Functional Nucleic Acids in High Throughput Screening and Drug Discovery

Seergazhi G. Srivatsan[1] and Michael Famulok[*,2]

[1]*Department of Chemistry and Biochemistry, University of California, San Diego, La Jolla, CA 92093-0358, USA*

[2]*LIMES Institute, Program Unit Chemical Biology and Medicinal Chemistry, c/o Kekulé-Institut für Organische Chemie und Biochemie, Gerhard Domagk-Str. 1, D-53121 Bonn, Germany*

Abstract: *In vitro* selection can be used to generate functional nucleic acids such as aptamers and ribozymes that can recognize a variety of molecules with high affinity and specificity. Most often these recognition events are associated with structural alterations that can be converted into detectable signals. Several signaling aptamers and ribozymes constructed by both design and selection have been successfully utilized as sensitive detection reagents. Here we summarize the development of different types of signaling nucleic acids, and approaches that have been implemented in the screening format.

Keywords: Aptamers, *in vitro* selection, aptamer displacement assays, ribozymes, allostery, aptazymes, riboswitches, signaling aptamers.

INTRODUCTION

High throughput screening (HTS), a technique in which libraries of compounds are tested to identify small molecules that interact with target proteins or alter catalytic activity is contemplated as a key drug discovery tool [1-3]. Therefore, assays that are robust, reliable and amenable to a HTS-format are highly desirable. In this regard, functional nucleic acids sensors, both natural and artificial have proven to be very sensitive tools in assessing nucleic acid-protein/small molecule interactions, as these biological events are most often associated with intricate structural and dynamic changes that are translated into an easy read-out signal [4-9]. An important feature that makes these natural and unnatural nucleic acids as valuable tools for screening approaches is their mode of recognition and binding. They use their secondary and tertiary structures to recognize the three-dimensional topology of the target, and simultaneously undergoing conformational changes upon ligand binding, sometimes referred to as induced fit or adaptive binding [10,11]. Thus, these conformational changes within the functional nucleic acid around the target provide a way to obtain structural information about the target in the form of signals.

Artificial nucleic acid sensors that have been adapted to HTS setup include aptamers and allosteric ribozymes [4-6]. Aptamers are single stranded nucleic acids possessing high binding affinity and specificity to its target molecule. Using *in vitro* selection protocol, SELEX (Systematic Evolution of Ligands by Exponential Enrichment), functional nucleic acids can be selected from a large random nucleic acid libraries (~10^5) for potentially any target molecule spanning small molecules to proteins [12-14]. *In vitro* selected nucleic acids can be easily tuned to exhibit higher stability, affinity and specificity towards a desired target [15]. Moreover, aptamers are amenable to chemical manipulations and hence, new functionalities can be introduced to augment their inherent propensity to bind targets. A chimera of functional domains, such as an aptamer and a catalytic domain, is referred to as aptazyme, reporter ribozyme, allosteric ribozyme or ribo-reporter [4,16]. Akin to natural allosteric enzymes, catalytic activity of allosteric ribozymes is also regulated by the binding of the effector molecule to the aptamer domain, distant from the ribozyme active site.

Several naturally occurring and therapeutically relevant nucleic acids targets have been implemented in screening formats in an effort to identify small molecule inhibitors. They include ribozymes [17], bacterial ribosomal assembly [18,19] and HIV-1 TAR [20], to name a few. Recently, a new class of functional nucleic acids called riboswitches was discovered [21,22]. Riboswitches have a modular architecture, consisting of an aptamer domain responsible for ligand binding and an "expression platform" that communicates the ligand-binding state from the aptamer to RNA elements that are involved in gene expression.

Functional nucleic acids can be readily modified to accommodate several functional groups such as fluorescent dyes, affinity tags or cross-linker molecules. Numerous reports demonstrate the ease of adapting nucleic acid sensors to almost any type of biophysical technique, such as fluorescence-, enzyme-linked, bead-, or radioactive-based assays. In particular, fluorescence-based methods like fluorescence intensity (FI), fluorescence resonance energy transfer (FRET), fluorescence polarization (FP) and fluorescence lifetime (FLT), typically provide information about a binding event in real-time and with great sensitivity [23-25]. Not surprisingly, functional nucleic acids used in screening approaches mostly employ fluorescence-based assays. The most commonly employed read-out method in HTS is a FRET between a fluorescent donor (D) and an acceptor or quencher molecule (Q). When donor and acceptor molecules are distant from each other, donor emission can be observed upon excitation of the donor. A ligand induced structural changes within the nucleic acid sensor can result in the donor and acceptor molecules coming in close proximity, and

*Address correspondence to this author at the LIMES Institute, Program Unit Chemical Biology and Medicinal Chemistry, c/o Kekulé-Institut für Organische Chemie und Biochemie, Gerhard Domagk-Str. 1, D-53121 Bonn, Germany; Tel: +49-228-735661; Fax: +49-228-735388;
E-mail: m.famulok@uni-bonn.de

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hence the acceptor emission is predominantly observed due to a FRET from the donor to acceptor [23,26,27].

Fluorescence intensity (FI) is a straightforward and conventional method in which structural changes sustained during the complex formation between nucleic acid-protein and nucleic acid-small molecule result in enhanced or diminished emission of the fluorescently tagged reporter nucleic acid. This method has been elegantly exploited in studying RNA folding and recognition, and has found important applications in numerous bioanalytical and discovery assays [28-31].

Although less prevalent in HTS protocols, fluorescence polarization (FP) is highly effective in monitoring complex formation and disruption in real time. This method uses the amount of depolarization incurred upon exciting the fluorescent molecule with polarized light as a measure of the binding event [23]. The degree of depolarization is directly proportional to the molecular weight of the excited molecule. The higher the molecular weight of the excited molecule, the lesser is the rate of rotation and tumbling of the molecule, resulting in higher FP. On the other hand, if the excited molecule is small, rotation and tumbling processes occur faster, and consequently, FP is reduced. A similar assembly can be applied in discovery assays, wherein a fluorescently modified nucleic acid-protein complex displays higher FP, and when an inhibitor molecule disrupts the complex, a reduced FP is observed corresponding to the free fluorescently modified nucleic acid.

SIGNALING APTAMERS AS SCREENING TOOLS

DNA Aptamers

Li and coworkers constructed a structure-switching signaling aptamer for monitoring enzyme activity and inhibitor screening. As a proof of concept they used an anti-ATP DNA aptamer [32], which can discriminate between closely related homologues such as adenosine (A), adenosine mono-, di- and triphosphate (AMP, ADP and ATP). A tripartite, fluorescence-quenching, two-stem duplex containing an antisense sequence embedded with fluorophore (F), an antisense sequence carrying a quencher, and an extended aptamer sequence was assembled (Fig. **1a**) [33]. The extended aptamer construct was designed to possess complementary regions for the above two short DNA sequences carrying the fluorophore and quencher in addition to the target binding motif. In the absence of target, the three DNA oligonucleotides assemble into tripartite duplex structure, thereby bringing the fluorophore and the quencher in close proximity for maximum quenching effect by FRET (Fig. **1a**). However, in the presence of target molecule, the aptamer domain binds to adenosine and the quencher-labeled antisense strand is set free, resulting in enhanced emission [33].

Drawing inspiration from the efficacy of the signaling aptamer to distinguish between adenosine and AMP, Li and coworkers speculated that their aptamer would serve as a suitable reporting system for nucleotide-dephosphorylating enzymes such as alkaline phosphatase (ALP). ALP is an enzyme which hydrolyzes the phosphate groups from ATP, ADP and AMP to adenosine. Preliminary studies revealed that the aptamer better discriminates between adenosine and AMP, and rather poorly between adenosine, ATP and ADP. Addition of ALP to the aptamer assembly in the presence of AMP led to a rapid increase in fluorescence intensity as a consequence of increasing concentration of adenosine [34] (Fig. **1a**). The potential utility of this aptamer as a signaling tool in screening small molecule enzyme inhibitors was also attempted. An inhibition assay performed in a 96-well plate, in the presence of known inhibitors demonstrated the feasibility of using the signaling aptamer in HTS [34].

The above signaling aptamer technology was also applied in the screening of small molecule inhibitors for a relatively challenging target, adenosine deaminase (ADA). ADA plays a pivotal role in the purine metabolism pathways, catalyzing the irreversible deamination of adenosine/deoxyadenosine to inosine/deoxyinosine. Importantly, ADA deficiency is known to cause certain severe combined immunodeficiency diseases [35]. Therefore, development of a homogeneous and sensitive assay amenable to HTS format will be of great significance. Elowe and coworkers developed a screening assay similar to the one discussed above, by using a signaling aptamer, which exhibited high affinity for adenosine and practically no affinity for inosine [36]. The aptamer was fluorescently modified at the 5' end with fluorescein and contained a complementary binding region for the quencher-labeled antisense strand (Fig. **1b**). The aptamer in the free form can only hybridize with the antisense strand containing the quencher resulting in overall quenching by FRET. In the presence of adenosine, the aptamer readily binds adenosine and higher fluorescence emission associated with free fluorescein-modified aptamer is observed. However, in the presence of ADA, adenosine is converted into inosine and hence, the DNA duplex form of the aptamer predominates, leading to a decrease in emission (Fig. **1b**). Adapting this assay to HTS, a collection of 44,400 compounds was tested for possible inhibitory activity. An Initial screen revealed 47 hits, which was trimmed down to 7 after testing in the presence of nonspecific inhibitors such as reducing agents, detergents and bovine serum albumin. However, after thorough characterization only one compound, MAC-0038732 was found to be a true ADA inhibitor [36].

Janjic and colleagues designed a competition binding HTS assay based on DNA aptamers that specifically recognize platelet-derived growth factor (PDGF) B-chain, and thus prevent the binding of the growth factor to its cognate receptor [37,38]. Twelve anionic organic compounds derived from napthalenesulphonic acid, which are known to inhibit the binding of PDGF to its receptor, were tested for their ability to displace the aptamer-PDGF complex in a radioactive filter binding assay. When tested in a functional assay, the binding affinities of all the ligands strongly correlated with their inhibitory potential, which further proves the ability of an aptamer probe in preserving the information about a biological event, in this case the inhibitory activity of small molecules [37].

Recently, a novel and general approach to create fluorescent signaling aptamers using a fluorescent nucleoside analogue was presented. Aptamers for human α-thrombin, immunoglobulin E, and PDGF were modified with fluorescent nucleoside analogue, 2-aminopurine in positions that undergo conformational changes upon binding with their respective targets [39]. 2-aminopurine is a highly emissive adenine analogue and its emission strongly depends on the

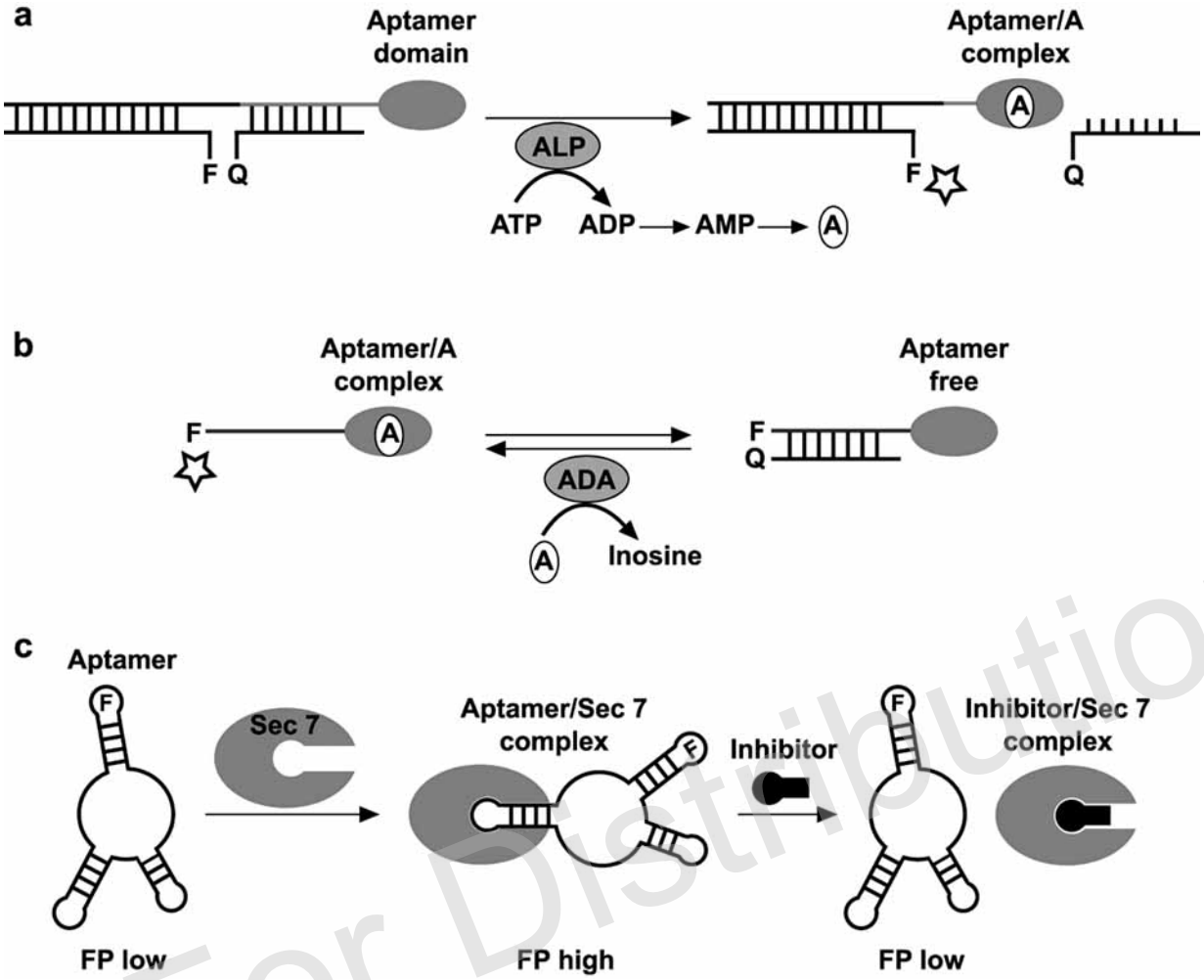

Fig. (1). Aptamers as screening tools. (**a**) Monitoring the activity of ALP in real time by using a signaling aptamer that reports the hydrolysis of an AMP to adenosine (A). In the absence of adenosine maximum quenching is observed, whereas in the presence of the hydrolysis product, the duplex structure is transformed into aptamer-A complex with concomitant release of the quencher (Q), and an enhancement in fluorescence signal corresponding to the free fluorophore (F). (**b**) A signaling aptamer that senses the presence of adenosine. As adenosine is transformed to inosine by ADA, the aptamer becomes available to complex with the quenching group (Q) resulting in fluorescence quenching by FRET. (**c**) Aptamer displacement screening assay by fluorescence polarization. The fluorescently-labeled aptamer exhibits low polarization in the unbound state. When bound to the Sec7 domain of cytohesin 1, fluorescence polarization becomes high. A small molecule that displaces the aptamer from the protein results in low fluorescence polarization.

base stacking interactions with other bases in the oligonucleotide [40]. Fluorescently modified aptamers clearly indicated a binding event with an increase in fluorescence signal of up to 30-fold [39]. Although extension of this approach to HTS was not attempted, it underscores the possibility of using such signaling aptamers in screening protocols.

RNA Aptamers

Theoretically speaking, it should be possible to generate signaling aptamer system from every aptamer. In the case of protein targets, aptamers can be readily applied to HTS format by utilizing fluorescence polarization (FP) method. FP can be effectively used in monitoring the equlibrium binding event by measuring the changes in depolarization incurred upon exciting the fluorescent molecule by polarized light. The amount of depolarization is directly related to the size or the molecular weight of the fluorophore [23]. Since aptamers are amenable to chemical modifications, it would be convenient to attach a fluorescent tag to the functional nucleic acid, preferably distant from the target binding domain. Fluorescently modified aptamers in the unbound state rapidly tumble, leading to a higher depolarization effect and hence, lesser fluorescence polarization. Therefore, the free aptamer will experience less polarization than the aptamer-protein complex. Such a scenario would be ideally suited for screening libraries of compounds to identify potential leads possessing the ability to displace the aptamer from the complex and consequently, resulting in reduced polarization corresponding to the free aptamer.

We applied this principle in a HTS format to identify cytohesin-specific small molecule inhibitors [41]. An aptamer specific for the Sec7 domain of the small guanine nucleotide exchange factors (GEFs) and cytohesin-1 Sec7 domain [42] was fluorescently modified, and a FP-based screening assay was conceived to identify cytohesin-specific small molecules that displaced the aptamer from its target protein (Fig. **1c**). A series of 1,2,4-triazole derivatives were

identified from a complex library of synthetic compounds. Initial hits were subjected to preliminary *in vitro* guanine nucleotide exchange assays to obtain binding data, and a promising lead compound, SecinH3 was chosen for further studies. The inhibition of cytohesins by SecinH3 in mice resulted in hepatic insulin resistance, one of the first steps in the development of type 2 diabetes [43]. Moreover, SecinH3 resulted in a phenocopy of the effects caused by mutations in the steppke gene, the single cytohesin homolog in *Drosophila melanogaster* [44]. Taken together, these results demonstrate the potential of chemical biology and aptamer based HTS in exploring the molecular basis of disease states [41].

ALLOSTERIC RIBOZYMES AS SCREENING TOOLS

Generation of Allosteric Ribozymes

Allosteric ribozymes normally consist of an aptamer domain and a catalytic motif. The catalytic activity of the ribozyme is allosterically modulated by conformational changes experienced by the aptamer upon ligand binding, which is communicated to the catalytic unit. Allostery appears to be a common mechanism in aptamer/ligand complexation and was first discovered in small molecule/aptamer complexes [11,45,46]. Most artificial allosteric ribozymes generated by design and selection methods, are derived from naturally occurring hammerhead and hairpin ribozymes [4,16].

Artificial allosteric ribozymes have been generated in three different ways; rational design, selection, and a combination of both. In a rational design approach, an aptamer with desired affinity is conjugated to the ribozyme, and the catalytic activity of the ribozyme is enhanced or diminished by the binding of the effector molecule to the aptamer. Modular rational design was used to create several allosteric ribozymes that are regulated by the binding of specific organic effectors to their cognate aptamer domain [47,48]. Using a similar approach, oligonucleotide-dependent hairpin ribozyme was also assembled [49].

Alternatively, appending a randomized region to the ribozyme and using allosteric selection, ribozymes with new effector specificities can be generated. In this approach, an entire allosteric domain of the ribozyme is randomized and an allosteric selection protocol is applied to isolate constructs whose cleavage activity is activated or deactivated in the presence of external effectors [50-52]. A protein dependent RNA-ligase ribozyme was also reported using an allosteric selection technique [53,54].

Thirdly, allosteric ribozymes can be generated using a three-domain construct composed of a ribozyme and an RNA aptamer that are linked *via* a small randomized bridge. This bridge domain or 'communication module' acts as a channel, which relays the binding status of the aptamer to the ribozyme, thereby regulating the activity of the ribozyme. Ribozymes made of a flavin mononucleotide (FMN)-specific aptamer and a hammerhead ribozyme were subjected to selection to evolve functional communication modules that could allosterically regulate the ribozyme activity [55,56].

Direct Screening

The cleavage activity of allosteric ribozymes can be modulated by small molecules or proteins that compete for the aptazyme or its target [4]. A screening protocol to test novel interaction partners of a target can be envisaged, if the cleavage activity can be translated into a real time read-out signal, such as fluorescence. We developed a FRET-based assay to monitor the cleavage activity of hammerhead ribozyme by using an external oligonucleotide substrate containing a fluorophore at the 5' end and a quencher at the 3' end [57]. Utilizing this approach, we moved on to rationally design a signaling Rev-responsive ribozyme by fusing a Rev binding element (RBE) of HIV-1 to the stem II of the hammerhead ribozyme [58]. Addition of Rev protein or a peptide corresponding to the RNA-binding arginine-rich motif epitope of Rev triggered a conformational switch, causing the ribozyme to be inactive towards the fluorescent substrate and thus, fluorescence quenching by FRET was observed (Fig. **2a**). However, the cleavage activity of the ribozyme was restored by the addition of a small molecule that competed with the RBE for Rev binding. In a similar strategy, a HIV-1 reverse transcriptase (HIV-1 RT) dependent reporter ribozyme was also constructed [59]. We chose a HIV-1 RT specific aptamer with pseudoknot structure because this motif is often used as a regulatory element in nature [60,61]. In the absence of HIV-1 RT the hammerhead ribozyme was active. However, in the presence of HIV-1 RT the activity was impaired due to the induction of a pseudoknot fold by the protein [59]. This reporter ribozyme was also transformed into a screening format and applied to identify a novel small molecule inhibitor for HIV reverse transcriptase (HIV-RT) that binds an unexploited site on this protein [62]. The compound, termed SY-3E4, selectively inhibited DNA-dependent polymerase activity, and was able to inhibit the replication of wild-type virus and a multidrug-resistant HIV-1 strain.

In another approach, we generated an aptamer inhibited ribozyme by appending an anti-Rev aptamer [63] to the 5' end of the hammerhead ribozyme [58]. A part of the aptamer was designed to hybridize to the hammerhead ribozyme to form a stem that prevented annealing of the fluorescent substrate and hence, its cleavage. Upon addition of Rev protein or peptide to the reporter system, the aptamer formed a defined structure around the target protein rendering the substrate-binding site available for annealing and cleavage of the FRET-labeled substrate. However, addition of the Rev specific organic molecule disrupted the aptamer-protein interaction and hence, the cleavage activity of the ribozyme by rehybridization of the aptamer. Consequently, maximum quenching by FRET was observed.

Both the Rev-responsive ribozyme and the aptamer-inhibited ribozyme discussed above were successfully applied for the screening of organic molecules binding to Rev protein. A model library of 96 structurally diverse antibiotics was screened with each of the signaling systems, and coumermycin A was identified as a novel Rev-binding lead compound that also inhibited the HIV-1 replication [58].

A simplified version of the reporter ribozyme was designed using hairpin [64] and hammerhead ribozymes [58]. These ribozymes, referred to as aptamer-hybridizing reporters contained sequences complementary to the anti-thrombin aptamer. Hybridization of the aptamer to the complementary region of the ribozyme resulted in a deactivated form of the ribozyme. On the contrary, addition of excess of the cognate

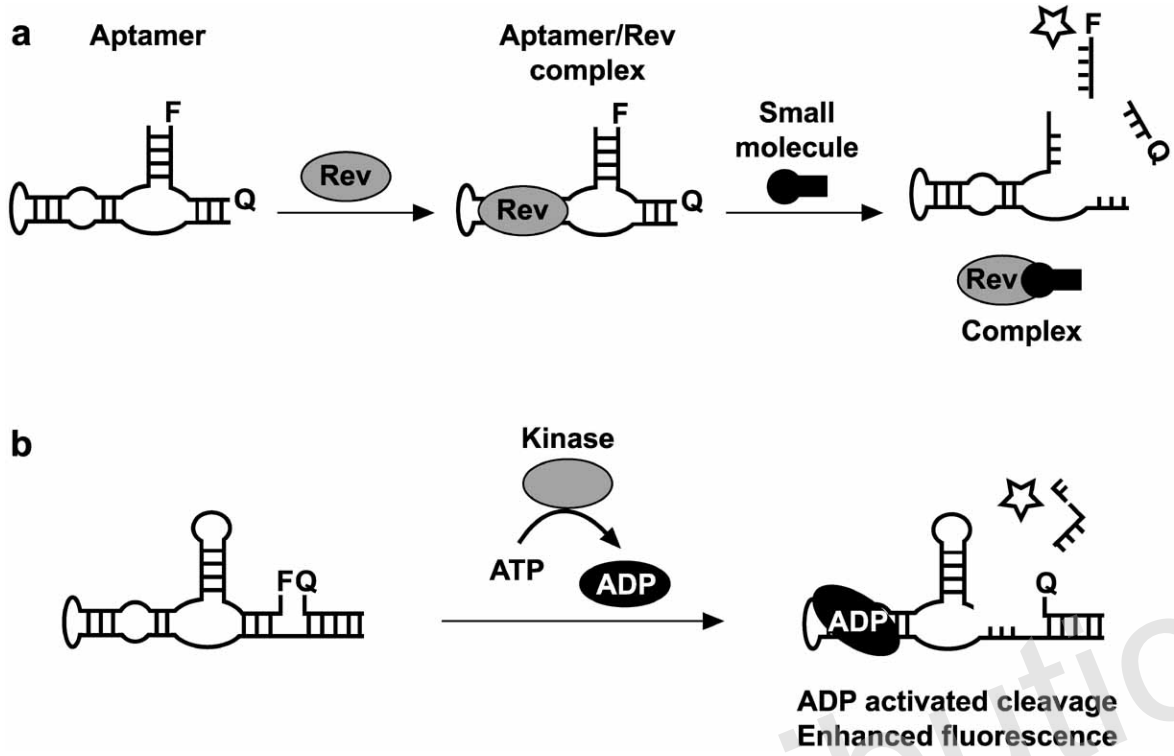

Fig. (2). Ribozymes as screening tools. (**a**) A Rev-responsive hammerhead ribozyme directly reports the cleavage status of the ribozyme. Binding of a small molecule to the Rev protein releases the protein from the ribozyme and thus triggers the cleavage of the doubly-labeled FRET probe, which causes fluorescence enhancement. (**b**) RiboReporter consisting of an ADP aptamer appended to the hammerhead ribozyme indirectly reports kinase activity by responding to ADP generated in the reaction. ADP binding activates ribozyme cleavage and leads to release of the fluorescently-labeled (F) oligonucleotide, which generates a fluorescence signal.

protein, thrombin restored ribozyme activity. This assay sensitively reported the α-thrombin-hirudin protein-protein interaction in a concentration-dependent manner [58]. These results show that thrombin-responsive ribozymes can be adapted to HTS protocols for identifying small molecules or peptides that function as better thrombin inhibitors.

Indirect Screening

Ribozymes can be designed to monitor the changes in the concentration of substrate or product in an enzymatic reaction. In this approach a suitable aptamer specific for either substrate or product, is linked to the ribozyme. Recently, a fluorescent read-out assay was developed by constructing a RiboReporter using a minimized ADP aptamer that detects ADP in a background of over 100-molar excess of ATP [65]. An aptamer appended to a hammerhead ribozyme through a random eight nucleotide connecting stem region was subjected to rounds of selection. After selection and characterization, RNA molecules whose catalytic activity was ADP dependent was isolated (Fig. **2b**). The highly robust ADP dependent RiboReporter sensor was used to monitor pERK2 protein kinase activity in a pilot screen of 77 drug-like molecules. The sensor was able to rediscover a known protein kinase inhibitor (staurosporine) present in the microplate [65].

NATURAL RNAS AS SCREENING TOOLS

Expanding knowledge of several new key biological roles played by RNA molecules, especially in the gene expression processes has fueled the growing interest in exploring RNA as disease intervention targets [67-69]. Assays similar to those used for protein targets have been implemented in search for compounds that interfere with the functions of therapeutically relevant RNA targets. Although, these naturally occurring functional RNA can be readily converted into probes, only few systems have been used in HTS formats in the pursuit of discovery of novel inhibitors [69].

Riboswitches

Many bacteria utilizing noncoding regions of mRNA called riboswitches as metabolite-dependent genetic control elements was a surprising discovery [21,22]. The concept of metabolite-dependent gene regulation has opened up a completely unexplored class of potential targets for the development of bactericides [70,71]. Upon interaction with an appropriate small molecule ligand or metabolite, the riboswitch undergoes structural reorganization that results in the modulation of genes that they code [72-77]. Structural probing studies indicate that riboswitches are composed of two domains: a natural aptamer that serves as a ligand-recognition element, and an 'expression platform' that acts as a conduit for communicating the ligand-binding state to RNA elements involved in the gene regulation [73-75].

A HTS compatible fluorescent-based assay to monitor the *cis*-cleaving activity of glmS riboswitch was recently reported by our group [78]. The glmS riboswitch is located in the 5'-UTR of glmS–mRNA that codes for glucosamine-6-phosphate synthetase [79] and its activity is regulated by

glucosamine-6-phosphate (GlcN6P), which is a metabolite of glmS-encoded protein itself. Identifying small molecules that activate this riboswitch in a fashion analogous to GlcN6P will be of significant importance because they are likely to exhibit antibiotic activity by stimulating the destruction of the mRNA, and hence, the protein required for the synthesis of the bacterial cell wall precursor molecule. An 81-nucleotide minimal ribozyme motif, which was fluorescently modified at the 5'-end for an FP-based assay was constructed (Fig. **3a**). Addition of GlcN6P triggered the *cis*-cleavage of the ribozyme resulting in the release of a 4-mer fluorescently modified short RNA from an 81-mer substrate. The high difference in the molecular weight was concomitantly translated into sensitive fluorescence (FP) response (Fig. **3a**). To test the compatibility of this assay in a HTS setup, a pilot screen using 88 compounds including GlcN6P was successfully established [78].

Blount and coworkers also developed a HTS assay for glmS ribozyme cleavage based on FRET [80]. To validate the screening assay, a focused library of GlcN6P analogues whose affinities for the ribozymes were determined by conventional radiolabeled method was used. A test screen containing 960 compounds revealed five active compounds, one of which was a GlcN6P analogue known to activate ribozyme activity. These results demonstrate the potential of riboswitch targets in the development of antimicrobials using HTS technique. Analogues that exhibit antimicrobial action against riboswitch targets are also known; examples include, S-(2-aminoethyl)-L-cysteine binds to lysine riboswitch [81] and pyrithiamine, a thiamine analogue binds to thiamine pyrophosphate responsive riboswitches in bacteria and fungi [82]. Similarly, short RNA hairpins isolated by *in vitro* selection to bind the thiM riboswitch only in the absence of the ligand may prove useful either as inhibitors themselves or as tools for setting up screening approaches to find thiamine pyrophosphate analogues [83].

Pre-miRNAs

Micro RNAs (miRNAs) are short, double-stranded, regulatory RNA constructs of ~21 base pairs in length. Mature miRNAs are generated from their inactive hairpin precursor molecules, the pre-miRNAs by a nuclease called Dicer that is specific for double-stranded RNA [84,85]. The mature miRNAs, in the form of RNA–protein complexes inhibit protein translation. The precise biological role and the mechanism of miRNA-mediated gene regulation are still unclear. However, compelling evidence indicates that various diseases, especially many types of cancer may be associated with specifically modified cellular miRNA expression

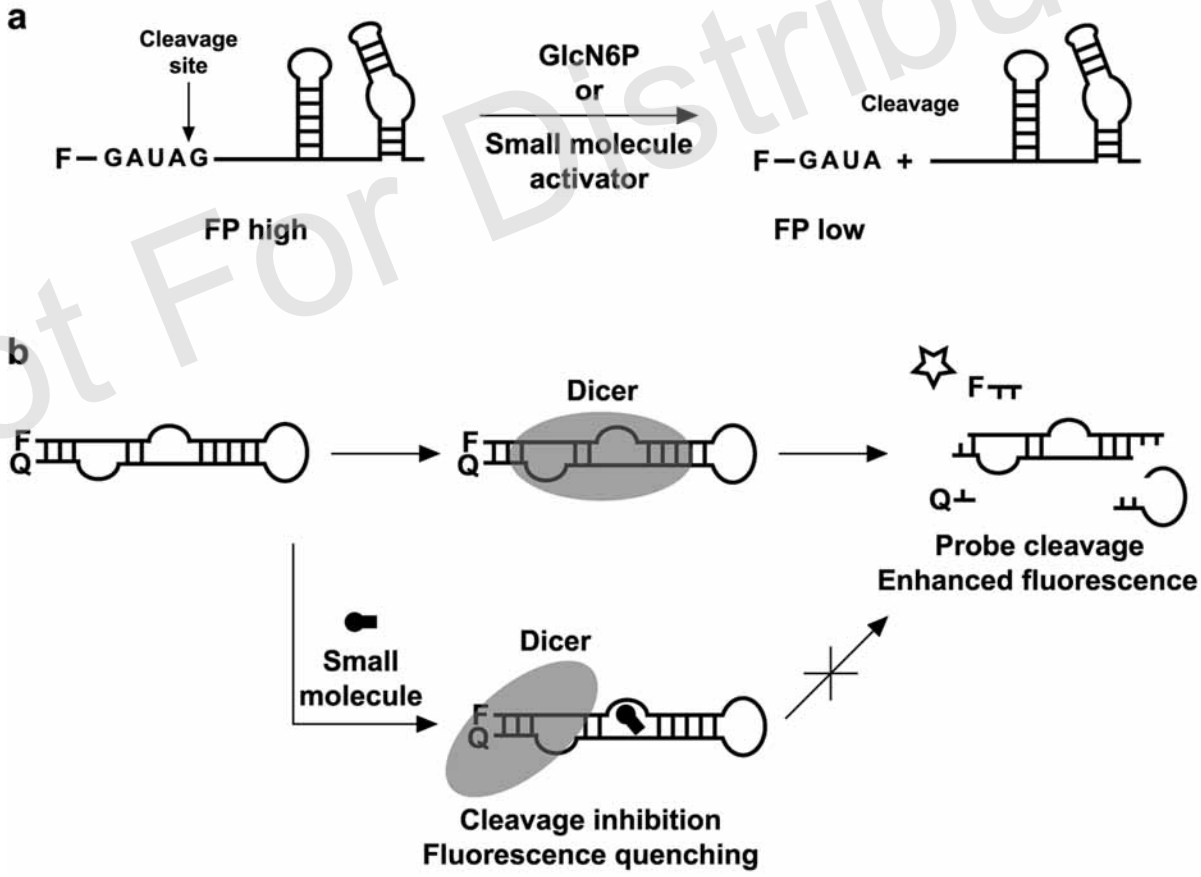

Fig. (3). Natural RNAs as screening tools. (**a**) A fluorescent-labeled glmS ribozyme and in *cis* cleavage induced by glucosamine-6-phosphate (GlcN6P) or a potential small molecule activator. The inactive full-length fluorescently-labeled ribozyme exhibits high fluorescence polarization. Activation of the ribozyme by GlcN6P triggers cleavage, releasing a short fluorescently-labeled fragment, which shows low polarization. (**b**) A homogenous assay for miRNA maturation. The doubly labeled pre-miRNA is hydrolyzed by Dicer leading to increased fluorescence. In the presence of a small molecule pre-miRNA binder, the Dicer-mediated cleavage is inhibited and hence, results in fluorescence quenching by FRET.

patterns [86,87]. Predictably, miRNAs are in the spotlight as targets for new therapeutic approaches [88].

Davies and Arenz devised a homogeneous fluorescence-based assay to monitor Dicer mediated miRNA processing [89]. A doubly labeled pre-miRNA, pre-let-7 RNA from *Drosophila melanogaster* [90] was constructed by ligating a 5'-fluorescein modified and a 3'-qencher modified strands (Fig. **3b**). Dicer-mediated hydrolysis of pre-miRNA would result in the dissociation of the fluorescence emitter and quencher, and thus increase in the fluorescence. Molecules that bind to pre-miRNA and inhibit Dicer-mediated cleavage would essentially result in fluorescence quenching by FRET (Fig. **3b**). Dodecapeptides derived form the amino acid sequence of Dicer as potential pre-miRNA binders were successfully tested for their ability to inhibit the mi-RNA maturation [89]. In principle, this assay can be adapted to HTS formats to fish out prospective inhibitors of miRNA maturation from a library of potential pre-miRNA/Dicer binders.

CONCLUSIONS

Screening or reporter assays based on functional nucleic acids have now been designed and applied for a variety of purposes, from high throughput screening to bio-sensing. There are two types of allosteric ribozymes amenable for screening: Firstly, there are ribozymes that selectively monitor substrate or product formation during an enzymatic reaction. These are used to screen for inhibitors of catalytic activity in an indirect way. Secondly, there are ribozymes whose cleavage activity is directly dependent on proteins and peptides. Small molecules or other proteins that disrupt aptazyme-target binding can adversely affect the cleavage activity of these allosteric ribozymes. If the cleavage activity is transduced into an easy read-out signal, such as fluorescence, screening of novel interaction partners of a target protein can be performed. Finally, fluorescence labelled aptamers or natural RNA/protein complexes can also be used directly for screening, provided that the formation or disruption of the complex triggers a signal that can be detected.

By combining high throughput screening with aptamer technology, mechanism of Darwinian evolution can be exploited for identifying new drugs or drug-leads. RNAs and other biomolecules continuously adapt their functional properties to external conditions by mechanisms of Darwinian evolution, in which superior features emerge from a combination of genetic alterations and survival of selective pressures and are passed on to subsequent generations. The same principles can be applied *in vitro* to select for aptamers from libraries with a molecular diversity of up to 10^{16} different structured nucleic acid sequences, the highest degree of diversity currently amenable to screening. Aptamers can serve as specific inhibitors of virtually any given protein target, as has been demonstrated many times. Many of the assays summarized here allow, in principle, to directly translate the information stored within an aptamer into a small molecule, providing new and largely unexplored opportunities for drug discovery.

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ABBREVIATIONS

A	=	Adenosine
ADP	=	Adenosine diphosphate
ADA	=	Adenosine deaminase
ALP	=	Alkaline phosphatase
AMP	=	Adenosine monophosphate
ATP	=	Adenosine triphosphate
D	=	Donor
F	=	Fluorophore
FI	=	Fluorescence intensity
FLT	=	Fluorescence lifetime
FMN	=	Flavin mononucleotide
FP	=	Fluorescence polarization
FRET	=	Fluorescence resonance energy transfer
GEFs	=	Guanine nucleotide exchange factors
GlcN6P	=	Glucosamine-6-phosphate
HIV	=	Human Immunodeficiency virus
HTS	=	High throughput screening
miRNAs	=	Micro RNAs
PDGF	=	Platelet-derived growth factor
Q	=	Quencher
RBE	=	Rev binding element
RT	=	Reverse transcriptase
SELEX	=	Systematic evolution of ligands by exponential enrichment
TAR	=	Trans-activating response

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