

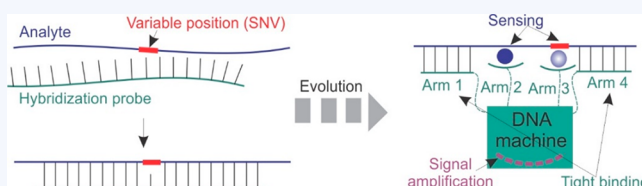
Evolution of Hybridization Probes to DNA Machines and Robots

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CONSPECTUS: Hybridization probes are RNA or DNA oligonucleotides or their analogs that bind to specific nucleotide sequences in targeted nucleic acids (analytes) via Watson–Crick base pairs to form probe–analyte hybrids. Formation of a stable hybrid would indicate the presence of a DNA or RNA fragment complementary to the known probe sequence. Some of the well-known technologies that rely on nucleic acid hybridization are TaqMan and molecular beacon (MB) probes, fluorescent *in situ* hybridization (FISH), polymerase chain reaction (PCR), antisense, siRNA, and CRISPR/cas9, among others. Although invaluable tools for DNA and RNA recognition, hybridization probes suffer from several common disadvantages including low selectivity under physiological conditions, low affinity to folded single-stranded RNA and double-stranded DNA, and high cost of dye-labeled and chemically modified probes.

Hybridization probes are evolving into multifunctional molecular devices (dubbed here “multicomponent probes”, “DNA machines”, and “DNA robots”) to satisfy complex and often contradictory requirements of modern biomedical applications. In the definition used here, “multicomponent probes” are DNA probes that use more than one oligonucleotide complementary to an analyzed sequence. A “DNA machine” is an association of a discrete number of DNA strands that undergoes structural rearrangements in response to the presence of a specific analyte. Unlike multicomponent probes, DNA machines unify several functional components in a single association even in the absence of a target. DNA robots are DNA machines equipped with computational (analytic) capabilities. This Account is devoted to an overview of the ongoing evolution of hybridization probes to DNA machines and robots. The Account starts with a brief excursion to historically significant and currently used instantaneous probes. The majority of the text is devoted to the design of (i) multicomponent probes and (ii) DNA machines for nucleic acid recognition and analysis. The fundamental advantage of both designs is their ability to simultaneously address multiple problems of RNA/DNA analysis. This is achieved by modular design, in which several specialized functional components are used simultaneously for recognition of RNA or DNA analytes. The Account is concluded with the analysis of perspectives for further evolution of DNA machines into DNA robots.



1. INTRODUCTION

“Sticks and stones picked up unaltered from the ground were probably the only implements used by earliest human.... Later on, tools became more specialized.”¹ “Tools may have allowed hominids to be more adaptable, extract food from a greater range of areas.”² Just like hunting tools in the past, hybridization probes undergo continuous evolution to benefit humankind.

The initial design of hybridization probes was inspired by the natural ability of two complementary DNA strands to hybridize to each other. Since the development of the first hybridization procedures by Hall and Spiegelman in 1961³ and by Bolton and McCarthy in 1962,⁴ nucleic acid hybridization has been widely accepted as a tool for recognition of specific RNA and DNA sequences. In this approach, a nucleic acid strand forms a complementary duplex with a single-stranded (ss) analyte (Figure 1A). In applications where single nucleotide variations (SNVs) need to be identified, the probe should form a hybrid only with the fully complementary (matched) analyte fragment, but not with a single base mispaired sequence. Early applications of hybridization probes include Southern⁵ and Northern⁶ blots, fluorescent *in situ* hybridization,⁷ polymerase chain reaction (PCR), and DNA microarrays. These techniques required

removing an excess amount of unhybridized probe by extensive washing or time intensive sample analysis by gel electrophoresis.

Introduction of instantaneous probes^{8–11} has enabled fast assays, in which the change in fluorescence is detected immediately after hybridization in solution, thus avoiding the need to separate the probe–analyte hybrid from the excess amount of the unbound probe. State-of-the-art instantaneous probes include Taqman¹¹ and MB probes^{11,12} used in quantitative real-time PCR assays (qPCR) (Figure 1B,C).

In addition, since 1967, hybridization-based approaches have been considered for therapeutic purposes.¹³ Modern therapeutically significant hybridization-based agents include antisense, small interfering RNA (siRNA), and clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated proteins (CRISPR/cas), which can be used for suppression of specific genes, gene knockout, and gene therapy (GT).¹⁴

Common challenges of modern hybridization probes include inadequate differentiation of SNVs at ambient temperatures (0–

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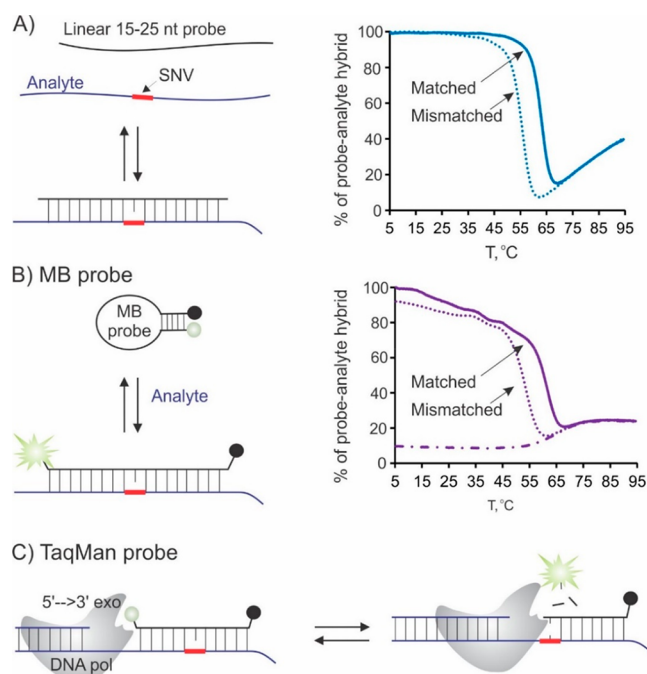


Figure 1. Principles of conventional hybridization probes. (A) General concept of a linear hybridization probe. A fully matched probe–analyte hybrid melts at a higher temperature than the corresponding mismatched complex, thus providing differentiation of single nucleotide variations (SNVs) within a certain relatively narrow temperature range (e.g., 56.7–71.5 °C, $\Delta T_m = 14.8$ °C).¹⁷ (B) A conformationally constrained molecular beacon (MB) probe produces fluorescence upon hybridization to analyte. The differentiation range was 52.4–69.5 °C ($\Delta T_m = 17.1$ °C) for the same analyte as in panel A.¹⁷ (C) TaqMan probe takes advantage of the 5' → 3' exonuclease (5' → 3' exo) activity of DNA polymerase (DNA pol) to cleave a fluorophore- and quencher-labeled DNA oligonucleotide during primer elongation. Panels A and B adapted with permission from ref 17. Copyright 2016 American Chemical Society.

40 °C), inability to analyze dsDNA or folded nucleic acids, and high cost of chemically modified strands. Invention of the MB probe has resulted in improved selectivity in comparison with linear probes, which is attributed to the presence of a stem–loop in its structure. This structural feature broadens the selectivity temperature range and shifts it to lower temperatures (compare right panels in Figure 1A,B).¹⁵ However, the MB probes cannot differentiate SNVs at ambient temperatures.^{11,12,15} Furthermore, being folded into a secondary structure themselves, MB probes have lower affinity to analytes than their linear probe counterparts (see section 3 for more discussion). All these challenges are further complicated by well-documented design problems including stem invasion and loop interference¹² to the degree that prevents designing MB probes for some practically significant sequences.¹⁶ Finally, the MB probe is an expensive commercial product as it requires double dye-labeling and double HPLC purification. At least some of these challenges are relevant to other commonly used hybridization probes.

Inspired by the developments of DNA nanotechnology,^{18,19} we hypothesized that the aforementioned challenges can be addressed by converting conventional probes into more sophisticated multicomponent probes and DNA machines, in which each specialized component addresses a particular challenge, and then all the components jointly participate in interrogation of a DNA or RNA analyte.

2. ADDRESSING THE SELECTIVITY ISSUE

Our endeavor in redesigning hybridization probes began with addressing the selectivity problem, which is associated with a typical probe length of >15 nt (e.g., 18–22 nt TaqMan probe or 20–25 bp siRNA). These lengths maximize the probability that a single binding site is targeted within a genome made of billions of base pairs. However, hybrids of >15 bp in length can be stable under physiological conditions even in the presence of one or several mismatches.²⁰ In practice, such hybrids will be formed even with the mismatched sequences, thus jeopardizing the ability of the probe to differentiate SNVs. This problem is addressed in commercially available assays by the use of qPCR instruments with DNA melting capabilities.²¹ The precise measurement of the hybrid's melting temperature (T_m) enables discrimination of a fully matched from a mismatched complex, which creates a temperature range useful for differentiating the two analyzed sequences (e.g., $\Delta T_m = 14.8$ °C in Figure 1A). However, the probe-based qPCR is applicable neither for point-of-care molecular diagnostics nor for recognition of nucleic acids under physiological conditions (e.g., RNA monitoring in live cells).²² The same reasons are responsible for the off-target effects of siRNA and CRISPR/cas agents.^{23–25}

Selectivity can be improved by using locked nucleic acids (LNAs) and peptide nucleic acids (PNAs), whose affinity to RNA and DNA is higher than that of the DNA-based probes.²⁶ In this case, shorter probes are able to maintain high affinity to the targeted analytes. A single base mispairing would destabilize a shorter hybrid to a greater extent than a longer one, thus ensuring destabilization of the mismatched complex. However, shorter sequences can bind to alternative nonspecific sites in a DNA background of a genome size. Moreover, PNAs and LNAs are expensive synthetic products.

An elegant solution to the selectivity problem is a binary (split) approach for the probe design.²⁷ It takes advantage of two strands (arms 1 and 2 in Figure 2A), which hybridize to adjacent positions of an analyte and produce a detectable signal. Arm 1 can be made short enough to form a stable complex only with a fully matched but not with a single-base mismatched sequence. High selectivity of the binary probes under ambient temperatures is well-documented.^{27–32} Importantly, there is no need to use expensive LNA or PNA binding arms.

Variations of binary DNA probes include adjacent hybridization, excimer-forming pairs, and chemical ligation, among others.²⁷ Figure 2B illustrates the design of binary aptameric probes, in which the two strands form a binding site for a fluorogenic dye after hybridization to the analyte. The dye binding to the aptamer increases its fluorescence multifold. DNA-based probes^{33,34} offer advantages in cost and stability over the RNA binary aptameric probes^{35,36} and can be used as a more selective and less expensive alternative to the MB and Taqman probes.

Conventional hybridization probes are selective only within narrow temperature ranges that may not cover ambient temperatures (Figure 1A,B). The selectivity range for the multicomponent probes is broader. For example, the MB-based X probe takes advantage of two adaptor strands that hybridize to both the MB probe and the analyte to form a DNA crossover (X) structure (Figure 2C).³⁷ The X structure is thermodynamically stabilized by the formation of base pairs with the analyte, as well as by the DNA four-way junction (4WJ). The X probe can differentiate SNVs in the range 5–40 °C.¹⁷ The observed melting profile (Figure 2C, right) contradicts that expected from

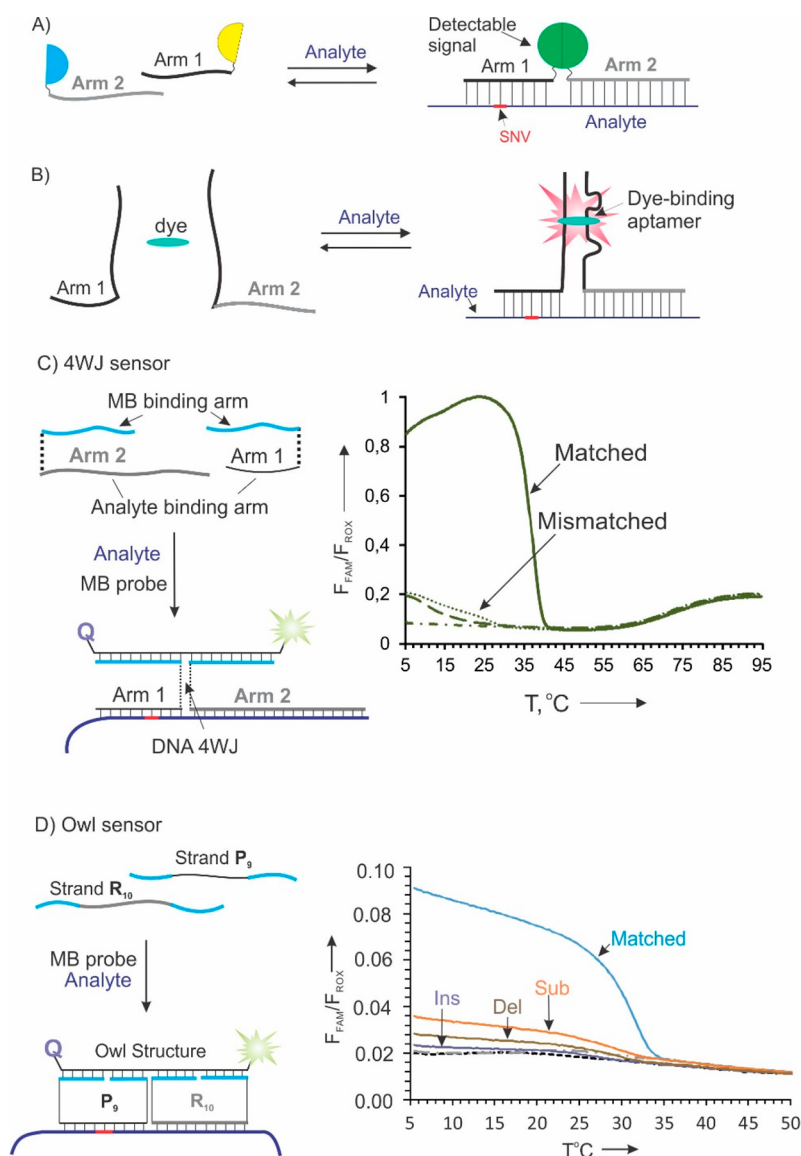


Figure 2. Binary (split) probes enable highly selective recognition of SNVs. (A) General design of a binary probe: arms 1 and 2 conjugated with signal-forming units (semicircles) hybridize to the analyte and form a detectable signal. Arm 1 binds only the fully matched analyte; signal is not produced if arm 1 is mismatched. (B) Aptamer-based binary probes: two strands hybridize to the analyte and form a dye-binding site; this increases fluorescence of the otherwise nonfluorescent dye. (C) MB-based X probe: two adaptor strands hybridize to the analyte and universal MB probe and form a fluorescent complex (bottom). X probe differentiates SNVs in a temperature range of 5–40 °C.¹⁷ Panel A adapted with permission from ref 17. Copyright 2016 American Chemical Society. (D) “Owl sensor” forms a fragile structure with the analyte. The adaptor strands R_x and P_y reversibly hybridize to the analyte and the universal MB probe forming a fluorescent “Owl Structure”, which differentiates the matched analyte from substitution (Sub), deletion (Del), or insertion (Ins)-containing sequences in a temperature range of 5–32 °C.³⁹ Adapted with permission from ref 39. Copyright 2018 Royal Chemical Society.

thermodynamic predictions.¹⁷ This behavior is a combination of both nonequilibrium hybridization conditions and the “kinetic inversion” effect, in which the fully matched analyte hybridizes with the probe faster than the mismatched one.¹⁷ Such low signal with the mismatched analyte cannot be achieved even by the most accurately optimized hybridization probe acting under equilibrium conditions.³⁸

Alternatively, high selectivity in a broad temperature range can be achieved by a probe that forms a fragile DNA structure when bound to an analyte (Figure 2D).³⁹ This structure would decompose into separate strands in response to a small imperfection, such as the presence of a mismatch.

Multicomponent probes can also be designed to tolerate SNVs^{40,41} or to detect a selected SNV, while being tolerant to

other SNVs in the analyzed sequences.⁴² The latter is important to maintain binding affinity to highly mutagenic viral sequences. For example, the DX probe forms >26 base pairs with the analyte, which forms a complex stable even in the presence of one SNV (Figure 3).^{40,43}

3. ANALYSIS OF SECONDARY STRUCTURE-FOLDED NUCLEIC ACIDS

Inefficient binding of the probe to folded analytes is a well-recognized problem in nucleic acid analysis.^{44–46} Indeed, if a targeted RNA sequence already participated in intramolecular base pairing, the probe would need to outcompete these existing interactions to bind the target. The situation is extreme for folded probes, like the MB probe, in which the MB stem–loop

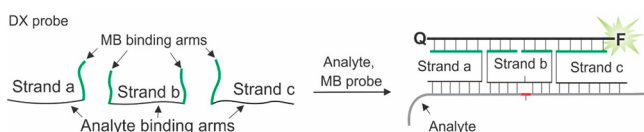


Figure 3. DX motif-forming sensor produces stable complex even with a mismatched analyte. The adaptor strands are unmodified oligodeoxyribonucleotides. The position of an SNV site is shown in red.

further stabilizes the dissociated state (Figure 4A). Search for accessible RNA fragments requires labor-intensive trial-and-error experiments, since computational prediction of RNA folding is still under development.⁴⁷

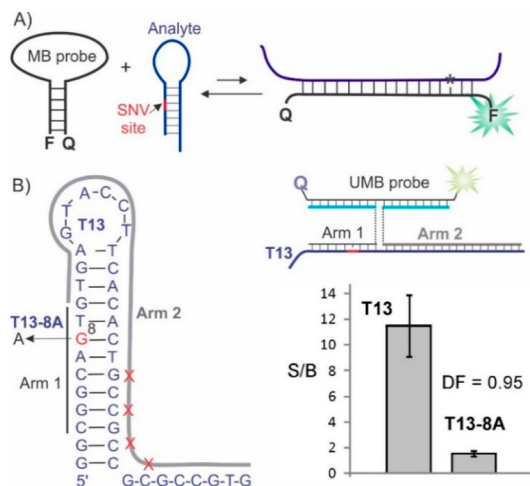


Figure 4. Detection of folded nucleic acids. (A) Hybridization of the MB probe with a stem–loop folded nucleic acid can be unfavorable. (B) Detection of a folded analyte by the X probe. (left) Model of folded T13 analyte. Black and gray lines indicate regions bound by the SNV-specific arm 1 and long unwinding arm 2, respectively. Red crosses indicate mismatched positions between T13 and arm 2. (right) X structure and signal-to-background ratio for the 4WJ probe in the presence of matched T13 and mismatched T13-8A.⁴⁸ Adapted with permission from ref 48. Copyright 2010 Wiley.

Approaches that address this problem use a structure-free DNA with pseudocomplementary properties,⁴⁴ or LNA and PNA that form thermodynamically more stable complexes than DNA–DNA and thus outcompete the intramolecular interactions.^{45,46} However, this approach requires expensive non-natural nucleotides.

It is possible to analyze folded nucleic acids using unmodified DNA probes. Figure 4B illustrates a folded target T13 with a SNV in its mismatched counterpart T13-8A. This challenging analyte was interrogated by both the MB probe and X probe.⁴⁸ The MB probe was unable to produce fluorescence signal even with the complementary T13, while the X probe produced high signal with T13 but not with T13-8A (Figure 4B, right). This difference in the performance was attributed to the ability of X probe to unwind T13 secondary structure. Indeed, arm 2 was designed to bind one side of the stem, the loop, and 4 nucleotides of the 5' side of the stem. This extended contact was able to unwind the analyte's secondary structure and open the SNV-containing site for its interaction with short arm 1. Importantly, arm 2 contained a series of nucleotide substitutions, which reduced both its internal folding and the affinity to arm 1. As a result, the introduced mismatches

increased signal-to-background ratio. The assay was performed at room temperature and did not require annealing.⁴⁸ Similar strategy for analyte-binding arm design was shown to be efficient for other types of multicomponent probes, including deoxyribozymes and G-quadruplex-forming binary probes.^{49,50}

4. LIMIT OF DETECTION

A typical limit of detection (LOD) for a conventional fluorescent probe is 0.1–1 nM, with some exceptional probes detecting down to 10 pM analyte.^{12,27} It is limited by the background fluorescence and the measurement error of ~15%. The concentration of DNA and RNA in a biological sample could be much lower. We, however, do not consider insufficient LOD a major complication of hybridization probes. Indeed, 0.1–1 nM LOD is sufficient for amplification-based assays, since a typical amplicon's concentration produced by PCR or isothermal amplification is high enough. Nevertheless, reducing LOD of amplification-free assays is the subject of large variety of multistage detection schemes.^{51,52}

We and others explored a protein-free assay that uses RNA-cleaving deoxyribozymes (Dz) for signal amplification.^{53–57} For example, some Dz can be split in two fragments (Dz_a and Dz_b in Figure 5A) to form a binary Dz (BiDz) probe.^{54,55} Upon

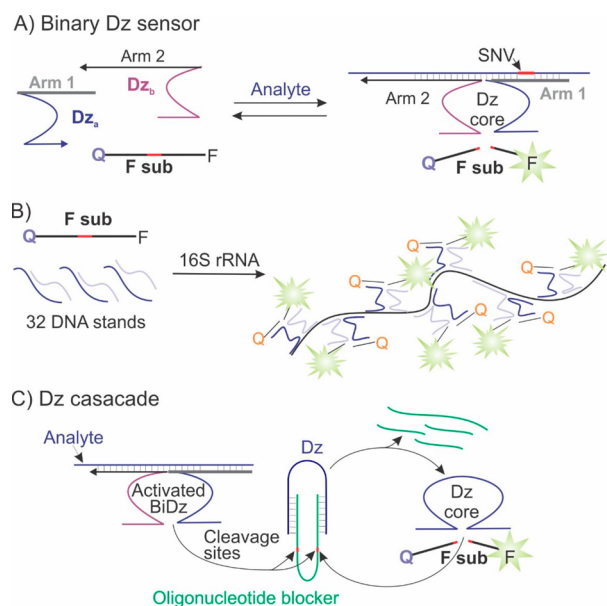


Figure 5. Design of deoxyribozyme (Dz)-based sensors. (A) Binary Dz (BiDz) probe re-forms catalytic core in the presence of a specific analyte. (B) Detection of 16S rRNA using “deoxyribozymes-on-a-string” complex.⁵⁹ *E. coli* 16S RNA was incubated with 32 DNA strands that formed 16 Dz cores, each of which was able to cleave the same F-sub. Adapted with permission from ref 59. Copyright 2013 Wiley. (C) Dz based cascade for signal amplification. Activated BiDz cleaves the oligonucleotide blocker and releases active Dz in solution, which either cleaves another blocker or F-sub. Adapted with permission from ref 60. Copyright 2014 American Chemical Society.

hybridization to a complementary analyte, the two strands form a catalytically active Dz core, which cleaves a fluorophore- and quencher-labeled substrate (F-sub), thus producing a fluorescent output. The signal is accumulated over time due to the catalytic turnover. Mokany et al. used this approach to design a BiDz sensor with a LOD of 5–10 pM.⁵⁵ To further reduce the LOD to ~0.3 pM, we used the entire length of *E. coli* 16S RNA to

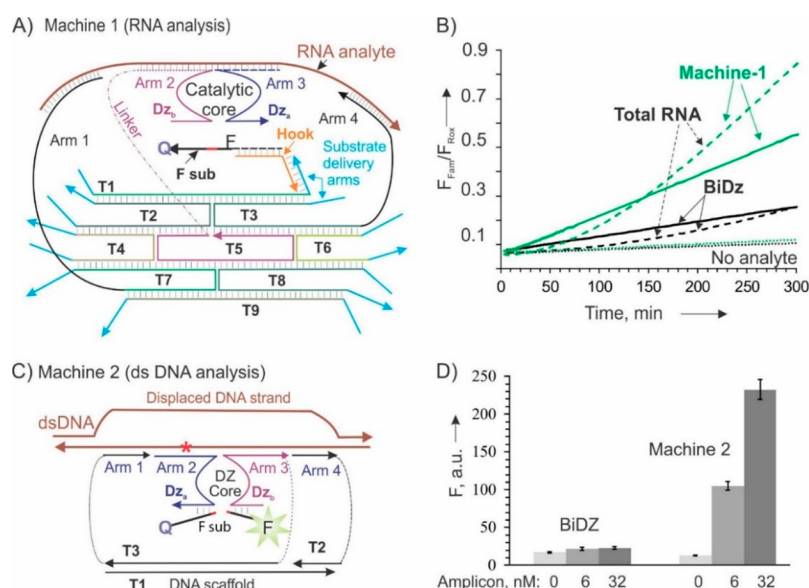


Figure 6. Design and performance of Dz-based DNA machines. (A) DNA machine 1 for the analysis of 16S rRNA consists of nine DNA strands (T1–T9) that form a DNA tile. Dz_b is covalently attached to T5. T3 and T7 are equipped with RNA-binding arms (arms 1 and 4). Strands T1–T5 are equipped with “substrate delivery arms” (cyan), which is complementary to the Hook strand bound to F-sub (shown only once). (B) Fluorescent response of DNA machine 1 and the tile-free BiDz probe in the absence (dotted curves) or presence of 100 pM DNA analyte (solid curves) or with total *M. smegmatis* RNA containing 16S rRNA (dashed curves).⁶⁶ Panels A and B are adopted with permission from ref 66. Copyright 2019 the Royal Society of Chemistry. (C) DNA machine 2 for dsDNA analysis consists of association of strands T1, T2, and T3 linked to Dz_b via arm 3, arm 1, and arm 4, respectively. Strand Dz_a is attracted to the association from solution in the presence of the analyzed DNA target. (D) Detection of HPV16 PCR amplicon by machine 2 or the correspondent BiDz probe.⁷⁰ Panels C and D are adopted with permission from ref 70. Copyright 2019 the Royal Society of Chemistry.

form multiple Dz cores (Figure 5B).⁵⁹ Another strategy to achieve low LODs in a protein enzyme-free system is to employ Dz-based cascades. Bone et al. inhibited catalytic activity by the oligonucleotide blocker Dz (Figure 5C), which could be cleaved at the two sites by an analyte-activated BiDz or by the Dz itself. The released Dz cleaves F-sub for fluorescent signaling. A single activated BiDz can start a cascade, in which the newly released Dz can activate another Dz molecule, thus increasing the number of agents for F-sub cleavage. The cascade enabled LOD down to 1 pM.⁵⁸ The LOD improvement was limited by the high background reaction in the absence of the analyte. Dz cascades can also be used to convert the Dz activity into color change, for the instrument-free signal detection.⁶¹ One advantage of the BiDz approach is its protein enzyme-free format, which promises to increase the probe shelf life. Another advantage is high SNV selectivity common for all binary probes.¹⁷

5. DNA MACHINES FOR NUCLEIC ACID ANALYSIS

DNA machines and conformational switches of various designs have been reported to date,^{62–64} with the MB probe arguably being the most elegant and useful. DNA machines with analytical characteristics exceeding that of MB probes are described below.

Our continuous attempts to increase the LOD of BiDz-based probes led to a design that would increase the diffusion rate of F-sub to the core of the activated BiDz probe (Figure 6A). The effort was based on the fact that under optimal *in vitro* conditions the Dz's catalytic activity is limited by substrate diffusion and binding to the Dz.⁵⁶ To increase Dz activity, we attached one strand of the BiDz probe (Dz_b in Figure 6A) to a DNA “antenna tile”.⁶⁵ The tile was equipped with “substrate delivery arms” consisting of 14 DNA fragments (cyan in Figure 6A)

complementary to the Hook/F-sub complex. LOD of such system was improved by a factor of 9.⁶⁵ This step was pivotal in understanding the utility of a DNA platform that can hold together separate functional units.

Next, we equipped the “DNA antenna tile” with a pair of additional RNA binding arms complementary to *M. smegmatis* 16S rRNA (arms 1 and 4 in Figure 6A).⁶⁶ Interestingly, this construct (DNA machine 1) produced higher signal over time in the presence of an RNA target than with a short DNA analyte. While for the BiDz probe this could be explained solely by tighter binding to RNA than to DNA due to higher stability of DNA–RNA hybrid, for DNA machine 1 this stability was even higher due to the additional arms 1 and 2. As a result, folded biological RNA could be detected at lower concentrations than short linear DNA, a result that contradicts common practice.^{42–45} Could the same approach be applied for the detection of dsDNA?

Interrogation of dsDNA by hybridization probes is a challenging task. In practice, the problem is solved by production of ssDNA fragments by asymmetric PCR⁶⁷ or via λ exonuclease cleavage of a DNA strand complementary to the targeted strand. These approaches require additional steps and cannot be applied for the analysis of dsDNA in cells. Notable alternative approaches use PNA and LNA “openers” to enable analysis of native dsDNA.^{68,69}

DNA machine 2 was designed to detect dsDNA using unmodified DNA strands.⁷⁰ Binding of a conventional probe to a targeted strand within dsDNA would require displacement of the complementary strand, which is thermodynamically unfavorable. The probe–dsDNA complex, however, can be stabilized by extended probe–analyte hybrids. Combination of the improved binding affinity with the high BiDz probe sensitivity enabled us to design a DNA machine that can detect

dsDNA amplicon. In DNA machine 2, there are four DNA-binding arms (arms 1–4) responsible for tight amplicon binding. This design resulted in significantly greater turn-on values for DNA machine 2 in comparison with the tile-free BiDz probe (Figure 6D). The formation of the DNA machine 2–dsDNA complex is thermodynamically unfavorable. However, Dz probe has an LOD of 10 pM, while amplicon concentration can reach 20 nM. In this case, formation of only ~0.5% of the complex would be sufficient for detection. DNA machine 2 maintained high selectivity toward SNVs.⁶⁷ We acknowledge that this approach lacks elegance as it is one-on-one direct competition with the displaced strand. Other DNA designs are required to enable dsDNA “opening”, especially under physiological conditions.

6. TOWARD DEVELOPMENT OF DNA MACHINES FOR CANCER THERAPEUTICS

It is tempting to apply the developed recognition principles for the design of therapeutic DNA machines. Despite certain success in FDA approved antisense and siRNA therapeutics,⁷¹ GT agents failed to deliver a clinically useful anticancer treatment. Besides the major problem of intracellular delivery, GT agents suffer from low selectivity and efficiency.^{23–25}

We integrated the binary Dz technology into an anticancer DNA machine to achieve cancer marker-dependent activation of Dz cleaving activity (Figure 7A).⁷² The machine consists of a

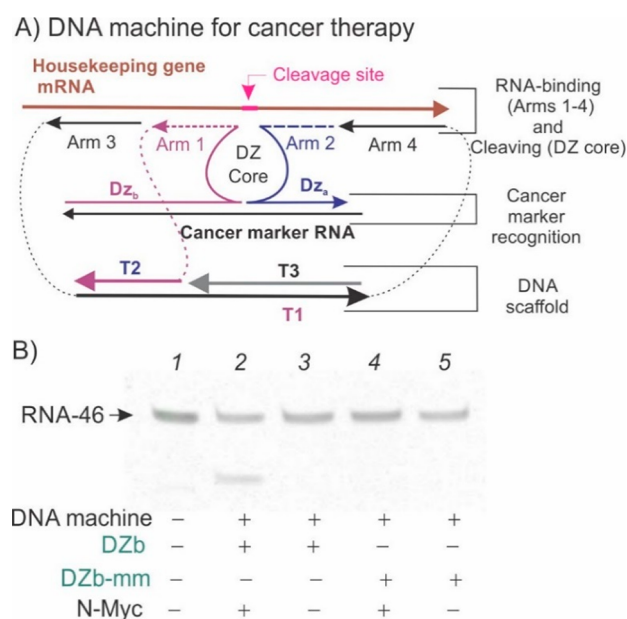


Figure 7. Design of the DNA machine for cancer marker-dependent RNA cleavage. (A) Complexes of DNA machine-1 with RNA-46 (a prototype for a housekeeping gene mRNA) and N-Myc (a cancer marker). (B) The DNA machine cleaves RNA-46 only in the presence of the fully matched N-Myc activator. Adapted with permission from ref 72. Copyright 2019 Wiley.

DNA scaffold that holds together cancer marker-sensing, RNA binding and unwinding (arms 3 and 4), and RNA cleaving functions. Dz_a and Dz_b fragments of the DNA machine recognize a cancer marker with high selectivity and translate the recognition event into cleavage of a vital housekeeping gene mRNA. The four RNA-binding arms act cooperatively to unwind the RNA secondary structure, but at the same time are short enough to efficiently release the RNA cleavage products.

The DNA machine cleaved only the fully complementary RNA target. Indeed, a single base mismatch between the DNA machine and the target completely abolished the cleavage activity (Figure 7B). In addition, the DNA machine recognized neuroblastoma cancer marker sequence (N-Myc) with selectivity to a single base mismatch.⁷² The major advantage of the design is that the DNA machine is activated by one RNA while it targets another, opening a route toward cleavage of a housekeeping gene exclusively in cancer cells. Future success of this technology depends on the ability to efficiently deliver the DNA machine to the cytoplasm of cancer cells.

7. PERSPECTIVES ON DNA NANOROBOTICS

A possible result of the ongoing evolution of hybridization probes could be DNA robots that sense biological markers, analyze them autonomously (without human interference), and fix the identified problem (e.g., kill malignant cells). Perspectives of molecular robotics have been discussed earlier.^{73–75} Some reports use the term “DNA robot” for DNA devices capable of recognizing several analytes.⁷⁴ However, such use of the term can be confused with the earlier introduced terms, for example, “sensors”, “structural switches”, or “DNA logic gates”. Others state that “systems having all three functions—sensing, computation, and actuation—have not been realized yet”.⁷⁵ I agree with this last quote. A DNA robot should have a structurally independent module devoted entirely to the computation and analysis function, which is separated from the sensing module due to its self-significance. This computational module could consist of two or more layers of DNA logic gates interconnected in circuits and should be able to communicate with sensing and actuating units of the DNA device. The evolution of DNA machines into robots will depend on the progress in assembling a first practically useful DNA nanoprocessor. Computational circuits based on DNA are under development.^{76,77} One major technical challenge is integration of DNA logic gates in long chains of communicating gates.⁷⁷ On the other hand, there is a lack of practically oriented tasks that would be addressed by a DNA-based processor more efficiently than by conventional approaches that use electronic computers.

8. CONCLUDING REMARKS

Hybridization probes are a highly reputable tool for nucleic acid recognition. Advances in our understanding of DNA and RNA structure, hybridization thermodynamics, DNA nanotechnology, and development of deoxyribozymes and aptamers in the last 20–30 years laid a foundation for building DNA machines that demonstrate improved characteristics in DNA and RNA recognition in comparison with currently used probes. There seems to be a gap between the probe invention and their practical applications. Future evolution of DNA machines into DNA robots depends not only on academic creativity, but also on practical motivations. Open and visionary cooperation of chemists, health care professionals, and visionary businessmen could become a driving force in evolution of “sticks” into “more specialized tools” to make “hunting” for biological RNA and DNA more successful and health care more efficient.

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Notes

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Biography

Dmitry Kolpashchikov received his Ph.D. in Bioorganic Chemistry from Novosibirsk Institute of Bioorganic Chemistry in 1999. He completed his postdoctoral training in the National Institute of Genetics (Japan) and at Columbia University in the City of New York prior to joining the Chemistry Department at the University of Central Florida in 2008. His research interests include biomedical applications of DNA nanotechnology, as well as all aspects of nucleic acid chemistry and thermodynamics.

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