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Review

DNA as a 'Nanomaterial'☆

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Abstract

DNA is recognized as a nanomaterial, not as a biological material, in the research field of nanotechnology. This article reviews recent research on nanowires, nanoarchitectures, computing, aptamers, biocatalysts, devices, and machines using DNA. In these works, the characteristics of DNA including facile synthesis by the solid-phase method, self-assembly, electro-conductivity, information elements, amplification, switching, molecular recognition, and catalytic functions, were appropriately applied. Multiple functions of DNA could be used simultaneously, and activated independently, by molecular switching. Therefore, the combinations of functional sequences of DNA can produce unique materials. It is obvious that the DNA molecule is one of the most promising functional nanomaterials. However, the application of DNA molecules is still under study because of the big gap that exists between theory and practice. We eagerly anticipate a 'coming out' of DNA due to breakthroughs in nanobiotechnology.

Keywords: DNA wire; DNA architecture; DNA computer; Aptamer; DNAzyme; DNA device; DNA machine

1. Introduction

The year 2003 is the 50th anniversary of the discovery of the double-helical structure of DNA. Many articles and special issues relating to DNA have been published. The decoding of the human genome sequence has set the stage for 'post-genomic' science. DNA makes up the genetic component of all eukaryotes, bacteria and many viruses. Many biotechnology techniques, including recombinant DNA, anti-sense DNA and RNA, anti-gene technology, DNA vaccines, etc., have been developed over the past 30 years. Recently, other studies on DNA have focused on its potential use as a nanomaterial, reviewed by Seeman [1]. Seeman quoted the words of Ronald Hoffman, "*The nucleic-acid 'system' that operates in terrestrial life is optimized (through evolution) chemistry incarnate. Why not use it . . . to allow human beings to sculpt something new, perhaps beautiful, perhaps useful, certainly unnatural.*" in *American Scientist*, 1994. In the present paper, we review some recent research concerning the application of DNA as a nanomaterial, in which several functions of DNA are described (Fig. 1).

2. DNA as a conductive nanowire

The conductivity of DNA has been considered an interesting subject since 1993, when reported by Murphy et al. [2]. They suggested it was feasible for DNA to be a molecular wire 'π-way' by photo-activation, following the introduction of metallic complexes onto both DNA terminals to act as both electron donors and electron acceptors. However, other experiments suggesting that DNA was almost insulative have been also reported. According to current understanding, DNA could show many conductivity features [3–5]. In fact, DNA would be an insulator, a good conductor, or a semiconductor with a large band gap, depending on the DNA sequence and the conditions in which the evaluation took place [3–5]. Artificial nucleic acid bases have been designed and studied as effective for DNA-mediated hole transport [6]. Other trials, including a

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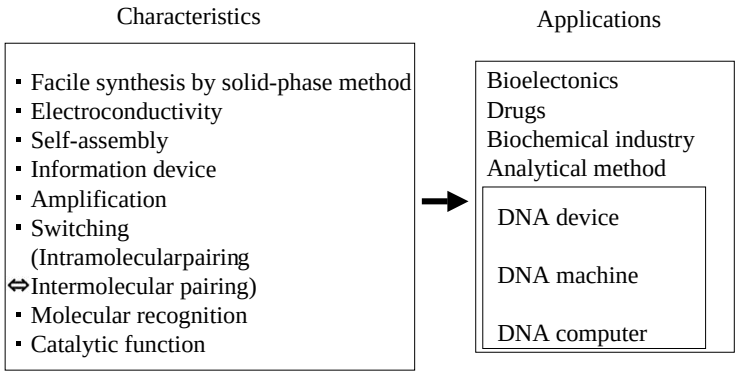

Fig. 1. Characteristics of DNA and potential fields in which it could be applied as a nanomaterial.

coordination of metal and a doping of iodine, have also been performed.

Preparation of a conducting nanowire derived from DNA has been reported [7,8]. Braun et al. [7] attached DNA of different sequences onto two microelectrodes respectively (Fig. 2a). A connection between the two electrodes was then made, with the DNA fragment complementary to both electrode-linked DNA sequences, using DNA-DNA hybridization. After that, a silver ion was doped to the DNA to be wired (Fig. 2b). Recently, molecular lithography using protein was reported in which the RecA protein, a single-stranded DNA-binding protein, was used for the resist process (of lithography) [9]. DNA has been employed not only for conductive materials, but also for photoelectric transfer materials [10].

The conductivity of DNA has already been used in the principle of biosensor development, including detection of SNPs (single nucleotide polymorphisms). Barton et al. developed an electrochemical biosensor for the detection of 'DNA mismatch' [11,12]. They used the principle that 'DNA mismatch' would disturb base stacking and reduce the efficiency for charge transfer. Other trials for the detection of SNPs have also been reported. One used the principle that the binding of number of intercalaters around a mismatch site would be decreased [13]. Others were based on the tendency of 'mismatch DNA' to be dissociated by an imposed potential [14]. A biosensor using DNA to measure biomolecules other than DNA was also reported [15]. The sensor was based on the idea that conductivity should be modulated by

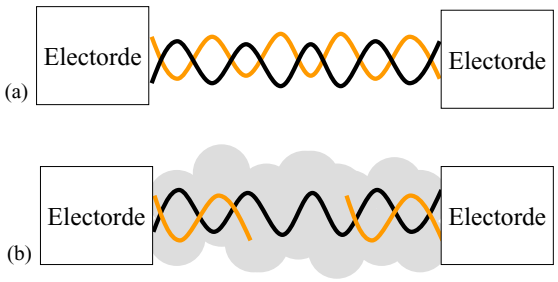

Fig. 2. Conductive DNA nanowire. (a) DNA only and (b) DNA with metals.

the intrusion of specific molecules into a double-stranded portion of DNA that was an introduced molecular recognition region.

3. DNA as a nanoarchitecture

Nanotechnology involves two strategies. One is the 'top–down' approach that atoms or molecules should be operated upon directly by a fine manipulation technique. The other is the 'bottom–up' approach, based on the self-integration of molecules. DNA is per se a programmed molecule with information and addresses. DNA could qualify to be an ultimate information material, with a fine integration density of around 0.34 nm clearance, based on the size of double-stranded DNA. DNA could also be assembled into macromolecules in centimeter levels by self-assembly. Therefore, DNA plays an important role in the 'bottom–up' approach in nanotechnology. Seeman succeeded in preparing several architectures using an overlap width for adhesion and branched structures (Fig. 3) [1]. Batalia et al. reported a frayed-wire network by self-assembly of DNA [16]. Recently Yan et al. designed a DNA nanostructure in which four-armed DNA 'tiles' in the shape of a plus sign assembled into two distinct lattice forms: nanoribbons and two-dimensional (2D) nanogrids [17]. Each tile was made of nine DNA strands, with one strand in the center participating in all four arms of the tile. By changing the ends of the DNA strands, they could control which lattice structure formed. The DNA nanogrid structure had large cavities that could be used as binding sites for other molecules.

DNA has been also used as a template in the architecture of nanoparticles. Soto et al. synthesized a photo-band gap in a photonic crystal by using DNA as an intercalating agent of nanoparticles [18]. Other trials have prepared several nanoparticle assemblies using 'DNA recognition protein' and 'DNA' [19,20]. Maeda et al. succeeded in the precise control of positioning of gold particles using DNA [21]. Warner and Hutchison constructed a linear structure and a ribbon shape from cationic gold particles and DNA [22]. Preparations of copper nanowire and platinum cluster aggregation using DNA as a template have also been reported

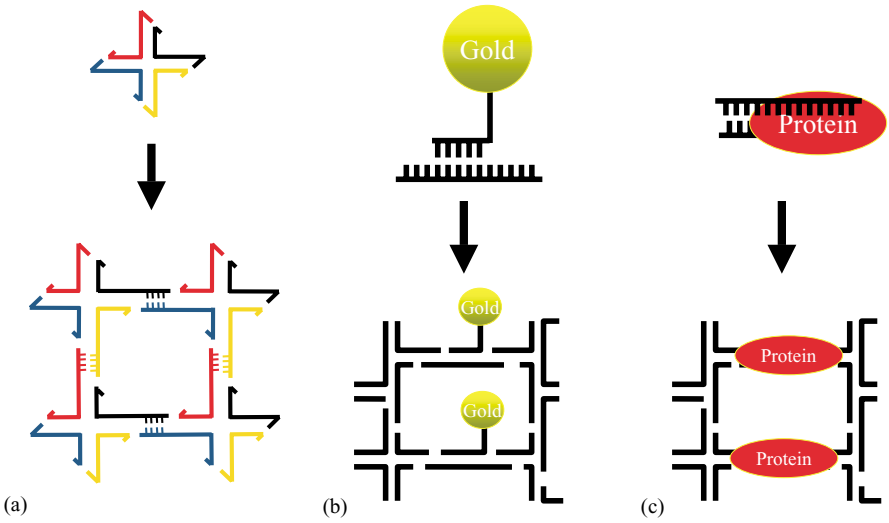

Fig. 3. DNA nanoarchitectures. (a) DNA only, (b) DNA template for gold particle array, and (c) DNA template for protein array.

[23,24]. Interaction of DNA with a carbon nanotube (representative of a nanomaterial) and a DNA-modified nanotube have been widely investigated [25–27].

A gold nanoparticle–DNA complex was investigated by Mirkin [28] and Harnack [29] for DNA sequencing and protein detection [30]. Other DNA sequencing methods using quantum dot [31], nano rod barcode [32], and electrochemical methods using several nanoparticle complexes [33–35] have been devised. A unique method for DNA sequencing has been invented by Saghatalian et al. [36]. They conjugated a reporter enzyme and a specific inhibitor to a single-stranded DNA spacer. In this single-stranded DNA complex, the activity of the reporter enzyme was inhibited. However, addition of a complementary DNA (cDNA) induced the formation of a double-helical structure to the single-stranded DNA spacer, causing the release of an inhibitor from the enzyme and thus resulting in an increase in enzymatic activity. These results support the conclusion that the DNA sequence can be monitored by enzymatic activity.

In another study, Mirkin and co-workers achieved a DNA nanolithography as a nanopatterning onto a secondary dimensional surface [37,38]. Hu et al. [39] succeeded in preparing several artificial DNA patterns on a solid substrate by an atomic force microscope (AFM) dissection. Liu et al. [40] accomplished the placement of single-stranded DNA onto a gold surface via sulfur, after removing a self-assembled resist pattern by AFM. They succeeded in preparing a nanopattern with 10 nm clearance.

Recently, Shionoya and co-workers succeeded in synthesizing an artificial DNA-based conjugate using metal ions instead of hydrogen bonds between the base pairs of the DNA double helix [41]. Several kinds of metals, with various functions, can give and receive electrons. Incorporation of metal ions into the DNA double helix would cause a drastic change in the natural function of DNA. The mode of DNA dissociation could be modulated by using several different metal ions. If all base pairs were linked with a metal ion, a wire could be formed in the double helix. This suggests that producing a molecular level-conductive wire is feasible. Trials for the creation of this new type of base pairing are proceeding. Another type of new base pairing was prepared by Schultz and co-workers [42]. These new base pairing modes will contribute to the increasing versatility of DNA as a nanomaterial, and are also expected to offer new templates.

4. DNA as a computer

In 1994, Adelman proposed a new calculation method named ‘DNA computing’ [43,44]. In the computer, ‘calculation’ was converted into ‘molecular operation’ based on a chemical reaction. The method was applied to solving ‘the directed Hamiltonian path problem’, an example of a complex combinatorial problem. To explain DNA computing, let us consider a seating arrangement of five persons at a round table as shown in Fig. 4. The possible combinations for seating are 120. However, assuming that two specific persons could not occupy seats side-by-side due to a bad relationship, the seating arrangement must take this into account. Computers usually calculate all combinations exhaustively to obtain the appropriate one. Five check points should be evaluated per combination. Consequentially, 5×120 calculations would be required. A DNA computer, however, would employ completely different tactics. It would allocate independent three-letter codes, such as ‘AAA’, ‘AAC’ or ‘AAG’, to each of the five people. Next, the DNA fragment corresponding to the 120 cases of combinations would be prepared. If the connection of the sequence ‘AAC’ and ‘AAG’ must be eliminated, the complementary sequence ‘TTG’ and ‘TTC’ would be added for hybridization with the target sequence. After the hybridized DNA fragments are removed, the remaining DNA fragments would be amplified and sequenced as answer

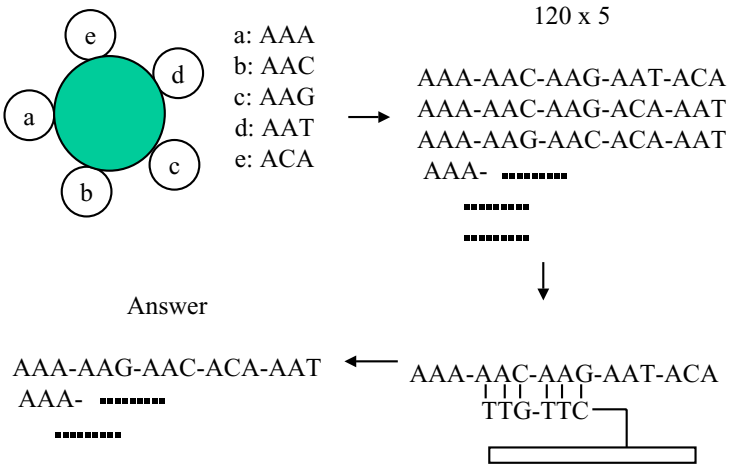

Fig. 4. A problem for DNA computing.

sequences. The calculation needs to be carried out only once.

The most remarkable feature of DNA computing is a super-synchronism based on a series of parallel chemical reactions of a large amount of molecules. Bacteria, whose replication speed is thought to be relatively fast, can generally replicate 500 base pairs every second. Thus, in computing terms, the replication speed for bacteria could be assumed to be 1000 bits/s. Such a low speed is obviously under the specification required for present computer technology. However, DNA computers can use an exponential enrichment strategy. This means the processing speed of a DNA computer should double as DNA is replicated. In fact, a DNA computer of 1000 bits/s would be one of 2000 bits/s after replication. Accordingly, 30 times replication would afford a processing speed of 1000 Gbits/s. A DNA computer would be composed of nanomolecules of 'DNA' and would require only a chemical reaction for processing. In light of this, its feasibility as an energy saving micro-memory device is being evaluated.

However, a large number of DNA fragments would be required to solve complicated problems, and it would be difficult to perform all designed chemical reactions. The processing speed of a DNA computer is relatively slow, as already mentioned. In addition, conventional DNA computers are specialized to perform exclusive tasks and would not be applicable for solving certain problems. To overcome this drawback, Sakakibara and Suyama applied the DNA calculation method to seek genes related to specific symptoms [45]. First, Suyama prepared a cDNA fragment to specific target genes. Subsequently they developed a DNA computing algorithm to elucidate the dynamic states of target genes in mice using cDNA fragments. They reportedly required only 3 h to complete the calculation. An application of DNA computing for the elucidation of SNPs is proceeding.

Recently, Benenson et al. reported tremendous results with a DNA computer device that includes input/output, software, hardware, and an energy source using DNA molecules and enzymes [46]. They described 'one spoon' solution to produce a DNA computer that would run one hundred thousand times faster than the fastest personal computer. The device comprises three components: an input molecule, to carry data to be calculated; a software molecule, involving a program routine; and a hardware molecule, to perform an actual calculation. The input molecule also provides enough energy for the enzymatic reaction, without the need of additional energy resources. They reported that the 'one spoon' solution included '1.5×10^{16}' DNA computer devices having a capacity of 3.3×10^{14} operations per second.

On the other hand, Stojanovic et al. proposed a logic circuit using DNAzymes. They prepared several logic gates, such as 'NOT', 'AND', and 'XOR', using the hammer-head DNAzyme with a molecular beacon attached to it [47]. They developed a molecular automation system, called MAYA, which encodes a version of the game of tic-tac-toe and interactively competes against a human opponent [48].

5. DNA as a molecular recognition element

Functions for molecular recognition, as for antibodies, can be imposed onto DNA. The methodology is called 'in vitro selection' or systematic evolution of ligands by exponential enrichment (SELEX), which is one of the combinatorial bioengineering techniques [49–51]. SELEX includes a series of processes that correspond to the processes in the Darwinian theory of evolution, which are 'spontaneous mutation', 'natural selection', and 'proliferation' (Fig. 5). A pool of random sequences of DNA would be constructed as a molecular library corresponding to 'spontaneous mutation'. The random DNA pool could be prepared by a repetition of a solid-state synthesis with a mixture of the four nucleotides A, T, G, and C. The library obtained would then be subjected to asymmetric PCR using only the forward

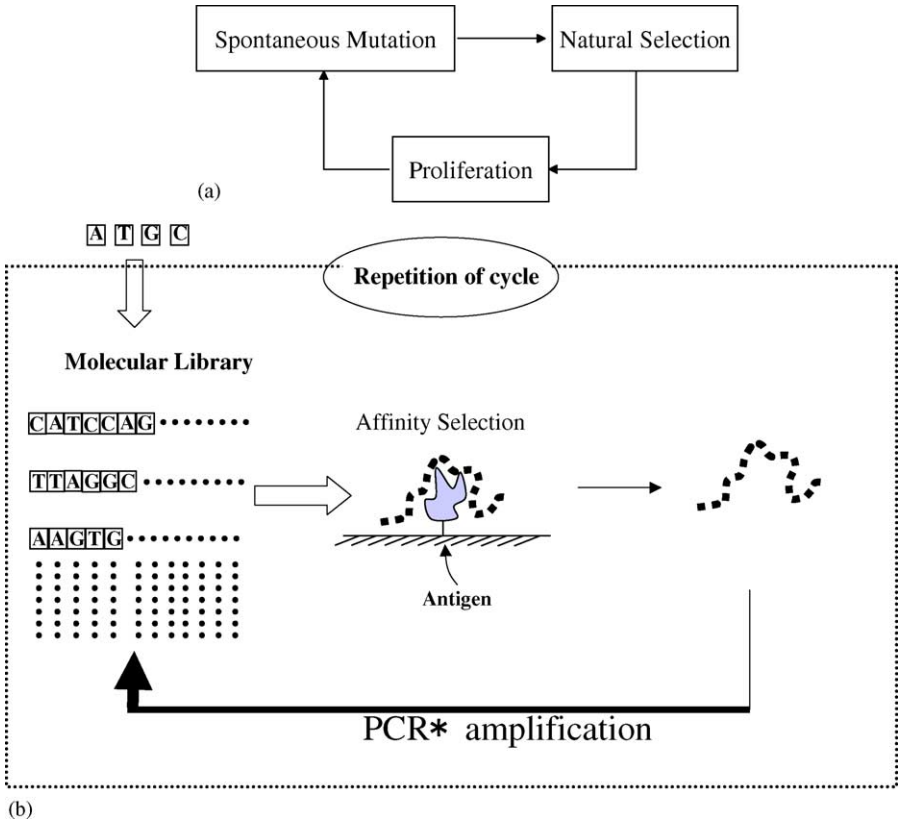

Fig. 5. Principles of (a) Darwinian evolution and (b) molecular evolutionary engineering.

primer for yielding a single-stranded DNA pool. (In the case of RNA, reverse transcription would be performed.) Each single-stranded oligonucleotide would fold to form an independent three-dimensional structure according to the intra-molecular hydrogen bonds and hydrophobic interactions in each molecule. The specific oligonucleotide that could recognize the target molecule would then be selected, through the process of 'natural selection'.

More specifically, the particular oligonucleotide that binds the target molecule would be concentrated by a washing process to remove unbound oligonucleotide. The bound oligonucleotide could then be specifically removed by competitive elution with target molecules. Perturbations, such as pH modulation, a change of salt concentration, and so on, could also be employed as a method for elution. The next step, 'proliferation', would be performed by PCR enrichment. Repetition of the above-mentioned three steps, 'spontaneous mutation', 'natural selection' and 'proliferation', would yield an oligonucleotide with higher affinity to the target molecule. Finally, the selected oligonucleotide would be subjected to sequencing analysis.

The oligonucleotide obtained by SELEX is called an aptamer, after the Latin *aptus*, 'to fit'. Aptamers that bind various types of target molecules have been reported [52]. Several trials of aptamers as substitutes for antibodies have been performed because of the following advantages: (1) they can be obtained without experimental animals; (2) they can be automatically synthesized according to the sequence required; and (3) site-specific chemical modifications of aptamers are quite easy. In the case of aptamer acquisitions, several repetitions of the SELEX process described above were employed. Eight to fifteen cycles would usually be required for aptamer acquisition, and one cycle needs 2 days. Recently, automatic machines for aptamer acquisition have been developed.

Several scientific groups have been developing a molecular sensor, or antibody drug, using aptamers [53,54]. An example of a medical drug is the recent development, by Rusconi et al., of an aptamer that acts as an antithrombogenic agent by targeting the blood coagulation factor IXa [55]. A characteristic of aptamer drugs is their ease of inhibition, by the addition of cDNA, when the drug is no longer necessary. Aptamers usually work as single-stranded DNA, therefore, double-stranded formation with cDNA reduces activity, thus working as an antidote (Fig. 6).

The biotech company, SomaLogic Co. (Colorado) founded by Gold, has been developing a photoaptamer micro-array chip for proteomics research using PhotoSELEX technology [56]. These 'DNA-based chips' for proteomics have some advantages compared with the usual antibody-based chips. In fact, the signal-to-noise ratio of the DNA chips would be higher than antibody chips because DNA aptamers would not be stained in the protein-staining step.

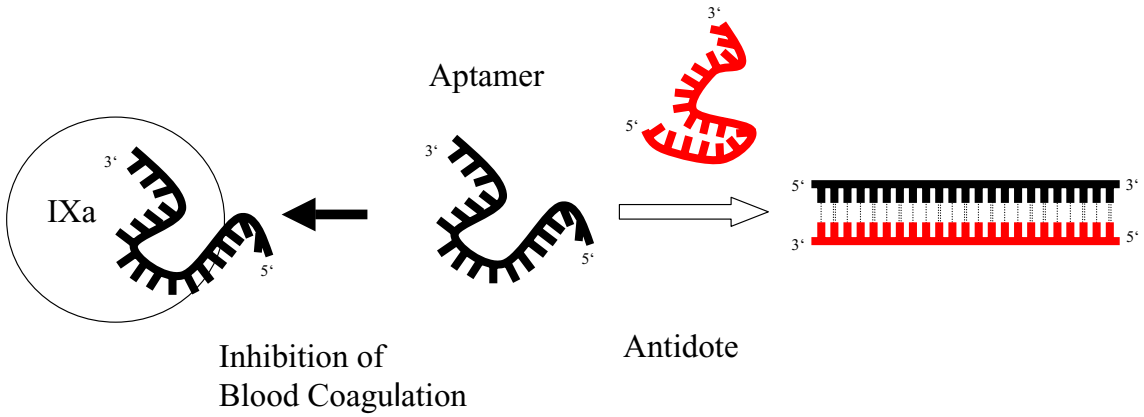

Fig. 6. An example of an aptamer drug. DNA works as an aptamer and is deactivated when the single-stranded structure is formed by intra-molecular interactions and it forms a double-helical complex with the complementary DNA strand, respectively.

6. DNA as a catalyst

Considering the example of 'catalytic antibody', it is reasonable to take into account the idea of catalysis using oligonucleotides that contain molecular recognition activity. Consequently, in addition to several naturally occurring ribozymes, some 'catalytic aptamers' have been reported. DNAzymes that catalyze 'DNA ligation' and 'RNA cleavage' have also been identified by in vitro selection technology. Recently, a DNAzyme that catalyzed 'RNA ligation' was reported. [57].

There are two methods for the preparation of catalytic DNA (Fig. 7a). One is the 'indirect method', similar to the preparation method for catalytic antibodies, in which specific DNA molecules that bind to a transition state analog as a target molecule are selected. For example, the method affords the methylporphyrin-binding of RNA or DNA. A methylporphyrin, whose planate structure was slightly deformed, was employed as a transition state analog for porphyrin metalation. The methylporphyrin-binding RNA/DNA obtained catalyzed the insertion of a copper ion into the center of the porphyrin [54]. The ribozyme and DNAzyme can demonstrate peroxidase activity by forming a complex with a hemin.

The second method for acquiring a DNAzyme is the 'direct method' (Fig. 7b). An example of the 'direct method' is as follows. Firstly, a pool of random sequences of DNA is mixed with a biotin-labeled substrate to create a reaction. DNA that can make a covalent bond with the substrate by autocatalysis is labeled with biotin. The resulting biotin-labeled substrate DNA can be recovered by avidin capturing. The captured DNA is then amplified and repetitively selected. This method can result in catalytic DNA that catalyzes an amide formation, an ester formation, or a Diels–Alder reaction. Such reactions were not known to be catalyzed by natural ribozymes.

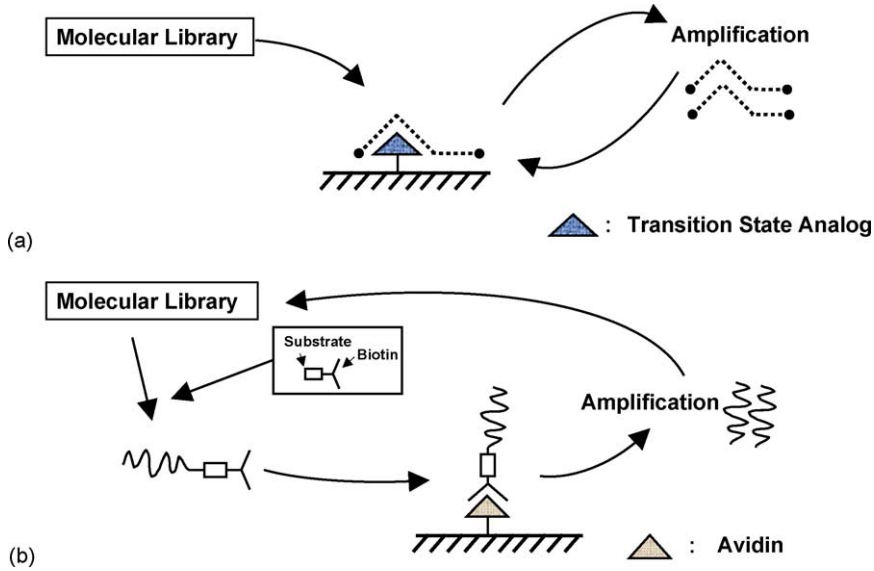

Fig. 7. Methods for searching for catalytic oligonulceotides by in vitro selection. (a) The indirect method using transition analog and (b) the direct method for exploiting catalytic DNA.

To seek out catalytic DNA that catalyzes amide formation, complexes formed from 'substrate containing carboxyl groups' and 'DNA molecules with an amino group in the library pool' were selected. The principle of this method is to seek out DNA molecules that can catalyze the reaction and bind themselves to the substrate. However, it was recently reported that catalytic DNAs selected by this method were available for catalyzing bond formation between a carboxylic acid and an amine group free from the DNA [58].

Biocatalysts have remarkable advantages such as high substrate specificities and high reactivity under mild conditions. However, biocatalysts also have serious drawbacks including a tendency to be easily deactivated by environmental changes. In vitro selection can yield biocatalysts that are active even in extreme environments. For example, the ribozyme catalyzing ligation that occurred only in neutral pH conditions evolved into one that could react in acidic conditions by the direct method of in vitro selection [59]. The procedure included the following steps. First, spontaneous mutations were imposed on ribozymes, catalyzing only in neutral pH, through error-prone PCR. The enriched RNA pool was used as the 'ribozyme library'. Next, the 'ribozyme library' was subjected to reaction with biotin-labeled substrates under acidic conditions. Positive clones that could react with the substrate should be labeled with biotin. Then these positive clones were recovered by capture using an avidin matrix, and were subjected to the next selection. Six repetitions of the selection yielded positive clones with 2000 times stronger activities in acidic conditions. This proved that appropriate biocatalysts could easily be prepared by the in vitro selection method under the desired conditions.

7. DNA as a nanodevice

Many types of nanodevice have been designed using the functions of DNA. Fig. 8 indicates an example of aptamer beacons that have been developed. The system uses the detection method fluorescence resonance energy transfer (FRET), which is based on the principle that fluorescence intensity should be changed based on the distance between a reporter fluorescence molecule and a quencher molecule. Recently, a nanodevice based on the visible light analytical system has been designed [60]. The combined system of both PCR and aptamer technologies has also been reported [61].

Moreover, integrated nanodevice systems including 'molecular recognition' and 'catalytic function' have been frequently reported [62–65]. Ellington's group developed an allosteric ribozyme with a switching function, which

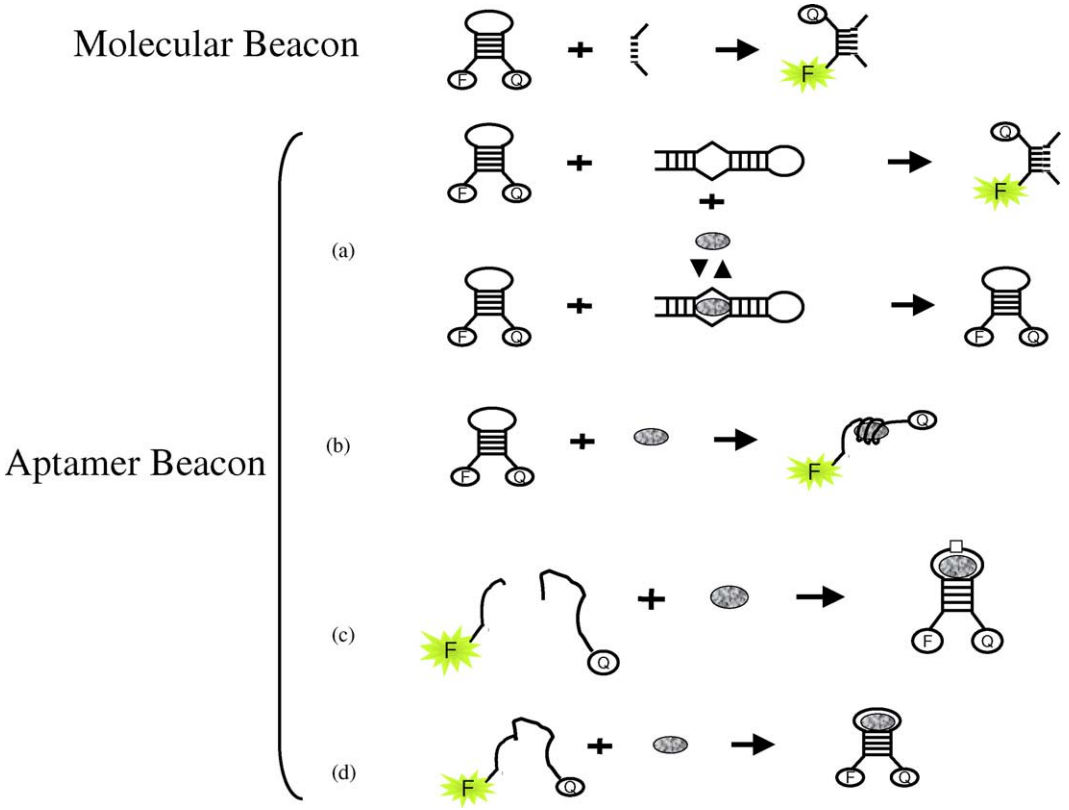

Fig. 8. A molecular beacon, which emits fluorescence when forming a complex with complementary DNA and aptamer beacons. (a) An aptamer beacon is quenched and emits fluorescence in the absence and in the presence of antigen, respectively, (b) an aptamer emits fluorescence in the presence of antigen by changing the secondary structure, (c) an aptamer recognizes antigen by self-assembly of two strands, (d) an aptamer beacon whose fluorescence is quenched in the presence of antigen.

they called an ‘aptazyme’ [62]. In the first stage of development, the molecular-recognizing motif was introduced to the ribozymes to design an allosteric ribozyme. However, the first allosteric ribozyme showed only a 10 to 100-fold modulating effect. After several studies, a new selection protocol was developed to afford a switching effect of more than 10,000-fold. On the other hand, Breaker’s group developed an ‘allosteric selection’ method to yield a ribozyme that varied its activity in response to the concentration of cGMP or cAMP [63]. They also reported a biosensor prototype, in which the hammer-head ribozyme, which would be activated only by some kinds of target molecules, was integrated and arrayed for the multiple sensing of target molecules [65].

A lead ion-monitoring sensor was reported by selection of the metal ion-dependent DNAzyme [66]. The principle of this sensor is that the DNAzyme can be modulated to cleave the bond between DNA and the fluorescence-labeled RNA substrate by the presence of lead ion. When lead ion was added to the DNAzyme solution, the observed fluorescence intensified. Recently, Mei et al. reported a DNAzyme with synchronized catalytic activity and fluorescence signaling [67].

In addition to these combinations of DNA functions, chemical modification of DNA was also performed. Because the number of naturally occurring nucleotides is restricted to four, newly synthesized unnatural nucleotides were used for in vitro selection of oligonucleotides. The trial mentioned above is expected to be an interdisciplinary way of producing functional polymers. To date, several nucleotide derivatives have been synthesized and subjected to in vitro selection (Fig. 9).

Ito et al. succeeded in creating an ATP-recognizing aptamer using biotin-labeled nucleotides [68]. The biotin moiety is usually introduced only at the terminal end of oligonucleotides, therefore its sensitivity is not very high. To accomplish a higher sensitivity, they used oligonucleotides that have multiple biotin-labeled side chains [68]. A similar attempt was undertaken using nucleotides with fluorescence groups attached, whereby fluorescence intensity was modulated by recognition of a target molecule [69]. The first attempt at producing catalytic oligonucleotides by introduction of unnatural nucleotides was the ribozyme that catalyzed the Diels–Alder reaction [70]. It was prepared by in vitro selection using a nucleotide to which the imidazole group was introduced. The selection of a ribozyme that catalyzed a zinc ion-dependent RNA cleavage reaction was also performed using imidazole-modified deoxyuridine instead of deoxyuridine [71]. Perrin et al. introduced an amino group into the unnatural nucleotide moiety in the DNA library for the screening of DNAzyme in addition to an imidazole group [72]. The DNAzyme obtained indicated a remarkable RNAase activity even without divalent ions.

Kawazoe et al. performed in vitro selection using an unnatural nucleotide to which an amino group instead of a hydroxyl group was introduced at the 2′ position. Using the library that included the unnatural oligonucleic acids, a ribozyme that catalyzed porphyrin metalation was obtained [73]. The aptamer also demonstrated peroxidase activity, i.e. reduction of hydrogen peroxide, by forming a complex with a hemin molecule [74]. Recently, Vester et al. applied their new technology, locked nucleic acid (LNA), to design a remarkably stable catalytic nucleic acid, which

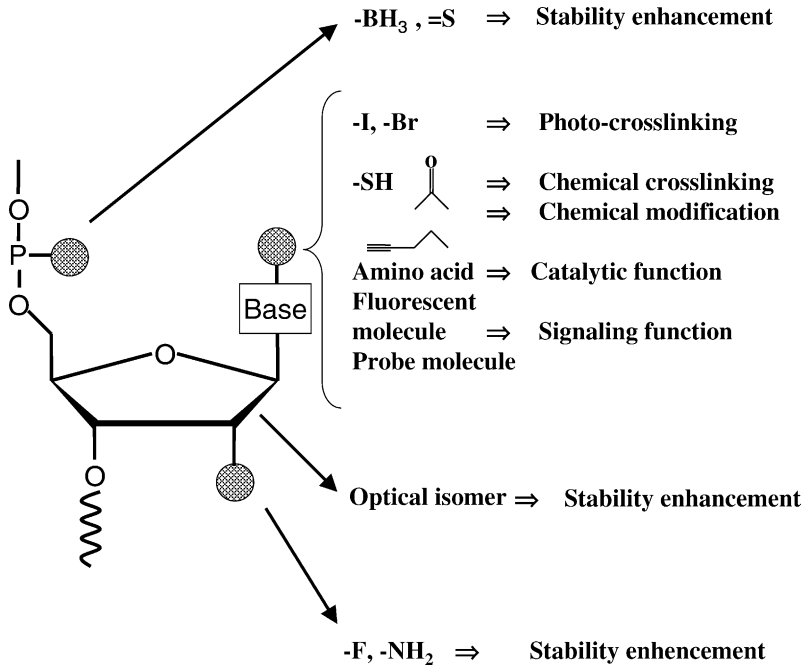

Fig. 9. Non-natural nucleotides, which were incorporated into DNA for construction of functional oligonucleotides.

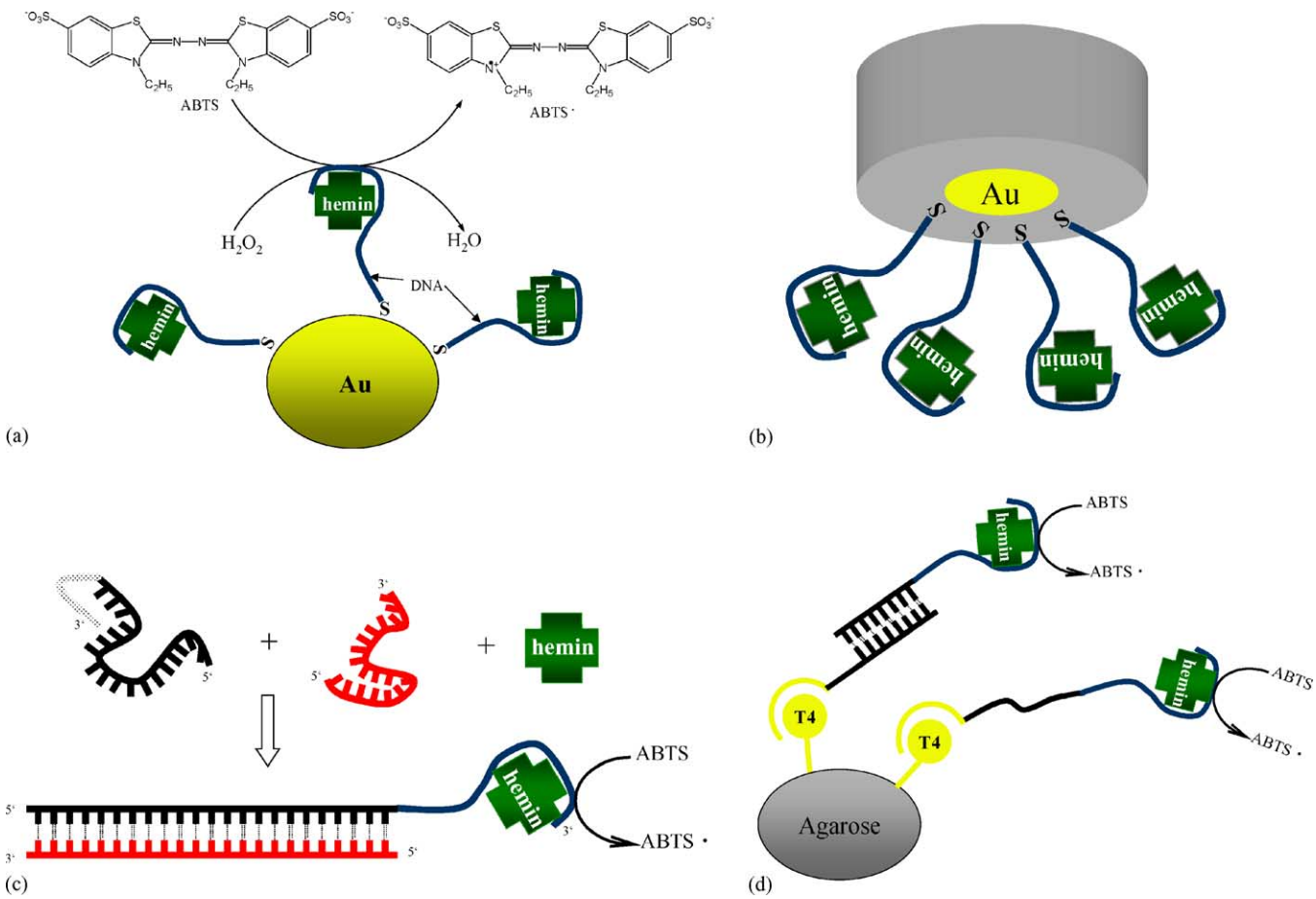

Fig. 10. Examples of molecular devices using DNAzymes. (a) Immobilized DNAzyme, (b) DNAzyme-immobilized electrode, (c) sensor containing DNAzyme, its activity is changed in the presence of the complementary DNA sequence, (d) enzyme immunoassay device containing both regions for recognition of thyroxine (T4) and DNAzyme.

they named 'LNAzyme' [75]. In the future, it is expected that various libraries that include unnatural nucleotides will be employed to yield a novel type of functional nucleic acid.

In a current trial, Ito and Hasuda are preparing some DNA devices as shown in Fig. 10. They evaluated a DNAzyme that immobilized onto a gold particle as an immobilized enzyme [76]. Ito [54] is also preparing a gold electrode onto which a 'DNAzyme' is self-assembled. This electrode could be applied as a biosensor. Troublesome operations that are required for the immobilization of enzymes, such as thiolation or functional group-activation, are not needed for DNAzyme immobilization because of the ease with which DNA can be finely modified. One remaining problem is low activity of the DNAzyme.

Ito [54] is also developing a new molecular nanodevice in which several functional DNA motifs could be connected by 'single stroke drawing'. These examples include two concepts. One is a DNAzyme that should indicate its activity through the formation of a complementary strand with a specific DNA sequence. The other is a DNAzyme device that consists of a molecular recognition motif and a reporter enzymatic motif as and antibody–enzyme complex for an enzyme immuno assay (EIA). Ito [54] has already prepared a 'DNAzyme' that includes both 'aptamer motif' and 'catalytic motif' as substitutions of an antibody and a reporter enzyme, respectively. Spacer DNA separates both motifs in order to prevent the DNAzyme from forming an undesired conformation. The usefulness of the DNAzyme as an analytical tool has been proven, although its sensitivity is not so high.

8. DNA as a nanomachine

Yurke et al. developed a DNA machine that worked with DNA fuel. The machine consisted of three components [77]. One was named the A chain (40 bases) and included two terminal 18-base domains. The second was the B chain (42 bases) that contained the complementary sequence to one terminal of the A chain. The third was the C chain (42 bases) that contained the complementary sequence to the other terminal of the A chain. The three component A, B, and C chains were assembled to construct a set of tweezers

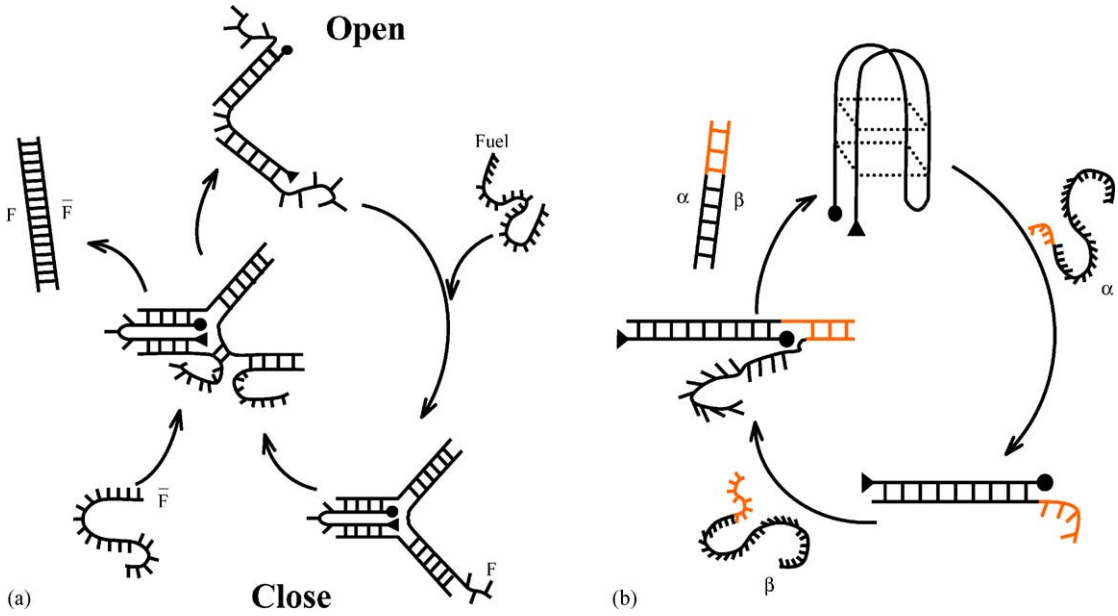

Fig. 11. Examples of DNA machines. (a) A DNA machine designed by Yurke et al. and (b) a DNA nanomotor designed by Li and Tan.

(Fig. 11a). A 24-base section of both the B and C chains projected from the edge of the tweezers. Another DNA fragment, the F chain, included complementary sequences to both B and C chains and could close the edge of the tweezers. Addition of the F' chain DNA fragment, which included a complementary sequence to the F chain, could make the tweezers re-open. Both F and F' chains therefore act as fuel. The added fuels were exhausted and thus the double-strand DNA, FF', was produced through one cycle of open-close. Analysis of intra-molecular fluorescence labeling showed that the clearance of the edge of the tweezers and the time for one cycle of 'open-close' was 6 nm and 13 s, respectively.

In other work, Li and Tan reported DNA as a nanomotor (Fig. 11b) [78]. The DNA nanomotor could be assembled into different conformations independently by hybridization with cDNA fragments. One possible conformation was an intra-molecular quadruplex strand structure and another was an inter-molecular double-stranded structure. Dynamic expansion and contraction of the DNA nanomotor, like a worm's motion, was observed. Seeman and co-workers [79] developed a DNA device from a combination of double-stranded DNA to form a quadruplex structure. Its topology was controlled by the addition of short pieces of DNA.

Recently, Feng et al. designed a DNA nanoactuator that could reversibly expand and contract a two-dimensional array of DNA strands [80]. They first devised a nanoactuator that had two states. The first state consisted of four DNA strands that formed a bulged three-armed DNA branch junction with a loop sticking into the center. Addition of an appropriate 'fuel strand' that was complementary to the loop sequence stretched the structure into a linear second state. The nanoactuator was then incorporated into a 2D DNA lattice. The cavities in the 2D lattice could then be switched reversibly, from 14 nm × 14 nm to 14 nm × 20 nm, without deterioration of the lattice.

The Gaub and co-workers used DNA as a programmable force sensor [81]. Direct quantification of biomolecular interactions by single-molecule force spectroscopy has evolved into a powerful tool for the life sciences. They introduced an approach in which the unbinding forces required to break inter-molecular bonds are measured in a differential format by comparison with a known reference bond (a short DNA duplex). In addition to a marked increase in sensitivity and force resolution, which enabled them to resolve single-base pair mismatches, the concept allowed for highly specific parallel assays.

9. DNA as a tag or molecular certificate

DNA can be applicable as a tag for discrimination or personal identification. Combinatorial chemistry is a good example for use of DNA as a molecular tag [82]. For example, a DNA tag was used in the construction of a combinatorial molecular library by a 'pool and split' method. DNA corresponding to treatment histories was connected onto library beads. A selected bead could be identified from the information contained in the DNA bound on the bead, via a practical assay, and the information of the DNA was easily enriched by PCR technology. Recently, a security company is offering an identification service for contracts or wills using DNA technology. A special ink, which includes a small amount of DNA derived from a specific client, could be used as a sign-up for this service. An ID system could thus be easily applied by verification of 'DNA ink'.

10. Conclusion

DNA has a remarkable character to which useful functions could easily be integrated. Multiple functions of DNA could be used simultaneously, and could be activated independently by molecular switching. It is obvious that the DNA molecule is one of the most promising functional nanomaterials. However, the application of DNA molecules is still under study because of a big gap between theory and practice. We are eagerly anticipating a 'coming out' of DNA due to breakthroughs in nanobiotechnology.

References

[1] N.C. Seeman, Nature 421 (2003) 427.
[2] C.J. Murphy, M.R. Arkin, J. Jenkins, N.D. Ghatlia, S.H. Bossmann, N.J. Turro, J.K. Barton, Science 262 (1993) 1025.
[3] G. Taubes, Science 275 (1997) 1420.
[4] M. Ratner, Nature 397 (1999) 480.
[5] N.W. Grinstaff, Angew. Chem. Int. Edn. Engl. 38 (1999) 3629.
[6] A. Okamoto, K. Tanaka, I. Saito, J. Am. Chem. Soc. 125 (2003) 5066.
[7] E. Braun, Y. Eichen, U. Sivan, G. Ben-Yoseph, Nature 391 (1998) 775.
[8] I. Willner, Science 298 (2002) 2407.
[9] K. Keren, M. Krueger, R. Gilad, G. Ben-Yoseph, U. Siven, E. Braun, Science 297 (2002) 72.
[10] T. Kawasaki, Kobunshi/High Polym., Japan 52 (2003) 143.
[11] E.M. Boon, D.M. Ceres, T.G. Drummond, M.G. Hill, J.K. Barton, Nat. Biotechnol. 18 (2000) 1096.
[12] T.G. Drummond, M.G. Hill, J.K. Barton, Nat. Biotechnol. 21 (2003) 1192.
[13] K. Yamashita, A. Takagi, M. Takagi, H. Kondo, Y. Ikeda, S. Takenaka, Bioconj. Chem. 13 (2002) 1193.
[14] http://www.nanogen.com/.
[15] R.P. Fahlman, D. Sen, J. Am. Chem. Soc. 124 (2002) 4610.
[16] M.A. Batalia, E. Protozanova, R.B. Macgregor Jr., D.A. Erie, Nano Lett. 2 (2002) 269.
[17] C.M. Soto, M. Srinivasan, B.R. Ratna, J. Am. Chem. Soc. 124 (2002) 8508.
[18] S.S. Smith, Nano Lett. 1 (2000) 51.
[19] C.S. Yun, G.A. Khitrov, D.E. Vergona, N.O. Reich, G.F. Strouse, J. Am. Chem. Soc. 124 (2002) 7644.
[20] H. Yan, S.H. Park, G. Finkelsein, J.H. Reif, T.H. Labean, Science 301 (2003) 1882.
[21] Y. Maeda, H. Tibet, T. Kawai, Appl. Phys. Lett. 79 (2001) 1181.
[22] M.G. Warner, J.E. Hutchinson, Nat. Mater. 2 (2003) 272.
[23] C.F. Monson, A.T. Woolley, Nano Lett. 3 (2003) 359.
[24] M. Mertig, L.C. Ciacchi, R. Seidel, W. Pompe, A. De Vita, Nano Lett. 2 (2002) 841.
[25] H. Gao, Y. Kong, D. Cui, C.S. Ozkan, Nano Lett. 3 (2003) 471.
[26] C.V. Nguyen, L. Delzeit, A.M. Cassell, J. Li, J. Han, M. Meyyappan, Nano Lett. 2 (2003) 1079.
[27] S.E. Baker, W. Cai, T.L. Lasseter, K.P. Weidkamp, R.J. Hamers, Nano Lett. 2 (2002) 1413.
[28] C.A. Mirkin, MRS Bull. 43 (2000).
[29] O. Harnack, W.E. Ford, A. Yasuda, J.M. Wessels, Nano Lett. 2 (2002) 919.
[30] J.-M. Nam, C.S. Thaxton, C.A. Mirkin, Science 301 (2003) 1884.
[31] M. Han, X. Gao, J. Su, S. Nie, Nat. Biotechnol. 19 (2001) 631.
[32] B. Reiss, L. He, I. Walton, R. Cromer, C. Keating, M. Natan, Science 294 (2001) 137.
[33] J. Wang, D. Xu, R. Polsky, J. Am. Chem. Soc. 124 (2002) 4208.
[34] J. Wang, R. Polsky, A. Merkoci, K.L. Turner, Langmuir 19 (2003) 989.
[35] J. Wang, G. Liu, A. Merkoci, J. Am. Chem. Soc. 125 (2003) 3214.
[36] A. Saghatelian, K.M. Guckian, D.A. Thayer, M.R. Ghadin, J. Am. Chem. Soc. 125 (2003) 344.
[37] Y.C. Cao, R. Jin, C.A. Mirkin, Science 297 (2002) 1536.
[38] R. Jin, G. Wu, Z. Li, C.A. Mirkin, G.D. Schatz, J. Am. Chem. Soc. 125 (2003) 1643.
[39] J. Hu, Y. Zhang, H. Gao, M. Li, U. Hartmann, Nano Lett. 2 (2002) 55.
[40] M. Liu, N.A. Amro, C.S. Chow, G. Liu, J. Am. Chem. Soc. 2 (2002) 863.
[41] K. Tanaka, A. Tengeiji, T. Kato, N. Toyama, M. Shionoya, Science 299 (2003) 1212.
[42] N. Zimmermann, E. Meggers, P.G. Schultz, J. Am. Chem. Soc. 124 (2002) 13684.
[43] L.M. Adleman, Science 226 (1994) 1021.
[44] http://hagi.is.s.u-tokyo.ac.jp/MCP/.
[45] Y. Sakakibara, A. Suyama, Genome Inform Ser Workshop Genome Inform. 11 (2000) 33.
[46] Y. Benenson, R. Adar, T. Paz-Elizur, Z. Livneh, E. Shapiro, Proc. Natl. Acad. Sci. U.S.A. 1001 (2003) 2191.
[47] M.N. Stojanovic, T.E. Mitchell, D. Stefonovic, J. Am. Chem. Soc. 124 (2002) 3555.
[48] M.N. Stojanovic, D. Stefanovic, Nat. Biotechnol. 21 (2003) 1069.
[49] A.D. Ellington, J.W. Szostak, Nature 346 (1990) 818.
[50] C. Tuerk, L. Gold, Science 249 (1990) 505.
[51] L. Gold, J. Biol. Chem. 270 (1995) 13581.
[52] http://aptamer.icmb.utexas.edu/index.html.
[53] Y. Ito, N. Kawazoe, Y. Imanishi, Methods 22 (2000) 107.
[54] Y. Ito, Polym. Adv. Technol. 15 (2004) 3.
[55] C.P. Rusconi, E. Scardino, J. Layzer, G.A. Pitoc, T.L. Ortel, D. Monroe, B.A. Sullenger, Nature 419 (2002) 90.
[56] http://www.somalogic.com/.
[57] A. Flynn-Charlebois, Y. Wang, T.K. Prior, I. Rashid, K.A. Hoadley, R.L. Coppins, A.C. Wolf, S.K. Silverman, J. Am. Chem. Soc. 125 (2003) 2444.
[58] B. Seeling, S. Keiper, F. Stuhlmann, A. Jaeschke, Angew. Chem. Int. Edn. Engl. 39 (2000) 4576.
[59] Y. Miyamoto, N. Teramoto, Y. Imanishi, Y. Ito, Biotechnol. Bioeng. 75 (2001) 590.
[60] M.N. Stojanovic, D.W. Landry, J. Am. Chem. Soc. 124 (2002) 9678.
[61] S. Fredriksson, M. Gullberg, J. Jarvius, C. Olsson, K. Pietras, S.M. Gustafsdottir, A. Ostman, U. Landegren, Nat. Biotechnol. 20 (2002) 473.
[62] M.P. Robertson, A.D. Ellington, Nat. Biotechnol. 17 (1999) 62.
[63] M. Koizumi, G.A. Soukup, J.N.Q. Kerr, R.R. Breaker, Nat. Struct. Biol. 6 (1999) 1062.
[64] N. Piganeau, A. Jenne, V. Thuillier, M. Famulok, Angew. Chem. Int. Ed. 39 (2000) 4369.
[65] Seetharaman, M. Zivarts, N. Sudarsan, R.R. Breaker, Nat. Biotechnol. 19 (2001) 336.
[66] J. Li, Y. Lu, J. Am. Chem. Soc. 122 (2000) 10466.
[67] S.H.J. Mei, Z. Liu, J.D. Brennan, Y. Li, J. Am. Chem. Soc. 125 (2003) 412.
[68] Y. Ito, A. Suzuki, N. Kawazoe, Y. Imanishi, Bioconj. Chem. 12 (2001) 850.
[69] S. Jhaveri, M. Rajendran, A.D. Ellington, Nat. Biotechnol. 18 (2000) 1293.
[70] T.W. Tarasow, S.L. Tarasow, B.E. Eaton, Nature 389 (1997) 54.
[71] S.W. Santoro, G.F. Joyce, K. Sakthivel, S. Gramatikova, C.F. Barbas, J. Am. Chem. Soc. 122 (2000) 2433.
[72] D.M. Perrin, T. Garestier, C. Helene, J. Am Chem. Soc. 123 (2001) 1556.
[73] N. Kawazoe, N. Teramoto, H. Ichinari, Y. Imanishi, Y. Ito, Biomacromolecules 2 (2001) 681.

[74] N. Teramoto, H. Ichinari, N. Kawazoe, Y. Imanishi, Y. Ito, Biotechnol. Bioeng. 75 (2001) 463.
[75] B. Vester, L.B. Lundberg, M.D. Sorensen, B.R. Babu, S. Douthwaite, J. Wengel, J. Am. Chem. Soc. 124 (2002) 13682.
[76] Y. Ito, H. Hasuda, Biotechnol. Bioeng. 86 (2004) 72.
[77] B. Yurke, A.J. Turberfield, A.P. Mills Jr., F.C. Simmel, J.L. Neumann, Nature 406 (2000) 605.
[78] J.J. Li, W. Tan, Nano Lett. 2 (2002) 315.
[79] H. Yan, X. Zhang, Z. Shen, N.C. Seeman, Nature 415 (2002) 62.
[80] L. Feng, S.H. Park, J.H. Reif, H. Yan, Angew. Chem. Int. Engl. 42 (2003) 4342.
[81] C. Albrecht, K. Blank, M. Lalic-Muelthaler, S. Hirler, T. Mai, I. Gilbert, S. Schiffmann, T. Bayer, H. Clausen-Schaumann, H.E. Gaub, Science 301 (2003) 367.
[82] S. Brenner, R.A. Lerner, Proc. Natl. Acad. Sci. U.S.A. 89 (1992) 5381.