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REVIEW

**Ciara K. O'Sullivan**

# Aptasensors – the future of biosensing?

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**Abstract** Aptamers are artificial nucleic acid ligands that can be generated against amino acids, drugs, proteins and other molecules. They are isolated from combinatorial libraries of synthetic nucleic acid by an iterative process of adsorption, recovery and reamplification. Aptamers, first reported in 1990, are attracting interest in the areas of therapeutics and diagnostics and offer themselves as ideal candidates for use as biocomponents in biosensors (aptasensors), possessing many advantages over state of the art affinity sensors. The properties of aptamers, their applicability to biosensor technology, current research and future prospects are addressed in this short review.

**Keywords** Aptamers · ELONA · Aptasensors · SELEX

**Abbreviations** *SELEX* systematic evolution of ligands by exponential enrichment · *Aptasensors* biosensors utilising aptamers as biocomponent · *ELISA* enzyme linked immunosorbent assay · *ELONA* enzyme linked oligonucleotide assay · *FITC* fluoroscein isothiocyanate

## Introduction

In 1990, within months of each other, the laboratories of G.F. Joyce (La Jolla, USA.) [1], J.W. Szostak (Boston, USA.) [2] and L. Gold (Bolder, USA.) [3] independently reported on the development of an in vitro selection and amplification technique, which has allowed the discovery of specific nucleic acid sequences that bind a wide array of non-nucleic acid targets with high affinity and specificity. The technique by which these oligonucleotide ligands are obtained was coined as SELEX – systematic evolution of ligands by exponential enrichment, and the resulting oligonucleotides are referred to as aptamers, derived from the latin *aptus*, meaning, 'to fit' [4]. The last decade has seen the selection of an extensive range of aptamers with abilities to bind to small molecules, peptides, proteins, cells etc. with selectivity, specificity and affinity equal and often superior to those of antibodies [5, 6, 7, 8, 9]. Aptamers possess the ability to discriminate targets on the basis of subtle structural differences such as the presence or absence of a methyl or hydroxy group or the D- vs. L-enantiomeric configuration of the target [10]. In parallel, the body of literature covering immunosensor development has exploded over the last twenty years with numerous generic transduction platforms reported. However, as outlined in Table 1, antibody generation, particularly for use in biosensors, has several fallbacks that are addressed by aptamers and it can be envisaged that aptasensors, using already developed or novel transduction platforms, will be increasingly exploited in the coming years.

C.K. O'Sullivan (✉)
Bioelectrochemistry and Bioengineering Group,
School of Chemical Engineering,
Universitat Rovira i Virgili, Tarragona 43006, Spain
e-mail: ckosulli@etse.urv.es

## Aptamer selection – The SELEX process

The SELEX process, depicted in Fig. 1, is a technique for screening very large combinatorial libraries of oligonucleotides by an iterative process of in vitro selection and amplification. Nucleic acid libraries are easily obtained via combinatorial chemistry synthesis. Each sequence synthesised represents a linear oligomer of unique sequence and the molecular diversity is dependent on the number of randomised nucleotide positions. Typically libraries of at least $10^{13}$–$10^{18}$ independent sequences are employed, with a variable region of 30 bases flanked by primers of 20–25 bases normally being employed.

In the process, a random sequence oligonucleotide library is incubated with a target of interest in a buffer of choice at a given temperature. In the initial cycles of selection, a tiny percentage (0.1–0.5%) of individual sequences interact with the target. These sequences are separated from the rest of the library by using techniques such as affinity chromatography or filter binding [11, 12]. This isolated population of sequences is then amplified to obtain an enriched library to be used for the proceeding se-

**Table 1** Advantages of aptamers over antibodies for analysis using enzyme linked oligonucleotide assays and aptasensors

| Antibodies | Aptamers |
|---|---|
| Limitations against target representing constituents of the body and toxic substances | Toxins as well as molecules that do not elicit good immune response can be used to generate high affinity aptamers |
| Kinetic parameters of Ab-Ag interactions cannot be changed on demand | Kinetic parameters such as on/off rates can be changed on demand |
| Antibodies have limited shelf life and are sensitive to temperature and may undergo denaturation | Denatured aptamers can be regenerated within minutes, aptamers are stable to long term storage and can be transported at ambient temperature |
| Identification of antibodies that recognise targets under conditions other than physiological is not feasible | Selection conditions can be manipulated to obtain aptamers with properties desirable for in vitro assay e.g. non-physiological buffer/T |
| Antibodies often suffer from batch to batch variation | Aptamers are produced by chemical synthesis resulting in little or no batch to batch variation |
| Requires the use of animals | Aptamers are identified through an in vitro process not requiring animals |
| Labelling of antibodies can cause loss in affinity | Reporter molecules can be attached to aptamers at precise locations not involved in binding |

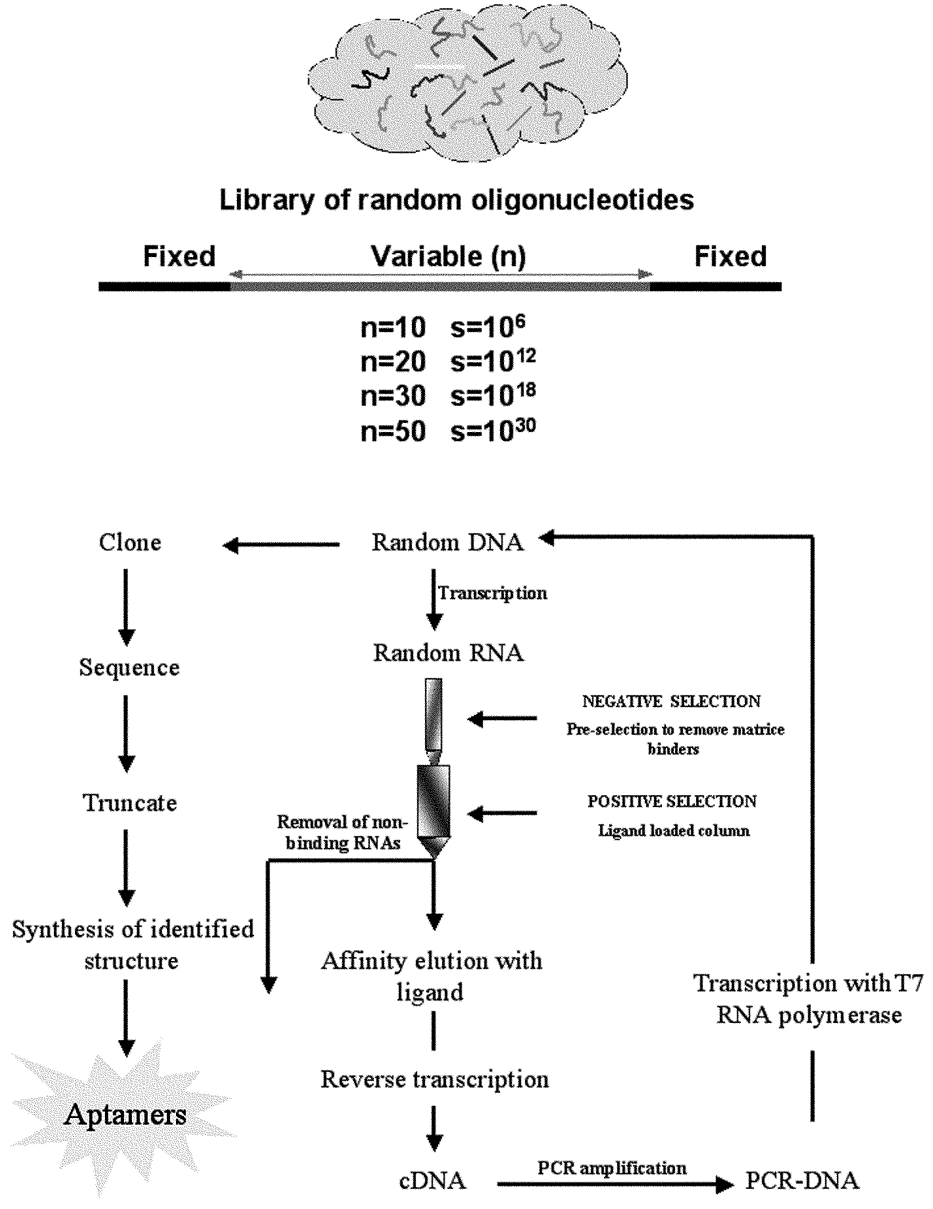


**Fig. 1** Schematic indicating steps involved in aptamer selection using the SELEX process

lection/amplification cycle. The efficiency of enrichment of high-affinity binders is governed by the stringency of selection of each round. To this end, negative selection (removal of aptamers that bind ligand support) and counter selection (removal of aptamers that bind to structures similar to that of the target) are employed. The length of incubation time of library with target used is manipulated to yield aptamers of desired kinetics. Upon achieving affinity saturation, typically after 8–15 cycles of selection/amplification, the enriched library is cloned and sequenced and individual sequences investigated for their ability to bind to the target (e.g. using surface plasmon resonance) [13]. Aptamers can then be truncated as desired, eliminating the fixed primer sequences as well as those identified not to be part of the consensus motif (i.e. sequence required for binding). When the desired sequence has been identified the aptamer can be produced in sizeable quantities by chemical synthesis [4].

## Nuclease resistant aptamers

Unmodified oligonucleotides, especially RNA, have a very short lifetime in biological fluids (typically <10 min) due to the action of nucleases [14]. Reports have been published detailing approaches to make oligonucleotide sequences resistant to nucleases by modifying the oligonucleotide backbone. However, most of these modifications result in structures not recognised by the enzymes used in the SELEX process. Recently however, modification of the 2' positions of pyrimidine nucleotides with amino/fluoro groups has been demonstrated to dramatically increase the half life of aptamers in biological fluids with the modified structures still being recognised as substrates for the enzymes utilised in the SELEX process [15, 16].

## Aptamers in analysis

a. ELONA – Enzyme linked oligonucleotide assay

As antibodies are used in ELISA, aptamers may be utilised, but with a much greater degree of flexibility in ELONAs. As depicted in Fig. 2, various formats can be exploited using, for example,

- aptamers as capture molecules and antibodies as reporter molecules
- antibodies as capture molecules and labeled aptamers against the antibody-analyte immunocomplex as reporter molecules
- aptamers as capture molecules and labeled aptamers against the aptamer-analyte complex as reporter molecules

The use of aptamers thus facilitates the detection of small and large molecular weight analytes in a sandwich-type format overcoming the limitations of competitive-type assays, allowing the development of robust, sensitive assays. The use of ELONA was patented by NexStar [17] (now Gilead Sciences, Foster City California) in 1997 and a handful of publications have also appeared [18].

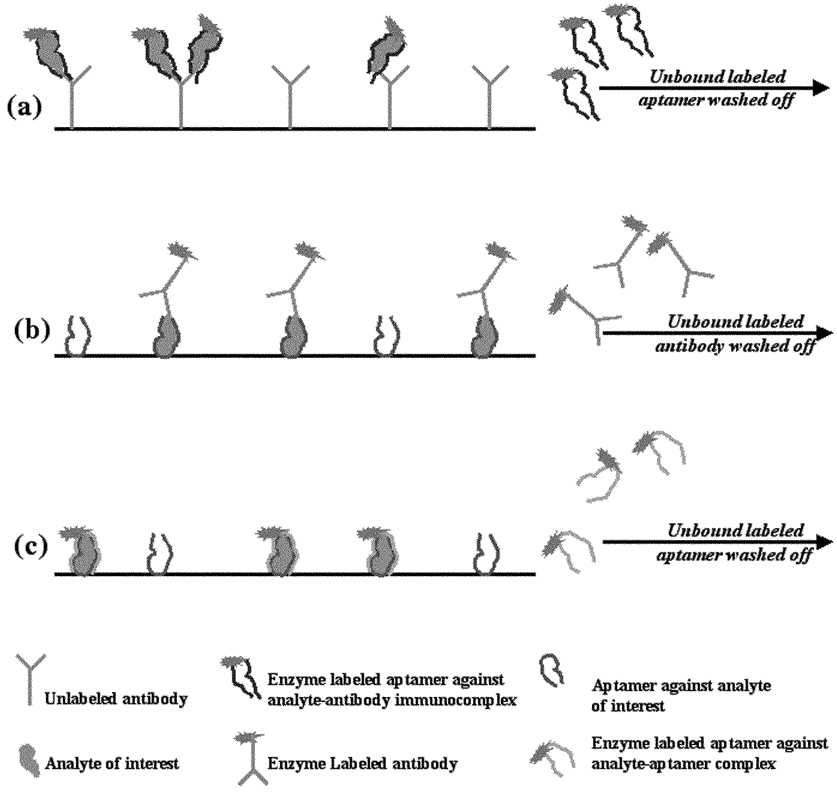


**Fig. 2** Schematic of possible assay formats for use in ELONAs and aptasensor

b. Aptasensors

The application of aptamers as biocomponents in biosensors offers a multitude of advantages over the state of the art in affinity sensing. These advantages include:

- $k_{on}$ and $k_{off}$ rates can be tailor designed according to the nature of transduction and the desired assay time
- small molecules can be detected using sandwich-type formats eliminating need for competitive type assays and the associated stringent assay requirements
- selection of aptamers can be performed in conditions akin to that of real matrix, particularly useful for environmental and food aptasensors
- modification of aptamers during immobilisation or when labelling with reporter molecules can be facilitated without affecting affinity
- aptamers can, if desired, be subjected to repeated cycles of denaturation and regeneration

Additionally, dependent upon the time required for aptasensoric detection, the use of nuclease resistant aptamers may be avoided, a notable advantage over ELONA.

At the time of preparing this review, reports of aptasensors have been limited, and have been almost exclusively based on the use of fluorescence transduction, the field of aptasensing still being in its infancy.

Kleinjung et al. (1998) [19] immobilised a biotinylated RNA aptamer selected to L-adenosine on an optical fibre surface derivatised with streptavidin. Real time measurement was obtained using total internal reflection fluorescence in a fibre optic format and the sensor utilised a competitive format using FITC-labeled L-adenosine as a reporter molecule. Quantitation in the submicromolar range with selective binding to L-adenosine and a chiral discrimination of 1700-fold was observed.

Potyrailo et al. (1998) [20] reported on the first aptasensor that offers one-step direct detection of analyte. An anti-thrombin DNA aptamer was labelled at the 5' end with FITC and the 3' end with an alkyl amine so as to immobilise it to a glass surface and fluorescence anisotropy was used to detect the bound labelled aptamer probe-analyte binding event. The assay was completed in 10 min and as little as 5 nM protein could be detected in an addressed volume of 5 nL.

Lee and Walt (2000) [21] also reported on the use of a fibre-optic biosensor using an aptamer receptor for the measurement of thrombin but used a competitive mode of assay. An anti-thrombin DNA aptamer was immobilised on the surface of silica microspheres the beads distributed in microwells on the distal tip of an imaging fibre The imaging fibre was coupled to a modified epifluorescence microscope system, and the distal end of the fibre was incubated with a fluorescein-labeled thrombin (F-thrombin) solution and thrombin was detected via competitive assay. The aptamer beads selectively bound to the target and could be reused without any sensitivity change. The fibre-optic microarray system had a detection limit of 1 nM, and each test could be performed in 15 min, including regeneration of the sensor.

Hesselberth et al. (2000) [22a,b] detailed the efforts of their group to select signalling aptamers for use in aptasensors *ab initio*, postulating that selecting for aptamers already labelled with reporter molecules would minimise any effect of post-selection modification on aptamer affinity, but have yet (at time of manuscript preparation), to publish conclusive results.

Stojanovic et al. (2001) [23a] reported a sensor for detection of cocaine by ingeniously engineering an instability in one stem of a three-way junction that formed the cocaine binding pocket with the short stems labelled with a fluorophore and a quencher. In the absence of cocaine, the stems are open but in its presence they close and the three-way junction forms. This major structural change brings fluorescence and quencher together, thus signalling the presence and concentration of analyte. The sensor was selective for cocaine over its metabolites and was operable in serum. The group had previously reported on extending this ability to rationally design aptamers and constructed bipartite aptamers that self-assemble in the presence of their ligands [23b].

c. The use of molecular beacons in aptasensing

Aptasensors (in contrast to immunosensors) lend themselves readily to the transduction of target binding using molecular beacons. Molecular beacons essentially contain two structural components, a loop and a stem, with the loop serving as a probe and the annealing of two complementary arm sequences that are flanked by the probe forms the stem (see Fig. 3). A fluorophore and fluorescent quencher are linked covalently at each end of the arm. The stem of the beacon brings the fluorophore and quencher into close proximity, resulting in no fluorescent signal. When the molecular beacon encounters a target molecule it forms a probe-target hybrid that is stronger and more stable than the stem in the hairpin, with the resulting conformational change forcing the arms apart, thus permitting the fluorophore to fluoresce [24]. The use of molecular beacons can be exploited for aptasensor development, with several modes of assay possible, the most straightforward being that the aptamer is incorporated in an immobilisable hairpin structure. Yamamoto and Kumar (2000) [25] have reported on the development of an immobilisable molecular beacon aptamer that fluoresces

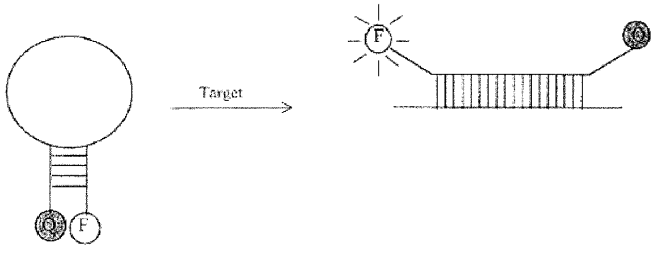


**Fig. 3** Schematic of action of fluorescent molecular beacon. F – fluorophore, Q – quencher

specifically in the presence of TAT-1 protein, derived from HIV-1 or HIV-2, but not in the presence of RNA binding proteins and are working towards its incorporation in an aptasensor.

## Conclusion and outlook

This review gives an overall introduction into the selection of aptamers and their potential uses in analysis via ELONAs and aptasensors, outlining their advantages over the state of the art in affinity based analysis and reviewing publications in the area. Despite the wealth of literature detailing the use of aptamers in therapeutics, the use of aptamers in in vitro diagnostics is a field that is still in its infancy with the main focus of literature currently available being on the detection of the aptamer-target binding event using fluorescence transduction. However, with the advent of automated platforms for aptamer selection coupled with the advances being made in increasing the stability of aptamers to nucleases, it is anticipated that aptamers will become increasingly accessible to researchers in the biosensor field.

The plethora of reported transduction platforms for affinity sensing can be readily adapted to the use of aptameric biocomponents. In addition, catalytic aptamers (aptazymes) have been reported, offering further modes of binding event detection. Moreover, it can be anticipated that the use of immobilisable molecular beacons will be exploited in the biosensor field (to date a handful of publications exist), a mode of detection highly suited to aptasensors facilitating reagentless one step analysis.

Overall, the potential of aptasensors is immense, and this exciting area is on the brink of exponential growth. The ability to develop affinity based detection systems of tailor designed characteristics, which can be applied to analysis of analytes, unlimited, for example, by size, toxicity and matrix effects, offers the field of biosensing the opportunity to explore new and dynamic routes of sensor development and the availability of commercial aptasensors by the end of the decade can definitively be anticipated.

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