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Aptamers: Current Challenges and Future Prospects

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Article Highlights

- Aptamer libraries are considered to contain the largest diversity with up to 10^{15} different molecules, which make them very attractive for leads discovery.
- Aptamers have exceptional advantages that outweigh antibodies.
- Better technologies that could detect a larger number of molecules with more specificity can be readily approached with aptamer technology.
- Interdisciplinary collaborations are needed in order to foster a prototype of a solid technological platform for aptamer discovery.
- Microchip-based technologies and Next Generation Sequencing systems may provide the foundation bases for a non-Selex aptamer discovery platform.

ABSTRACT

Introduction: Aptamers are oligonucleotide molecules raised *in vitro* from large combinatorial libraries of nucleic acids and developed to bind to targets with high affinity and specificity. Whereas novel target molecules are proposed for therapeutic intervention and diagnostic, aptamer technology has a great potential to become a source of lead compounds.

Areas Covered: In this review the authors address the current status of the technology and highlight the recent progress in aptamer-based technologies. They also discuss the current major technical limitations of aptamer technology and propose original solutions based on existing technologies that could result in a solid aptamer-discovery platform.

Expert opinion: Whereas aptamers have shown to bind to targets with similar affinities and specificities to those of antibodies, aptamers have several advantages that could outweigh antibody technology and open new opportunities for better medical and diagnostic solutions. However, the current status of the aptamer technology suffers from several technical limitations that slowdown the progression of novel aptamers into the clinic and makes the business around aptamers challenging.

1. INTRODUCTION

Aptamers have received extensive interest since they were discovered twenty five years ago by Tuerk and Gold [1]. Aptamers are short (generally 15 to 80 nucleotides) single-stranded molecules (ssDNA or ssARN) with the ability to fold in stable three-dimensional structures that allow them to interact with target molecules either through electrostatic interactions, hydrogen bonding, Van der Waals forces, base stacking or a combination of these.

Currently, the adopted methodology for the discovery of new potential therapeutic candidates for medical research, diagnostics or basic research utilizes the power of combinatorial chemistry that relies on the screening of large chemical libraries. Interestingly, aptamers are identified from synthetic ssDNA or ssRNA libraries, generally through a process termed SELEX (Systematic Evolution of Ligands by EXponential enrichment) [1]. Aptamer libraries are considered to contain the largest diversity with up to 10^{15} different molecules, which make them very attractive for leads discovery. Aptamer libraries surpass in diversity to combinatorial small-molecule chemistry [2], phage display [3], and ribosome display [4] among others.

So far, studies on aptamer functionality have demonstrated that antibodies are no longer the only entities that may be developed to bind to targets with high affinity and specificity. However, aptamers should not be seen only as a rival of antibodies. Aptamers have exceptional advantages that outweigh antibodies including; 1) nucleic acid chemistry that allows unlimited amplification of single aptamers enzymatically, 2) easy to synthesize in automated instruments, 3) easy to perform controlled modification, and 4) high flexible

structure. Aptamers have been selected to bind a plethora of targets such as small molecules, peptides, proteins and cells [5] and their use as therapeutic agents or analytical tools are under intense studies [6]. However, only a single aptamer has been approved for clinical use [7] since its discovery, and the introduction of aptamers into the market has not been very successful. Although, several aptamers are currently being assessed at different clinical phases [8], aptamer developments are facing skepticism and private investors are approaching with caution. In addition, the unexpected adverse events observed in a phase III clinical trial of REG1 (Regado Biosciences, Inc), a reversible anticoagulant aptamer targeting Factor IXa, may significantly slowdown the progression of new therapeutic aptamers and will make the business around aptamers more challenging. However, setbacks can be overcome as many other well-established technologies did in the past. The exploitation of the therapeutic potential of monoclonal antibodies envisioned since their discovery in 1975 [9] had to overcome many failures and wait 27 years until the approval of the first humanized antibody, Daclizumab (Roche).

Antibodies are among the most commonly used tools in the biological sciences and clinical diagnostics. In addition, interest in antibody-based therapies has grown incrementally over the last decades. Antibodies have been shown to be very effective on their capturing capacity for other molecules and it is clear that antibodies brought better biomarker assays to the clinic and better medicines to patients. However, produced antibodies are available to detect only a small percentage of the human proteome and the urge for antibody validation has recently started to be discussed [10, 11]. There is still a large opportunity for better technologies that could detect a larger number of molecules with more specificity and bring novel assays to the clinic, and this demand can be readily approached with aptamer technology.

Contrary to the skepticism of private investors, the academic interest on aptamers has intensified over the years. A Pubmed search on aptamers results in over 5000 articles with an

increasing number of publications over the years. Although aptamers have been raised to a diverse set of clinically relevant molecules, the majority of the publications have focused on only a few well-characterized molecules. Academics don´t have the pressure of generating products and revenues in the short-term and in this sense, they are showing in original articles how aptamers can be used to provide novel solutions in health and diagnostics without the need of validation in different clinical contexts. In our opinion, there are several limitations that are slowing down the progress of aptamer technology and some of them were well addressed in other critical reviews [12-14]. External factors that limit aptamer technology could be partially assigned to the enormous investment that companies allocate to monoclonal antibodies bioprocesses, thus reducing their interest on other technologies that threatens their current developments. On the other hand a search in the World International Patent Organization (WIPO) database, patents titled with the word aptamer resulted in 1564 patents. While patent protection is a good instrument to promote innovation, sometimes the patent landscape becomes a minefield and investors wants to be sure that their technology will not be blocked for commercializing their development by a third party patent. However, investors are not the main culprits and the major limitation resides mainly on the actual aptamer discovery process, which has many components that add significant uncertainty on the success of the methodology such as PCR bias, PCR artifacts [15, 16] and background binders. Most academic laboratories are still using SELEX as their primary strategy for aptamer discovery and as Schütze et al. [17] wrote, “SELEX is like a black box that remains closed for many rounds of selections with the inherent risk of identifying only unspecific artifacts”.

The advancement of technological instrumentation has been the major driver of several biotechnological achievements. Next Generation Sequencing (NGS) is a clear example of how the interaction of different disciplines managed to successfully develop an instrument

that made a breakthrough in all the areas of genomics. NGS merged the knowledge provided by life science researchers, engineers and material scientists. Taking the case of Illumina´s successful story, molecular biologists and biochemists provided the foundations of Bridge PCR and Sequencing by Synthesis, material scientists provided knowledge for the fabrication of ordered nanowell arrays and engineers designed the automation to assemble all the processes in a single instrument. Without these interdisciplinary collaborations, NGS wouldn´t be possible. Therefore, the aptamer field should learn from the history of NGS and if private investors are not showing interest on aptamers at the moment, efforts between academic labs should be focused in generating interdisciplinary collaborations in order to foster a prototype of a solid technological platform for aptamer discovery. From our point of view, existing technologies that have been built for this specific purpose could be easily adapted for the sake of aptamer discovery.

This review summarizes the latest achievements with aptamers and highlights the major advantages of aptamers over other technologies. This article discusses the major limitations of the aptamer technology and proposes a roadmap for building a technological platform for aptamer development that could result in a solid platform for aptamer generation.

2. WHY APTAMERS?

There are many reviews that highlight the advantage of aptamers over existing technologies[18, 19]; hence we will focus on- and extend the most relevant aspects of aptamers.

2.1 Diversity.

Current trends in searching for novel therapeutic drugs have focused on the preparation of chemical libraries as potential sources of new leads for drug discovery. Aptamers are selected against a target of interest from a large combinatorial nucleic acid library containing up to 10^{15} (1 peta) nucleic acid species [20]. This library contains an immense structural diversity and it is the largest combinatorial library at the moment [21].

2.2 In vitro selection.

The production does not require any animals or specific cell lines, thus they can be raised against toxic molecules or non-immunogenic targets with high affinity and specificity. Raising antibodies is dependent on living organisms and are very sensitive to their environment, while aptamers are more resistant to pH and temperature changes and the selection condition can be modified accordingly to the final usage of the aptamer.

2.3 Immunogenicity.

Aptamers display low to no immunogenicity when administered in preclinical doses 1000-fold greater than doses used in animal and human therapeutic applications [22, 23]. However, the halt of a phase III clinical trial of REG1 in 2014 due to an excess of severe allergic reactions, suggests that low immunogenicity and toxicity may not be generalizable to all aptamers.

2.4 Synthesis and bioavailability.

Aptamers are chemically synthesized by solid-phase technology, which renders large-scale production cost-effective and reproducibility between different batches when compared to biological drugs. Moreover, they withstand long-term shelf storage at room temperature while preserving their activity. Bioavailability and pharmacokinetics of nucleic acid ligands can be controlled using modified nucleotides, which are added during synthesis. In addition,

antidote control provides a safe means to regulate drug action and minimize side effects. While developing antidotes for antibodies and small drugs is challenging, it is not for aptamers [24]. Although REG1 didn't pass a phase III trial, the origins of its antigenicity remains unknown and is still a great example on how aptamers can be developed with a rationally designed antidote control [25].

2.5 Nanocarriers.

Knockdown of specific genes, such as oncogenes by RNA interference (RNAi) is a promising therapeutic technique for treating diseases associated with protein overexpression. Small interfering RNAs (siRNAs) can reduce gene expression via the RNAi pathway [26]. However, one major challenge in using RNAi *in vivo* is the delivery of the molecule to the correct tissue or cells and its technical difficulty to cross cell-plasma membranes. siRNAs-linked aptamers that target specific cell surface proteins are easy to synthesize and have a great potential to make RNAi attractive for therapies. McNamara et al. [27] generated an aptamer RNA against the human PSMA protein linked to a siRNA that targets tumor-overexpressed genes PLK1 and BCL2, which are associated with tumor survival. The aptamer portion of these chimeras induced cell internalization and delivered the siRNA cargo. These chimeras were able to bind selectively to PSMA positive cells and reduce gene expression of targeted genes. In addition, the PSMA-BCL2 chimera substantially decreased tumor volume in a xenograft mouse model of prostate cancer when injected intratumorally and intraperitoneally [28]. More recently, this research group elaborated a cell-internalization SELEX to obtain aptamers that are internalized by specific cell types [29]. This method allowed raising of an aptamer-siRNA chimera to $HER2^+$ cells and aptamers against human papillomavirus type 16 associated cancers [30] without knowing their mechanism of entrance. The usage of aptamers as potent systemic transporters has been extended to many other targets [31] including to deliver siRNA and suppress HIV replication [32].

2.6 Nanostructures.

DNA is an ideal material for the construction of objects at a nanometer-scale [33]. The specificity of A-T and G-C base pairing and the physicochemical nature of DNA allow the assembly of three-dimensional DNA-based nanostructures in solution with precise architectures and high efficiency [34]. These characteristics motivate the design of DNA nanostructures as simple machines, drugs and gene delivery vehicles. The first attempt to construct a DNA scaffold conjugated to an aptamer consisted in the assembly of DNA tiles into highly ordered DNA lattices containing anti-thrombin aptamers placed orderly in defined patterns [35] to selectively position the proteins. These types of developments could lead to novel biosensing applications. Yi Shu et al. [36] exploited the properties of bacterial virus phi29 DNA packaging RNA (pRNA) molecule to assemble a hexameric ring nanostructure capable of delivering multiple therapeutics into specific cells. The addition of different receptor-binding ligands to pRNA double-stranded 5´/3´end helical domain resulted in chimeric molecules such as pRNA/aptamer [37, 38]. Zhou et al. [37] managed successfully to reduce HIV infectivity in a cultured cell line by constructing a pRNA dimer fused to a HIV gp120-binding aptamer and a siRNA targeting the expression of HIV tat/rev regulatory elements. Whereas the anti-HIV gp120 aptamer targeted the chimeric pRNA to the surface of HIV-infected cells for its internalization, the siRNA element was necessary for the inhibition of HIV replication.

DNA nanostructures can also be manipulated to create molecular machines of different complexities. These molecular devices can be controlled to release different kind of cargos at specific targets by conformational switching [39]. This conformational change is induced by a molecular effector or by intermolecular hybridization with complementary nucleic acids. A simply DNA-aptamer machine was described by Dittmer et al. [40] using the well-characterized aptamer anti thrombin. This molecular device was able to sequester the

thrombin protein and release it upon binding to a complementary DNA sequence in a reversible fashion. Another device with a more complex nanoarchitecture was constructed consisting of cage-like structure with the configuration of a barrel containing an aptamer-based lock [41]. This barrel was capable to hold locked a variety of payloads. The aptamer lock was used to keep the cage in its closed conformation using a complementary DNA sequence, which in the presence of the aptamer-target such as PDGF, would open the cage and deliver the payloads to cells due to the formation of an energetically favored aptamer-ligand complex.

More recently, stimuli-responsive DNA microcapsules were developed. In this study, Liao et al. [42] constructed spherical microcapsules consisting of oligonucleotide layers crosslinked by anti-ATP aptamer sequences. The presence of the ATP-aptamers resulted in DNA-shell disassembly upon ATP binding, thus releasing the encapsulated bioactive molecules.

These kind of developments open new pathways for novel applications such as smart drugs and stimuli-responsive DNA-based nanocapsules that provide selective control of load delivery and release.

2.7 Nanosensors.

In our opinion, aptamer-based biosensor technology is the fastest-growing aptamer field and promises to continuously emerge with novel clinical or companion drug diagnostic and personalized theranostic assays. Aptamer-based biosensors has been recently reviewed [6] and the increased interest in the application of aptamers to biosensors resides mainly in the physicochemical properties of aptamers. Many academic laboratories exploit the capacity of aptamers to change their conformation upon ligand binding to develop label-free analytical assays. Existing assays for water and environmental analysis, clinical diagnosis and food safety are mostly time consuming and require sophisticated equipment. Aptamer-based

biosensors could lead to on site and real-time assays among other analytical techniques. As mentioned above, aptamer activity can be modulated by competitive interaction with a target molecule or hybridization with a complementary nucleotidic sequence; aptamers are modified with ease and can be labeled with dyes and functional groups either to obtain a signal or for immobilization on solid supports.

3. APTAMER CURRENT LIMITATIONS

Original aptamer developments coming from the academic field are continuously showing the great potential of this technology, however, as any early technology it carries its own advantages and limitations. While the advantages where discussed above, in our opinion, two major limitations are slowing down the progress of aptamer developments; 1) the usage of PCR during SELEX, and 2) the reduced chemical information of unmodified nucleic acids.

3.1 The PCR problem.

PCR has become widely used in molecular biology, applied microbiology and medical diagnostic assays where in many cases, the template is a mixture of homologous nucleotide sequences. In these cases it is assumed that the products after PCR are homogeneous. On the contrary, one of the many steps of SELEX requires the PCR amplification of huge diverse DNA templates generating a reaction mixture with complex chemical kinetics that are difficult to control. PCR amplification of an aptamer library usually produces many artifactual products and a bias for some nucleotide-sequences is observed [15, 17, 43] that render aptamer-selection procedure inefficient, time consuming and tedious. Several factors might be generating these biased and artifactual PCR products; 1) not all the aptamer sequences are equally accessible to primer hybridization following denaturation, 2) templates

are not extended by the polymerase with same efficiency, and 3) single-stranded templates hybridize with each other acting as initiation primers. Evidence suggests that a DNA sequence with a rich content of guanosine and cytosine influence PCR amplification [44]. Although template folding might also influences the bias in a PCR by hindering the primer priming regions or affecting polymerase progression, this is not well documented yet. Hence, it is possible to assume that the result of the PCR is based on PCR selection and aptamer best binders may be lost during this procedure. There are two strategies to reduce the effects of the PCR: 1) to perform single-molecule PCR, and 2) reduce as much as possible the number of SELEX cycles.

PCR can be significantly improved when performed on a single DNA template and this can be achieved by compartmentalization of the reaction in miniature reactors. Even more, next generation sequencing systems such as the Ion Torrent (Life Technologies) and Illumina´s sequencers use PCR-compartmentalization strategies for large genome sequencing. While the former uses emulsion-PCR [45], the latter amplifies single-DNA templates in defined clusters by bridge-PCR [46]. Emulsion-PCR is much more easy to operate in an academic laboratory and it has been applied for aptamer discovery to reduce by-product formation [47-49]. Basically, emulsion-PCR consists in the generation of a water-in-oil emulsion containing all the PCR-elements and where a single DNA template is placed in each droplet by limited dilution. Thus, amplification occurs in the droplets reducing PCR product-artifacts coming from intertemplate hybridization.

Decreasing the number of SELEX rounds is another approach to reduce PCR bias and artifacts. Many processes are under development to make aptamer discovery more efficient, and have been reviewed elsewhere [50, 51]. These processes include automated robotic SELEX platforms [52], capillary electrophoresis SELEX [53], non-equilibrium capillary electrophoresis of equilibrium mixtures (NECEEM) [54], microfluidics [55-58], graphene

oxide separation [59], capillary transient isotachophoresis [60], micro free flow electrophoresis [61], and high--put SELEX [48]. While research efforts are being applied for resolving the hurdles of aptamer development, only a few aptamer-oriented commercial incubators have been raised such as Somalogic (Boulder, CO, USA), Noxxon (Berlin, DE) and Apta Biosciences (Nanos, SG). Somalogic and Apta Biosciences have created aptamer-discovery platforms based on modified nucleotide-bases that help to increase the chances of obtaining high affinity aptamers against many different targets [62, 63]. On the other hand, the strength of Noxxon resides in their proprietary spiegelmer technology that overcomes aptamer instability in serum using aptamer enantiomers [64, 65], although, their technology requires access to the chiral mirror-image of the intended targets, which can be a difficult task for large protein targets.

3.2 Chemical information.

Nucleotide analogs have been used clinically as antiviral and antitumor drugs since the 1950s and with the advent of antisense oligonucleotides and *in vitro* evolution of aptamers, chemically altered nucleotides have found new uses. Initially, modified nucleotides have been used in aptamers to circumvent their susceptibility to nuclease degradation [66], however the utility of novel nucleosides to increase the chemical diversity of aptamers was rapidly elucidated. The natural four bases found within either RNA or DNA do not contain abundant chemical information when compared to amino acids, limiting aptamer-targets interactions and the efficiency of aptamer selection. Hence, modifying the nucleotide base, sugar, and/or the sugar-phosphate backbone of the aptamers, it is possible to transfer some properties of the amino acids to oligonucleotides such as hydrophobic groups and positive charges among others [67]. However, for SELEX, not all nucleotide chemical modifications are compatible with DNA/RNA polymerases [68] and new engineered polymerases are being developed that can be applied for aptamer discovery [69, 70]. Whereas polymerases for

sugar-modified nucleic acids have been created [70], modification of the nucleobases at the C5-position of the pyrimidines and the N7 of 7-deaza-purines are preferred since these positions have been shown to be good substrates for polymerases.

Somalogic was a pioneer in translating the opportunities of modified nucleotides into its commercial Slow Off-rate Modified Aptamer (SOMAmer) platform [62]. SOMAmers are aptamers containing nucleotides modified at the C5-position, which are used in SELEX. This allowed Somalogic to create a commercially efficient aptamer-discovery platform. Apta Biosciences has also exploited the benefits of novel nucleotide modifications and has created aptamers containing amino acid-like side chains [63]. Clearly, chemical modifications introduce new functionalities to aptamers, increase affinity for their targets, and enhance the aptamer discovery process; however, it has to be borne in mind that how the addition of chemical complexity affects aptamer properties such as denaturalization and antigenicity is still elusive.

During the past 25 years, great progress in aptamer research has been made. Aptamers have the potential to supplant current antibody-based technologies and promise to introduce novel diagnostic assays and therapies. However, PCR bias and the intrinsic low chemical diversity of natural oligonucleotides still present obstacles that discourage the consolidation of aptamer-based companies. Nevertheless, a handful of companies have been established that provide different technological platforms for the discovery and commercialization of aptamers and services including Base Pair Biotechnologies (Houston, TX, USA), Aptagen (Jacobus, PA, US) Neoventures Biotechnology (London, ON, CA), Somalogic (Boulder, CO, USA), Aptamer Sciences (Gyeonbuk. KR), Apta Matrix (Syracuse, NY, USA), AM Biotech (Houston, TX, USA), OTC Biotech (San Antonio, TX, USA), Apta Biosciences (Nanos, SG), Aptamer Group (York, UK) and Noxxon (Berlin, DE) among others. Interestingly, these companies claim that they have overcome some of the aptamer technology limitations using

process automation, high-throughput screening, NGS, and/or modified nucleotides. While many high quality aptamers have been selected manually, the future of aptamer high-throughput screening in the pharmaceutical industry may hinge on automation of SELEX

4. Expert Opinion

In general, there is a good agreement between aptamer researchers that an optimal commercial technological platform for aptamer discovery should avoid extensive rounds of selections, but is it possible to develop a technological platform without SELEX?

Microchip-based technologies such as DNA microarrays could provide the answer to this question. These devices contain an array of oligonucleotide elements bound to the surface of a glass, semiconductor chip, plastic plate or film and the location of each element bound to the surface is predefined. DNA microarrays are widely used to investigate large number of genes, however, with the advent of aptamers, DNA/RNA arrays have been found to be useful to identify aptamers [71-74]. In conventional microarrays, a fluorescently labeled-ligand is used to detect molecular recognition events and since these arrays are able to hold only thousands of molecules, their use in aptamer selection requires designing or working with a simplified aptamer library. Despite that DNA microarray technology is considered a high-throughput technique, from the aptamer technology perspective it is not and adaptation of DNA microarray technology for non-SELEX aptamer discovery will require arrays to harbor over millions of random molecules in predefined locations. There are different strategies to fabricate DNA microarrays and not all of them can be applied to generate a representative aptamer library on a solid support. Robotic spotting, for example, requires initially the synthesis of individual sequences in a solid-phase synthesizer making it economically prohibitable. On the other hand, automated on-chip fabrication or in situ synthesis of

thousands of oligomers in parallel seems to be a cost-effective approach. An aptamer discovery platform should be scalable and automated as much as possible; hence we think that on-chip fabrication can easily fulfill both requirements. Methodologies for in-situ fabrication include photolithography, micromirror, ink-jet and microfabricated electrode arrays [75]. We find the latter the most interesting technology for aptamer discovery. Its plasticity to be updated with electronic circuits, for example, to measure real-time molecular interactions by measuring microcurrent variations, makes it ideal for the discovery of aptamers to be used in sensing devices. In addition, in-situ fabrication can work with aptamer libraries that contain a variety of modified nucleotides to increase their chemistry diversity. The major obstacle in constructing this chip is mainly monetary, especially for academic laboratories. Considering that the cost of a DNA Chip array that holds 90K sequences is on average 400 USD$, in order to create a library of 9 million sequences will cost 40,000 USD$. Hence, it is not only costly but also $9x10^6$ sequences are far from the 10^{15} sequences contained in a standard library to be used for Selex, although this reduction in the number of molecules could be compensated using modified nucleotides to increase chemical diversity. Nevertheless, from a business point of view, these numbers should not be discouraging, and it is worth mentioning that the completion of the Human Genome Project costed over billions of dollars to sequence one human genome and we can now do the same for just 1000 USD$.

NGS technology is another well-established technology that could also provide the foundation bases for a novel aptamer discovery platform, although it will be less compatible with modified nucleotides due to the fact that most NGS systems are still dependent on enzymatic polymerases. Illumina and Life Technologies NGS systems are widely used for genome sequencing. Basically, a DNA library is prepared by random fragmentation of DNA, followed by *in vitro* ligation of adaptor sequences. Then, bead emulsion PCR or bridge PCR is used to generate spatially clustered clonal amplicons or an array of sequences that are

subject for sequencing by synthesis. Data are acquired by imaging of the full array or sensing hydrogen ions at each cycle [76]. Interestingly, what makes NGS a breakthrough is the array (also known as flow cell) that allows holding tens of millions of DNA sequences for parallel sequencing. However, contrary to a DNA chip array, locations of the sequences in a NGS array are not predefined. The Illumina flow cell is a planar optically transparent surface similar to a microscope slide, which contains a lawn of oligonucleotides anchors bound to its surface [77] that are complementary to the adaptors previously ligated enzymatically to genomic DNA fragments. It is easy to envision how this type of array could be implemented in a non-SELEX aptamer discovery platform. Instead of working with an aptamer library flanked with primer priming regions, it is possible to have a library flanked by adaptors for flow cell anchoring and for bridge PCR in order to generate millions of clusters, where each cluster contains a group of clonal aptamers. As mentioned above, location of each sequence is unknown, hence a typical procedure should be as follows: 1) incubation of aptamers attached to the flow cell with a fluorescently labeled target molecule, 2) acquiring data by flow cell imaging, 3) array sequencing, and 4) superimposing the sequencing results obtained from step 3 and images obtained from step 2 in order to retrieve those DNA sequences that interacted with the target molecule for further biochemical assays. This procedure can be readily applied since Illumina´s systems already collect fluorescent data from every cluster during sequencing and correlation between data obtained from an aptamer cluster bound to a fluorescently labeled ligand and clustered-aptamer sequence should not be a limitation. On the other hand, Life Technologies NGS (LT-NGS) systems are slightly different from Illumina´s technology, however LT-NGS systems have also the adaptability for aptamer discovery. LT-NGS flow cell consists of a CMOS sensor array chip that can monitor and measure hydrogen ions liberated during sequencing chemistry of millions of DNAs in parallel [78]. Basically, a bead carrying DNA sequences previously amplified by emulsion

PCR are deposited by limited dilution in microwells where the CMOS sensor resides at the bottom of the wells to monitor the sequencing chemistry. Contrary to Illumina's technology, these systems don´t work with an image data collector, hence, their adaptation for aptamer discovery will require intense modifications of such systems.

It is reasonable to expect that assembling and testing such technological platforms will be costly but we hope this review could stimulate further attempts to address this crucial issue.

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