Searching for Molecular Solutions – Cited Notes

CHAPTER 6

These Files contain details on all references to this ftp site within **Chapter 6** of *Searching for Molecular Solutions*. The page numbers of the book where the reference is made are shown in the Table below, the corresponding page number for this file, and the title of each relevant section.

Contents:

Book Reference Page Numbers	Page Number in this File	Section	
		No.	Title
212 219	2	17	Aptamer and Ribozyme Applications

Section 17:   *Aptamer and Ribozyme Applications*

Cited on p. 212 and 219 of *Searching for Molecular Solutions*

This section provides some detail on non-clinical applications of RNA and DNA aptamers and ribozymes. In particular, this embraces the arena of 'switchable' ribozymes in conjunction with aptamers, a big growth area in functional nucleic acid research.

An overview of nucleic acid enzymes and their overlap with aptamers is provided in Fig. 6.Na below. The category of 'Other Catalytic Applications' refers to enzymatic activities not related to nucleic acid cleavage.
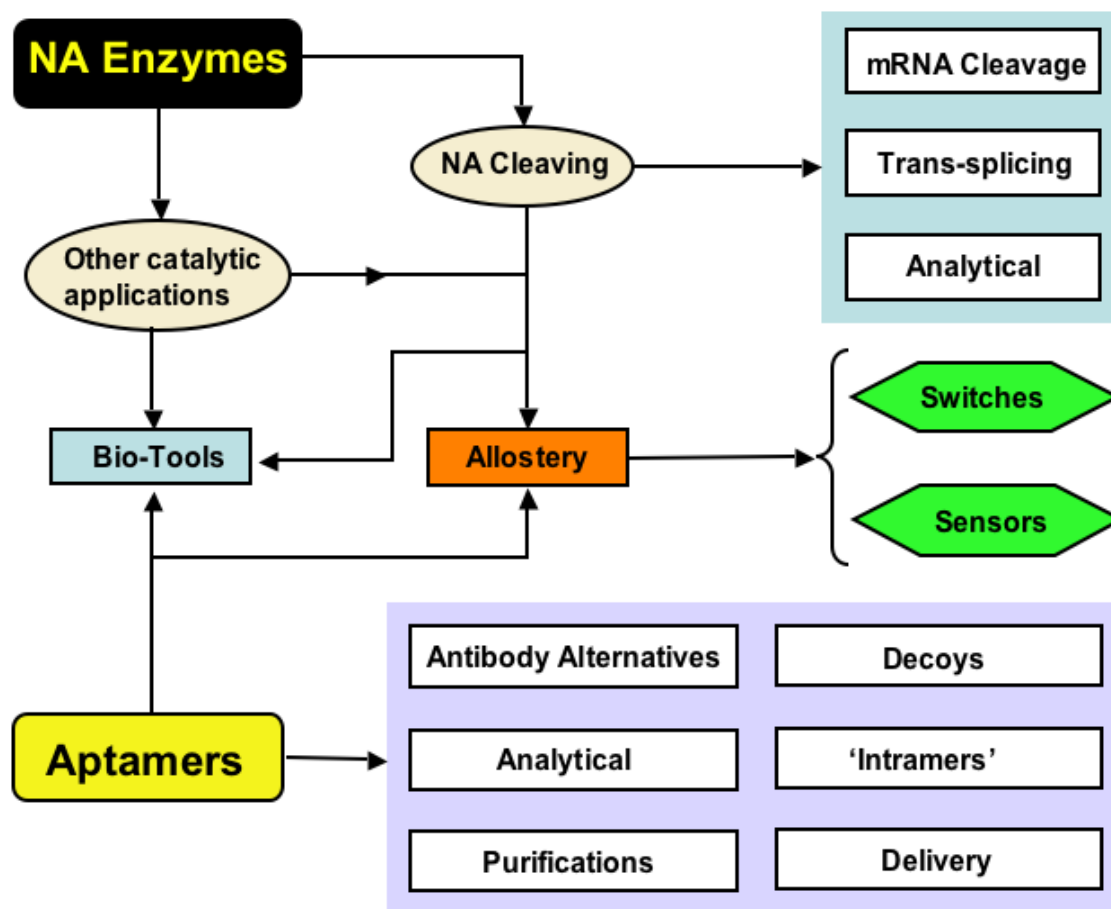
Fig. 6.Na.

Applications of nucleic acid (NA) enzymes and aptamers. For aptamers, 'Antibody Alternatives' refers to any role as a specific binding molecule which could in principle be performed by antibodies also; includes diagnostic applications. 'Intramers' are aptamers expressed intracellularly. Some ribozyme applications such as trans-splicing are briefly noted in Chapter 9 of *Searching for Molecular Solutions.*

Tools for Science, Technology and Beyond

It takes a lot less time, money and effort to validate a molecule as a scientific tool than to get a new molecule approved as a drug in humans, so we should hardly be surprised that reagents as versatile as nucleic acid aptamers should have first found applications in the laboratory. Applying *in vitro* evolution with nucleic acids to achieve an empirical molecular solution to a defined binding or catalytic problem can provide highly useful tools to rationally probe many scientific questions. A good example of this kind of process is the systematic generation of amino acid-binding aptamers for analyzing theories of the origin of the genetic code ♥ [1].

By their natures, aptamer applications in general will inevitably be associated with an intermolecular binding reaction of some kind, which will necessarily include interactions which are conventionally dealt with by means of antibodies. Here, though, we can initially make note of some particular binding applications of scientific or technical value which, while not necessarily off-limits to antibodies, are promising for nucleic acid aptamers.

As specific recognition molecules, aptamers can potentially be used in a variety of molecular purification or analytical processes. Aptamer-based affinity chromatographic [2] or electrophoretic / microfluidic [3] separations have proven effective, including for the resolution of molecular enantiomers (stereoisomers) [4-6]. Aptamers against specific carbohydrate motifs in a common chromatographic matrix (Sephadex) can usefully be applied as affinity tags for RNAs [7]. Indeed, it can be asserted that aptamers (and *in vitro* evolution of recognition molecules in general) are transforming the practice of traditional affinity chromatography [8]. Another broad aptameric analytical application of great potential is in the field of microarrays. Nucleic acid and protein microarrays have gained wide application

---

♥ Some more details regarding this area of work are provided in the file SMS–Extras-Ch5/Section A7 ('Genetic Code Choice'); from the same ftp site.

for diverse needs in molecular and cellular biology, as noted in *Searching for Molecular Solutions*. Aptamers can be applied towards a special type of microarray, still of course based on nucleic acid, but with an entirely distinct mode of recognition. Signals from conventional DNA or RNA arrays rely on Watson-Crick hybridization between nucleic acid strands at each point within the array and complementary RNAs or DNAs in the sample for analysis. In contrast, an aptamer array recognition mode is at the level of molecular shape interactions, in the same manner as for antibodies. Initial technical difficulties associated with stably printing appropriately-folded aptamers on surfaces have been largely overcome, and aptamer arrays have been compared favorably with analogous antibody arrays [9-11].

But that issue brings us back to comparisons between the binding and recognition characteristics of antibodies and aptamers in general. Before we launch into a discussion of this, it is important to realize that aptamers are but one class of potential antibody alternatives among several other distinct contenders. This is considered in more detail in Chapter 7 of *Searching for Molecular Solutions*, where antibodies are once more the central preoccupation. But aptamers are the theme here, so we should indeed consider how aptamer-based molecular recognition can compete in a seemingly protein-dominated world.

As an example, the regulation of gene expression has historically been regarded as the province of specific DNA (or RNA) binding proteins and associated protein cofactors. More recently, a hitherto hidden world of critical RNA-based regulation has been revealed, in the form of microRNAs and other short regulatory RNA molecules [12]. In an analogous manner, artificial modulation of expression can be addressed at the protein level (as for example with 'designer' zinc-finger proteins ♣), or by artificial small interfering RNAs (considered briefly in *Searching for Molecular Solutions* Chapter 9). To date, small natural regulatory RNAs

---

♣ Considered in SMS–CitedNotes-Ch4/Section 8B; from the same ftp site.

ultimately rendezvous with their nucleic targets in *trans* by base - complementarity [♥], but the example of natural riboswitch aptamers for gene regulation in *cis* and the case for *trans*-acting natural aptamers were also noted, in Chapter 6. Artificial evolved aptamers have been isolated which bind various components of the transcriptional apparatus [13,14], and RNA aptamers with significant transcriptional activation function have been identified [15,16]. Nucleic acid aptamers can then functionally modulate systems usually regarded as either directly (transcription factors) or indirectly (RNAi) the province of proteins.

Another example where antibodies and aptamers can be compared comes from their respective intracellular applications ('intrabodies' and 'intramers' respectively [♣]), as noted in Chapter 6 of *Searching for Molecular Solutions*. The general issue of alternative recognition molecules is also considered in Chapter 7. So leaving aside the relative merits of aptamers and antibodies (and other potential competitors), we can continue to look at some of the areas where aptamers show practical promise.

Aptamers, Switches and More

Although nucleic enzymes and aptamers converge in allosteric applications, the latter have numerous uses in their own right. As a rough guide to the relative research interest in both domains of functional nucleic acids, we can look at the citation rate for relevant articles from 1982 (the year of self-splicing RNA discovery) until 2007, 25 years later. While the nucleic acid enzyme citation rate appears to have more or less reached a plateau around 2000, the aptamer rate has dramatically increased in the same time period (Fig. 6.Nb). Much of this

---

[♥] This generally involves several steps of protein-mediated processing and assistance prior to the final hybridization with target nucleic acid.

[♣] More detail on intramers is provided in SMS–Extras-Ch7/Section A10; from the same ftp site.

increase is associated with the numerous applications of functional DNA and RNA aptamers (Fig. 6.Na).
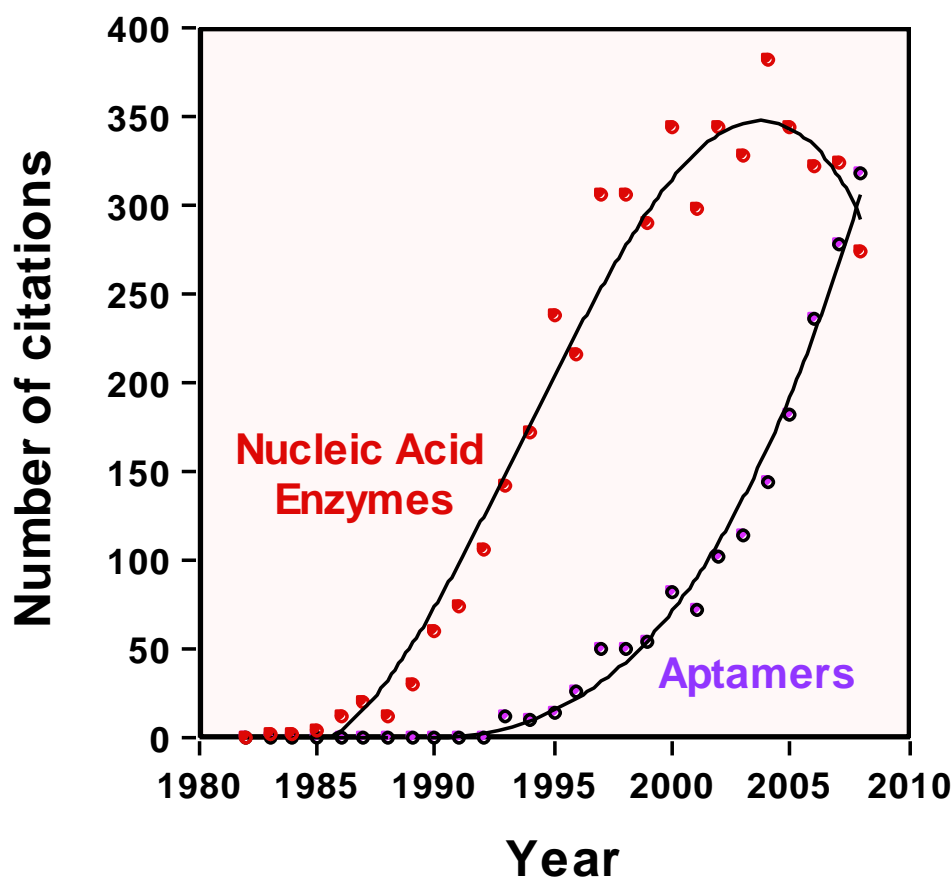

Fig. 6.Nb.

Citation rates for 'ribozyme(s)' / 'RNA enzyme(s)' / 'DNA enzyme(s) / deoxyribozyme(s) (red circles) *vs.* 'aptamer(s)' NOT 'peptide aptamer(s)' (purple circles); X-axis shows years 1982-2008; Y-axis shows number of citations in PubMed.

Advances in technology very often open up new avenues for exploitation of previously-existing processes, and this is very much applicable to the field of

functional nucleic acids. Let's then look at some of the burgeoning applications of aptamers, including the overlapping uses of aptamers and ribozymes which involve allostery.

Aptazymes, Aptaswitches and Aptasensors

The general domain of evolved or engineered pathways, switches, and circuits is not of course restricted to nucleic acid intermediaries, and a complex biocircuit could involve in principle any combination of protein, nucleic acid, and small-molecular ligand participants. Here, we can focus on some aspects relevant to functional nucleic acids. The key factor here is allostery, which was noted at both the protein and nucleic acid levels in Chapter 6 of *Searching for Molecular Solutions*.

Just as the term 'enzyme' is no longer the exclusive preserve of proteins, so too can we note that 'allostery' as a term must be likewise extended. (Nevertheless, many current textbook definitions of allosteric effects inaccurately continue to refer to proteins only). The central aspect of an allosteric effect in any molecule is a conformational change upon ligand binding such that functional properties are altered. An associated aspect of this definition is that ligand-binding *per se* certainly does not prove allostery, and one must take care to note the differences between allosteric nucleic acid effector molecules and those which make use of ligands as direct functional cofactors (Chapter 6). The differences upon ligand binding between conventional riboswitches (resulting in conformational changes) and the cofactor-using *glmS* ribozyme-riboswitch (not associated with conformational change) is another case in point also considered in Chapter 6.

But a true allosteric interaction will engender a conformational change, and in turn the nature of the resulting functional alterations determines the type of 'switchable' molecule we are dealing with. The catalytic function of an allosteric ribozyme (or 'aptazyme') can be turned either up or down by an associated

ligand interaction, and likewise for an allosteric aptamer's binding function [♥]. As with allostery in general, there is no requirement for a relationship between the effector ligand (controlling conformational change) and the function which is subject to conformational regulation. As such, the nature of the controlling effector ligand is essentially arbitrary provided it is compatible with the functional domain *per se* and can engender the required conformational switch [♣].

Unfortunately, these dictates are evidently not trivial in themselves, since some aptamers pre-selected for the binding of certain ligands have proven refractory for use in generating ribozyme allostery, at least using strategies which have been successful in other cases [18,19]. The observation that some ligand / aptamer combinations (such as theophylline and flavin mononucleotide and their respective aptamers) are more cooperative than others for evolving and engineering directed allostery has led to their widespread adoption for such purposes. The nature of the functional nucleic acid itself is also relevant. For example, in contrast to small ribozymes it initially proved more difficult to devise allostery for large natural catalytic RNAs [19]. With Group I self-splicing introns, success was eventually achieved by judicious choice of the aptamer domain, combined with the use of suitable insertion points for the aptamer within the Group I sequence itself. With this engineered regulatable self-splicing RNA, ligand-dependent intron removal was demonstrable *in vivo* [20].

A broad class of allosteric nucleic acids can be termed *sensors*, since the allosterically-modulated function controls the production of a readable signal. In principle, this can be any type of signal whatsoever, but some of most useful signals in practice result from various ingenious deployments of fluorescent

---

[♥]Allostery is not confined to functional RNAs, since DNAzymes can be likewise allosterically regulated [17]. Hence when speaking in general terms, 'allosteric nucleic acid enzymes' is more accurate than solely referring to ribozymes.

[♣]Although a wide variety of ligands can be used for allostery *per se*, other properties of ligands which determine reversibility of binding may be important comsiderations, as we will see below.

labels, which we will get to shortly. A switch can act as logic channel beyond a simple ON / OFF configuration, as in the case of a Boolean NOR gate provided by a tandem riboswitch where binding of either of two ligands sends an OFF signal [21]. This and some other basic logic gates are depicted in Fig. 6.Nc.

YES

A	OUT
0	0
1	1

ON

OR

A	B	OUT
0	0	0
1	0	1
0	1	1
1	1	1

ON

NOT

A	OUT
0	1
1	0

OFF

NOR

A	B	OUT
0	0	1
1	0	0
0	1	0
1	1	0

AND

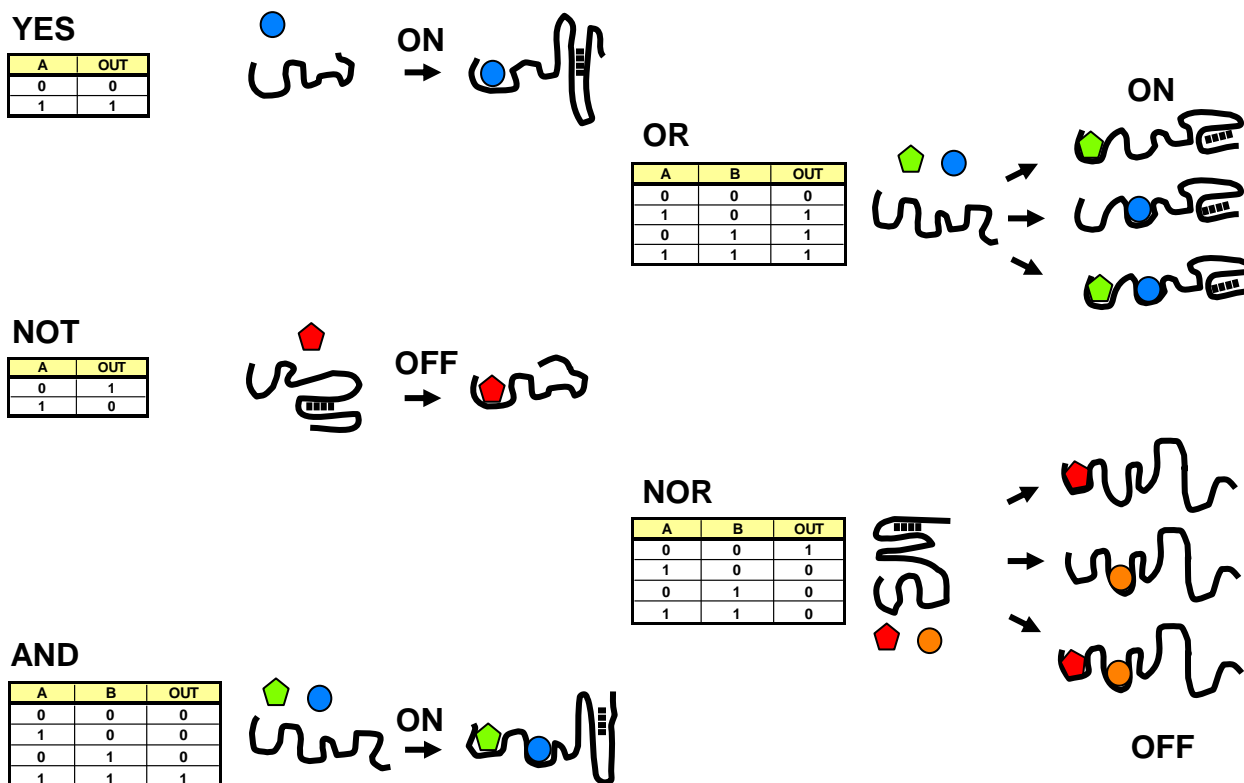| A	B	OUT
0	0	0
1	0	0
0	1	0
1	1	1

ON

OFF

Fig. 6.Nc.

Depiction of simple Boolean logic as applied to allosteric nucleic acid switches. The 'Truth Tables' show one or two conditions (A, B) and OUT (read-out / outcome) with digital 0, 1 values where 0 = 'false' (no binding or functional activity) and 1 = 'true' (binding or positive function). For nucleic acid schematics, short parallel lines denote ordered functional structure; linear shapes represent two different ligands for conditions A, B. (Note: This is not intended to imply that the opposite allosteric states in each case are completely disordered, only that the function has changed). **On left**, YES, NOT and AND switches; **On right**, OR and NOR switches. In each case, only possible binding

states relevant to functional change are shown. (for example, for AND, binding of only one of the two ligands is an insufficient condition for generation of functional activity.

As we have also seen earlier, another interesting aspect of artificial switchable nucleic acids is the interplay between empirical and rational design. Exploitation of empirical approaches in many different contexts often allows one to leap-frog over otherwise-intractable problems into new fields where rational design can be applied. Functional nucleic acids can provide us with classic examples of this, whether through the adoption of a bacterial riboswitch (derived through natural evolution) or an artificial ribozyme (derived through *in vitro* evolution). In both cases, difficult design problems (specific ligand binding or catalysis by nucleic acids) have been circumvented through an empirical strategy. Diversification and selection by nature or humans thus enables the acquisition of a diverse tool-box of functional nucleic acids, which in theory can easily be recombined in a 'mix-and-match' manner to 'rationally' obtain combinatorial functions.

Yet things do not necessarily fall into place quite so simply. Joining of two aptamers requires careful attention to the bridging region between the two functional domains, which itself can be sought by *in vitro* selection [22]. And to generate allostery, one is seeking to not only to combine two functions, but to engineer an arrangement where one strongly influences the function of the other. There must be, in effect, 'communication' between the two functional domains, and the intervening nucleic acid sequences between such linked domains are accordingly often termed a 'communication module'. Moreover, the effector aptamer ligand-binding domain can modulate the second functional domain in either a positive or negative manner. And as we have seen, more complex arrangements are also possible (Fig. 6.Nc).

Even prior to the discovery of riboswitches, the notion of artificial functional nucleic acid allostery was a potent motivator [23]. Initial experiments were

successful in generating allostery with an aptamer domain directed against ATP and a hammerhead ribozyme, where in specific cases ATP was capable of strongly inhibiting ribozyme catalysis [23]. This appeared to be a relatively simple instance of spatial proximity determining the ability of the aptamer + ATP to sterically interfere with the hammerhead ribozyme domain [23,24]. Certainly other modes of communication between general allosteric effector and controlled functional domains are possible, and *in vitro* selection has been used to define minimal sequence motifs which enable either positive ('YES') or negative ('NOT') switching ♥ [18]. One such mechanism has been termed 'slip-structure', involving the formation of alternate local base pair 'registers' in the bridge region following binding of allosteric ligand, which in turn impacts upon the folding of the ribozyme domain [18].

Allosteric nucleic acid systems are often built upon previous work where the relevant functional components were identified, and allosteric design is therefore essentially modular in nature, but with the important caveat that the junctional communication regions must often be fine-tuned. While the idea of defined communication modules between aptamer effector domains and ribozymes is supported by experimental evidence [18], specific junctional communication structures do not appear to be transferable from one class of ribozymes to another [19]. Selection processes for allostery-compatible communication modules still therefore have a place, especially where novel functional activities are concerned. As is so often the case, attention to the design of selection processes in this regard is of great importance, to eliminate non-allosteric (inactive or constitutively active) variants.

It is possible to use allostery itself as a basis for selection of allosteric ribozymes in the ingenious approach (unsurprisingly) termed 'allosteric selection' [26,27]. Here RNAs are expressed where a random tract abuts a ribozyme domain whose

---

♥ Here we can also note a parallel at the protein level, where *in vitro* selection has been used to define optimal joining regions between zinc fingers for 'designer' DNA sequence binding [25].

activity is ablated. If the ribozyme is re-activated through binding of a ligand to specific sequences found within the randomized library, then self-cleavage will occur, and this is a selectable event by virtue of the physical change in the size of the RNA molecule (Fig. 6.Nd). Among other applications, this kind of approach has been used to select ribozymes which are allosterically-responsive to divalent metal ions [28]. With some of the ways of obtaining nucleic acid allostery in mind, let's now look at several specific instances of artificial allosteric nucleic acid switches, with varying degrees of *in vitro* selection and explicit rational design….
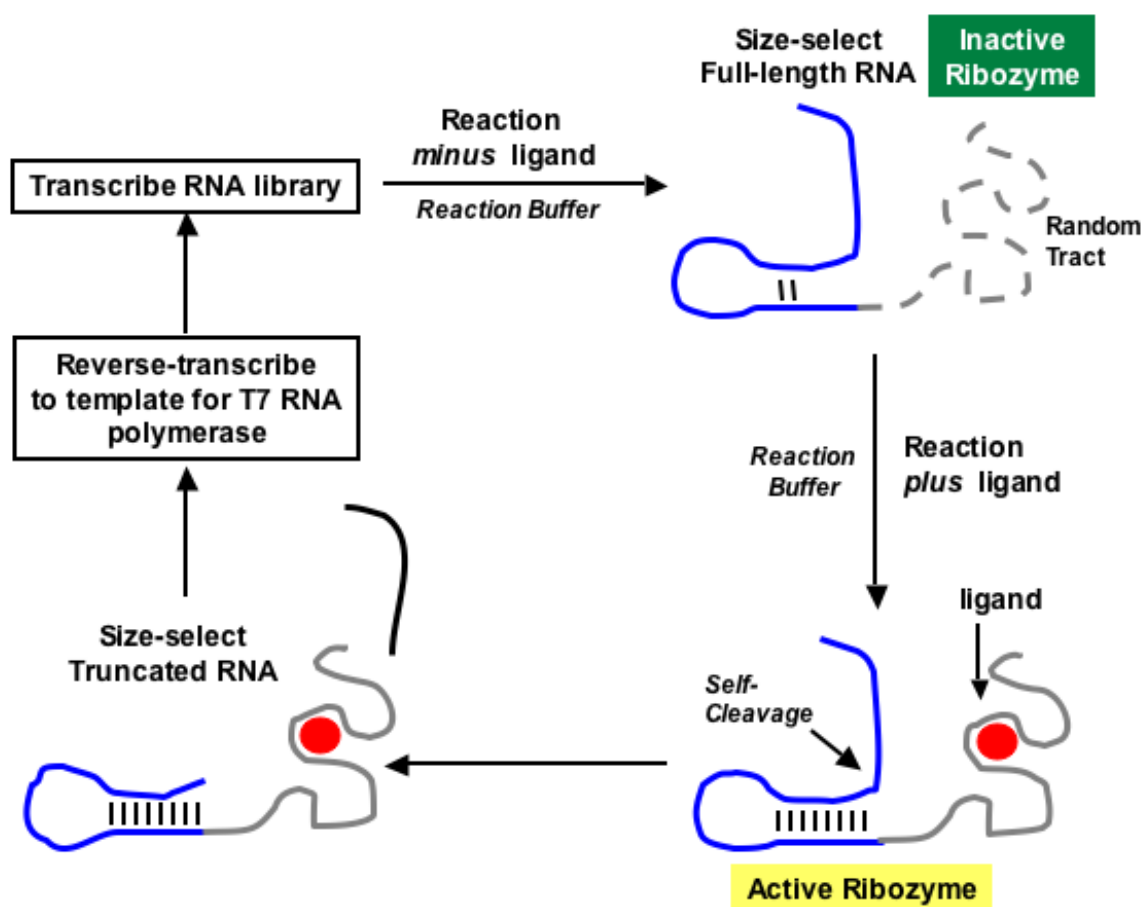

Fig. 6.Nd.

Fig. 6. Nd. Principle of allosteric selection with a ribozyme. An RNA library consisting of a ribozyme inactivated by an abutting randomized segment is incubated without a desired allosteric ligand, and full-length (inactive) RNAs are size-selected by gel electrophoresis. Repeating this in the presence of the ligand of interest allows specific ribozymes within the library which are allosterically re-activated to self-cleave. Since the product is distinguishable in size from the uncleaved RNA, pools of candidates can be size-selected and subjected to repeated cycles by reverse-transcription and renewed RNA transcription by T7 RNA polymerase.

Some Simple Switches, YES? Or NOT?

In Chapter 6 of *Searching for Molecular Solutions* it was noted that while most natural riboswitches are 'OFF' (NOT) signals, it is indeed possible to find 'ON' (YES) switches as well. Human ingenuity can engineer artificial nucleic acid switches in either direction, through the power of allostery. Hammerhead ribozymes appropriately conjoined with a flavin mononucleotide (FMN)-binding aptamer can be modulated positively or negatively upon ligand binding [18,29,30]. In a different natural RNA-cleaving ribozyme (the hairpin variety), another level of switching with FMN-mediated allostery has been demonstrated. As a redox-active cofactor, FMN can interconvert between two forms: a planar (flat) oxidized molecule (FMN_{OX}), and a non-planar (bent) molecule upon its chemical reduction (FMN_{RED}). A hairpin ribozyme allosterically activated by FMN_{OX} fails to respond to FMN_{RED}, in effect setting a switch ligand which is itself switchable by manipulation of local oxidation-reduction conditions [31]. This kind of result is particularly important towards achieving a switch which is rapidly and completely *reversible*, which is not automatically obtained with artificial nucleic acid allostery. (That is to say, isolating a good ON or OFF switch does not mean that either can necessarily be rapidly returned to the ligand-free unswitched state after ligand

binding has occurred. An aptamer recognition domain binding its ligand with very high affinity will dissociate and 'reverse' slowly ♥).

The FMN redox system is thus a specific instance of switch control by means of an isomerizable ligand (a ligand capable of assuming two different molecular forms in a controlled manner). Another example of this is the control of an allosteric hammerhead ribozyme using ligands capable of isomerization in response to light, to achieve reversible ligand-induced functional modulation [32].

When we talk about 'ligand-binding', we tend to think of small molecule bound by a larger one, and all the cases of nucleic acid allostery we have considered thus far certainly fit this view. But there is no inherent reason why the activating molecule for an aptamer recognition domain of an allosteric construct should be small. From this point of view, a nucleic acid molecule (oligonucleotide) itself can act as a ligand for effecting an allosteric change in a functional nucleic acid. In principle, it is very easy to arrange such an interaction, by rendering the molecules mutually 'sticky' for each other through regions of base complementarity. For this purpose, a DNA oligonucleotide ligand could hybridize to a functional RNA, and equally so could an RNA ligand be used to bind a functional DNA molecule. Shortly we will see some useful switch logic-related applications of oligonucleotide-mediated allostery. But taking this a step further, an oligonucleotide ligand itself can be designed as an allosteric aptamer, which in turn is altered upon ligand binding. This is depicted in Fig. 6.Ne for a DNA aptamer and a ribozyme, where aptamer : ribozyme hybridization destroys activity of the latter. (Owing to its mediation by sequence complementarity where one region of the aptamer is the opposite sense to a region of the ribozyme target, this kind of system can also be termed 'antisense switching' [33]).

♥In this context, it should be noted that optimal binding affinities in evolving biosystems will converge on a level which has the best functional outcomes, which will often be only mid-range in terms of the maximum possible affinities. In that vein, we find that natural riboswitches are reversible and 're-usable' [31].

DNA aptamer

**Active
Ribozyme**

*Complementary
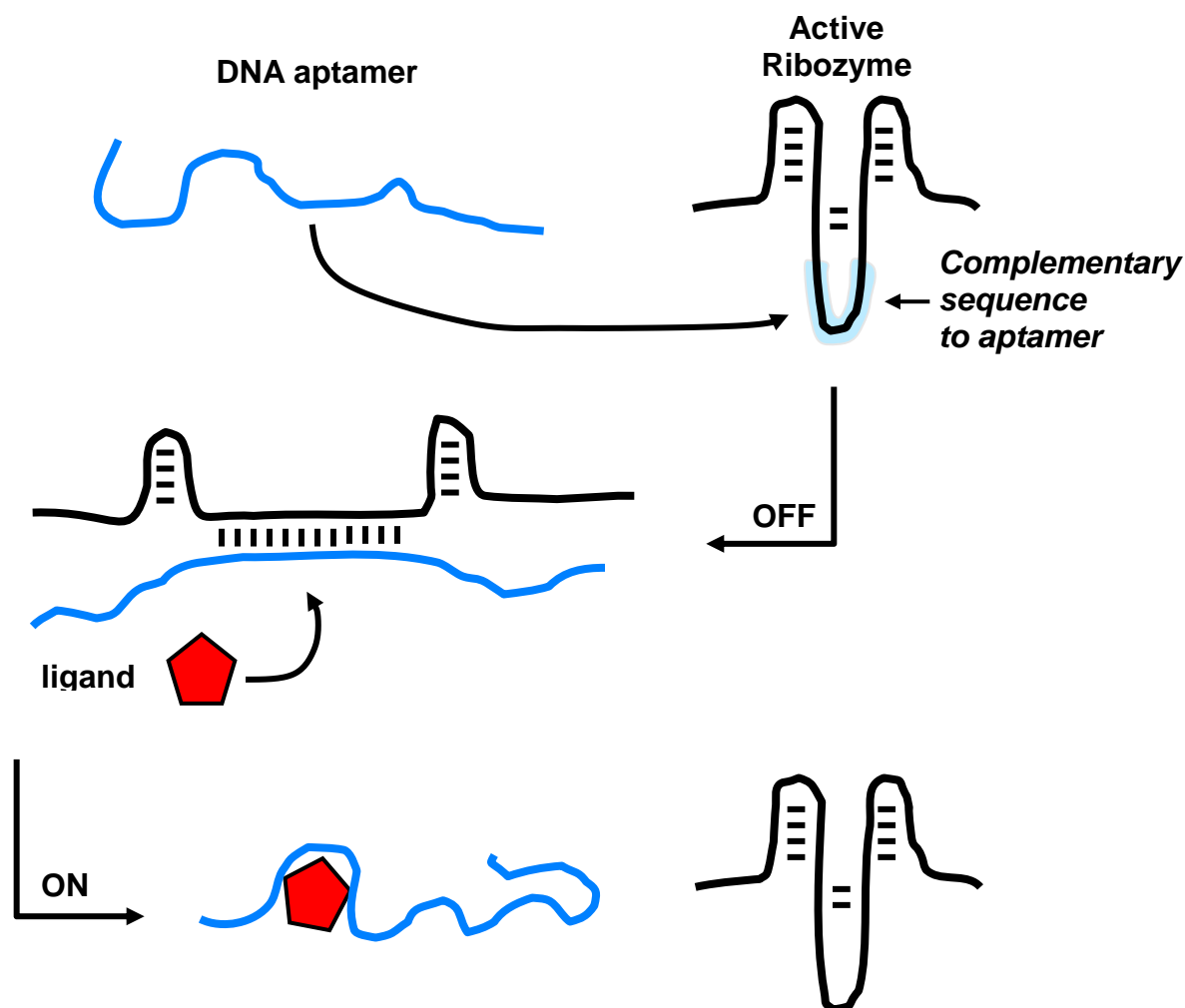sequence
to aptamer*

OFF

ligand

ON

Fig. 6.Ne

Allosteric interactions between a nucleic acid aptamer (shown as DNA; blue lines) and a ribozyme (black lines). Base-pairing between regions of complementarity on both molecules are thermodynamically more stable than the normal ribozyme folded structure itself, inactivating it. Binding of a ligand to the aptamer changes its conformation in such a way that the base-pairing with the ribozyme is no longer favorable and it dissociates, restoring ribozyme activity.

Binding of the allosteric ligand molecule to the aptamer changes its conformation, with regain of function by the ribozyme. In cases such as Fig. 6.Ne, the aptamer oligonucleotide is an allosteric ligand itself for the ribozyme, and the aptamer in turn binds another ligand with a resulting conformational change. This combined effect could be therefore viewed as a case of 'double-allostery' [♥]. A real case of this of allosteric dual-control has been described for hairpin ribozyme regulation by hybridization to an aptamer, with associated aptamer control by binding to the protein thrombin [34]. In the world of proteins, where of course allostery was first recognized, we can find some complex regulatory examples which are analogous to the dual-allosteric control concept. One such case is the regulation of the important transcription factor NF-κB [35].

As these and earlier issues serve to reinforce, a multifunctional nucleic acid molecule capable of allostery can be considered from a number of different vantage points. We can inspect the ligand-binding aptamer recognition domain, the controllable functional effector domain (whether a ribozyme or other), the bridging region, the nature of the ligand, and the nature of the ligand-induced changes which perturb function. While this exercise is not quite in parallel with the legendary blind men examining an elephant, focusing exclusively on only one aspect of the function of such allosteric molecules may blind us to others. But practicalities dictate that we must continue with a reductionist approach here, so let us for the moment look at additional nucleic acid functions which have been subjected to allosteric control.

In strong contradistinction to allosteric natural aptamers (as with riboswitches), no evidence for natural allosteric control of ribozymes has been produced at this time, although its existence in the early RNA World can be assumed. (The ribozyme function associated with the *glmS* riboswitch was initially thought to be subject to allosteric regulation, but (as noted above and in Chapter 6) this is now

---

[♥] This in theory could be extended even further to a third level if the specifc aptamer differentially recognized the oxidized / reduced forms of flavin mononucleotide (FMN) noted above.

believed to be a case of cofactor-assisted catalysis). Allosteric control of artificially-selected nucleic acids with diverse functions (beyond nucleic acid cleavage) has been also successfully attained, as exemplified by positive allosteric regulation of both ribozyme and DNAzyme ligases[17,19,36]. Ribozymes capable of catalyzing the important organic chemical Diels-Alder reaction have also been successfully coupled with a positive allosteric aptamer domain (using the small caffeine-related molecule theophylline as the target ligand [37,38].

But in this general context, we need not be fixated on nucleic acid catalysis as the modulatable function, since any nucleic acid function in principle can come under the influence of allostery. In many such cases, in effect one aptamer specificity is used as the ligand-binding control element and (instead of a ribozyme) a second aptamer provides the functional read-out, under the allosteric control of the first aptamer domain. These are more simply designated as 'allosteric aptamers' [39], the concept of which has already been noted in Fig. 6.9 of *Searching for Molecular Solutions*. With this in mind, we can look at some interesting examples.

It may be remembered that earlier we recounted RNA aptamers capable of modulating eukaryotic transcription, and this has been extended towards the generation of allosterically-regulated aptamers for control of gene expression. One strategy in this regard is to arrange for the selected aptamer to be tethered upstream of the normal transcriptional start site for a gene of interest. How does one do this? Since a variety of natural proteins binding specific RNA structures are known (such as the RNA phage MS2 coat protein [40] and HIV Rev [41,42]), an appropriate protein domain binding a specific RNA motif can be fused to a DNA-binding protein known (or engineered) to bind to a sequence near the promoter of interest. Alternatively, the RNA-binding motif can be fused to a cofactor protein associating with a DNA-binding protein relevant to the regulatory system of interest. Random RNA sequences can then be fused to the 'anchoring' RNA motif and selected for activity *in vivo* [43]. In turn, another ligand-binding aptamer

domain can be joined with the transcriptionally-active aptamer RNA for the purposes of establishing allosteric regulation. This has been achieved in yeast cells using an aptamer with specificity for Malachite Green, which was noted in *Searching for Molecular Solutions* (Fig. 6.7), although using a structurally-related compound (tetramethylrosamine) as the *in vivo* ligand inducer [44]. This general concept, in the form of an allosteric ON switch, is depicted in Fig. 6.NfA. In principle, a reverse OFF switch could also be engineered in this kind of system, but an alternative approach is to select not for a negatively regulatable expression-enhancing aptamer but rather one which can actively *silence* expression [45].
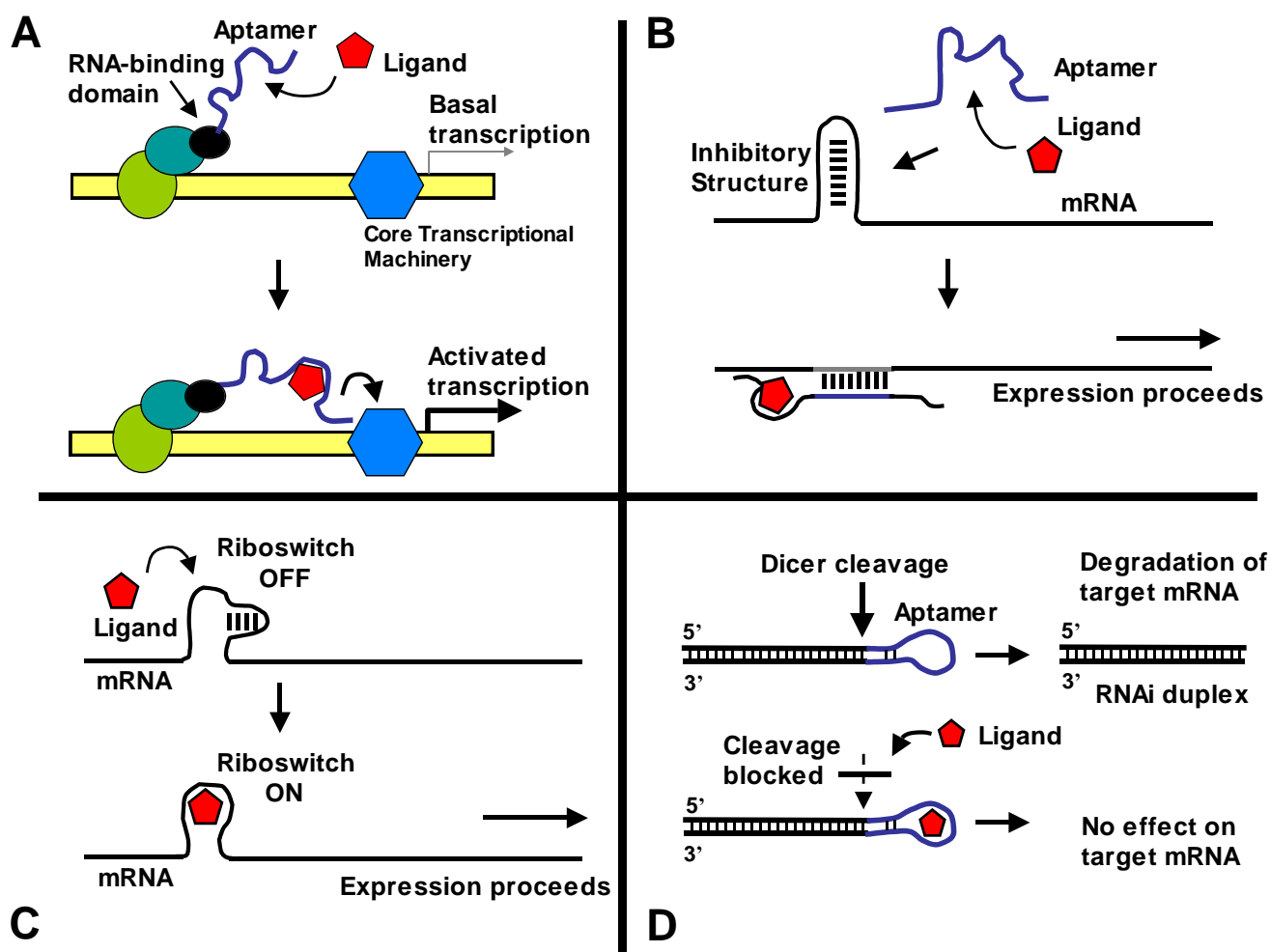

Fig. 6.Nf

Fig. 6.Nf. Some aptamer-based artificial riboregulation strategies. **A**, At the level of transcriptional initiation. This system tethers an RNA aptamer upstream of a target gene's core transcriptional initiation machinery via an engineered bifunctional protein composed of a specific DNA-binding domain (to target a sequence adjacent to the promoter of interest), which is fused to an RNA-binding domain. The aptamer segment itself is joined to the RNA-binding domain's specific target RNA motif, which in this manner provides the desired local tethering function, while leaving the aptamer segment free to interact with proximal transcriptional machinery. The aptamer is activated on ligand binding. **B**, At the level of ongoing transcription or translation with a *trans*-acting aptamer. An inhibitory structure in an mRNA is disrupted upon aptamer binding, which in turn is enabled by aptamer activation through specific ligand. Upon disruption of the inhibitory structure, normal function is restored. **C,** Also at the level of ongoing transcription or translation with a *cis*-acting aptamer. An artificial riboswitch impedes transcription or translation until a conformational change is induced by ligand binding, **D**, At the post-transcriptional level where a short hairpin RNA incorporates an aptamer into the hairpin region, but is still processable by Dicer enzyme to enter the siRNA pathway. Ligand blnding by the aptamer impedes cleavage and thus blocks the molecule from functioning as an siRNA on a specific target mRNA. As shown here, **A**, **B**, and **C** are ON switches and D is an OFF switch, but in principle all could be designed as the reverse (where the functional state is only ON in the absence of the ligand for A-C and in the presence of the ligand for D). Strategy A can also be 'reversed' by using an aptamer that functions as a gene silencer rather than an activator.

Artificial *trans*-acting aptamers can be designed to work by the disruption of mRNA secondary structures which impede expression, or by generating such structures upon interacting with a target mRNA ♥, for ON or OFF switches respectively. Fig. 6.NfB depicts an allosterically-activated *trans*-acting aptamer which disrupts an inhibitory structure and promotes expression [33,46]. (Note also that this is conceptually similar to the *trans*-acting allosteric aptameric control of a

---

♥As noted in Chapter 6, with natural riboswitches, mRNA secondary structures can act as transcriptional pause sites, or impair translational initiation or elongation in a number of ways.

ribozyme noted above (Fig. 6.Ne). Natural RNA aptamers which work in *cis* are now familiar to us as riboswitches, and many workers have used this tactic to construct artificial riboswitch variants for wide applicability. (Another simplified depiction of a riboswitch ON/YES signal is provided in Fig. 6.NfC).

A powerful demonstration of artificial riboswitch control in *E. coli* has come from studies of chemotaxis, which is the ability of organisms to track and follow solution gradients of metabolically useful compounds, or avoid toxic ones. Bacterial chemotaxis has been much-studied as a paradigm for signal transduction between surface chemoreceptors and flagella, and is of great interest also as a highly parsimonious information-processing system [47,48]. (We might remember the subject of bacterial flagella from Chapter 2 of *Searching for Molecular Solutions*, from the rather different viewpoint of their 'design features' which have made them something of a *cause célèbre* in the struggle against 'Intelligent Design'). Chemosignaling to the proteins controlling the flagellar motor can alter the direction of flagella rotation and thereby act as a means for guiding movement directionality, and one *E. coli* control protein (of six in this pathway) is a phosphatase enzyme encoded by the *CheZ* gene [48]. Provision of an artificial riboswitch for a non-metabolizable compound (theophylline) in the 5' region of CheZ mRNA allowed positive control over CheZ expression through the theophylline ligand [49]. In turn, this indirectly enabled modulation of flagella rotation, in turn eliciting a 'pseudotaxis' response to theophylline. Here bacterial cells could be guided towards higher concentrations of the riboswitch ligand, but entirely by-passing the natural cell-surface chemoreceptors [49]. It is interesting to note also that an alternative approach would have been to change a protein chemoreceptor specificity in favor of the artificial ligand, but this would be a considerably more difficult challenge [49].

In the context of riboswitch expression control, we are also reminded of the (as yet) special case of the *glmS* riboswitch whose mechanism is ribozyme based. Some artificial systems using the control of ribozymes embedded into transcribed

mRNAs have been generated, where the ribozyme *cis* cleavage of mRNA can be allosterically controlled by oligonucleotides or specific ligands [50,51]. While these have a 'ribozyme-switch' mechanism in common with the natural *glmS*, the mechanism of the latter (as we have noted) is fundamentally different in using the ligand as a catalytic cofactor rather than as an agent of conformational change.

Control of gene expression is possible at many different levels, and previously we have mentioned post-transcriptional control by specific targeting of mRNAs through harnessing the natural RNA inhibition (RNAi) system. Beyond this, it has been shown that aptamers can be used as switching elements for RNAi activity itself [♥]. This has been possible owing to knowledge that a specific ribonuclease (termed 'Dicer') is required for the processing of short hairpin RNAs to form short inhibitory RNAs (siRNAs), which by judicious sequence choice can be targeted to virtually any mRNA of interest. Embedding an allosteric aptamer sequence into such a hairpin can afford control over the ability of Dicer to cleave (depicted in Fig. 6.NfD), and in turn the ability of the whole molecule to actively enter the RNAi pathway [52].

The ON/OFF RNA-based switches that we have looked thus far are only a sampling of a very large and mushrooming field. As with our previous considerations of competing biotechnologies, time will tell to what extent specific examples (such as the aptamer-controlled RNAi above) become widely adopted. It is very likely, though, that some of these systems will serve as springboards for the development of switches and circuits with increasing levels of sophistication, just as isolated ligand-binding aptamers and ribozymes themselves have lead to the development of allostery through their combinatorial arrangement. With this in mind we can then briefly look at some additional types of switch logic, along the lines of Fig. 6.Nc.

[♥] The use of aptamer segments for targeting RNAi delivery was referred to in Chapter 9 of Searching for Molecular Solutions (Fig. 9.6).

….AND other Logic Switches

Beyond simple YES/NOT 'decisions', more complex molecular logic switches are indeed possible. (We have already noted the precedent for a natural riboswitch NOR logic gate earlier). Again, this is enabled by the existence of nucleic acid aptamers, ribozymes and 'communication modules' (themselves largely obtained through natural or *in vitro* evolutionary selection) which can be combined in a modular fashion to obtain new allosteric functions. Once these are available, though, rational choices as to how they should be strung together can indeed be made.

Switches for AND or OR functions (Fig.6.Nc) could then in principle be obtained by two recognition aptamers and one functional 'read-out' aptamer or ribozyme. In one study, joining in series two defined aptamers (theophylline and flavin mononucleotide [FMN]) and a hammerhead ribozyme (each separated by defined communication modules) enabled cooperative binding and ribozyme activation [53]. In this case ribozyme activation required structural changes wrought by FMN binding, but the FMN interaction with the RNA aptamer was itself dependent on conformational changes elicited by prior binding of the theophylline ligand [53]. This could be regarded as a logical AND switch ♥ (Fig. 6.Nc), since 2-signal 'true' input is required for output (activation).

One aspect of nucleic acid chemistry which is not available to proteins is nucleotide Watson-Crick base-pairing, and as we noted earlier, it is relatively simple to arrange for two single-stranded nucleic acids to mutually interact through regions with such hydrogen-bonding complementarity. It is not so simple, of course, to anticipate all the functional consequences of such bimolecular interactions if at least one of these single-stranded molecules normally

---

♥To be a little pedantic, this falls short of an ideal AND switch because in this study [53] the requirement for both ligands was not absoulte, although the activity with either ligand alone was very small in comparison to that seen with both.

possesses a highly specific folded shape. Yet prediction of RNA folding is sufficiently advanced that much can be done in this regard. A sophisticated application of this kind of knowledge is to predict RNA structures which will have specific consequences for allostery when appended to a ribozyme domain. From this starting point, an appropriate oligonucleotide can be designed as an allosteric 'ligand', and a *complementary* RNA sequence placed in the context of a ribozyme for anticipated specific structural and functional results (upon hybridization with the designed oligonucleotide as the inducer of allosteric modulation). For computer-aided rational design of ribozyme allostery, there are many factors to account for, but it is particularly important to ensure that energy barriers between different allosteric states are within an appropriate range [54]. This means in practice that secondary structures in the ribozyme are not rendered so stable that transitions from one state to another require a prohibitive energy input to proceed, or are so unstable that effective control is abrogated. The take-home message here is that rational crafting of oligonucleotide-mediated ribozyme allostery has enabled more complex AND and OR switches to be achieved [54].

We have spent some time thinking about nucleic acid allostery, but before moving on we should examine in more detail the large field of nucleic acid biosensors which we alluded to earlier. These too are allosterically-based, but their diversity and growing importance justifies a little additional attention.

Making Apt Sense of Sensors

A sensor of course is a device which responds to a specific input with a measurable signal, and this broad definition can be applied in a vast number of ways. As opposed to more familiar macroscopic sensors, artificial sensing at the molecular level is of relatively recent vintage. And useful biosensors are very recent indeed, but now constitute an exploding field with huge diversity and utility. The essence of biosensors lies in the exploitation of the exquisite

recognition functions of specific types of biological molecules, which potentially could be used to sense and measure virtually any target molecule. Molecular sensing has a huge variety of potential applications, ranging from the measurement of diverse solutes, water quality monitoring, biosafety, and biological threat detection (whether natural or man-made). Sensing systems can also be adapted for the screening of candidate drugs by high-throughput screening ♥.

In this subsection we will look at sensors based on functional nucleic acids, although of course protein-based sensors (principally using antibody domains) are also highly significant. The molecular recognition function of an RNA or DNA biosensor must be fulfilled by an aptamer domain ('aptasensors'), but in both principle and practice, either aptamers or ribozymes can be harnessed for transduction of a measurable signal as a result of specific ligand or substrate binding. (In other words, either a conformational or catalytic change can used for signal generation).

A diversity of approaches can also be applied at the level of the specific process used for the signal. In general terms, activation of an enzyme pathway leading to an easily-measurable product is one such strategy, but fluorescence measurements are also very prominent, especially for nucleic acid systems. Since the intention here is to provide an overview of general principles, let's concentrate on the latter fluorescent signal systems. And there is considerable diversity within this area itself, in any case. In order to inspect fluorescent applications for nucleic acid biosensors, first we must think about the phenomenon of fluorescence itself and what it can offer. Fluorescent molecules or specific chemical groups attached to larger molecules (fluorophores) absorb light of a specific wavelength (unique to that molecule), called the absorption or excitation wavelength. To return to the 'ground state' from the excited state, such

---

♥ As noted in Chapter 8 of Searching for Molecular Solutions, and continued in SMS–CitedNoted/Section 21; from the same ftp site.

molecules release the energy as light at a characteristic longer emission wavelength.

The key to the multi-varied usefulness of fluorescence for molecular analyses and signaling lies in the fact that fluorescent effects can be greatly modulated through altered local chemical environments, in an number of different ways. This has been known for a long time, and is routinely used by molecular biologists for visualizing nucleic acids. In a footnote within Chapter 6 of *Searching for Molecular Solutions*, it was briefly noted that the planar compound ethidium bromide is famous for its interaction with DNA through a process known as intercalation, which lacks sequence specificity. This effect would be vastly less used were it not for the fact that in the bound state, the ethidium bromide undergoes a marked change in fluorescence ♥, associated with alteration of its local hydrophobic environment [55]. In turn, this fluorescence allows nucleic acids to be tracked and monitored in a variety of contexts, especially during electrophoretic separations.

In the earlier section on nucleic acid switches, we thought in terms of a dichotomy between recognition domains binding ligands, and functional domains which were modulated and switched by resulting allosteric effects. Certainly this can be equally well applied to aptamer-based sensing, but in this context another option exists. We must recall that structural changes in aptamers after ligand binding (or 'induced fit') are more the rule than the exception. While conformational changes in a *single* aptamer are difficult to apply towards constructing molecular switches, they can be used relatively simply for molecular sensing purposes when fluorescence technology is brought into play. Owing to ligand-induced conformational change, a fluorescent label at a specific site in an RNA aptamer may encounter an altered chemical environment after ligand binding, and in turn modulation of fluorescence may occur. This has been

♥When bound with DNA, the excitation peak for ethidium bromide is the ultraviolet, and the emission peak is in orange-red part of the visible spcctrum.

experimentally validated, with *in vitro* selection applicable for the identification of sites which are most amenable to this approach [56]. A conceptually similar approach has been demonstrated with DNA aptamers incorporating fluorescently-labeled nucleotide analogs, which undergo changes in fluorescence following base-stacking alterations resulting from ligand binding [57]. Numerous other approaches towards aptasensors with single ligand recognition domains exist, which we will get to shortly. But let's first look at a system which should be familiar from the preceding nucleic acid switch section, which also involves an aptamer which we have considered in more detail than others.

Unlike intercalators such as ethidium bromide, the interaction of Malachite Green with its RNA aptamer (Fig. 6.7 of Chapter 6) is highly dependent on the folding and shape conferred by the specific aptamer sequence. Even so, the binding of this dye alters its chemical environment in a manner also associated with increased emission fluorescence [58], and this property can be exploited for the generation of aptamer-based sensors [59]. This kind of 'aptasensor' (which is but one among many) in general can be divided into recognition aptamer domains binding a ligand of interest (the 'analyte' ♥), and a 'reporter' aptamer domain which is allosterically activated through the sensing ligand domain, and in turn binds another molecule whose fluorescence is thereby activated and measurable (Fig. 6.Ng). With two levels of molecular recognition involved, this has also been termed a 'chimeric aptamer' approach [60].

♥ Any compound which is to be analyzed, the physical target of a chemical measurement.
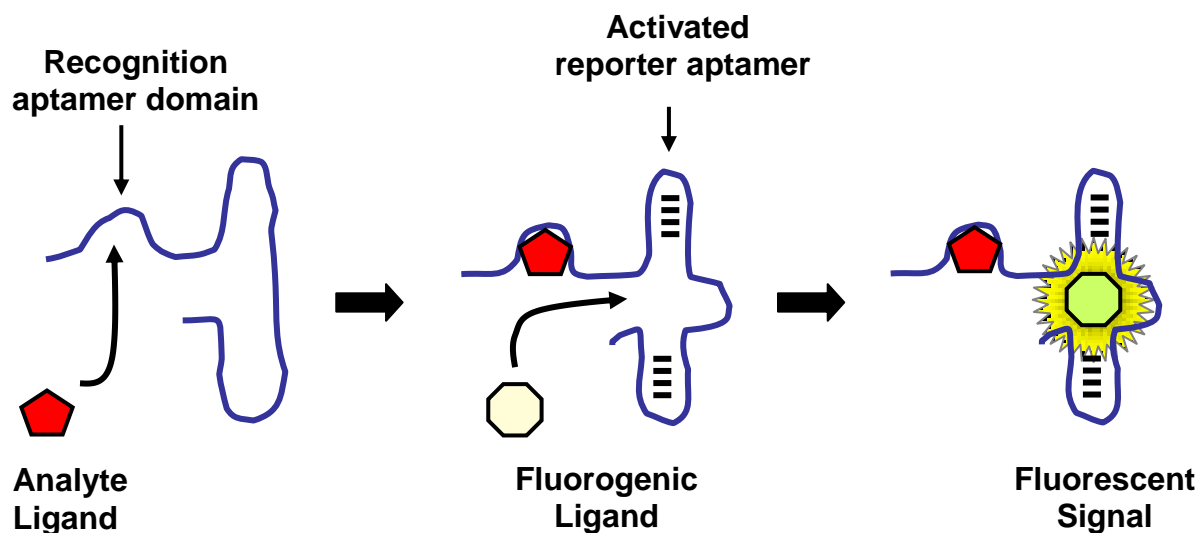
Fig. 6.Ng

Aptasensor based on fluorescent activation of a reporter ligand. The molecule to be analyzed (the analyte) binds the aptamer recognition domain, enabling (via allostery) the subsequent binding of another molecule to the reporter domain which then has enhanced fluorescence. ('Fluorogenic' here denotes having fluorescent potential).

Fluorescent excitation energy can be channeled in other ways besides direct and rapid emission from the fluorophore, which has proven to be very useful for humans interested in molecules. Fluorophores can interact with each other in a manner which is heavily dependent on their physical proximity in space. When fluorophores are within approximately 2-10 nanometers of each other, they can potentially participate in the phenomenon of Fluorescence Resonant Energy

Transfer, or FRET [♥]. Provided this spatial positioning and certain other conditions are fulfilled [♣], the excitation energy of a 'donor' fluorophore at its normal wavelength can be transferred to a proximal 'acceptor' fluorophore, which then emits light at its own characteristic emission wavelength (Fig. 6.Nh). This FRET effect generates quenching of the donor's fluorescence and (in effect) a shift in the fluorescent excitation wavelength of the acceptor. Some chemical groups can act as FRET acceptors, but return to their low energy ground states by emitting heat rather than visible light (and hence receive the label of 'dark quenchers'). When fluorophores are in very close apposition, another quenching mechanism comes into play, termed 'static' (or 'contact') quenching which does not depend on overlap between the excitation and emission spectra of the two fluorescent moieties [61,62].

The special properties of FRET, and the ability to measure it, mean that it is extremely useful for monitoring conformational changes within biological macromolecules, or association / dissociation between interacting molecules. By its nature, a FRET application requires the participation of two chemical groups (Fig. 6.Nh), and can involve detection of the groups being brought close together or pulled apart as a result of conformational changes. These can be measured by changes in donor /acceptor fluorescence, or through the modulation of quenching and (in a turn) a fluorescent signal. Most aptasensor systems based on a FRET process make use of 'de-quenching' to give a positive read-out of a restored fluorescent signal, but sometimes a loss-of-fluorescence read-out has been used [60,63].

[♥] Though widely defined in this way, this acronym can be worrisome to those who uphold accuracy. In fact, the energy transfer in question is not fluorescence *per se* (no photons are involved; it is a non-radiative transfer process), as opposed to the consequences of the energy transfer itself. Fortunately, though, the FRET acronym can be preserved by referring to it as Förster Resonant Energy Transfer, in honor of Theodor Förster, a pioneer of this work.

[♣] For efficient FRET, the emission spectrum of the donor must overlap the excitation (absorption) spectrum of the acceptor [61].
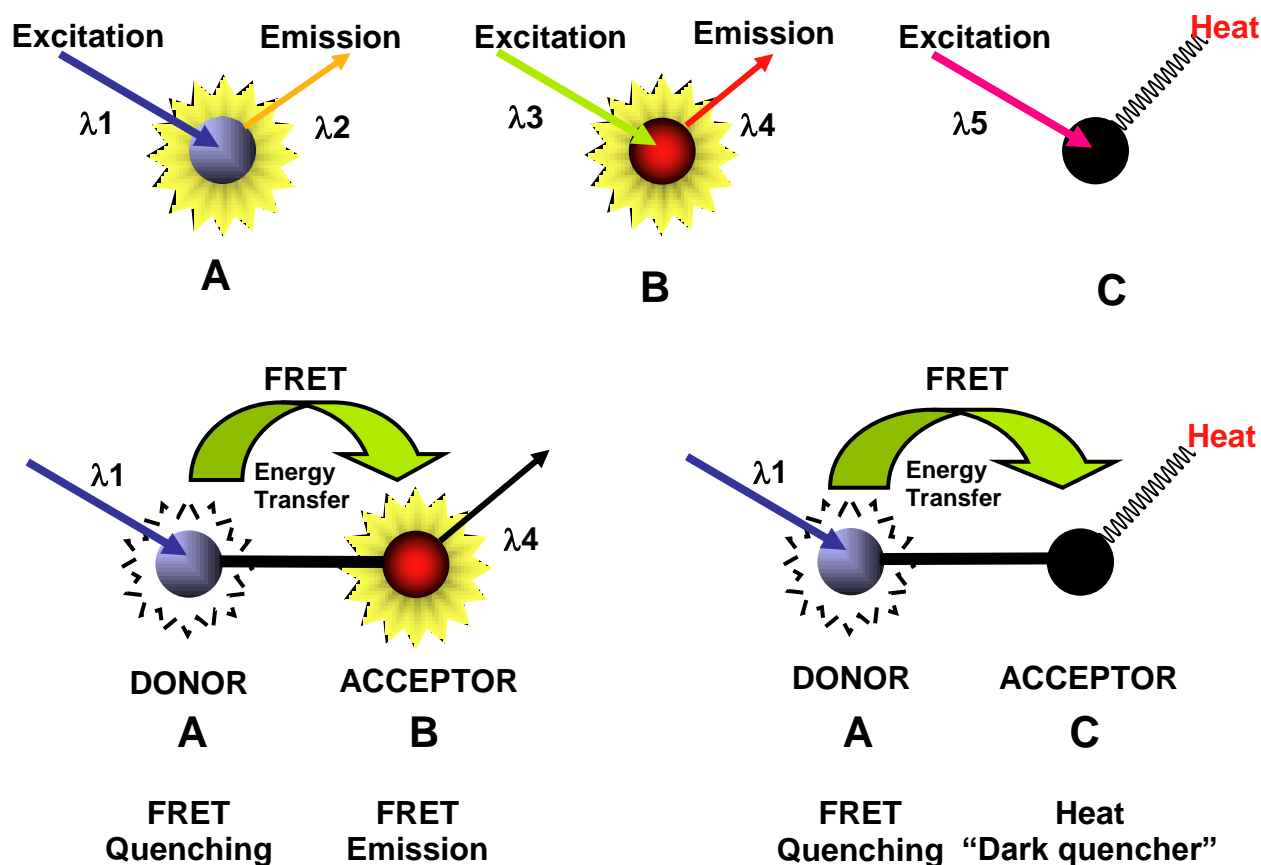
Fig. 6.Nh

FRET effects. Two fluorescent molecules or functional groups (fluorophores, represented by spheres A and B) are shown, each with separate excitation and emission wavelengths (λ1 - λ4). A third molecule or group C can absorb light but emits energy (returns to ground state) through emission of heat. If A and B are at a distance of <~10 nm (10^{-8} meters) , FRET can occur. When A acts as the FRET donor to B, quenching of A fluorescence in response to light of the normal excitation for A occurs, with accompanying emission from B at the characteristic B emission wavelength. In the same arrangement with C replacing B, A is quenched but no light is released from C, hence its label as a 'dark quencher'.

With dual fluorophores (or fluorophore / 'dark quencher' pairs, Fig. 6.Nh), there are a variety of strategies for generation of aptasensors. A fluorophore and a quencher brought into proximity by strand complementarity can be separated by hybridization to a target sequence, producing a more stable duplex as the outcome. This event is then detectable through the resulting upsurge in fluorescence, and has been termed 'molecular beacon' technology [64] (depicted in Fig. 6.NiA).
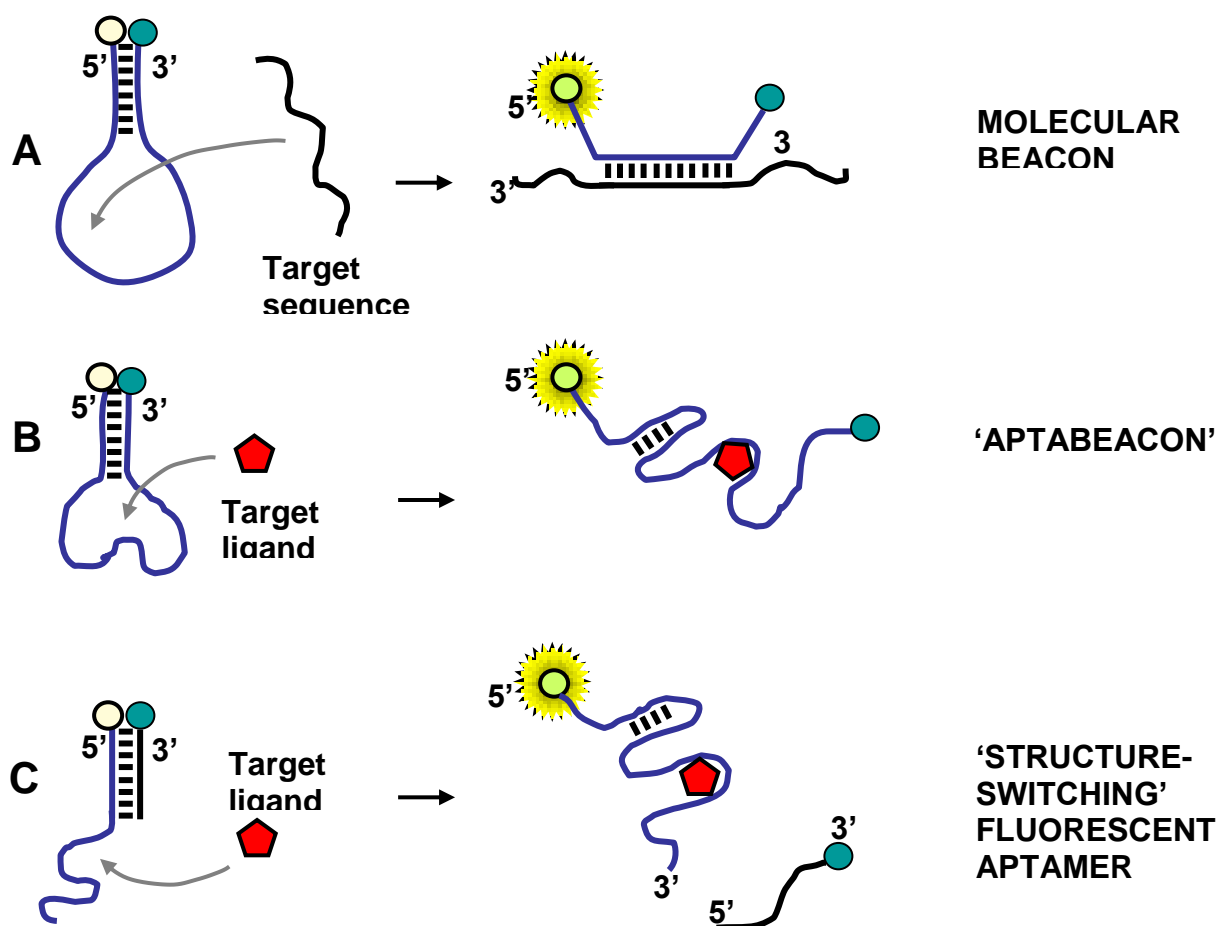

Fig. 6.Ni

Fig. 6. Ni. Some aptamer-based fluorescent sensing strategies using physical separation of fluorophore and quencher. **A**, Standard (non-aptamer) molecular beacon where separation is based on hybridization **B**, Separation based on aptamer / ligand-induced conformational change (single molecule). **C,** Separation based on aptamer / ligand-induced conformational change (bimolecular).

The same effect can be mimicked by designing an aptamer where binding of ligand elicits a conformational change separating the quencher from the fluorophore, thus producing a fluorescent sensor for a target compound of interest (Fig. 6.NiB). For this effect to operate, the quencher need not be covalently linked to the strand bearing the fluorophore (Fig. 6.NiC). This has been termed a 'structure switching' signaling process [60,63], although a structural change following ligand binding to aptamer is fundamental to all aptamer-based sensors.

Many variations on the themes shown in Fig. 6.Ni can be envisaged and have been produced [60,63]. For example, it is possible to engineer the physical separation of fluorophore and quencher to occur via the agency of activated ribozymes [65,66]. Another concept for nucleic acid detection is to rely on reconstitution of separated aptamer fragments by complementarity with a target nucleic acid template. Here once again the Malachite Green aptamer has been exploited, by virtue of its fluorescence when in the aptamer-bound state (Fig. 6.Nj) [67]. Aptamer reconstitution is also readily compatible with FRET technologies, for either the generation or suppression of fluorescent signals [60,63].
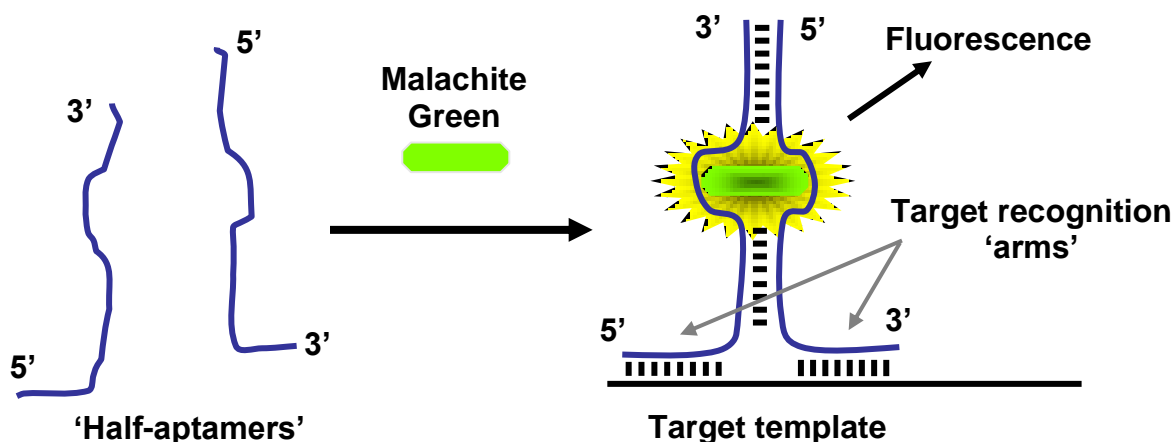
Fig. 6.Nj

Binary arrangement with Malachite Green aptamer. Separate 'half-aptamer' strands are brought together via recognition of a target nucleic acid strand as shown, which allows assembly of an aptamer ligand recognition site, binding of Malachite Green, and induction of fluorescence [67].

A fluorescence-based approach which has particular promise for *in vivo* applications is the application of 'excimer' technology. Certain fluorescent molecules in close apposition form 'excited-state dimers' which strongly fluoresce at an altered emission wavelength. One such molecule, pyrene, has been exploited for derivation an aptamer-based excimer sensor system [68]. In its physical arrangements for signal generation , this kind of excimer system can be regarded as the reverse of quenching strategies, as the fluorescent excitation occurs only when the compound of interest is (noncovalently) 'dimerized' by close spatial proximity. Hence for a positive signal read-out, an aptamer with appended pyrene molecule must undergo a conformational change on ligand binding which juxtaposes the pyrene molecules [68], rather than pulling them apart

as for many FRET systems (Figs. 6.Nh, 6.Ni). As well as a strong change in emission wavelength upon aptamer binding, the pyrene excimer fluorescence is characterized by much longer fluorescent lifetime than other biological molecules which can contribute to *in vivo* background fluorescence [68].

We can see that even when we look at fluorescent signal detection methods alone, we are faced with a profusion of alternative artificial biosensor systems, and 'more than one way to skin a cat' seems something of an understatement for this field. If the target molecules for sensor signaling are nucleic acids themselves, non-aptamer systems exist, as we have noted with molecular beacons (Fig. 6.Ni), and improved alternatives without aptamers have been developed [69,70]. We must also not forget that protein-based sensors exist and also are under active development. It is natural to wonder which approach is best, but as usual a simple answer cannot be given when context-dependent factors are important. Different sensors will also vary in the stringency of the demands placed upon them according to the environments in which they are expected to operate, as (for example) an *in vitro* as opposed to an *in vivo* setting.

One can certainly define factors by which alternate sensor options can be judged, such as signal strength, ligand recognition and signal specificity (false signal control), the ability to multiplex (the ability to run analyses for multiple separate targets at once), and the ability to operate *in vivo*. And some general statements can be made, such as the observation that 'de-quenching' of an efficient fluorophore (in a dual fluorophore / quencher FRET arrangement) often appears to provide a stronger signal than that obtained from single fluorophores as a result of an altered chemical environment. As we have observed in other biotechnological contexts, 'technological Darwinism' will in theory see a winnowing of approaches which are globally less effective in favor of superior competitors, but it is unlikely that any single sensor strategy will come to outrank all others, at least as judged by the field so far.

References:

1.   Yarus, M., Caporaso, J. G. & Knight, R. Origins of the genetic code: the escaped triplet theory. *Annu Rev Biochem* **74**, 179-98 (2005).

2.   Hutanu, D. & Remcho, V. T. Aptamers as molecular recognition elements in chromatographic separations. *Adv Chromatogr* **45**, 173-96 (2007).

3.   Mosing, R. K. & Bowser, M. T. Microfluidic selection and applications of aptamers. *J Sep Sci* **30**, 1420-6 (2007).

4.   Michaud, M. et al. A DNA aptamer as a new target-specific chiral selector for HPLC. *J Am Chem Soc* **125**, 8672-9 (2003).

5.   Michaud, M. et al. Immobilized DNA aptamers as target-specific chiral stationary phases for resolution of nucleoside and amino acid derivative enantiomers. *Anal Chem* **76**, 1015-20 (2004).

6.   Ruta, J., Ravelet, C., Baussanne, I., Decout, J. L. & Peyrin, E. Aptamer-based enantioselective competitive binding assay for the trace enantiomer detection. *Anal Chem* **79**, 4716-9 (2007).

7.   Srisawat, C., Goldstein, I. J. & Engelke, D. R. Sephadex-binding RNA ligands: rapid affinity purification of RNA from complex RNA mixtures. *Nucleic Acids Res* **29**, E4 (2001).

8.   Clonis, Y. D. Affinity chromatography matures as bioinformatic and combinatorial tools develop. *J Chromatogr A* **1101**, 1-24 (2006).

9.   Stadtherr, K., Wolf, H. & Lindner, P. An aptamer-based protein biochip. *Anal Chem* **77**, 3437-43 (2005).

10.   Cho, E. J., Collett, J. R., Szafranska, A. E. & Ellington, A. D. Optimization of aptamer microarray technology for multiple protein targets. *Anal Chim Acta* **564**, 82-90 (2006).

11.   Li, Y., Lee, H. J. & Corn, R. M. Fabrication and characterization of RNA aptamer microarrays for the study of protein-aptamer interactions with SPR imaging. *Nucleic Acids Res* **34**, 6416-24 (2006).

12.   Eckstein, F. Small non-coding RNAs as magic bullets. *Trends Biochem Sci* **30**, 445-52 (2005).

13.   Thomas, M. et al. Selective targeting and inhibition of yeast RNA polymerase II by RNA aptamers. *J Biol Chem* **272**, 27980-6 (1997).

14.   Fan, X., Shi, H. & Lis, J. T. Distinct transcriptional responses of RNA polymerases I, II and III to aptamers that bind TBP. *Nucleic Acids Res* **33**, 838-45 (2005).

15.   Sengupta, D. J., Wickens, M. & Fields, S. Identification of RNAs that bind to a specific protein using the yeast three-hybrid system. *Rna* **5**, 596-601 (1999).

16.   Saha, S., Ansari, A. Z., Jarrell, K. A. & Ptashne, M. RNA sequences that work as transcriptional activating regions. *Nucleic Acids Res* **31**, 1565-70 (2003).

17. Levy, M. & Ellington, A. D. ATP-dependent allosteric DNA enzymes. *Chem Biol* **9**, 417-26 (2002).

18. Soukup, G. A. & Breaker, R. R. Engineering precision RNA molecular switches. *Proc Natl Acad Sci U S A* **96**, 3584-9 (1999).

19. Robertson, M. P. & Ellington, A. D. Design and optimization of effector-activated ribozyme ligases. *Nucleic Acids Res* **28**, 1751-9 (2000).

20. Thompson, K. M., Syrett, H. A., Knudsen, S. M. & Ellington, A. D. Group I aptazymes as genetic regulatory switches. *BMC Biotechnol* **2**, 21 (2002).

21. Sudarsan, N. et al. Tandem riboswitch architectures exhibit complex gene control functions. *Science* **314**, 300-4 (2006).

22. Burke, D. H. & Willis, J. H. Recombination, RNA evolution, and bifunctional RNA molecules isolated through chimeric SELEX. *Rna* **4**, 1165-75 (1998).

23. Tang, J. & Breaker, R. R. Rational design of allosteric ribozymes. *Chem Biol* **4**, 453-9 (1997).

24. Tang, J. & Breaker, R. R. Mechanism for allosteric inhibition of an ATP-sensitive ribozyme. *Nucleic Acids Res* **26**, 4214-21 (1998).

25. Wang, B. S. & Pabo, C. O. Dimerization of zinc fingers mediated by peptides evolved in vitro from random sequences. *Proc Natl Acad Sci U S A* **96**, 9568-73 (1999).

26. Soukup, G. A., Emilsson, G. A. & Breaker, R. R. Altering molecular recognition of RNA aptamers by allosteric selection. *J Mol Biol* **298**, 623-32 (2000).

27. Soukup, G. A., DeRose, E. C., Koizumi, M. & Breaker, R. R. Generating new ligand-binding RNAs by affinity maturation and disintegration of allosteric ribozymes. *Rna* **7**, 524-36 (2001).

28. Zivarts, M., Liu, Y. & Breaker, R. R. Engineered allosteric ribozymes that respond to specific divalent metal ions. *Nucleic Acids Res* **33**, 622-31 (2005).

29. Araki, M., Okuno, Y., Hara, Y. & Sugiura, Y. Allosteric regulation of a ribozyme activity through ligand-induced conformational change. *Nucleic Acids Res* **26**, 3379-84 (1998).

30. Araki, M., Okuno, Y. & Sugiura, Y. Expression mechanism of the allosteric interactions in a ribozyme catalysis. *Nucleic Acids Symp Ser*, 205-6 (2000).

31. Strohbach, D., Novak, N. & Muller, S. Redox-active riboswitching: allosteric regulation of ribozyme activity by ligand-shape control. *Angew Chem Int Ed Engl* **45**, 2127-9 (2006).

32. Lee, H. W., Robinson, S. G., Bandyopadhyay, S., Mitchell, R. H. & Sen, D. Reversible photo-regulation of a hammerhead ribozyme using a diffusible effector. *J Mol Biol* **371**, 1163-73 (2007).

33. Bauer, G. & Suess, B. Engineered riboswitches as novel tools in molecular biology. *J Biotechnol* **124**, 4-11 (2006).

34. Najafi-Shoushtari, S. H. & Famulok, M. DNA aptamer-mediated regulation of the hairpin ribozyme by human alpha-thrombin. *Blood Cells Mol Dis* **38**, 19-24 (2007).

35. Xiao, W. Advances in NF-kappaB signaling transduction and transcription. *Cell Mol Immunol* **1**, 425-35 (2004).

36. Robertson, M. P. & Ellington, A. D. In vitro selection of an allosteric ribozyme that transduces analytes to amplicons. *Nat Biotechnol* **17**, 62-6 (1999).

37. Helm, M., Petermeier, M., Ge, B., Fiammengo, R. & Jaschke, A. Allosterically activated Diels-Alder catalysis by a ribozyme. *J Am Chem Soc* **127**, 10492-3 (2005).

38. Amontov, S. & Jaschke, A. Controlling the rate of organic reactions: rational design of allosteric Diels-Alderase ribozymes. *Nucleic Acids Res* **34**, 5032-8 (2006).

39. Soukup, G. A. Aptamers meet allostery. *Chem Biol* **11**, 1031-2 (2004).

40. Valegard, K., Murray, J. B., Stockley, P. G., Stonehouse, N. J. & Liljas, L. Crystal structure of an RNA bacteriophage coat protein-operator complex. *Nature* **371**, 623-6 (1994).

41. Bartel, D. P., Zapp, M. L., Green, M. R. & Szostak, J. W. HIV-1 Rev regulation involves recognition of non-Watson-Crick base pairs in viral RNA. *Cell* **67**, 529-36 (1991).

42. Giver, L. et al. Selective optimization of the Rev-binding element of HIV-1. *Nucleic Acids Res* **21**, 5509-16 (1993).

43. Buskirk, A. R., Kehayova, P. D., Landrigan, A. & Liu, D. R. In vivo evolution of an RNA-based transcriptional activator. *Chem Biol* **10**, 533-40 (2003).

44. Buskirk, A. R., Landrigan, A. & Liu, D. R. Engineering a ligand-dependent RNA transcriptional activator. *Chem Biol* **11**, 1157-63 (2004).

45. Kehayova, P. D. & Liu, D. R. In vivo evolution of an RNA-based transcriptional silencing domain in S. cerevisiae. *Chem Biol* **14**, 65-74 (2007).

46. Davidson, E. A. & Ellington, A. D. Engineering regulatory RNAs. *Trends Biotechnol* **23**, 109-12 (2005).

47. Parkinson, J. S., Ames, P. & Studdert, C. A. Collaborative signaling by bacterial chemoreceptors. *Curr Opin Microbiol* **8**, 116-21 (2005).

48. Baker, M. D., Wolanin, P. M. & Stock, J. B. Signal transduction in bacterial chemotaxis. *Bioessays* **28**, 9-22 (2006).

49. Topp, S. & Gallivan, J. P. Guiding bacteria with small molecules and RNA. *J Am Chem Soc* **129**, 6807-11 (2007).

50. Yen, L. et al. Exogenous control of mammalian gene expression through modulation of RNA self-cleavage. *Nature* **431**, 471-6 (2004).

51. Ogawa, A. & Maeda, M. Development of a new-type riboswitch using an aptazyme and an anti-RBS sequence. *Nucleic Acids Symp Ser (Oxf)*, 389-90 (2007).

52. An, C. I., Trinh, V. B. & Yokobayashi, Y. Artificial control of gene expression in mammalian cells by modulating RNA interference through aptamer-small molecule interaction. *Rna* **12**, 710-6 (2006).

53. Jose, A. M., Soukup, G. A. & Breaker, R. R. Cooperative binding of effectors by an allosteric ribozyme. *Nucleic Acids Res* **29**, 1631-7 (2001).

54. Penchovsky, R. & Breaker, R. R. Computational design and experimental validation of oligonucleotide-sensing allosteric ribozymes. *Nat Biotechnol* **23**, 1424-33 (2005).

55. Morgan, A. R., Lee, J. S., Pulleyblank, D. E., Murray, N. L. & Evans, D. H. Review: ethidium fluorescence assays. Part 1. Physicochemical studies. *Nucleic Acids Res* **7**, 547-69 (1979).

56. Jhaveri, S., Rajendran, M. & Ellington, A. D. In vitro selection of signaling aptamers. *Nat Biotechnol* **18**, 1293-7 (2000).

57. Katilius, E., Katiliene, Z. & Woodbury, N. W. Signaling aptamers created using fluorescent nucleotide analogues. *Anal Chem* **78**, 6484-9 (2006).

58. Babendure, J. R., Adams, S. R. & Tsien, R. Y. Aptamers switch on fluorescence of triphenylmethane dyes. *J Am Chem Soc* **125**, 14716-7 (2003).

59. Stojanovic, M. N. & Kolpashchikov, D. M. Modular aptameric sensors. *J Am Chem Soc* **126**, 9266-70 (2004).

60. Nutiu, R. & Li, Y. Aptamers with fluorescence-signaling properties. *Methods* **37**, 16-25 (2005).

61. Marras, S. A., Kramer, F. R. & Tyagi, S. Efficiencies of fluorescence resonance energy transfer and contact-mediated quenching in oligonucleotide probes. *Nucleic Acids Res* **30**, e122 (2002).

62. Johansson, M. K. Choosing reporter-quencher pairs for efficient quenching through formation of intramolecular dimers. *Methods Mol Biol* **335**, 17-29 (2006).

63. Nutiu, R. & Li, Y. Structure-switching signaling aptamers: transducing molecular recognition into fluorescence signaling. *Chemistry* **10**, 1868-76 (2004).

64. Piatek, A. S. et al. Molecular beacon sequence analysis for detecting drug resistance in Mycobacterium tuberculosis. *Nat Biotechnol* **16**, 359-63 (1998).

65. Stojanovic, M. N., de Prada, P. & Landry, D. W. Catalytic molecular beacons. *Chembiochem* **2**, 411-5 (2001).

66. Navani, N. K. & Li, Y. Nucleic acid aptamers and enzymes as sensors. *Curr Opin Chem Biol* **10**, 272-81 (2006).

67. Kolpashchikov, D. M. Binary malachite green aptamer for fluorescent detection of nucleic acids. *J Am Chem Soc* **127**, 12442-3 (2005).

68. Yang, C. J., Jockusch, S., Vicens, M., Turro, N. J. & Tan, W. Light-switching excimer probes for rapid protein monitoring in complex biological fluids. *Proc Natl Acad Sci U S A* **102**, 17278-83 (2005).

69. Sando, S. & Kool, E. T. Nonenzymatic DNA ligation in Escherichia coli cells. *Nucleic Acids Res Suppl*, 121-2 (2002).

70. Silverman, A. P. & Kool, E. T. Quenched autoligation probes allow discrimination of live bacterial species by single nucleotide differences in rRNA. *Nucleic Acids Res* **33**, 4978-86 (2005).