Published in final edited form as:

Bioconjug Chem. 2019 January 16; 30(1): 2–12. doi:10.1021/acs.bioconjugchem.8b00810.

Designed and Evolved Nucleic Acid Nanotechnology: Contrast and Complementarity

Tulsi Ram Damase, Peter B. Allen*

University of Idaho, Dept. of Chemistry, 001 Renfrew Hall, 875 Perimeter Dr, Moscow, ID 83844-2343, USA

Abstract

In this review, we explore progress on DNA aptamers (evolved DNA), DNA circuits (designed DNA), and the newest projects that integrate both. Designed DNA nanotechnology includes static nanostructures, dynamic nanodevices, and reaction networks (sometimes called DNA circuits). DNA circuits are dynamic DNA reactions that perform computations and sequence-specific amplification. Directed evolution can be used to produce DNA that can recognize specific targets. Aptamers are evolved nucleic acids; they are produced artificially with an *in vitro* selection process. DNA aptamers are molecular recognition elements made of single-stranded DNA (ssDNA) with the potential to interact with proteins, small molecules, viruses, and even cells. Designed molecular structures can incorporate aptamers for applications with immediate practical impact.

Graphical Abstract

Keywords

DNA; DNA circuits; Aptamer; Aptamer based DNA circuits

*Corresponding Author: pballen@uidaho.edu.

The authors declare no competing financial interest.

INTRODUCTION

DNA aptamers (evolved DNA), DNA circuits (designed DNA), and the newest projects that integrate both all rely on the specific properties of DNA. The structure of DNA is predictable: Watson-Crick base pairing is strong, specific, and well characterized thermodynamically. Synthetic DNA binds with high affinity to its reverse complement DNA (synthetic or natural). This forms the famous double-stranded, double-helical structure. Single stranded nucleic acid (DNA and RNA) molecules fold on themselves according to the same base pair rules. This can form wildly diverse structures^{1,2}. Natural and artificial selection can drive the evolution of nucleic acid molecules to useful structures. However, humans can also design machines that use these rules to perform tasks. The integration of evolved and designed DNA will allow for more practical application of both.

We review examples of evolved nucleic acid structures including natural, folded RNA (e.g., ribosomal RNA) and aptamers. Aptamers are evolved, single-stranded oligonucleotides that bind to a target with high specificity and affinity^{3,4}. We contrast these with designed DNA including DNA circuits. DNA circuits are used in recognition and signal amplification for DNA and RNA analytes. Non-biological applications of designed DNA also include structural DNA based nano-devices. Finally, we show examples of the work integrating evolved and designed elements. The first step to integrate these approaches is to extend designed DNA circuits to recognize various biomarkers using evolved DNA aptamers.

NUCLEIC ACID STRUCTURE

Deoxyribonucleic acid (DNA)

DNA (deoxyribonucleic acid) is a biomolecule that carries genetic information for all known living organisms. The genetic information flows from DNA to RNA to protein. This is the concept of ‘central dogma of molecular biology’. DNA is a polynucleotide chain composed of phosphate groups, five carbon sugar and nitrogen-containing bases (see Figure 1A). The sugar in DNA is deoxyribose. The bases are adenine(A), cytosine(C), guanine(G) and thymine(T). Adenine and guanine are two-ring bases called purines. Cytosine and thymine are single-ring base called pyrimidines. ‘A’ pairs with ‘T’ and ‘G’ pairs with ‘C’. The DNA polynucleotide chain has an alternating sugar phosphate backbone⁵.

DNA hybridization

DNA is an asymmetric and directional molecule. DNA sequences are written by convention from 5’ to 3’ (see Figure 1B). In this review, we represent the 3’ end of a DNA with an arrow (see Figure 1B). Two strands that are complementary to each other in an antiparallel fashion can hybridize with each other. For instance, 5’-GGCATTCCG-3’ hybridizes to 5’-CGGAATGCC-3’ (see Figure 2A) but not to 5’-CCGTAAGGC-3’ (see Figure 2B)⁶. Base stacking also contributes to the thermodynamic stability of DNA hybridization. Two strands of ssDNA hybridize to form base pairs and base stacks. If the complementary length is ~18 nucleotides or higher, the interaction is thermodynamically favored at room temperature. If the interaction is complementary over ~56 base pairs, then the interaction is comparable in strength to a covalent bond⁷. In solution, associated DNA molecules “breathe” such that any

given base pair may be temporarily unbound (see Figure 2C). Base breathing or “fraying” is prominent at the termini of helices and plays an essential role in DNA circuit reactions (strand displacement and branch migration)⁶.

Folded nucleic acid structures

The double helix is very famous, but nucleic acids can form many other structures. RNA is well known to fold into functional 3D shapes to perform its biological roles. All eukaryotic and prokaryotic ribosomes contain ribosomal ribonucleic acid (rRNA) with structural and catalytic functions. The secondary structure of 5S rRNA consists of RNA helices, loops, and a three-way junction⁸. Figure 3A shows the tertiary structure from cryo-electron microscopy⁹. Figure 3B shows a computer model predicted secondary structure based on the thermodynamics of base pairing. Many other examples exist in nature of folded RNA structures. The Shine-Dalgarno (SD) sequence¹⁰ is a purine-rich sequence found in many mRNAs of bacteria. The 3D structure of this RNA helps ribosomes bind to the mRNA.

DNA is chemically synthesized as single stranded oligonucleotides that can also fold into complex structures. Single-stranded DNA assembles with its complementary sequence to form double helices. This predictability allows DNA to be “programmed” to form complex multidimensional nanostructures. Nadrian Seeman (1982)¹³ demonstrated the earliest designed DNA structures. Early forms of designed DNA structures like those by Seeman and Zheng were exclusively built from short, synthetic oligonucleotides. For example, see Figure 4 which shows a DNA tensegrity triangle. Zheng and Seeman¹⁴ designed tensegrity triangles to self-assemble from component oligonucleotides. The crystal structure from Duana et al.¹⁵ and domain level schematic of a tensegrity triangle are shown in Figure 4. This structure has been determined with X-ray crystallography (Figure 4A) and is simple enough to represent in detail with a domain level diagram (Figure 4B). DNA origami is the more modern form of designed DNA that uses a long, single-stranded scaffold DNA and short, complementary oligonucleotide staples to assemble larger structures. Paul Rothemund (2006)¹⁶ developed the modern approach to scaffolded DNA origami at the 100 nm scale. He constructed discrete DNA nanostructures from a long ssDNA scaffold guided by short DNA strands called staples. Shih et al. (2004)¹⁷ reported the creation of DNA-based octahedra using a long (1669nt) ssDNA and five short oligonucleotides (40nt). Hao Yan (2005)¹⁸ presented the construction of finite size DNA nanoarrays. Peng Yin (2017)¹⁹ introduced unimolecular ssDNA origami. ssDNA can fold like proteins into programmed nanostructures and may have more practical uses than multicomponent DNA folding^{1,2,19,20}. Wang et al. (2013)²¹ reported RNA-DNA hybrid origami using long RNA scaffolds and short staple DNA strands. Rinker et al. (2008)²² demonstrated an example of integration of aptamers and DNA origami. They studied distance-dependent, multivalent ligand-protein binding effects. Aptamers against thrombin were immobilized on DNA nanostructures at various distances.

EVOLVED STRUCTURES

Aptamers

Aptamers have gained significant attention since 1990. Aptamers are discovered by SELEX (Systematic Evolution of Ligand by Exponential Enrichment) technique^{3,4}. Aptamers are

short single-stranded DNA or RNA molecules that uniquely fold and bind target molecules. Aptamers bind to targets with high specificity and affinity (picomolar to micromolar range dissociation constants). Aptamers have a complementary range of targets when compared with antibodies. For example, antibodies don't recognize toxins because they do not cause strong immune responses (or kill their host) whereas aptamers recognize toxins because aptamer selection is not tied to the immune system^{23,24}.

Aptamers bind to target molecules, and in some cases have affinities approaching that of monoclonal antibodies (see Table 1). Currently, many antibody-based drugs are in clinical use and hundreds of antibody-based drugs are in clinical trials. Although monoclonal antibodies can bind to target molecules with high specificity and affinity, they have many limitations. The high production cost of monoclonal antibodies makes them less available for research. Monoclonal antibodies show less penetration into tumor tissues than aptamer because they are large in size^{23,25}.

Aptamers are non-toxic and non-immunogenic. The production cost of aptamers is low because of chemical synthesis (rather than recombinant expression in cells). They have high penetration power into tissues due to their small size. They have few side effects because the immune system does not directly recognize them. Their storage and transportation are robust because of their thermal stability. The ease of chemical modifications of aptamers without affecting their target specificity makes them attractive therapeutic probes in medical applications^{27,29,30}.

Aptamers have shown promise as therapeutics. There is only one US Food and Drug Administration (FDA) approved aptamer-based drug (Macugen / Pegaptanib, 2004) along with a few aptamer-based drug candidates (for instance, AS1411, NOX-A12, ARC183, etc.) in clinical trials after 28 years of research^{25–27,29,30}.

SELEX technology

Aptamers are generated through an *in vitro* process called SELEX, developed in 1990^{3,4}. See schematic outline in Figure 5. In this process, a randomized DNA library is exposed to target. The single-stranded DNA library contains a 30–80 base random sequence region flanked by primer binding sites. Those sequences that are bound to the target are retained, amplified and utilized in a further round of selection. After multiple rounds of selection, individual aptamer candidates are cloned and sequenced to get high-affinity aptamers from the pool. The overall *in vitro* selection process is time-consuming. At each round of selection, the pool should be characterized and purified. Unwanted amplification products must be removed: primer dimers (where the random region is lost to produce an undesired, short product) are a common problem; background binders that bind the target's support are also a pernicious source of impurities. Without advanced techniques, it can take months to move from a random pool to the identification of a highly specific and high-affinity aptamer. Modern selections can be much faster³¹.

Since 1990, the SELEX process has modified in a variety of ways to increase the efficiency of SELEX (for instance, counter-SELEX³², capillary electrophoresis-SELEX³³, microfluidic-SELEX³⁴, toggle-SELEX³⁵, and many more). Cell-SELEX has been used to

generate aptamers^{36–39}. This technology uses whole living cells as targets to select aptamers. Next-generation sequencing has made SELEX less time-consuming and improved the success rate^{40–42}. For a recent review of more modern SELEX methods, see Zhuo et al. (2017)⁴³. Tolle et al. (2015)⁴⁴ introduced click-SELEX to select modified aptamers. Aptamers with chemically modified DNA have shown promise. Their affinity is generally stronger than unmodified chemistry. Concerns over safety of chemically-modified nucleotides have limited the use of modified aptamers as therapeutics^{45,46}.

Evolved deoxyribozymes

Deoxyribozymes are DNA oligonucleotides that act as a catalyst for RNA cleavage or ligation. Deoxyribozymes are evolved artificially and obtained through an *in vitro* selection process similar to SELEX. Breaker and Joyce (1994)⁴⁷ reported a specific DNA sequence that catalyzes the cleavage of RNA. The selection was designed such that catalytically active oligonucleotides were physically separated from the inactive pool. Diverse DNA molecules were synthesized with a RNA linker terminated with a biotin⁴⁸. The pool was then immobilized on an avidin coated solid support. Molecules capable of self-cleavage of the RNA linker were recovered by elution. The isolated DNA was then amplified and enriched in further rounds. A similar approach was used to select DNA ligating ribozymes. Successful deoxyribozymes self-ligate an RNA, generating a longer product. These ligated products are separable by PAGE. Purtha et al. selected such a ligating ribozyme⁴⁹ which was then crystallized by Ponce-Salvatierra et al.⁵⁰ (see Figure 6). Artificially evolved deoxyribozymes with defined catalytic activities have potential future *in vitro* diagnosis applications and *in vivo* therapeutic applications^{51–53}.

SELEX and the philosophy of *in vitro* evolution of DNA

Performing SELEX requires careful consideration of the functional properties of the DNA. These are not based on the specific molecular properties (e.g. molecular structure or conformation). The researcher's task is to ensure that only by binding to the target (or performing some other desired task) can an oligonucleotide be carried forward through each round. Any other activity that allows the molecule to survive to the next round must be considered and expunged. For example, it is usually necessary in aptamer selections to add steps to remove molecules that bind to plastic (e.g. DNA that binds directly to the magnetic bead surface instead of the target molecule attached to the magnetic bead). A good SELEX experiment produces aptamers that are strong (enough) and specific (enough) to survive the SELEX process. The process is agnostic to mechanism, structure, and even binding strength (beyond a critical threshold). The structures and binding mechanism are only known in a small subset of evolved nucleic acids. The philosophy of *in vitro* selection is about function, not molecular properties.

DESIGNED DNA REACTIONS

Biology uses regulatory networks to control cellular behavior. Biological regulatory networks are highly complex and hard to reproduce. Designed DNA reaction networks (i.e., DNA circuits) with the potential to detect and regulate nucleic acids and proteins could be a powerful tool in biology. Construction of synthetic DNA circuits that mimic biological

circuits is challenging. Over the last 18 years, the field has exploded with the advent of robust design elements and inexpensive synthetic DNA.

Designed DNA circuits rely on the ideas and concepts of computation and logic gates. The field grew from simple roots to highly complex systems from 2000 to 2018. Lee et al. (1970)⁵⁴ discovered “branch migration” and “strand displacement”. In 2000, Yurke et al.⁵⁵ used the phenomenon of strand displacement and branch migration to construct a DNA based molecular machine. This proved to be a highly designable reaction that could function with almost any sequence. The field has progressed rapidly, using this basic reaction to tackle ever more difficult tasks. Benenson et al. (2004)⁵⁶ designed a DNA-based biomolecular computer to diagnose disease related messenger RNA *in vitro* and release an ssDNA drug as output. Seelig et al. (2006)⁵⁷ designed hybridization based digital logic circuits. Designed circuits implement AND, OR, and NOT gates using oligonucleotides as input and output. Signal restoration (i.e. amplification) makes circuits sensitive enough to detect target in the presence of noise. Yin et al. (2008)⁵⁸ demonstrated diverse bimolecular self-assembly pathways including triggered assembly of quantized binary molecular trees. Zhang and Winfree (2009)⁵⁹ characterized the kinetics of DNA strand displacement reaction using toehold exchange. Quian et al. (2011)⁶⁰ demonstrated multilayer circuits using reversible strand displacement processes and computed responses to a complex series of inputs. This work integrated ~100 different designed oligonucleotides into a single circuit. Chen et al. (2013)⁶¹ did a detailed study of circuit leakage in amplification cascades and developed unique protocols to designed DNA amplifier circuits with minimal leakage. Srinivas et al. (2017)⁶² demonstrated an enzyme free, dynamic chemical oscillator using DNA strand displacement cascades. The result was an oscillating reaction analogous to the Belousov–Zhabotinsky and related chemical oscillators⁶³. Thubagere et al. (2017)⁶⁴ demonstrated an autonomous, cargo-sorting, DNA-based molecular robot. This achievement was widely reported by the popular press. The design of dynamic DNA-DNA reaction networks is a growing and dynamic field.

DNA Strand Displacement

The diverse achievements of DNA circuits rely on the DNA strand displacement reaction. DNA strand displacement is a process in which two strands hybridize with each other and displace a third strand^{1,59}. In toehold-mediated strand displacement reactions, a prehybridized duplex is composed of an output strand and a substrate strand with an unpaired single stranded region (see Figure 7A)⁵⁹. The short single stranded region is known as a toehold. An input strand initiates binding in the toehold region and initiates branch migration. Branch migration is a non-biased, random walk of DNA base pairs breaking and reforming^{54,55,57,65}. In a strand displacement reaction, the input strand displaces an output strand irreversibly from the prehybridized duplex. In toehold exchange strand displacement reactions, both the reactant and product have toeholds to initiate branch migration (see Figure 7B)⁵⁹. This allows for continuous equilibration of reactants and products. The formation of products is thermodynamically possible because products have more base pairs and stacking energy. The formation of products is kinetically possible because of the toehold and molecular breathing.

DNA circuits

DNA circuits are dynamic DNA devices made of DNA strands. DNA circuits can be built from a collection of DNA strand displacement reactions. The output of one reaction can be the input of another reaction. This network of connected reactions can amplify signals, perform computations and Boolean logic, and produce a detectable output.

DNA circuits can amplify a DNA or RNA input signal. In catalytic DNA amplifier circuits, DNA input is recycled for multiple rounds of catalytic generation of output. In some cases, exponential amplifiers catalytically generate further catalyst. The entropy driven amplifier (EDA) circuits and catalytic hairpin assembly (CHA) are good examples of non-enzymatic DNA amplifier circuits.

Zhang et al.⁶⁶ initially designed the entropy driven amplifier (EDA) circuit (see Figure 8A). The addition of each input molecule generates more than one output molecule. The input strand first binds to the toehold of spine and displaces the blocker. This exposes a toehold for fuel. The fuel binds to the toehold and displaces the input strand and signal strand. The total number of base pairs before and after the reaction is not changed in this reaction. In this process one free input strand releases two free output strands and double stranded product. Therefore, the overall reaction is driven by the increase in entropy derived from the increase in the number of free molecular species. In 2017, Damase and Allen⁶⁷ adapted the EDA circuit to generate release and sensor particles. Release particles release signal oligonucleotides and the released DNA is captured by sensor particles. This is an analogy of cell-to-cell communication.

Pierce and Yin⁵⁸ initially developed the catalytic hairpin assembly (CHA) circuit (see Figure 8B). In this reaction, a pair of hairpins are designed to be complementary to each other. Spontaneous hybridization between two hairpins (H1 and H2) is kinetically unfavorable. The reaction is kinetically trapped as complementary toeholds are occupied as dsDNA. The input strand binds to the toehold of H1 and exposes a single-stranded region. The exposed single-stranded region binds the toehold of H2 and triggers branch migration. Finally, the thermodynamically favorable duplex of H1/H2 is formed and input oligonucleotide is displaced. The displaced input oligonucleotide can then react with more H1 molecules in the system, resulting in signal amplification⁶⁵.

The hybridization chain reaction (HCR)^{58,69} is a designed, enzyme-free alternative to detect DNA analytes, developed in the Pierce lab. The reaction is based on recognition and hybridization between two kinetically trapped hairpins in the presence of initiator. Two hairpins are designed to hybridize with each other in presence of initiator. They form long concatemers (see Figure 9). The long concatemers can be used as a reporter for detection. The initiator opens one hairpin and exposes a single-stranded region. This single stranded region is a toehold for a second hairpin. The second hairpin binds to the toehold and forms a nicked double stranded DNA. The double stranded DNA has an exposed toehold that is identical to the initiator. The chain reaction continues until hairpins are exhausted. Pierce (2014) reported that HCR amplifiers can detect five types of target mRNA simultaneously in zebrafish embryos⁷⁰.

Nanomachines, Walkers and “DNA nanorobots”

Inspired by biological motor proteins, scientists around the world are building synthetic motors made of designed nucleic acids. Researchers have demonstrated the beauty of DNA walkers in mimicking cell transportation systems. Proof-of-concept data have been demonstrated showing that the technology might be used for drug transportation and release. The potential future biomedical application of walkers could be biomarker detection and signal amplification. Futurists imagine such nanomachines walking inside the human body to find targets and tag them for diagnosis or therapeutic delivery. Emerging technologies have begun to approach this vision. Li et al. (2018)⁷² have demonstrated a DNA nanorobot, using DNA origami, programmed to deliver thrombin aptamer to tumor sites and inhibit tumor growth.

DNA walkers are autonomous DNA nanomachines driven by strand displacement reactions (or other DNA-DNA reactions). Sherman et al. (2004)⁷³ demonstrated DNA-based molecular walker similar to walking proteins kinesin and myosin V. The walker walks on a track using strand displacement reaction. The hybridization of the input DNA strand provides the driving force of the molecular walker. Shin et al. (2004)⁷⁴ also demonstrated a molecular walker composed of two partially complementary ssDNA strands. Kinesin movement along a tubule inspired the development of this example molecular machine. The Shin walker walks on a predefined track with the help of attachment fuel strands and detachment fuel strands. Jung et al. (2016) developed a stochastic DNA walker that autonomously traversed the surface of DNA coated microparticles driven by the CHA reaction⁷⁵. In 2017, Damase and Allen⁶⁷ adapted the EDA circuit to develop DNA based molecular walker that could transfer from one particle to another. The DNA walker was released from “release particles” with the addition of fuel. “Sensor particles” captured the walker which then walked randomly on the surface of sensor particles. The sensor particles reported this activity through an increase in fluorescence emission (see schematic in Figure 10). Quing et al. (2018)⁷⁶ demonstrated the molecular DNA hopper carrying cargo at single molecule level on a protein track.

DNA circuits and the philosophy of designed DNA

Designing a DNA circuit requires sequences that obey predictable molecular rules. The design process generally assumes that DNA adopts a B-form double helix. This gives good predictions for the thermodynamics of hybridization¹². It also gives good kinetic predictions for the kinetics of OSD reactions⁷⁷ which have been combined systematically into amazingly complex designed reactions. Other, less common structures (e.g. G-quadruplexes and pseudoknots) are not incorporated into computer aided design software like NuPACK¹² or the rate data of OSD reactions. Nonetheless, such alternative structures are well understood. They have been leveraged to slow undesired reactions (“leakage”) in DNA circuits⁷⁸. The philosophy of DNA circuits relies on knowledge of specific molecular properties. This knowledge allows the prediction and design of desired behavior from abstract principles.

INTEGRATION OF EVOLVED AND DESIGNED DNA STRUCTURES

Although the philosophies of evolved and designed DNA are very different, both are made of DNA and there are advantages in the combination of the two approaches. Designed structures can 1) assemble objects in space at the nano-scale and 2) perform dynamic and computational tasks. Both can be expanded with the capabilities of evolved DNA. In the first case, DNA can be designed to link nano-scale objects together. Nanostructures such as DNA origami can be used as an addressable scaffold to arrange non-designed elements like aptamers, antibodies, or protein. In the second case, DNA reactions can integrate with aptamers to interact with non-nucleic acids. There is not yet a method to design DNA to bind to a non-nucleic acid target; that interaction must be evolved in a process like SELEX. Aptamers can guide DNA circuits and extend their ability to interact with various analytes such as small molecules, proteins, and other biological entities.

Aptamer Nanostructure Colocalization

Designed nucleic acid structures can hold multiple aptamers. In some cases, the spacing and orientation can be precisely controlled. Likewise, other nanostructures can be held together by designed DNA-DNA interactions⁷⁹ including superlattices of gold nanoparticles⁸⁰. This field has become quite mature, with dozens of examples and applications in photonics and optical detection. We give a few highlights here.

Borghei et al. (2016)⁸¹ developed a cancer cell detection method based on simple, designed hybridization between aptamers and two types of gold nanoparticles functionalized with ssDNA (DNA-AuNPs). Both designed DNA probes bind to the aptamer in absence of target. Designed DNA binding induces nanoparticle aggregation and produces a color change. Target cells bind to aptamers and remove them from the solution. The result is no self-assembly and an unchanged, red color (see Figure 11).

Aptamers can be linked to nanostructures by designed hybridization. In one example from 2018, Zhao et al.⁸² demonstrated a nanostructure that held three aptamers, a drug (doxorubicin), and an siRNA therapeutic (against anaplastic lymphoma kinase). They used this assembly to treat a model of anaplastic large cell lymphoma(ALCL). The aptamer-nanomedicine bound to ALCL cells via the aptamer. The cells internalized the aptamer-linked nanostructure and the cargo (doxorubicin and siRNA) were released.

There are many examples of designed hybridization linking aptamers to nanostructures. A designed DNA origami held dozens of aptamers in order to spatially organize thrombin and platelet derived growth factor (PDGF)⁸³. Zhu et al (2013) reported the integration of designed DNA called nanoflowers and cancer targeting aptamers to selectively recognize cancer cells⁸⁴. Norouzi et al. (2018) presented aptamer-integrated DNA assembly to detect cancer cells⁸⁵. The DNA assembly consisted of a concatemer linked with three-way junctions. The integrated aptamer guided the assembly to detect cancer cells. DNazymes embedded in the aptamer-functionalized DNA assembly produced a colorimetric signal.

Aptamers at the interface between DNA circuits and Biology

Aptamers can be integrated with more dynamic DNA nanotechnology and DNA circuits. While there are fewer examples (see Table 2), it is an exciting field with growing possibilities. Bompiani et al. (2012)⁸⁶ reported an excellent example to show the beauty of integration of evolved DNA and designed DNA. Thrombin-aptamer is an anticoagulant drug and its reverse complement is an “antidote.” This contrasts with warfarin, a small molecule anticoagulant. The effect of warfarin is continuous until the drug leaves the body. With the thrombin aptamer, the drug’s effect can be shut off with the addition of the reverse complement antidote. This designed, DNA-based antidote modulates the anticoagulant property of the aptamer and improves its safety⁸⁶.

The modulation of the thrombin aptamer was extended to include dynamic control using a DNA circuit (see Figure 12). The DNA circuit was designed using two different aptamers (TA-29 and TA-15). TA-29 binds to heparin exosite without inhibitory function. TA-15 binds to the fibrinogen exosite with strong inhibitory function. The thrombin input displaces TA-29 from duplex. The displaced DNA input is consumed by the threshold controller. The additional, displaced TA-29 also binds to the heparin exosite without inhibition. When threshold controller DNA is depleted, DNA input will generate TA-15. TA-15 will bind to fibrinogen exosite and inhibit blood coagulation⁹⁵. This means that inhibition only occurs when the activity exceeds the desired level encoded into the circuit.

In the thrombin activity regulator, the output was an inhibitory aptamer TA-15. In the same system, an aptamer acted as an input to the DNA circuit as well. The non-inhibitory aptamer, TA-29, detected the target. This regulated the DNA circuit based on a concentration triggered threshold. Aptamer TA-29 might be considered a transducer to convert thrombin concentration to an oligonucleotide input to which the circuit could respond.

Such aptamer transducers have also been demonstrated for small molecule targets. Zhu et al. (2014) reported a DNA circuit integrated with an aptamer to detect the small molecule adenosine triphosphate (ATP)⁹⁰. The scheme is outlined in Figure 13. In the presence of target (ATP), an aptamer (M) binds to the ATP. The bound ATP-aptamer complex assumes an open conformation. This exposes a toehold to a second DNA oligonucleotide (R). M and R react to produce an output strand, S. Two guanosine-rich oligonucleotides bind output S to assemble a G-quadruplex structure. The resulting split G-quadruplex binds to protoporphyrin IX (PPIX) and becomes fluorescent.

In summary, integration of aptamers and DNA circuits allows nucleic acid nanomachines to manipulate and respond to proteins and small molecules. Given the diverse aptamers in the literature, this might be expanded to viruses and cells as well. Aptamers provide specific biorecognition and DNA circuits provide precise and autonomous control. Integrating aptamers as inputs to a DNA logic circuit could compute a response based on multiple biomarkers. When a circuit generates an active aptamer as a product, the aptamer can act on biology as a regulation element. These two approaches have complementary applications in synthetic biology and biomedicine.

SUMMARY AND CONCLUSIONS

The DNA nanotechnology field has produced impressive and beautiful achievements including precisely designed nanostructures and nanodevices. Few practical examples yet exist of the application of DNA nanotechnology to biological needs in basic or biomedical science. The design of DNA to interact with other DNA is well understood, but the design of DNA to interact with protein or small molecules remains beyond the current capacity of computational prediction. If dynamic DNA technology could respond to protein analytes, it could be applied to analytical and diagnostic problems. The newest demonstrations in the literature show the integration of DNA circuits with biological regulatory networks. The interface between these two worlds of constructed and natural molecules is evolved DNA.

Aptamers are evolved DNA that bind to specific targets such as proteins. They are strong and specific, but (like antibodies) often require further amplification to act as detectors for low-concentration analytes. DNA circuits can be integrated with aptamers to solve this problem. Unlike enzymatic reactions, DNA-DNA reaction components can be easily dried, stored, transported and operated under harsh conditions. An aptamer can generate a signal in response to a specific target, and DNA circuits can amplify the signal. This integration strategy suggests a robust detection platform. This strategy could be significant in developing point-of-care devices.

DNA circuits and aptamers might eventually be used to interact with biological regulatory networks. This could be applied to learn about cells' internal dynamic regulation as well as to modify disease states. Aptamers can act as the "output" of a DNA circuit to affect a biological system according to an internal program. Thrombin aptamers have been demonstrated as anticoagulant drugs⁸⁶. The DNA circuit for regulating thrombin⁹⁵ set thresholds for activating and deactivating a thrombin aptamer. Further development might produce a self-regulating therapeutic. The system could continuously maintain an active concentration high enough to derive therapeutic benefit but not so high as to cause side-effects.

Aptamers on both the input and output side of NA circuits might allow for a more complete integration of these non-natural networks with biological regulation. The Saito lab⁹⁶ and Aldaye lab used intracellular RNA circuits with the stated goal of applying these reactions within cells for synthetic biology. Integration with evolved elements may allow designed regulatory networks to respond to expression patterns rather than transcription patterns. Ultimately, evolved and designed reaction patterns may help to enable synthetic regulation.

This all shows that integrated DNA-aptamer-circuits have powerful applications. However, it will be challenging to integrate the underlying philosophies. The abstract principles of DNA circuits (i.e. base pairing and known, non-canonical structures) must be expanded to include the diverse and idiosyncratic properties of aptamers.

ACKNOWLEDGMENTS

Research reported in this publication was supported by the National Institute of General Medical Sciences of the National Institutes of Health under Award Number P20GM104420. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

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Figure 2:

Schematic showing hybridization and base breathing (A) DNA hybridization between two complementary strands. (B) No DNA hybridization between non-complementary strands. (C) DNA base breathing.

Figure 3: Structure of 5S rRNA (A) 3D view of 1C2X 5S rRNA structure fitted to a cryo-electron microscopic map at 7.5 angstroms resolution⁹ visualized using NGL Viewer¹¹. (B) Secondary structure of 5S rRNA predicted and visualized with NUPACK¹².

Figure 4:

Early example of designed DNA structure. (A) Self-assembled 3D DNA crystal (4B8D Tensegrity triangle¹⁵ visualized with NGL Viewer¹¹). (B) Schematic representation of tensegrity triangle adapted from Zheng et al.¹⁴. Numbers represent short, complementary domains. T, G, A and C represent unpaired bases.

Figure 5:

Different steps of a typical SELEX process. A random pool is applied to an immobilized target. Undesired DNA is removed. Desired DNA that binds to target is recovered, amplified with PCR, and the pool is regenerated. After several rounds of this cycle, the pool converges on DNA with high-affinity to the target. This DNA is then sequenced, re-synthesized, and characterized.

Figure 6:
Deoxyribozyme structure (A)3D view of 5CKK Crystal structure of 9DB1*
deoxyribozyme⁵⁰ visualized using NGL Viewer¹¹. (B) Secondary structure of 9DB1*
deoxyribozyme from NUPACK¹².

Figure 7: Schematic showing DNA strand displacement via 3-way branch migration. (A) Toehold mediated strand displacement reaction^{1,59,65}. (B) Strand displacement using toehold exchange^{1,59,65}.

Figure 8: Schematic of DNA circuits. (A) Entropy driven Amplifier circuits^{65,66}. (B) Catalytic Hairpin Assembly reaction^{58,65,68}.

Figure 9:
Schematic of Hybridization chain reaction based on work by Evanko et al⁷¹.

Figure 10:

Schematic of DNA walker release and capture. (A) Release particle release walker on addition of fuel strand. (B) Sensor particle captures walker. The captured walker walks on the surface of sensor particles using the EDA reaction. The fuel strand displaces one leg of a catalyst. The displaced leg hybridizes with a second substrate before the remaining leg is displaced by fuel.

Figure 11: Schematic of aptamer-based aggregation of gold nanoparticles functionalized with ssDNA to detect cancer cells based on work by Borghei et al. The aggregation is induced by designed DNA hybridization of aptamer and ssDNA of gold nanoparticles⁸¹.

Figure 12:
Schematic illustrations of regulation of thrombin using aptamer-based DNA circuits based on Han et al⁹⁵.

Figure 13:
Schematic illustrations of aptamer-based DNA circuit to detect ATP based on Zhu et al⁹⁰.

Table 1:

Aptamers vs Antibodies

Properties	Aptamers	Antibodies
Low cost	+	
Binding Strength		+
Synthetic	+	
Non-immunogenic	+	
Easy site-specific modification	+	
Slow renal clearance		+
Number of FDA approved drugs	1 ^{26,27}	> 60 ²⁸

Table 2:

Integration of dynamic DNA Circuits and Evolved DNA

Authors	Evolved DNA	Designed DNA	Year
Fan et al. ⁸⁷	Thrombin aptamer, TBA15	DNA circuit	2018
Borghei et al. ⁸¹	AS 1411 for cancer cells	DNA hybridization	2016
Ang et al. ⁸⁸	Thrombin binding aptamer	DNA circuit	2016
Martini et al. ⁸⁹	Thiamine pyrophosphate (TPP) aptamer	Strand displacement	2015
Zhu et al. ⁹⁰	ATP aptamer	DNA circuit	2014
Zhu et al. ⁹¹	sgc8 & TDO5 aptamers	HCR (DNA nanotrain)	2013
Li et al. ⁹²	Aptamer against homodimer BB of platelet derived growth factor (PDGF-BB)	Strand Displacement	2013
You et al. ⁹³	Sgc8c, Sgc4f, and TC01 for cancer cells	Strand Displacement	2014
Li et al. ⁹⁴	Two thrombin binding aptamers	Strand Displacement	2013
Han et al. ⁹⁵	TA-29 and TA-15 for thrombin	DNA circuit	2012
Bompiani et al. ⁸⁶	Thrombin aptamer	DNA hybridization	2012