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Aptamers as Novel Reagents for Biomarker Discovery Applications

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Abstract

Detection of biomarkers indicating disease development is critically important for the early detection of disease, particularly in complex diseases such as cancer where early detection may determine the treatment protocol and outcome. Monoclonal antibodies have been routinely used in protein detection and several biomarker discovery platforms are built using antibodies as capture and detection reagents. However, several limitations with antibodies have led to the search for new reagents. Oligonucleotide-based reagents, called aptamers, are emerging as promising alternatives to antibodies. Aptamers show several advantages over antibodies, and several aptamer-based biomarker discovery applications are increasingly being reported. In this paper, we discuss the recent developments in aptamer technology and its application in biomarker discovery.

Keywords: Aptamers; Thioaptamer; Bead-based selection; Cancer biomarkers; X-aptamers

Introduction

Biomarkers are broadly defined as indicators of a biological state. The majority of biomarkers are recognition molecules found in blood and other body fluids, and serve as critical screening tools in disease detection, diagnostics and prognostics. Since a variety of proteins and other metabolites are secreted into the blood from localized diseased tissues and the blood flows to all tissues and quickly registers health status throughout the human body, screening blood (plasma or serum) for the detection of biomarkers is amongst the best, non-invasive method for biomarker detection [1]. This is particularly significant in cancer detection and diagnostics, as early detection often largely determines the disease prognosis and patient survival. With biomarkers, detection can be made at the very early stages of the disease, even when a person shows no other symptoms [2]. Despite the recent advances in surgical techniques and targeted therapy, the survival rates for several cancers (except a few notable childhood cancers) largely remain unchanged over the last two decades [3]. This is mainly due to the diagnosis made after the cancer has advanced and spread from the point of origin to other tissues and organs. Detecting biomarkers from serum samples provide a non-invasive, cost-effective method for the early detection of disease development. However, several challenges lie ahead in the field of biomarker discovery.

Biomarkers are often found at low-abundance, and therefore the detection method should be highly sensitive and accurate with very minimal false positive or false negative results. The detection method should also be sensitive enough to distinguish between different post-translational modifications and isoforms of the same protein. Over the last 50 years, monoclonal antibodies have been widely used in protein detection applications [4,5]. Several mAb-based platforms have been developed for biomarker discovery. Although the monoclonal antibodies were game-changers in protein detection and pose desirable qualities such as high affinity and specificity, there are several limitations with using mAbs in biomarker discovery. In the vast majority of biomarker discovery platforms, mass spectrometry is used as the analytical method of choice to quantitate the target protein. Since the biomarker is very low in concentration relative to that of the antibody, the abundant signals from the antibody will interfere with the detection signals of the biomarker protein, particularly after the digestion of antigen/antibody complex. Therefore, non-protein based reagents that do not produce interfering peptide sequences are highly desired.

Aptamers are emerging as attractive alternatives for monoclonal antibodies in diagnostic, therapeutic and targeting applications. Aptamers' promise was first demonstrated by their high-affinity binding to a target after *in vitro* combinatorial library screening, known as SELEX (Systematic Evolution of Ligands by Exponential enrichment) [6,7]. Since then aptamers have been extensively sought and studied as therapeutics [8,9], diagnostics [10-12] and as biosensors [10,13-15].

Aptamers are structurally distinct, small RNA or DNA oligonucleotides that mimic antibodies and exhibit high (nM) binding affinity and selectivity towards their targets [10,11,16]. Although they are 1/10th the molecular weight of antibodies, they provide complex tertiary structures with sufficient recognition surface area to rival or even surpass the binding affinities of antibodies. Aptamers offer significant advantages over antibodies, such as: 1) longer shelf life; 2) simple and inexpensive process for their synthesis and modification; 3) high accuracy, purity and reproducibility in synthesis; and 4) cell or animal free production processes. While antibodies can be generated only against antigens (proteins) + aptamers can be generated against virtually any kind of biomolecules, including proteins, lipids, sugars and even small molecules. Aptamers are capable of distinguishing between different modified forms and isoforms of the same protein, a significant advantage for biomarker discovery. Furthermore, aptamer's affinity can also be tuned by optimizing their recognition sequence and/or manipulating binding reaction conditions. Through established solid-phase chemical synthetic methods and site-directed chemistries, aptamers are convenient to label, conjugate, and immobilize [17] making aptamers ideal molecular recognition tools [17-19].

Chemical Modification of Aptamers

Since native oligonucleotides are susceptible to rapid digestion by nucleases present in body fluids and cellular extracts, chemical

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modifications are required to increase their resistance to degradation by cellular enzymes. Several strategies have been developed to increase the stability of aptamers without compromising the binding affinity and specificity towards their targets. These strategies include the chemical modification of the phosphate backbone, sugars and/or the bases, end-capping at the 3' or 5' termini [20] and locked nucleic acids [21].

Among the chemical modifications reported for oligonucleotides, the most common are the sulfur substitution of the phosphate backbone (for both DNA and RNA) and the modification of the 2' position of the ribose sugar (for RNA). The 2'-sugar modifications found to increase the resistance and the overall stability of the functional oligonucleotides are the O-Me and Fluoro substitutions, and the locked nucleic acid (LNA). The LNA modification contains an intramolecular 2'-O *to* 4'-C methylene bridge, and has been reported to exhibit enhanced secondary structure stability [22-24]. "Thio"aptamers, in which one (monothio) or both (dithio) of the non-bridging phosphoryl oxygens are replaced with sulfur, are attractive choices for aptamer technology (Figure 1). We have selected both monothio and dithio substituted aptamers against several targets [25-32]. Thioaptamers pose several advantages over normal aptamers. Sulfur substitution of the backbone not only increases the resistance for cellular nucleases, but can if suitably substituted, increase the binding affinity of the aptamers towards their targets. Based on our results from theoretical calculations, we suggested that the increased affinity results from sulfur being a soft anion, and do not coordinate as well to hard cations like Na^+, unlike the hard phosphate oxy-anion. The thio-substituted phosphate esters then act as bare anions and since energy is not required to strip the cations from the backbone, these agents can in principle bind even tighter to proteins [33]. Thioaptamers are easy to synthesize by chemical or enzymatic methods, and their sequences can be read out by PCR methods. The monothio aptamers can be PCR amplified, as the *Taq* DNA polymerase is capable of incorporating up to three different monothio-dNTPs. For RNA aptamers, T7 RNA polymerase is shown to be capable of incorporating (αS)-rNTPS to produce monothio phosphate containing RNA molecules [34]. Both DNA and RNA dithioaptamers can be chemically synthesized by the standard solid-phase synthetic methods using commercially available thio-phosphoramidites (Glen Research).

Oligonucleotide aptamers are negatively charged polymers, and therefore would be weak-binders to negatively charged, acidic proteins because there are few cationic groups to neutralize anionic surfaces on the protein. As described above, backbone modifications with dithiophosphates and monothiophosphates provide some additional variability to enhance protein-nucleic acid interactions, but we still have limited variability in the functional groups on the bases. This limitation can be overcome by introducing positively charged groups to the oligonucleotide bases. The 5th position of the dT residue can be easily modified to introduce additional functional groups. Positively charged, and/ or hydrophobic groups (even bulky groups such as pyrene) can be introduced at this position.

To be effective capture reagents, aptamers must recognize a broad surface area of the target proteins. The binding affinity and the specificity of the aptamers can be further increased by introducing additional functional groups on the oligonucleotide chain. This can be achieved by introducing a wide range of substituents (X) to the 5-position of dU (Figure 1). We have found that the addition of functional groups at the 5 position are well tolerated by *Taq* and other polymerases in amplifying the selected sequences. These next-generation aptamers, called "X"-aptamers, represent a self-folding nucleic acid scaffold that can present multiple small organic moieties selected from large combinatorial bead libraries and that is easily read out by PCR. The X-aptamers represent a protein-binding ligand class that combines the best features of nucleic acids, proteins and organic drugs and that is readily chemically synthesizable. Furthermore various synthetic methods can be used to introduce multiple, complex drug-like molecules into the X-aptamers (W. He, unpublished). Thus we can create enormously more complex libraries of X-aptamers in which every base along the chain can have a differentamino-acid-like side chain or even a complex drug moiety. Rather than having simply 4 bases or even 20 amino acids, we have virtually an unlimited range of chemical functional groups that can be introduced into an aptamer that by its selection will fold into a unique tertiary structure scaffold to present to the target protein multiple drug-like hits and amino acid-like side chains with an enormously more complex range of substituents.

Aptamer selection methods

Unlike antibodies, aptamers are selected by *in-vitro* methods, by screening a large library of oligonucleotides against the target to find the best binding candidates. The selection methods can be broadly divided into two categories (Figure 2). The SELEX or in vitro selection method [35], involves iterative cycles of screening (Figure 2a). The other is a newer, bead-based method where the oligonucleotide library is synthesized on non-cleavable microbeads and the high-affinity binders are identified in a single-step screening [36] (Figure 2b).

In a typical SELEX experiment, an initial oligonucleotide library

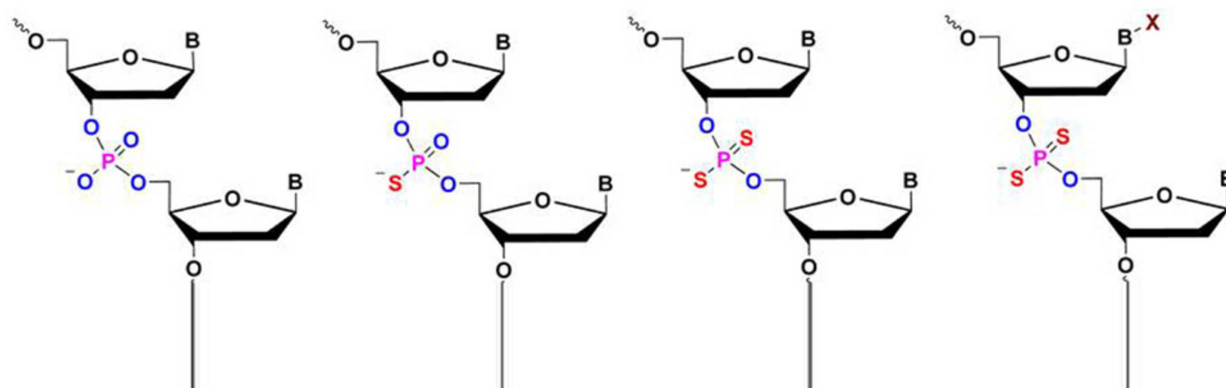

Figure 1: Chemical structures of modified aptamers. (A) Normal oligonucleotides (B) Mono-thio substituted thioaptamer (C) Di-thio substituted thioaptamer (D) X-modified thioaptamer.

containing ca. 10^{14} unique sequences is designed and chemically synthesized. The sequence has a 30-40 nucleotide random region, flanked by PCR primer regions (18-22 residues each). This DNA pool is first denatured at 95°C and subsequently cooled in a binding buffer to fold into stable structures and subsequently incubated with the target. The bound sequences are partitioned from the unbound and weakly bound sequences by membrane filtering or other such separation methods. The bound aptamers are eluted from the targets, amplified by PCR and taken to the next cycle of the screening. At each iterative cycle, the stringency of the selection is progressively increased by increasing the ratio of aptamers pool to the target in order to increase the competition between the aptamers and the target [37]. The iterative cycle of the selection process is continued until convergence towards a single or few sequences with high affinity to the target is obtained. The PCR of the aptamer pool is sub-cloned and plasmids with individual aptamers inserts are isolated and sequenced and analyzed. The selected aptamer sequences are synthesized and their binding affinities and specificities characterized. Most aptamers are selected by similar manual protocols, including thioaptamers. However, automation of the SELEX protocol is also described for several targets [38-41].

Aptamers can also be selected against whole cells. The Cell-SELEX method is used to select aptamers targeting cancer and other diseased cells [42-45]. The Cell-SELEX is also the method of choice for aptamer selection in instances where a clear target is not known, or the target is hidden or shielded from the surface. Aptamers are selected against the cell surface where the target protein is present in their native conformation and/or with post translational modifications retaining their biological function. The Cell-SELEX method may generate a pool of aptamers against many protein targets on the cell surface, generating a molecular signature to the cells that can be useful in cancer diagnosis and treatment. In the selection process, similar to the traditional SELEX, an initial random library is incubated with target cells and control cells. The library is first screened against the control (normal) cells to filter out the sequences that bind to the control cells. The unbound sequences are separated and screened against the diseased cells. The selection cycles are continued similar to the SELEX procedure until the selected sequences show convergence.

The enrichment of the pool can be monitored with a fluorescently labeled DNA pool and the cells sorted by flow cytometry or manually by fluorescent microscopy. The pool is sub-cloned and the sequences of the enriched pool identified to determine any convergence of the pool towards the target cells. Selected sequences from a converged pool are synthesized either chemically using the standard phosphoramidite chemistry or enzymatically by PCR from the plasmids with fluorescent or dye labels at either the 5' or 3' end and their binding affinities and Kd values be determined by flow cytometry. Several high affinity aptamers have been identified successfully using the Cell-SELEX method indicating that aptamers can be generated with complex targets such as tumor cells and tissues [44]. The targets can also be identified with the aptamers in the tissue samples. A fluorophore labeled aptamer can also bind to tissue containing the target protein.

Bead-based selection

While a wide-range of chemical modifications can be incorporated

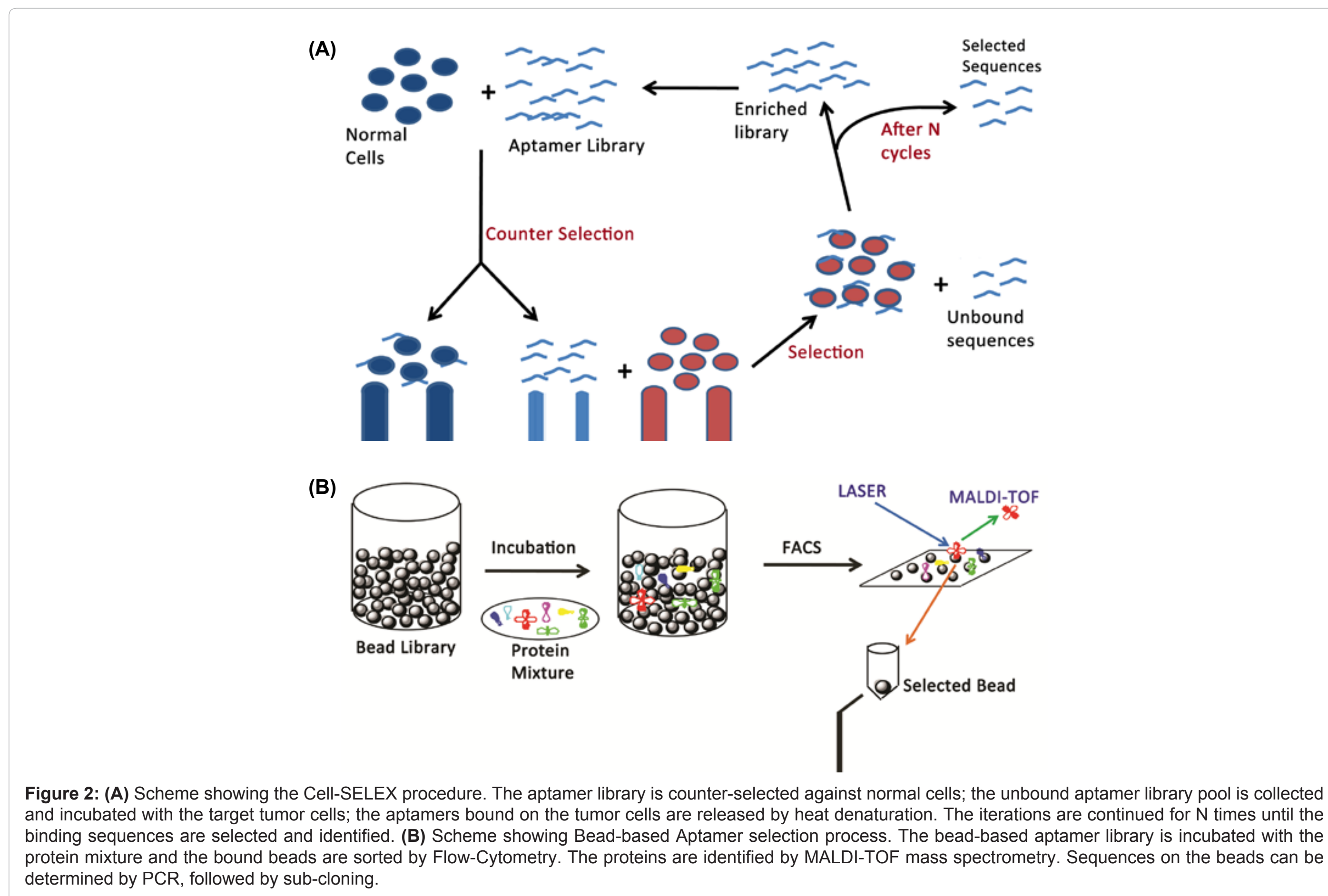

Figure 2: (A) Scheme showing the Cell-SELEX procedure. The aptamer library is counter-selected against normal cells; the unbound aptamer library pool is collected and incubated with the target tumor cells; the aptamers bound on the tumor cells are released by heat denaturation. The iterations are continued for N times until the binding sequences are selected and identified. **(B)** Scheme showing Bead-based Aptamer selection process. The bead-based aptamer library is incubated with the protein mixture and the bound beads are sorted by Flow-Cytometry. The proteins are identified by MALDI-TOF mass spectrometry. Sequences on the beads can be determined by PCR, followed by sub-cloning.

into the oligonucleotides to increase the diversity of the library, some of the modified oligonucleotides cannot be amplified by PCR methods, posing problems for aptamer selection by SELEX or other enzymatic amplification methods. Bead-based methods have been developed to overcome this limitation. Since bead based oligonucleotides are not PCR-amplified, the modified sequences need only to serve as a template for the polymerase to readout the sequence into a normal backbone oligonucleotide. We have developed a unique 'split and pool' synthesis method to create a combinatorial library of oligonucleotides, on micron-scale beads, with any type of backbone modification [36]. Selection of aptamers from the bead-based library is carried out through a distinct process from the SELEX process. The bead based library is incubated with the fluorescently tagged protein, and the protein-bound aptamer beads are sorted by flow-cytometry (FACS) [36]. Alternatively, proteins can be labeled with biotin and the bound aptamer beads can be sorted by magnetic selection using streptavidin coated magnetic nanoparticles (Invitrogen). The sequences of the aptamers on the selected beads can then be read-out by PCR amplification and sub-cloning. The bead-based process is much faster (hours) and the high affinity binders can be selected within one round. The added advantages in the bead-based process are 1) diverse chemical modifications (such as X-aptamers and dithiophosphate backbone modifications) can be introduced into the oligonucleotides 2) incubating soluble target with the immobilized thioaptamer library and therefore no direct competition between strong and weak binding candidates [27,35].

Aptamer-based biomarker discovery platforms

Since the first report in 1996, of using oligonucleotides to detect proteins in immunoassays [46], significant progress has been made in utilizing aptamers in detection assays [53]. A sandwich assay called ELONA, oligonucleotide-based aptamers replaced antibodies as either capture or detection reagents [47,48,58]. Recently, several groups have reported aptamer-based biomarker discovery platforms with multiplexing capabilities. Gold and co-workers have recently described biomarker discovery system that is capable of simultaneously measuring thousands of proteins from serum or plasma samples. Using their system, they discovered 58 potential biomarkers for chronic kidney disease [49]. The same group using their patented aptamers, called SOMAmers [50,51], reported a large scale study of screening serum samples to discover biomarkers for non-small cell lung cancer. These two reports showcase the significant improvements in the development of highly sensitive, aptamer-based biomarker discovery platforms. Aptamer-facilitated biomarker discovery (AptaBiD) technology [52] was reported to detect biomarkers differently expressed on cell surfaces. Through multi-round selection of single-stranded DNA aptamers, biomarkers are isolated from cells and subsequently identified by mass spectrometric methods [54-57].

Novel aptamer-based technologies continue to evolve and there is little doubt that they will provide enormous opportunities in the future for both biomarker discovery and detection.

References

1. Pepe MS, Etzioni R, Feng Z, Potter JD, Thompson ML, et al. (2001) Phases of biomarker development for early detection of cancer. J Natl Cancer Inst 93: 1054-1061.
2. Etzioni R, Urban N, Ramsey S, McIntosh M, Schwartz S, et al. (2003) The case for early detection. Nat Rev Cancer 3: 243-252.
3. Jemal A, Siegel R, Xu J, Ward E (2010) Cancer statistics, 2010. CA Cancer J Clin 60: 277-300.
4. Ayoglu B, Häggmark A, Neiman M, Igel U, Uhlén M, et al. (2011) Systematic antibody and antigen-based proteomic profiling with microarrays. Expert Rev Mol Diagn 11: 219-234.
5. Nelson BP (2009) Multiplexed antibody arrays for the discovery and validation of glycosylated protein biomarkers. Bioanalysis 1: 1431-1444.
6. Tuerk C, Gold L (1990) Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. Science 249: 505-510.
7. Ellington AD, Szostak JW (1990) In vitro selection of RNA molecules that bind specific ligands. Nature 346: 818-822.
8. Keefe AD, SupriyaPai S, Ellington AD (2010) Aptamers as therapeutics. Nat Rev 9: 537-550.
9. Lee JF, Stovall GM, Ellington AD (2006) Aptamer therapeutics advance. Curr Opin Chem Biol 10: 282–289.
10. Jayasena SD (1999) Aptamers: an emerging class of molecules that rival antibodies in diagnostics. Clin Chem 45: 1628-1650.
11. Brody EN, Gold L (2000) Aptamers as therapeutic and diagnostic agents. J Biotechnol 74: 5-13.
12. Tombelli S, Minunni M, Mascini M (2007) Aptamers-based assays for diagnostics, environmental and food analysis. Biomol Eng 24: 191–200.
13. Wang W, Chen C, Qian M, Zhao XS (2008) Aptamer biosensor for protein detection using gold nanoparticles. Anal Biochem 373: 213–219.
14. Radi AE, Lluıs J, Sanchez A, Baldrich E, O'Sullivan CK (2005) Reusable Impedimetric Aptasensor. Anal Chem 77: 6320-6323.
15. Bang GS, Cho S, Kim BG (2005) A novel electrochemical detection method for aptamer biosensors. Biosens Bioelectron 21: 863–870.
16. Proske D, Blank M, Buhmann R, Resch A (2005) Aptamers--basic research, drug development, and clinical applications. Appl Microbiol Biotechnol 69: 367-374.
17. Wang HQ, Wu Z, Tang LJ, Yu RQ, Jiang JH (2011) Fluorescence protection assay: a novel homogeneous assay platform toward development of aptamer sensors for protein detection. Nucleic Acids Res 39: e122.
18. Cho EJ, Lee JW, Ellington AD (2009) Applications of aptamers as sensors. Annu Rev Anal Chem (Palo Alto Calif) 2: 241-264.
19. Zhang X, Yadavalli VK (2011) Surface immobilization of DNA aptamers for biosensing and protein interaction analysis. Biosens Bioelectron 26: 3142-3147.
20. White RR, Sullenger, BA, Rusconi, CP (2000) Developing aptamers into therapeutics. J Clin Investigations 106: 929-934.
21. Crinelli R, Bianchi M, Gentilini L, Magnani M (2002) Design and characterization of decoy oligonucleotides containing locked nucleic acids. Nucleic Acids Res 30: 2435-2443.
22. Schmidt KS, Borkowski S, Kurreck J, Stephens AW, Bald R, et al. (2004) Application of locked nucleic acids to improve aptamer in vivo stability and targeting function. Nucleic Acids Res 32: 5757-5765.
23. Vester B, Wengel J (2004) LNA (locked nucleic acid): high-affinity targeting of complementary RNA and DNA. Biochemistry 43: 13233-13241.
24. Wahlestedt C, Salmi P, Good L, Kela J, Johnsson T, et al. (2000) Potent and nontoxic antisense oligonucleotides containing locked nucleic acids. Proc Natl Acad Sci U S A 97: 5633-5638.
25. King DJ, Ventura DA, Brasier AR, Gorenstein DG (1998) Novel combinatorial selection of phosphorothioate oligonucleotide aptamers. Biochemistry 37: 16489-16493.
26. King DJ, Bassett SE, Li X, Fennewald SA, Herzog NK, et al. (2002) Combinatorial selection and binding of phosphorothioate aptamers targeting human NF-kappa B RelA(p65) and p50. Biochemistry 41: 9696-9706.
27. Yang X, Bassett SE, Li X, Luxon BA, Herzog NK, et al. (2002) Construction and selection of bead-bound combinatorial oligonucleosidephosphorothioate and phosphorodithioateaptamer libraries designed for rapid PCR-based sequencing. Nucleic Acids Res 30: e132.
28. Kang J, Lee MS, Watowich SJ, Gorenstein DG (2007) Combinatorial selection of a RNA thioaptamer that binds to Venezuelan equine encephalitis virus capsid protein. FEBS Lett 581: 2497-2502.
29. Kang J, Lee MS, Copland JA 3rd, Luxon BA, Gorenstein DG (2008) Combinatorial selection of a single stranded DNA thioaptamer targeting TGF-beta1 protein. Bioorg Med Chem Lett 18: 1835-1839.

30. Somasunderam A, Thiviyanathan V, Tanaka T, Li X, Neerathilingam M, et al. (2010) Combinatorial selection of DNA thioaptamers targeted to the HA binding domain of human CD44. Biochemistry 49: 9106-9112.

31. Somasunderam A, Ferguson MR, Rojo DR, Thiviyanathan V, Li X, et al. (2005) Combinatorial selection, inhibition, and antiviral activity of DNA thioaptamers targeting the RNase H domain of HIV-1 reverse transcriptase. Biochemistry 44: 10388-10395.

32. Mann AP, Somasunderam A, Nieves-Alicea R, Li X, Hu A, et al. (2010) Identification of thioaptamer ligand against E-selectin:potential application for inflamed vasculature targeting. PLoS One 5: e13050.

33. Volk DE, Power TD, Gorenstein DG, Luxon BA (2002) Anab initio study of phosphorothioate and phosphorodithioate interactions with sodium cation. Tet Letters 43: 4443-4447.

34. Smith JS, Nikonowicz EP (2000) Phosphorothioate substitution can substantially alter RNA conformation. Biochemistry 39: 5642-5652.

35. Yang X, Li N, Gorenstein DG (2011) Strategies for the discovery of therapeutic Aptamers. Expert Opin Drug Discov 6: 75-87.

36. Yang X, Li X, Prow TW, Reece LM, Bassett SE, et al. (2003) Immunofluorescence assay and flow-cytometry selection of bead-bound aptamers. Nucleic Acids Res 31: e54.

37. Jhaveri S, Olwin B, Ellington AD (1998) In Vitro Selection of phosphorothiolated aptamers. Bioorg Med Chem Lett 8: 2285-2290.

38. Cox JC, Ellington AD (2001) Automated Selection of Anti-Protein Aptamers. Bioorgan Med Chem 9: 2525–2531.

39. Stoltenburg R, Reinemann C, Strehlitz B (2007) SELEX--a (r)evolutionary method to generate high-affinity nucleic acid ligands. Biomol Eng 24: 381-403.

40. Eulberg D, Buchner K, Maasch C, Klussmann S (2005) Development of an automated in vitro selection protocol to obtain RNA-based aptamers: identification of a biostable substance P antagonist. Nucleic Acids Research 33: e45.

41. Blank M, Blind M (2005) Aptamers as tools for target validation. Curr Opin Chem Biol 9: 336–342.

42. Shangguan D, Li Y, Tang Z, Cao ZC, Chen HW, et al. (2006) Aptamers evolved from live cells as effective molecular probes for cancer study. Proc Natl Acad Sci USA 103: 11838–11843.

43. Cerchia L, Duconge F, Pestourie C, Boulay J, Aissouni Y, et al. (2005) Neutralizing Aptamers from Whole-Cell SELEX Inhibit the RET Receptor Tyrosine Kinase. PLoS Biology 3: e123.

44. Morris KN, Jensen KB, Julin CM, Weil M, Gold L (1998) High affinity ligands from *in vitro* selection: Complex targets. Proc Natl Acad Sci USA 95: 2902–2907.

45. Daniels DA, Chen H, Hicke BJ, Swiderek KM, Gold L (2003) A tenascin-C aptamer identified by tumor cell SELEX: Systematic evolution of ligands by exponential enrichment. Proc Natl Acad Sci USA 100 : 15416–15421.

46. Drolet DW, Moon-McDermott L, Romig TS (1996) An enzyme-linked oligonucleotide assay. Nat Biotechnol 14: 1021-1025.

47. Rye PD, Nustad K (2001) Immunomagnetic DNA aptamer assay. Biotechniques 30: 290-292.

48. Baldrich E, Acero JL, Reekmans G, Laureyn W, O'Sullivan CK (2005) Displacement enzyme linked aptamer assay. Anal Chem 77: 4774-4784.

49. Gold L , Ayers D, Bertin J, Bock C, Bock A, et al. (2010) Aptamer-Based Multiplexed Proteomic Technology for Biomarker Discovery. PLoS ONE 5: e15004.

50. Kraemer S, Vaught JD, Bock C, Gold L, Katilius E, et al. (2010) From aptamer-based biomarker discovery to diagnostic and clinical applications: an aptamer based, streamlined multiplex proteomic assay. Naure Preceding 4642.1.

51. Ostroff RM, Bigbee WL, Franklin W, Gold L, Mehan M, et al. (2010) Unlocking biomarker discovery: large scale application of aptamer proteomic technology for early detection of lung cancer. PLoS ONE 5: e15003.

52. Berezovski MV, Lechmann M, Musheev MU, Mak TW, Krylov SN (2008) Aptamer-facilitated biomarker discovery (AptaBiD). J Am Chem Soc 130: 9137–9143.

53. Agnew HD, Rohde RD, Millward SW, Nag A, Yeo WS, et al. (2009) Iterative in situ click chemistry creates antibody-like protein-capture agents. Angew Chem Int Ed Engl 48: 4944-4948.

54. Ahn JY, Lee SW, Kang HS, Jo M, Lee DK, et al. (2010) Aptamer Microarray Mediated Capture and Mass Spectrometry Identification of Biomarker in Serum Samples. J Proteome Res 9: 5568–5573.

55. Cole JR, Dick LW Jr, Morgan EJ, McGown LB (2007) Affinity capture and detection of immunoglobulin E in human serum using an aptamer-modified surface in matrix-assisted laser desorption/ionization mass spectrometry. Anal Chem 79: 273-279.

56. Keller KM, Breeden MM, Zhang J, Ellington AD, Brodbelt JS (2005) Electrospray ionization of nucleic acid aptamer/small molecule complexes for screening aptamer selectivity. J Mass Spectrom 40: 1327–1337.

57. Zhao Y, Widen SG, Jamaluddin M, Tian B, Wood TG, et al. (2011) Quantification of Activated NF-κB/RelA Complexes using ss DNA Aptamer Affinity – Stable Isotope Dilution Selected Reaction Monitoring—Mass Spectrometry. Mol Cell Proteomics 10: M111.008771.

58. Yang X, Gorenstein DG (2004) Progress in thioaptamer development. Curr Drug Targets 5: 705-715.