a large number of random sequences (1015 to 1016) [20-25]. Then this library is incubated with specific target molecule for binding. Then the unbound target molecules are washed away from that bound target molecule. Which are then eluted from that target molecule and amplified by polymerase chain reaction. this selection process is repeated for several times until the resulted sequences are highly enriched. The SELEX technology generates aptamers with a high binding affinity and specificity. [26-29] Aptamers are short single-stranded nucleic acid oligomers with a specific and complex three-dimensional structure [30] Based on their three-dimensional structures, aptamers can bind well to a wide variety of targets. Binding of the aptamer to the target is due to structural compatibility, electrostatic interactions, van der Waals interactions, and hydrogen bonding [31]. Since the discovery of aptamers , many researchers have used SELEX technique for selection of aptamers having high affinity and specificity for their target molecule[32-35]. Many of the selected aptamers shows affinities comparable to those observed for antibodies. Recently, researchers have moved to a microfluidic chip/system to perform SELEX that can be optimized, giving significant advantages in terms of increased speed and reduced costs [36].

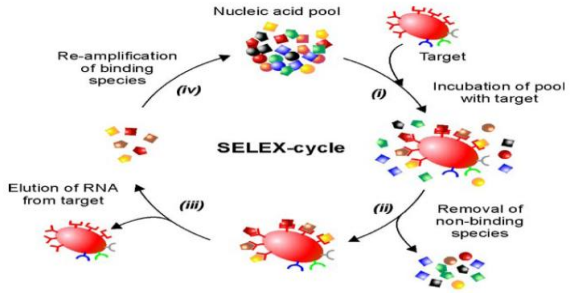

Figure5: SELEX technique for screening of Aptamers [101]

RECENT ADVANCES IN SCREENING METHODS OF APTAMERS

Typically, the SELEX method is an iterative process of incubation, separation, and nucleic acid amplification. Multiple rounds of selection are generally necessary to screen aptamers with a sufficient specificity and a high binding affinity, which requires more sample/reagent consumption and time (3 in CE MICROFUIDIC CHIPS). In order to accelerate this lengthy screening process, a wide variety of microfluidic incubation, separation and amplification techniques have been explored as a means to enhance the efficiency of aptamer selection, including capillary electrophoresis (CE), sol-gel isolation and magnetic-bead-based selection.

CE Microfluidic Chips

A library of ssDNA is incubated with the target molecules. Capillary electrophoresis is used to separate bound sequences. Binding nuclear acids are amplified by PCR and purified giving an enriched ssDNA pool which suitable for further rounds of selection. High-affinity aptamers are typically obtained after two to four rounds of selection.[37,38] Furthermore, the high partitioning efficiency of the CE-SELEX method in comparison with the traditional SELEX method decreases the number of rounds of SELEX to 1–3 rounds. In general, the incubation time of the CE-SELEX is less than an hour at room temperature. The short incubation time also maintains the activity of the targets. [39]

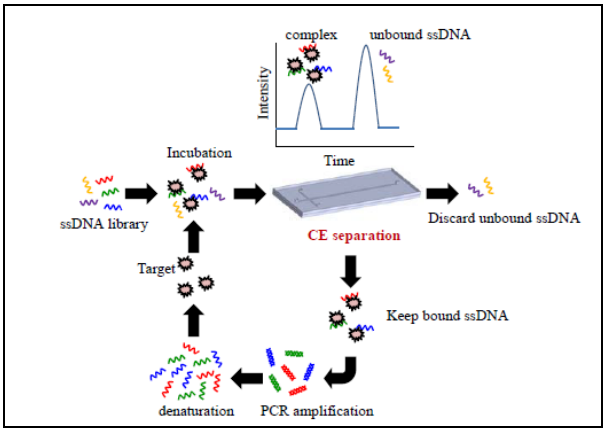

Figure6: CE-SELEX[37-39]

Sol-Gel Microfluidic Chips for Screening of Aptamers

A library of ssDNA is incubated with sol-gel arrays of proteins in a microfluidic system for efficient selection of ssDNA aptamers against target molecules. [40] The sol-gel microfluidic chips greatly improved selection efficiency, reducing the number of selection cycles needed to produce high affinity aptamers. Thus, it can lead to the isolation of aptamers specific to many of the target proteins, and improve the selection of aptamers to these specific proteins.[41]

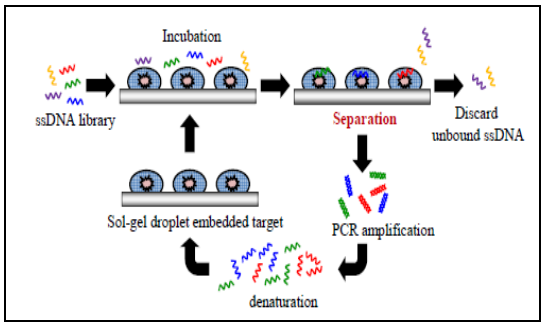

Figure7: SELEX processes in sol-gel microfluidic chips.[40,41]

3. Magnetic-Bead-Based Microfluidic Chips for Screening of Aptamers (42-44)

The microfluidic selection process begins with the incubation of the random ssDNA library with target proteins conjugated to magnetic beads. After incubation, the partitioning step to separate the target-bound aptamers from the unbound nuclear acids is performed in the microfluidic chip. [42] Stringent washing conditions than are imposed in the microchannel to continuously elute weakly- and unbound nuclear acids from the microfluidic chip. [43] After the separation, the external magnets are removed, and the beads carrying the selected aptamers are released from the device. The entire separation process with trapping, washing, and bead elution performs on the chip. Finally, the selected Aptamers are amplified via PCR. The use of magnetic beads to select aptamers in a microchannel has improved the efficiency of the SELEX method.[44]

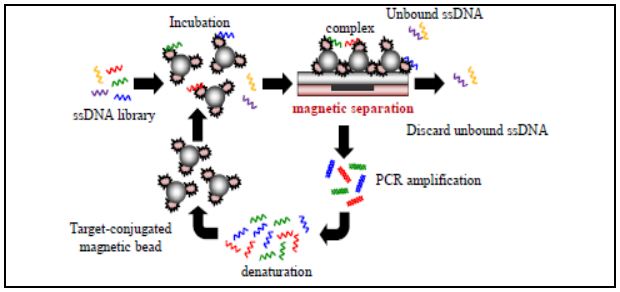

Figure8: SELEX processes in magnetic-bead-based

Microfluidic chips

Advantages of Aptamers [45]

- Can disrupt protein- protein interactions.
- Aptamers therapeutics which are used are given by the subcutaneous route.
- They are stable at room temperature so their storage at room temperature is possible.
- Aptamers have wide therapeutic margins, more stable, have modulated pharmaco-kinetic activity.
- Very low toxicity and low immunogenic activity.
- Can produce chemically and in readily scalable process.
- Small sized aptamers can easily and efficiently get entered into the biological compartments.
- Aptamers reversibly get denatured and phosphodiester bond is more stable chemically.

Disadvantages of aptamers [46-47]

- Pharmacokinetic properties and other systemic properties are very hard to determine and are variable. [46]
- They have small size, so they can easily pass from the renal filtration, thus they have a very short half-life. Some un-modified aptamers are also highly susceptible to serum degradation. This technology is now covered by a single intellectual property portfolio. [47]

Strategies to overcome Aptamers limitations (48-50)

Aptamers are collected for an activity and persistence under different physiological conditions while selecting or doing the structure activity relationship and medicinal chemistry study conducted after discovery. [48]

By adding conjugation partner we can increase circulating half-life like polyethylene glycol.[49]

Chemical changes made into the sugars enhance nuclease resistance. [50]

DIFFERENCE BETWEEN APTAMER AND ANTIBODY.(51-58)

- Aptamers are capable of greater specificity and affinity than antibodies [51]
- They can easily be modified chemically to yield improved, custom tailored properties. For instance, a reporter and functional groups and PEG can easily be attached to the aptamer in a deterministic way. In fact, they can even be combined with antibodies [52,53].
- Similarly, their ADME properties can be readily tuned by conjugation to other groups (PEG, etc.).
- Their small size leads to a high number of moles of target bound per gram, and they may have improved transport properties allowing cell specific targeting and improved tissue penetration [54].
- They are much more stable at ambient temperature than antibodies yielding a much higher shelf life.[55]
- They can tolerate transportation without any special requirements for cooling, eliminating the need for a continuous cold chain. [56-58]

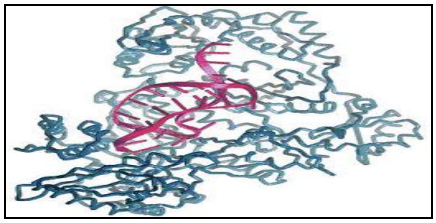

Figure9: Aptamers vs. Antibody[102]

Prospective Applications of Aptamers in the field of science

Therapeutic Potential of Aptamers [59-82]

Aptamers are getting developed as therapeutics in a variety of indications: the treatment of cancer [59], inhibiting proteins involved in Alzheimer disease [60], against apparently folded pathological isoforms of prion proteins [61,62] that cause Creutzfeldt-Jakob disease, against Mycobacterium tuberculosis [63], and against hepatitis C virus (HCV) [64-68].

Some of the best examples of therapeutic aptamers that have progressed through preclinical to clinical development include antithrombin aptamer (ARC183) [69,70], anti-platelet-derived growth factor (PDGF) aptamer (ARC127 [71-73] and an anti-von Willebrand factor aptamer (ARC1779) [74,75], for use as an anticoagulant during coronary artery bypass graft surgery, treatment of proliferative diseases such as intimal hyperplasia and thrombotic thrombocytopenic purpura and other thrombotic microangiopathies. These aptamers showed no acute toxicities, no evidence of genotoxicity, and no adverse effects in preclinical and clinical evaluations.

The most advanced aptamer in the potential treatment of cancer is AS1411. AS1411 aptamer binds nucleolin on the surface of cancer cells and induces apoptosis. [76,77] Another aptamer, called SM20, isolated against plasminogen activator inhibitor-1, has demonstrated in vitro therapeutic potential as an antimetastatic agent and could possibly be used as an adjuvant to traditional chemotherapy for breast cancer [78]. There are several aptamers that have been recently isolated for potential treatment of other cancers such as glioblastoma [(79)], T cell leukemia [79-81], and epithelial cancer cells in the breast, colon, lung, ovaries and pancreas [82]. Clearly, as aptamer research burgeons and more enter clinical trials, aptamers are likely to make a direct and significant contribution in the treatment of infectious and acute diseases, and chronic diseases such as cancer.

Aptamers as Templates for Rational Drug Design and Small molecule Lead Compounds [83-87]

Aptamers can also be used indirectly as templates for structure-based rational drug design and for biochemical screening of small molecule lead compounds. Generally, aptamers have a predilection of binding functional sites on target proteins in a manner similar to small molecule drugs. This property allows structural elucidation of the aptamere protein complex to provide insights on the identity of the active site that could then be used for rational drug design. Co-crystal structures of aptamer-thrombin have provided valuable insights into the molecular recognition mechanisms adopted by aptamers to their respective targets. [83,84]

The structure function relationship of gp120 binding and HIV-1 neutralizing aptamer has shown that the Aptamers sterically blocks the active site of the protein, and also interferes with protein activity by allosteric or non-competitive inhibition. Taken together, these biophysical properties of Aptamers make them desirable tools for structure-based rational drug design and for biochemical screening. [85]

Aptamers can also be used in high-throughput screening of libraries of small molecules, where the displacement of target bound aptamer by a small molecule in competition binding could be an effective method to identify hits. In recent studies, peptide aptamers have also been used for high-confidence validation of therapeutic targets and for guiding the discovery of small molecule drugs. [86,87]

Diagnostic and Bio sensing Potential of aptamers [88-92]

For aptamer-based diagnostics or biosensors, binding of an aptamer to its target molecule must be reported by attaching a signal transduction mechanism to the aptamer sequence. Allosteric aptamer-based fluorescence resonance energy transfer (FRET) for detection of molecular targets offers an excellent choice because of the convenience of detection, sensitivity and availability of numerous fluorophores and quenchers of nucleic acids.

A study has described linking an RNA aptamer isolated against C reactive protein, which is a biomarker for inflammation, sepsis and tissue necrosis, to a secondary antibody labelled with a dye or enzyme that is easily measured in an immunoassay [89]. This aptamer-based sandwich immunoassay provides the unique potential of detecting C reactive protein in serum samples of low-risk patients (1e3 mg/l) as well as high-risk patients (>500 mg/l). Through innovation and with the development of automated high-throughput isolation of aptamers, the aptamer-based sandwich immunoassays have evolved to high-throughput microarray-based diagnostics. [90,91,92]

POTENTIAL OF APTAMERS IN TARGETED DELIVERY OF DRUGS [92-95]

Nanoparticle-aptamer technology is dynamic and its application extends beyond diagnostics to targeted delivery of drugs. One of the common uses of nanoparticle aptamer bioconjugates is for targeted delivery of drugs to cancer cells.

Drugs are loaded inside the nanoparticles that are conjugated to the antigen-binding recognition element which bind specifically to antigen expressing cells. The nanoparticles are rapidly internalized once bound on the surface of diseased cells. The drugs encapsulated in the nanoparticles are released inside the cells and destroys the integrity of cells. [93]

Aptamer conjugated magnetic nanoparticles are proposed for in vivo isolation of specific cells and also for magnetic resonance imaging (MRI) techniques. Aptamer-magnetic nanoparticles were used to isolate mesenchymal stem cells and endothelial progenitor cells for isolating "ready to transplant" cells as promising methods. [94]

Nanomaterials for drug delivery are required to be stable and biocompatible. Current technology can supply materials that are capable of partially fulfilling these properties. Thus, combinations of surface coatings with complementary properties are proposed for developing better functioning delivery systems. In one example, gold nanorods were encapsulated within thin and uniform layer of silica shell for enhanced stability and a layer of PEG was used for biocompatibility. The composite nanoparticles were functionalized with PSMA aptamers and tested for their ability to target the nanoparticles to prostate cancer cells. [95]

CONCLUSION

With remarkable target specificity and sensitivity, versatile biophysical and pharmacokinetic properties, opportunities for alternative formulations and schedule of administration, improvements in process chemistry and manufacturing economics, including economies of scale, aptamers have found themselves a substantial niche and are becoming established as a promising new class of medicines. Nanoparticle-aptamer bioconjugates provide exciting prospects in medical nanotechnology for future disease treatments. The advancements in nanomaterial field together with cell- Selex procedures offer the controlled release polymer systems conjugated to aptamers tweaked to the any target diseased cell. Therefore, it is possible to produce a diverse range of specific and selective nanoparticle-aptamer bioconjugates.

REFERENCES

1. D Kiga et al., "nRNA Aptamer to the Xanthine/Guanine Base with a Distinctive Mode of Purine Recognition," *Nucleic Acids Research* 26 (1998): 1755–1760.
2. M Sassanfar and JW Szostak, "An RNA Motif That Binds ATP," *Nature* 364 (1993): 550–553.
3. Aptamers : The New Frontier In Drug Development? By larry gold,phD. and errol De souza, phD . Drug development and biotechnology health care, April-2007
4. (4)Deng, Q.; Watson, C.J.; Kennedy, R.T. Aptamer affinity chromatography for rapid assay of Adenosine in microdialysis samples collected *in vivo*. *J. Chromatogr. A* 2003, *1005*, 123-130.
5. Jayasena SD (1999) Clin Chem 45(9):1628–1650

6. 5 Kiga et al., "nRNA Aptamer to the Xanthine/Guanine Base with a Distinctive Mode of Purine Recognition," *Nucleic Acids Research* 26 (1998): 1755–1760.
7. Cox JC, Rudolph P, Ellington AD. Automated RNA selection. Biotechnol Prog 1998;14:845e50
8. F, Lurz R, Erdmann VA, et al. Selection of RNA aptamers to the Alzheimer's disease amyloid peptide. Biochem Biophys Res Commun 2002;290:1583e8.
9. . Gilch S, Schatzl HM. Aptamers against prion proteins and prions. Cell Mol Life Sci 2009;66:2445e55.
10. Neves, M.A.D.; O. Reinstein, M.Saad, P.E. Johnson (2010). "Defining the secondary structural requirements of a cocaine-binding aptamer by a thermodynamic and mutation study". *Biophys Chem*153: 9–16. doi:10.1016/j.bpc.2010.09.009. PMID 21035241.
11. Macaya, R. F., Schultze, P., Smith, F. W., Roe, J. A., Feigon, J. (1993), Thrombin-binding DNA aptamer forms a unimolecular quadruplex structure in solution. Proc. Natl. Acad. Sci. USA, 90, 3745-3749.
12. Bock, L. C., Griffin, L. C., Latham, J. A., Vermaas, E. H., Toole, J. J. (1992), Selection of single-stranded DNA molecules that bind and inhibit human thrombin. Nature, 355, 564-566.
13. Kyung-Nam Kang, Yoon-Sik Lee, RNA Aptamers: A Review of Recent Trends and Applications. Advances in Biochemical Engineering/Biotechnology Volume 131, 2013, pp 153-169
14. Long, S.; M. Long, R. White, B. Sullenger (2008). "Crystal structure of an RNA aptamer bound to thrombin". *RNA* 14 (2): 2504–2512. PMID 18971322
15. Colas, P., Cohen, B., Jessen, T., Grishina, I., McCoy, J. and Brent, R. (1996) *Nature*, 380, 548-550.
16. Geyer, C.R. and Brent, R. (2000) *Methods Enzymol.*, 328, 171-208.
17. Ellington, A.D.; Szostak, J.W. *In vitro* selection of RNA molecules that bind specific ligands. *Nature* 1990, *346*, 818–822.
18. Jayasena, S.D. Aptamers: An emerging class of molecules that rival antibodies in diagnostics. *Clin. Chem.* 1999, *45*, 1628–1650.
19. Tuerk, C.; Gold, L. Systematic evolution of ligands by exponential enrichment-RNA ligands to bacteriophage-t4 DNA-polymerase. *Science* 1990, *249*, 505–510.
20. Keefe, A.D.; Szostak, J.W. Functional proteins from a random-sequence library. *Nature* 2001,*410*, 715–718.
21. Mann, D.; Reinemann, C.; Stoltenburg, R.; Strehlitz, B. *In vitro* selection of DNA aptamers binding ethanolamine. *Biochem. Biophys. Res. Commun.* 2005, *338*, 1928–1934.
22. Yang, Y.; Yang, D.; Schluesener, H.J.; Zhang, Z. Advances in SELEX and application of aptamers in the central nervous system. *Biomol. Eng.* 2007, *24*, 583–592.
23. Tombelli, S.; Minunni, A.; Mascini, A. Analytical applications of aptamers. *Biosens. Bioelectron.* 2005, *20*, 2424–2434.
24. Sun, W.; Du, L.; Li, M. Advances and perspectives in cell-specific aptamers. *Curr. Pharm. Des.* 2011, *17*, 80–91.
25. Song, K.M.; Lee, S.; Ban, C. Aptamers and their biological applications. *Sensors* 2012, *12*, 612–631.
26. Stoltenburg, R.; Reinemann, C.; Strehlitz, B. SELEX-A (r)evolutionary method to generate high-affinity nucleic acid ligands. *Biomol. Eng.* 2007, *24*, 381–403.
27. Wang, Y.X.; Ye, Z.Z.; Si, C.Y.; Ying, Y.B. Application of aptamer based biosensors for detection Of pathogenic microorganisms. *Chin. J. Anal. Chem.* 2012, *40*, 634–642.
28. Feng, H.; Beck, J.; Nassal, M.; Hu, K.H. A SELEX-screened aptamer of human hepatitis B virus RNA encapsulation signal suppresses viral replication. *PLoS One* 2011, *6*, e27862.
29. Hwang, S.Y.; Sun, H.Y.; Lee, K.H.; Oh, B.H.; Cha, Y.J.; Kim, B.H.; Yoo, J.Y. 5'-Triphosphate- RNA-independent activation of RIG-I via RNA aptamer with enhanced antiviral activity. *Nucleic Acids Res.* 2012, *40*, 2724–2733.
30. Hyeon, J.Y.; Chon, J.W.; Choi, I.S.; Park, C.; Kim, D.E.; Seo, K.H. Development of RNA aptamers for detection of Salmonella Enteritidis. *J. Microbiol. Methods* 2012, *89*, 79–82.
31. Ylera, F.; Lurz, R.; Erdmann, V.A.; Furste, J.P. Selection of RNA aptamers to the Alzheimer's disease amyloid peptide. *Biochem. Biophys. Res. Commun.* 2002, *290*, 1583–1588.
32. Hybarger, G.; Bynum, J.; Williams, R.F.; Valdes, J.J.; Chambers, J.P. A microfluidic SELEX prototype. *Anal. Bioanal. Chem.* 2006, *384*, 191–198.
33. Lou, X.; Qian, J.; Xiao, Y.; Viel, L.; Gerdon, A.E.; Lagally, E.T.; Atzberger, P.; Tarasow, T.M.; Heeger, A.J.; Soh, H.T. Micromagnetic selection of aptamers in microfluidic channels. *Proc. Natl. Acad. Sci. USA* 2009, *106*, 2989–2994.
34. Mosing, R.K.; Bowser, M.T. Microfluidic selection and applications of aptamers. *J. Sep. Sci.* 2007, *30*, 1420–1426.
35. Park, S.M.; Ahn, J.Y.; Jo, M.; Lee, D.K.; Lis, J.T.; Craighead, H.G.; Kim, S. Selection and elution of aptamers using nanoporous sol-gel arrays with integrated microheaters. *Lab Chip* 2009, *9*,1206–1212.
36. Qian, J.; Lou, X.; Zhang, Y.; Xiao, Y.; Soh, H.T. Generation of highly specific aptamers via micromagnetic selection. *Anal. Chem.* 2009, *81*, 5490–5495.
37. Bowser, M.T.; Mendonsa, S.D.; Mosing, R. CE-SELEX: *In vitro* selection of DNA aptamer using capillary electrophoresis. *Abstr. Paper Am. Chem. Soc.* 2005, *229*, U139.
38. Tok, J.; Lai, J.; Leung, T.; Li, S.F.Y. Selection of aptamers for signal transduction proteins by capillary electrophoresis. *Electrophoresis* 2010, *31*, 2055–2062.
39. Mosing, R.K.; Mendonsa, S.D.; Bowser, M.T. Capillary electrophoresis-SELEX selection of aptamers with affinity for HIV-1 reverse transcriptase. *Anal. Chem.* 2005, *77*, 6107–6112.
40. Ahn, J.Y.; Jo, M.; Dua, P.; Lee, D.K.; Kim, S. A sol-gel-based microfluidics system enhances the efficiency of RNA aptamer selection. *Oligonucleotides* 2011, *21*, 93–100.
41. Ahn, J.Y.; Lee, S.; Jo, M.; Kang, J.; Kim, E.; Jeong, O.C.; Laurell, T.; Kim, S. Sol-gel derived nanoporous compositions for entrapping small molecules and their outlook toward aptamer screening. *Anal. Chem.* 2012, *84*, 2647–2653
42. Lou, X.; Qian, J.; Xiao, Y.; Viel, L.; Gerdon, A.E.; Lagally, E.T.; Atzberger, P.; Tarasow, T.M.;Heeger, A.J.; Soh, H.T. Micromagnetic selection of aptamers in microfluidic channels. *Proc. Natl.Acad. Sci. USA* 2009, *106*, 2989–2994.
43. Cho, M.; Xiao, Y.; Nie, J.; Stewart, R.; Csordas, A.T.; Oh, S.S.; Thomson, J.A.; Soh, H.T. Quantitative selection of DNA aptamers through microfluidic selection and high-throughput sequencing. *Proc. Natl. Acad. Sci. USA* 2010, *107*, 15373–15378.
44. Qian, J.; Lou, X.; Zhang, Y.; Xiao, Y.; Soh, H.T. Generation of highly specific aptamers via micromagnetic selection. *Anal. Chem.* 2009, *81*, 5490–5495.
45. Amy C. Yan. Et al. APTAMERS: PROSPECTS IN THERAPEUTICS AND BIOMEDICINE. Frontiers in Bioscience 10, , May 1, 20051802-1827
46. Healy, J. M. *et al*. Pharmacokinetics and biodistribution of novel aptamer compositions. *Pharm. Res.* 21, 2234–2246 (2004).
47. Amy C. Yan. Et al. APTAMERS: PROSPECTS IN THERAPEUTICS AND BIOMEDICINE. Frontiers in Bioscience 10, 1802-1827, May 1, 2005
48. (48) Guo, K. T. *et al*. CELL-SELEX: novel perspectives of aptamer-based therapeutics. *Int. J. Mol. Sci.* 9, 668–678 (2008).
49. Healy, J. M. *et al*. Pharmacokinetics and biodistribution of novel aptamer compositions. *Pharm. Res.* 21, 2234–2246 (2004).
50. Burmeister, P. E. *et al*. 2′-Deoxy purine, 2′-*O*-methylpyrimidine (dRmY) aptamers as candidate therapeutics. *Oligonucleotides* 16, 337–351 (2006).
51. Jayasena SD: Aptamers: An Emerging Class of Molecules That Rival Antibodies in Diagnostics. *Clinical Chemistry* 1999, 45:1628-1650.

52. Ferreira CS, Papamichael K, Guilbault G, Schwarzacher T, Gariepy J, Missailidis S: DNA aptamers against the MUC1 tumor marker: design of aptamer-antibody sandwich ELISA for the early diagnosis of epithelial tumors. *Analytical and bioanalytical chemistry* 2008, 390:1039-50.
53. Burbulis I, Yamaguchi K, Yu R, Resnekov O, Brent R: Quantifying small numbers of antibodies with a "near-universal" • protein-DNA chimera. *Nature Methods* 2007, 4:1011-3.
54. McCauley TG, Hamaguchi N, Stanton M: Aptamer-based biosensor arrays for detection and quantification of biological macromolecules. *Analytical biochemistry* 2003, 319:244-50.
55. Cao Z, Tong R, Mishra A, Xu W, Wong GCL, Cheng J, Lu Y: Reversible Cell-Specific Drug Delivery with Aptamer-Functionalized Liposomes. *Angew. Chem. Int. Ed.* 2009, 48:6494-6498.
56. De Rosa G, La Rotonda MI: Nano and Microtechnologies for the Delivery of Oligonucleotides with Gene Silencing Properties. *Molecules* 2009, 14:2801-2823.
57. Ferreira CSM, Cheung MC, Missailidis S, Bisland S, Gariepy J: Phototoxic aptamers selectively enter and kill epithelial cancer cells. *Nucl. Acids Res.*2009, 37:866-876.
58. Yan AC, Levy M: Aptamers and aptamer targeted delivery. *RNA biology* 2009, 6:316-320.
59. M. *et al*. OX40-deficient mice are defective in The cellproliferation but are competent Kopf in generatingB cell and CTL Responses after virus infection.*Immunity* **11**, 699–708 (1999).
60. (60) Barbas AS, White RR. The development and testing of aptamers for cancer. Curr Opin Investig Drugs 2009;10:572e8.
61. (61) Ylera F, Lurz R, Erdmann VA, et al. Selection of RNA aptamers to the Alzheimer's disease amyloid peptide. Biochem Biophys Res Commun 2002;290:1583e8.
62. (62) Gilch S, Schatzl HM. Aptamers against prion proteins and prions. Cell Mol Life Sci 2009;66:2445e55.
63. Sayer NM, Cubin M, Rhie A, et al. Structural determinants of conformationally selective, prion-binding aptamers. J Biol Chem 2004;279:13102e9.
64. Chen F, Zhou J, Luo F, et al. Aptamer from whole-bacterium SELEX as new therapeutic reagent against virulent Mycobacterium tuberculosis. Biochem Biophys Res Commun 2007;357:743e8.
65. Nishikawa F, Funaji K, Fukuda K, et al. In vitro selection of RNA aptamers against the HCV NS3 helicase domain. Oligonucleotides 2004;14:114e29.
66. Fukuda K, Toyokawa Y, Kikuchi K, et al. Isolation of RNA aptamers specific for the 39 X tail of HCV. Nucleic Acids Symp Ser (Oxf) 2008;52:205e6.
67. Kikuchi K, Umehara T, Nishikawa F, et al. Increased inhibitory ability of conjugated RNA aptamers against the HCV IRES. Biochem Biophys Res Commun 2009;386:118e23.
68. Konno K, Fujita S, Iizuka M, et al. Isolation and characterization of RNA Aptamers specific for the HCV minus-IRES domain I. Nucleic Acids Symp Ser (Oxf) 2008;52:493e4
69. Bock LC, Griffin LC, Latham JA, et al. Selection of single-stranded DNA molecules that bind and inhibit human thrombin. Nature 1992;355:564e6.
70. Lee WA, Fishback JA, Shaw JP, et al. A novel oligodeoxynucleotide inhibitor of thrombin II. Pharmacokinetics in the cynomolgus monkey. Pharm Res 1995;12:1943e7.
71. Green LS, Jellinek D, Jenison R, et al. Inhibitory DNA ligands to platelet-derived growth factor B-chain. Biochemistry 1996;35:14413e24.
72. Cosmi B. ARC-1779, a PEGylated aptamer antagonist of von Willebrand factor for potential use as an anticoagulant or antithrombotic agent. Curr Opin Mol Ther 2009;11:322e8.
73. Diener J, Daniel Lagasse HA, Duerschmied D, et al. Inhibition of von Willebrand Factor-mediated platelet activation and thrombosis by Anti-von Willebrand Factor A1-domain aptamer ARC1779. J Thromb Haemost 2009;7:1155e62.
74. Akiyama H, Kachi S, Silva RL, et al. Intraocular injection of an aptamer that binds PDGF-B: a potential treatment for proliferative retinopathies. J Cell Physiol 2006;207:407e12.
75. Bates PJ, Laber DA, Miller DM, et al. Discovery and development of the G-rich oligonucleotide AS1411 as a novel treatment for cancer. Exp Mol Pathol 2009;86:151e64.
76. Teng Y, Girvan AC, Casson LK, et al. AS1411 alters the localization of a complex containing protein arginine methyltransferase 5 and nucleolin. Cancer Res 2007;67:10491e500.
77. Blake CM, Sullenger BA, Lawrence DA, et al. Antimetastatic potential of PAI-1-specific RNA aptamers. Oligonucleotides 2009;19:117e28.
78. Liu Y, Kuan CT, Mi J, et al. Aptamers selected against the unglycosylated EGFRvIII ectodomain and delivered intracellularly reduce membrane-bound EGFRvIII and induce apoptosis. Biol Chem 2009;390:137e44.
79. Sefah K, Tang ZW, Shangguan DH, et al. Molecular recognition of acute myeloid leukemia using aptamers. Leukemia 2009;23:235e44.
80. Huang YF, Shangguan D, Liu H, et al. Molecular assembly of an aptamer-drug conjugate for targeted drug delivery to tumor cells. Chembiochem 2009;10:862e8.
81. Appert A, Nam CH, Lobato N, et al. Targeting LMO2 with a peptide aptamer establishes a necessary function in overt T-cell neoplasia. Cancer Res 2009;69:4784e90.
82. Ferreira CS, Cheung MC, Missailidis S, et al. Phototoxic aptamers selectively enter and kill epithelial cancer cells. Nucleic Acids Res 2009;37:866e76.
83. Padmanabhan K, Padmanabhan KP, Ferrara JD, et al. The structure of alphathrombin inhibited by a 15-mer single-stranded DNA aptamer. J Biol Chem 1993;268:17651e4.
84. Long SB, Long MB, White RR, et al. Crystal structure of an RNA aptamer bound to thrombin. RNA 2008;14:2504e12.
85. Marisa Joubert, personal communication 2009).
86. Bardou C, Borie C, Bickle M, et al. Peptide aptamers for small molecule drug discovery. Methods Mol Biol 2009;535:373e88.
87. Bouquier N, Fromont S, Zeeh JC, et al. Aptamer-derived peptides as potent inhibitors of the oncogenic RhoGEF Tgat. Chem Biol 2009;16:391e400.
88. Pultar J, Sauer U, Domnanich P, et al. Aptamer-antibody on-chip sandwich immunoassay for detection of CRP in spiked serum. Biosens Bioelectron 2009;24:1456e61.
89. Collett JR, Cho EJ, Ellington AD. Production and processing of aptamer microarrays. Methods 2005;37:4e15.
90. Platt M, Rowe W, Wedge DC, et al. Aptamer evolution for array-based diagnostics. Anal Biochem 2009;390:203e5.
91. Lao YH, Peck K, Chen LC. Enhancement of aptamer microarray sensitivity through spacer optimization and avidity effect. Anal Chem 2009;81:1747e54.
92. , R., J. Wiskirchen, K. Guo, B. Neumann, R. Kehlbach, J. Pintaske, V. Voth, T.Walker, A. M. Scheule, T. O. Greiner, U. Hermanutz-Klein, C. D. Claussen, H. Northoff, G. Ziemer and H. P. Wendel (2007). "Aptamer-based isolation and subsequent imaging of mesenchymal stem cells in ischemic myocard by magnetic resonance imaging. Rofo-Fortschritte Auf Dem Gebiet Der Rontgenstrahlen Und Der Bildgebenden Verfahren 179(10): 1009-1015.
93. Sobolewska, B. W., M. Avci-Adali, B. Neumann, T. O. Greiner, A. Stolz, D. Bail, T. Walker, A. Scheule, G. Ziemer and H. P. Wendel (2010). "A novel method for isolation of endothelial progenitor cells for cardiac stem cell therapy. Kardiochirurgia I Torakochirurgia Polska 7(1): 61-65.
94. Hu, X. and X. Gao (2011). "Multilayer coating of gold nanorods for combined stability and biocompatibility.

Physical Chemistry Chemical Physics 13(21): 10028-10035.
95. Ozalp, V. C., F. Eyidogan and H. A. Oktem (2011). "Aptamer-Gated Nanoparticles for Smart Drug Delivery. Pharmaceuticals 4(8): 1137-1157.
96. http://www.genelink.com/newsite/products/images/aptamer_structure.jpg
97. http://www.biochemistry.ucla.edu/biochem/Faculty/Feigon/tba.jpg
98. http://upload.wikimedia.org/wikipedia/commons/thumb/4/46/Aptamer_biotin.png/220px-Aptamer_biotin.png
99. http://www.rsc.org/ej/CC/2013/c2cc36348h/c2cc36348h-f3.gif
100. http://www.leaddiscovery.co.uk/images/aptamer.gif
101. http://bio349.biota.utoronto.ca/20069/bio349jerry1/images/background_fig2.jpg
102. http://bio349.biota.utoronto.ca/20069/bio349jerry1/images/aptameradvantage_fig1.jpg