DNA-based machines†

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Nucleic acids include substantial information in their base sequence and their hybridization–complexation motifs. Recent research efforts attempt to utilize this biomolecular information to develop DNA nanostructures exhibiting machine-like functions. DNA nano-assemblies revealing tweezers, motor, and walker activities exemplify a few such machines. The DNA-based machines provide new components that act as sensitive sensors, transporters, or drug delivery systems.

Introduction

DNA is probably the most exciting molecule that nature optimized over billions of years of evolution. Besides its central function in carrying the genetic information, and thus, acting as the key element of proliferation, DNA is possibly the most promising biomolecule for future applications in material science and nanotechnology. The unique functions of DNA originate from its composition, structure, and physical and chemical properties. The specificity of the A–T and G–C H-bonded Watson–Crick interactions provides a means to construct diverse DNA compositions of programmed sequences and structures. Furthermore, the directionality of the double-strand formation introduces an instructive pattern for controlled duplex formation. Other self-assembled structural motifs of DNA are also available, such as the G-quadruplex[1] or the i-motif.[2] Alternatively, DNA duplex structures with artificial ligands as base-pairing units are available.[3,4] The diversity of structural patterns of DNA and modified DNAs, together with automated techniques to synthesize substantial quantities of oligonucleotides pave the way to use DNA and its derivatives as a powerful building block for the assembly of 2D- and 3D-nanostructures of dictated shapes and geometries. Fig. 1 schematically summarizes several DNA self-assembly motifs demonstrating the possible variability and complexity of DNA composites. Indeed, ingenious DNA architectures were reported, and examples include lattices composed of DNA tiles[5] or rods consisting of DNA triangles.[6]

The structural features encoded in the duplex also provide instructive information related to the chemical properties and the reactivity of DNA. Intercalation of molecular components in-between specific bases or their association with minor or

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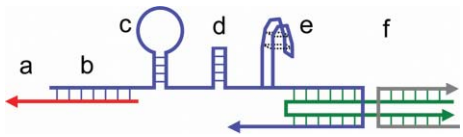

Fig. 1 Schematic presentation of structural motifs of DNA. a: sticky ends, b: duplex, c: hairpin, d: stem structure, e: G-quadruplex, f: crossover region.

major groove regions allows the localization of molecular units at pre-defined locations. The chemical, optical, or electrochemical activation of these molecular units can then be used to trigger perturbations in the DNA structure. Furthermore, various enzymes may be applied as biocatalytic "nano-tools" to manipulate DNA. For example, the ligation of DNA strands, the replication of a duplex, or the elongation of a single strand can be achieved using ligases, polymerases, and telomerase, respectively. Sequence-specific domains within double-stranded DNA provide instructive information for the selective binding of endonucleases or nicking enzymes that catalyze the cleavage of sequence-specific domains and facilitate the separation of duplex structures. These biocatalytic transformations not only yield new DNA structures, but also generate new versatile components containing sticky ends that act as secondary assembly units.

Not surprisingly, DNA structures have been employed as templates for the "bottom-up" construction of nanostructures and nanocircuitry. Biotin-labeled DNA was used as a template for the assembly of Au nanoparticles conjugated with avidin[7] or streptavidin,[8] intercalator-labeled Au nanoparticles were associated with DNA by intercalation,[9] nucleic acids were metallized to yield different types of metal nanowires,[10,11] and protein–DNA assemblies were used to fabricate nanodevices such as transistors.[12]

Beyond the use of DNA as a passive element that evolves functional nanostructures, exciting opportunities exist in the utilization of DNAs as dynamic biomolecules that duplicate machine functions. The configuration of DNA might be controlled *via* hybridization, and duplex DNAs could change their configuration upon scission by endonucleases, thus leading to the dynamic control of DNA structures. Indeed, impressive progress was accomplished by applying tailored DNA as functional nanostructures that mimic machinery functionalities. Systems duplicating activities of tweezers,[13] walkers,[14] gears,[15] and more[16,17] were developed. The present article aims to describe some of the recent advances in the area of DNA machines. We wish to emphasize that this topic is not only an intellectual scientific curiosity, but to demonstrate that the subject of DNA-based machines has bright future perspectives in the development of sensors, molecular transporters, and controlled drug delivery systems for the targeted and slow release of therapeutics.

DNA machines

The inherent dynamic properties of double-stranded DNA, and the thermally induced sequence-dependent (number of base-pairs and composition) separation of double-stranded nucleic acids allow the dynamic, controlled dehybridization and re-hybridization of pre-tailored nucleic acid structures. These dynamic functions of DNA may be employed to design DNA biomolecular devices that duplicate "machine-like" functions that perform linear or rotary motion, act as walkers, function as motors, rotors, or switches. These DNA machines may have important future implications in the development of nanorobotics, for the controlled delivery and transport, such as drug delivery, and in the fabrication of DNA sensors. We realize that the relation of "machine properties and functions" to molecular structures of DNA might introduce some controversy, and that the term "machine" might be differently defined. We view the "DNA machine" systems as biomolecular assemblies that perform machine-like functions, where several consecutive functions at the molecular level are triggered by external chemical inputs ("fuels"), and lead to dynamic two-dimensional and three-dimensional structural changes, and to the formation of chemical by-products ("waste products").

The G-quadruplex–duplex DNA transformations represent machine-like structural conversions driven by nucleic acid fuels. This has been demonstrated (Fig. 2) by the use of G-quadruplex DNA (**1**) that includes at its two ends a donor–acceptor pair, where the mutual FRET process signals the existence of the G-quadruplex structure.[18] Its hybridization with (**2**) opens the G-quadruplex while generating the respective duplex structure. The spatial separation of the donor–acceptor pair inhibits the FRET process. The addition of a nucleic acid (**3**) that exhibits enhanced affinity for (**2**) opens the duplex (**1**)–(**2**) while regenerating the G-quadruplex, accompanied by the reappearance of the donor–acceptor FRET signal, and the stable (**2**)–(**3**) duplex remains as a waste product. Related G-quadruplex–double strand DNA transitions were similarly used to perform reversible dynamic transformations of DNA structures.[18b]

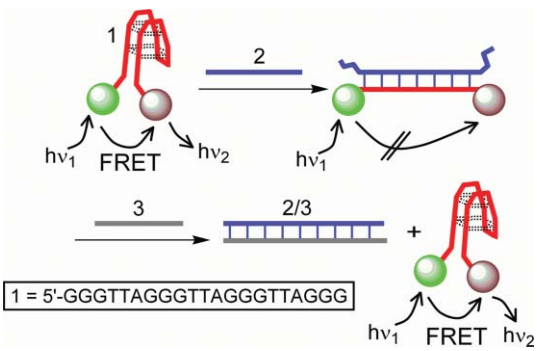

Fig. 2 G-Quadruplex–duplex transitions as a result of hybridization processes and their imaging by fluorescence resonance energy transfer (FRET).

Such conformational changes of single-stranded oligonucleotides can also be triggered by changes in the buffer composition of the reaction, instead of the addition of fuel DNA. Liedl and Simmel[19] describe a DNA molecule being reversibly switched between a random coil (induced at a pH below 6) and the i-motif (pH above 6), a quadruplex structure composed of cytosine residues, similar to the G-quadruplex. The changes in buffer pH are caused by a chemical oscillator, and take place periodically for several hours. The changes in DNA secondary structures are again monitored using fluorophore- and quencher-labeled strand ends.

The pH-controlled change of the DNA structure has been used to cycle single-stranded DNA immobilized on a microcantilever between the i-motif and duplex with a complementary oligonucleotide containing 3 mismatches.[20] The use of cantilevers as

surface supports allowed for the quantification of the forces resulting from the structural changes of the immobilized biomolecules.

A DNA machine duplicating the functions of tweezers was demonstrated[13] by the use of three strands of DNA that included tailored complementarity, Fig. 3. The ends of strand **A** were labeled with the fluorophore *tetrachlorofluorescein phosphoramidite* (indicated by the red ball) and a quencher (*carboxytetramethylrhodamine*, grey), and the segments of strand **A** were hybridized to each end of strands **B** and **C** to form two stiff double-stranded arms of the tweezers (structure **4**), to each of which a single-stranded overhang is tethered. In this open conformation, the fluorophore and quencher are spatially separated, and the fluorophore is not quenched. Addition of the DNA strand **F**, that is complementary to the single-stranded tethers of **B** and **C**, results in hybridization and leads to closure of the "open" tweezers to a compact configuration (state **5**), where quenching of the fluorophore proceeds. This effect is reversed by the subsequent addition of strand **F′**, that is fully complementary to **F**. The energetically favoured **F–F′** is removed as double-stranded waste, and the tweezers is regenerated to its starting configuration. The tweezers operated for more than eight cycles between the opened and closed states of the machine, which was monitored by the deactivation and reactivation of the fluorescence signal, respectively, Fig. 3(B).

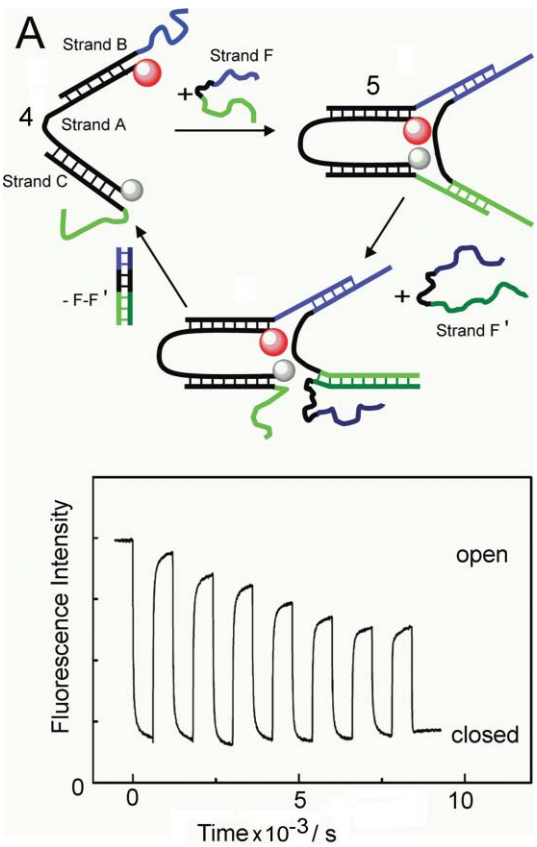

Fig. 3 (A) A DNA-based tweezers driven by the hybridization of nucleic acids. (B) Cyclic opening and closing of the tweezers configuration shown in (A), followed by fluorescence spectroscopy. (Reprinted by permission from Macmillan Publishers Ltd.: [Nature] ref. 13, copyright 2000).

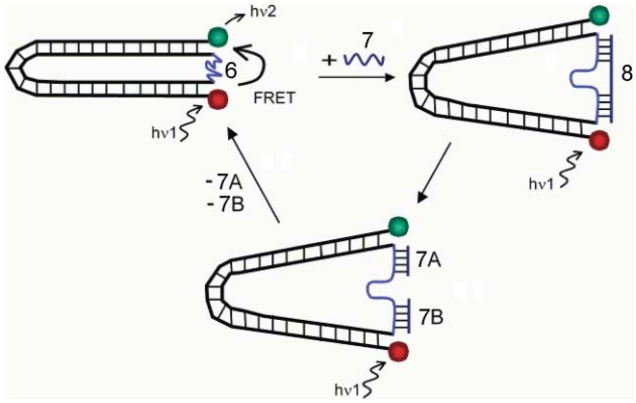

Fig. 4 A DNA-based tweezers driven by a DNAzyme biocatalyst cleaving an RNA substrate. The transformations of the tweezers between the "open" and the "closed" states are followed by fluorescence resonance energy transfer (FRET).

A biocatalytic approach to the cycling of a DNA tweezers using a DNAzyme was also described,[21] Fig. 4. In this system, the tweezers arms consist of two double-stranded DNA domains that are joined at their open sites by a single-stranded DNA sequence capable of cleaving RNA (**6**). The open sites are labeled with a donor–acceptor pair, between which FRET can occur. In the absence of a DNAzyme substrate, the single-stranded linker sequence forces the assembly into a compact structure, placing the donor–acceptor pair in close proximity, and thus leading to an efficient FRET process. Upon addition of an RNA (or an RNA–DNA chimera) target strand (**7**), the DNAzyme–substrate duplex (**8**) is formed, and the new double helix forces the fluorophores apart, causing a decrease of FRET. Subsequently, the DNAzyme hydrolyzes the substrate strand. Its fragments (**7A**, **7B**) are released due to a lower stability of the double-strand configuration of a decreased number of base pairs, and the machine returns to the closed conformation. Repetitive additions of substrate strands and their cleavage by the DNAzyme cycle the machine between the open and closed states, respectively.

A related machine for the controlled binding and release of proteins[22] was demonstrated by the application of the thrombin aptamer that binds, in a single-stranded G-quadruplex structure, to the protein thrombin. Treatment of the aptamer–thrombin complex with a nucleic acid that is complementary to the aptamer sequence separated the thrombin–aptamer complex by forming the energetically favoured duplex of the aptamer–complementary nucleic complex. Further addition of a nucleic acid that exhibits enhanced affinity for the complementary nucleic acid as a result of increased base-pairing, regenerated the free aptamer for thrombin binding and yielded a double-stranded waste product.

A DNA machine of enhanced complexity that relies on the powers of DNA transcription into RNA was recently accomplished. A DNA tweezers was switched from the open to the closed conformation by an mRNA fuel oligonucleotide, which was biocatalytically generated from a template DNA strand encoding the mRNA by an RNA polymerase.[23] The successful change in tweezers structure was detected using fluorescence and gel electrophoresis. This approach was further refined by placing the gene encoding the RNA fuel strand under the control of either a negative (LexA) or a positive regulator (LacI).[24] Both concepts rely

on bacterial expression control systems, in which the presence of an outside effector molecule either stops (LexA) or starts (LacI) transcription of a gene. The template DNA was designed to contain the respective binding sites for the regulator molecule upstream of the fuel gene. Fluorescence experiments verified that in the presence of the positive regulator, and the absence of its negative counterpart, a significantly higher percentage of tweezers molecules adopted the closed conformation. Although both systems cannot cycle the tweezers through both possible conformations, and the re-opening of a closed tweezers is reliant upon the manual addition of a complementary opening oligonucleotide, the idea of using genetic regulatory mechanisms to trigger DNA machines is intriguing.

Most of the machines found in the macro-world consist of "gears" and "wheels" revolving against one another. The macroscopic features of these machines were duplicated at the molecular level,[15] using two circular DNA units, **A** and **B**, as the moving elements, Fig. 5. Three complementary nucleic acids, that included single-stranded tethers **P1A**, **P2A**, and **P3A**, as sticky ends, were hybridized to unit **A**. Similarly, strands **P1B**, **P2B**, and **P3B**, each containing sticky ends, were hybridized with unit **B**. Hybridization of the sticky ends of **P1A** and **P1B** with **L1** interconnected the "wheel" units. Further hybridization of the sticky ends of **P2A** and **P2B** with **L2** generated the double-bridged "wheel" system. The subsequent hybridization of **L1** with the fuel nucleic acid **R1** disconnected the primary bridge while rotating component B around unit **A**. Thus, by stepwise hybridization–dehybridization of the sticky ends the molecular wheels rotated one against the other.

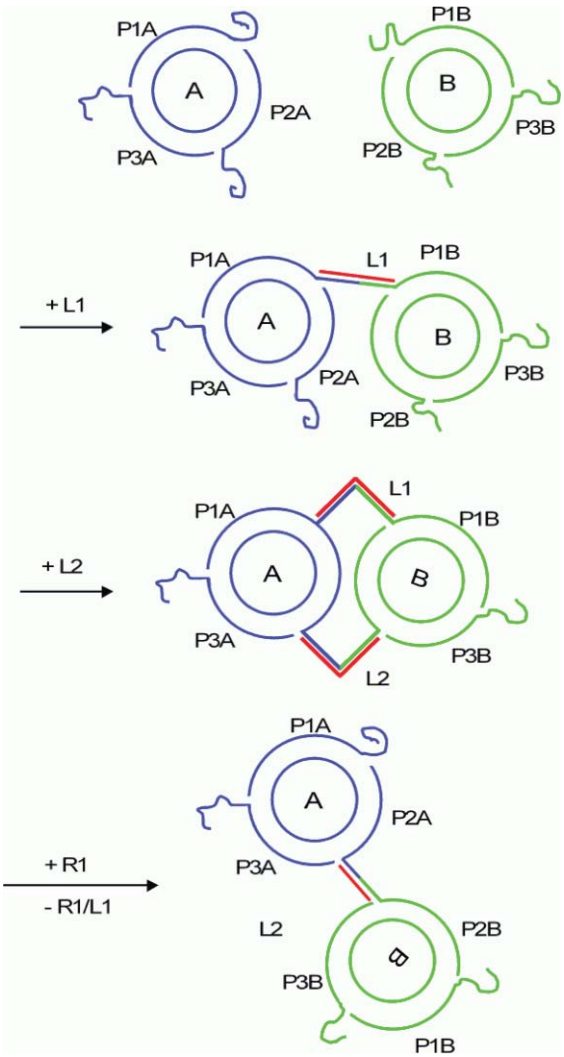

Fig. 5 A DNA-based gear driven by hybridization–dehybridization steps.

The controlled linear motion of molecular motors in a defined direction is one of the most fascinating features of nanobiotechnology. Myosin moves along cytoskeletal networks to confer order on the cell's backbone and contracts the muscular apparatus,[25] while kinesin secures the placement of organelles and proteins.[26] Polymerases move along their DNA templates in order to faithfully copy the information contained therein. The artificial design of bio-nanosystems capable of mimicking this behavior and motion of biomolecules on a supporting structure in a defined direction is a promising, but daunting challenge. Several studies have addressed the design of DNA-based "walkers" as directional motors.

The DNA motor "walker" moves along a nucleic acid track, onto which it remains attached throughout the operation of the device. The walker is temporarily bound to a single-stranded stepstone section of the track. Successive dissociation and re-association to the next single-stranded anchor constitutes the overall single-stepped motion of the walker. The operation of a four-step DNA walker[14] is depicted in Fig. 6. The track includes four different nucleic acids **I** to **IV** as footholds. The walker consists of a double-stranded DNA with two single-stranded ends, acting as legs. The stepstone **I** and one of the walker's legs act as sticky ends for the hybridization with nucleic acid **A1**, leading to the fixation of the walker on the track. The subsequent hybridization of the walker's second leg and the foothold **II** with the nucleic acid **A2** leads to the directional motion of the leg to perform the step. The introduction of a further nucleic acid **D1**, to which hybridization of **A1** is energetically favoured, yields the double-stranded waste product **A1**–**D1**, and the translocation of the walker to the second foothold position. This enabled, by the appropriate design of hybridizing nucleic acids, the stepwise translocation of the walker to the end of the track and back to its original starting position. Careful labeling of the walker's legs and footholds with fluorophore and quencher units enabled the real-time visualization of the directional motion of the walker, results that were further confirmed by electrophoretic experiments.

A related DNA walker that involved a biped system composed of two duplexes acting as the moving element was reported,[27] Fig. 7. The legs of the biped are interconnected by flexible nucleic acid bridging units, and each of the biped components is terminated by single-stranded tethers, **F1** and **F2**. The track consists of three footholds **A**, **B**, **C**, composed of duplex DNAs interconnected by nucleic acid bridges. Each of the footholds is terminated by a single-stranded DNA, providing sticky ends for the single-stranded tethers of the biped's legs **F1**, **F2**. The linkers **Y1** and **Y2** lack similarity or complementarity, and link the biped to the footholds **A** and **B**. Addition of the unset strand **X1** forms the duplex **X1**–**Y2**, resulting in the release of one of the biped's "legs". The re-hybridization of the released leg to foothold **C** *via* the set strand **Y3** is followed by the release of the other biped leg from foothold **A** using the unset strand **X2**. Subsequently, the released biped leg is bound to foothold **B** using the set strand **Y4**, resulting in the one-step movement of the walking element. Although these two walkers prove directional linear motion of the DNA, their

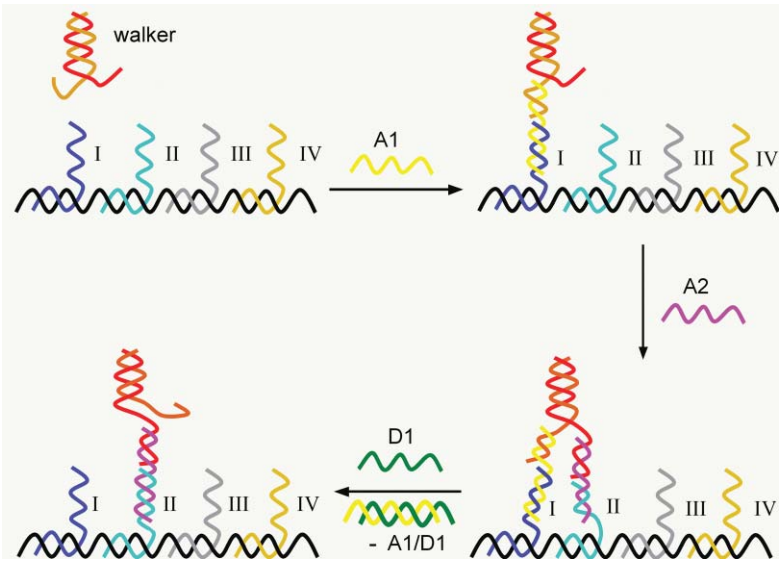

Fig. 6 A DNA "walker" on a four-footholds "track" triggered by hybridization–dehybridization events.

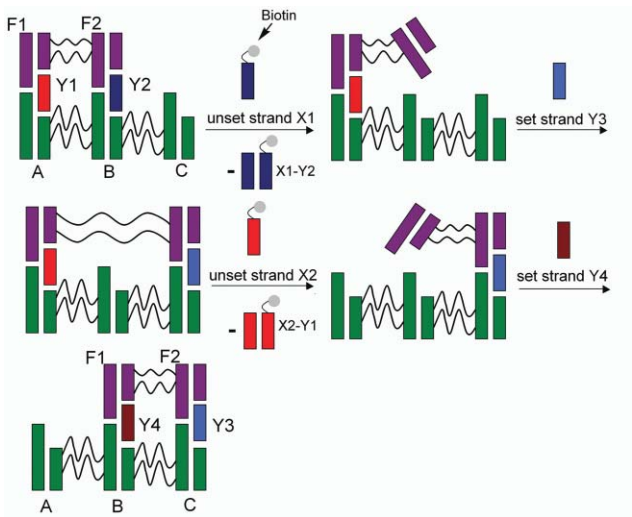

Fig. 7 A DNA walker triggered by the addition of set and unset strands. The unset strands yield duplex waste and are modified with biotin to facilitate their removal from the reaction mixture.

operation relies on external triggers, and precise tuning of the addition of the correct binding or unbinding oligonucleotides to the system.

Directed autonomous directional motion of a DNA walker without the need to add set and unset oligonucleotide strands was accomplished by the addition of biocatalysts, specifically, endonucleases and nicking enzymes. The availability of sequence-specific endonucleases that stimulate non-symmetric scission of double-stranded DNA or of nicking enzymes that cleave specifically one strand in the hybridized structure, may be applied to generate sequence-tailored sticky ends, or dictate the cleavage of duplex DNA, thus providing driving forces for programmed directional hybridization and motion of DNA on a track. An autonomously moving walker[28] that relies on the coupling of DNA ligase and two different restriction enzymes to confer directionality was designed. The track consisted of a linear double-stranded DNA, displaying three single-stranded footholds **A**, **B**, **C**, that protrude from the surface, Fig. 8. The lower part of the footholds forms a single-stranded hinge region, while the upper part is hybridized to complementary DNA strands that include single-stranded overhangs acting as a sticky end. The walker is formed by the ends of the first foothold **A**. The units **A** and **B** attach to one another by the hybridization of the sticky ends, yielding

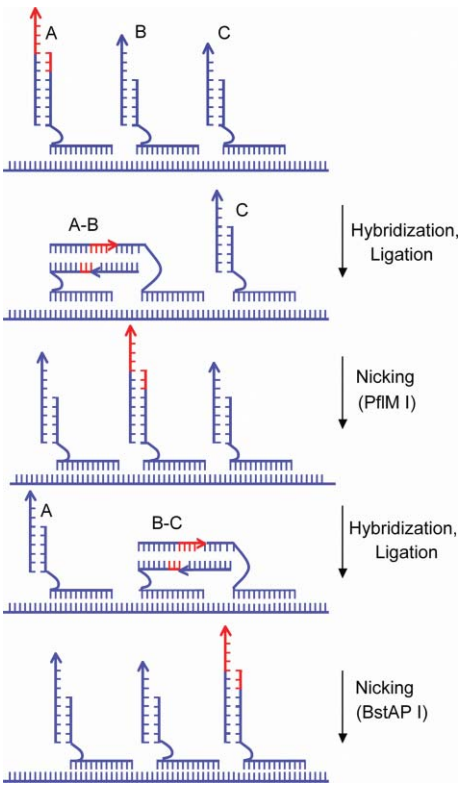

Fig. 8 A directional DNA "walker" activated by enzymatic ligation and specific scission processes. The "walker" nucleic acid is colored in red.

an interconnected complex **A–B** that is covalently linked by the T4 ligase, to yield the double-stranded recognition sequence for the first nuclease PfIm I. Scission of the DNA duplex on the **A** side of the walker leads to the separation of the **A**–**B** duplex and positions the walking nucleic acid at the end of foothold **B**. The subsequent hybridization of the DNA walker with the sticky end of foothold **C** is followed by the ligase-catalyzed ligation to form the **B–C** duplex. This duplex is designed to include the specific sequence that is cleaved by the second endonuclease, BstAP I. Its scission results in the positioning of the walker on the foothold **C**. Gel electrophoresis demonstrated the motion of the walker on a three-foothold model track. The use of two nucleases with differing recognition sites prevents the backward motion of the walker unit and allows the operation of the "walking machine" in the presence of all three enzymes employed. The drawback of this, rather impressive, concept is the fact that during the operation of the device, the walker must be bound at both ends of the sequence through hybridization; therefore the transport of a cargo cannot easily be realized. In fact, the motion of the device has little similarity to the image of walking; instead, it has been described as a 'pass-the-bucket' system.[29]

The 'burned bridges' approach was applied to develop a DNA walking system[30] by the directional powered motion of a nucleic acid on a track, using the nicking enzyme N. BbvC 1B, Fig. 9. The nicking biocatalyst recognizes a consensus sequence in double-stranded DNA and nicks one strand. The walker consists of an unmodified single-stranded oligonucleotide, named "*cargo*", which moves along a track of DNA that includes identical repetitive units of single-stranded footholds, the *stators.* In step 1, the walker hybridizes to one of these stators ($\mathbf{S_2}$), leading to a duplex formation with the pre-designed sequence for the nicking site of N. BbvC 1B. The duplex is cut in the middle of the stator strand (step 2), creating a single-stranded overhang of the cargo strand, which re-hybridizes with the end of the stator next in line ($\mathbf{S_3}$), Fig. 9, step 3. Branch migration leads to the displacement of the nicked stator ($\mathbf{S_2}$) by the next stator ($\mathbf{S_3}$), and the cargo is moved one step along the track, forming the next DNA duplex, Fig. 9, step 4. After nicking, the step backwards to $\mathbf{S_2}$ is prohibited, since the previously used stator is shortened and cannot bind the single-stranded overhang of the cargo. That is, the bridge backward is burned. The successful operation of this elegantly simple system was demonstrated on a three-stator test track using fluorescence labeling.

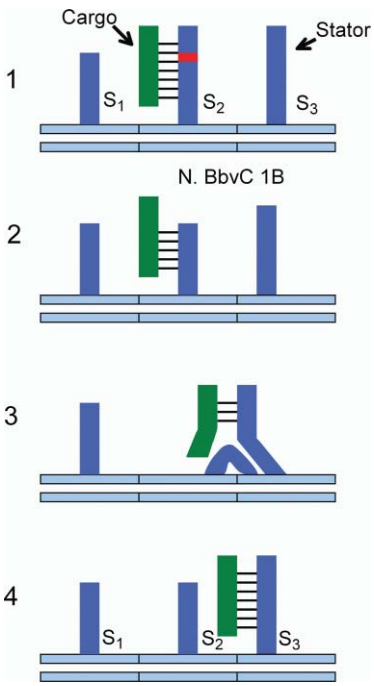

Fig. 9 A directional DNA "walker" designed according to the "burned bridges" paradigm and its potential use for the nanotransportation of a "cargo". The recognition sequence of the nicking enzyme is highlighted in red.

A different approach to design a directional walker has employed the use of a DNAzyme capable of cleaving RNA at a sequence-specific site,[31] Fig. 10. The track consists of an RNA strand (**9**), to which RNA segments containing a single-strand tether are hybridized (**10a**, **10b**). The single-stranded RNA is hybridized to the hairpin-structured DNAzyme (**11**) through its two binding arms consisting of 7 and 15 bases, respectively. This configuration leaves a single-stranded loop in the DNAzyme structure, which forms the active site. The DNAzyme cleaves the RNA (**10a**, position indicated by arrow), leading to the dehybridization of the short arm of the hairpin DNAzyme. The subsequent hybridization of the short arm with the next foothold in line (**10b**) followed by the branch migration of the other arm of the DNAzyme cause the transfer of the DNAzyme from (**10a**) to the next RNA segment (**10b**). By the sequential scission of the RNA units, the autonomous directed walking of the DNAzyme across the RNA track is accomplished. In contrast to the previous walkers that required addition of dehybridizing components or the presence of endonucleases/nicking enzymes as catalysts, this later system includes a built-in catalytically active unit that triggers directional motion.

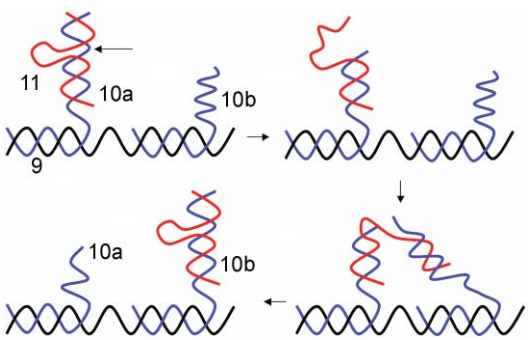

Fig. 10 A DNA "walker" on an RNA track triggered by DNAzyme scission and strand migration.

Albeit the autonomous vectorial motion of the "walker DNAs" is an admirable scientific advance, the available systems reveal a fundamental drawback related to their single-cycle "walking" operation. Multiple rounds of walking or transfer of the walker to another track are still scientific goals. Furthermore, the design of biomolecular walkers with controlled, externally triggered directionality, or multiple walkers following one another's footsteps are still scientific challenges. In fact, nature provides impressive complexity in its motor systems. Nonetheless, the immense recent progress in the field suggests that man-made systems of higher

complexity and enhanced functionality will be developed in the near future.

DNA machines as sensors

The sensitive detection of DNA is of fundamental interest in bioanalytical science. The rapid and sensitive analysis of nucleic acids has important implications for the detection of pathogens in the areas of clinical diagnostics, homeland security, and environmental control, as well as for the analysis of genetic disorders, tissue matching, and forensic applications. Substantial research efforts were directed in recent years towards the development of electronic[32] or optical[33] detection methods for DNA. Nonetheless, the Polymerase Chain Reaction (PCR) remains the key and central procedure for the amplified analysis of DNA, although the method is error-prone, requires long analysis time-intervals, and lacks quantitative assays of the analyzed DNA. The replication of the analyte DNA is the fundamental reaction of the PCR process, and the addition of the nucleotides mixture, acting as "fuel", and polymerase activate the polymerization and the synthesis of an ordered sequence of bases on the primer associated with a DNA template, thus mimicking some functions of a machine. The thermal separation of the replicated nucleic acids and their templates regenerates the machine.

The paradigm of the autonomous operation of DNA-based machines may be extended to DNA sensing or transporting with the challenging vision towards the future, suggesting such systems as analytical tools that could substitute PCR. The availability of sequence-specific endonucleases or nicking enzymes, together with polymerases that link nucleotides in specific order allows, in principle, the scission or separation of double-stranded nucleic acids, or the displacement of sequence-regulated nucleic acids from template DNAs. Accordingly, machines that generate catalytic nucleic acids or protein-specific aptamers can be designed, and eventually machines that replicate catalytic nanostructures may be envisioned.

Nucleic acids that alter their configuration upon hybridization provide a versatile tool to trigger a mechanical transformation. The most explored nucleic acid structure that changes its steric configuration upon hybridization, scission, or polymerization is the nucleic acid "hairpin", Fig. 11. Ingenious "hairpin" DNA structures modified with photonic reporter units are opened upon hybridization with the analyte DNA,[34] or upon the association of proteins[35] to the single-stranded loops of the nucleic acid hairpin. The activation of the photonic functions of the opened hairpin structure, *e.g.* activation of fluorescence or blocking intramolecular FRET, provide physical means to follow the opening of the hairpin structure, Fig. 11(A). Similarly, optically labeled hairpin structures were successfully applied as primers for the PCR analysis of DNA,[36] following several different strategies, such as the one in Fig. 11(B). A hairpin (**H**) is modified with a fluorophore (**F**) and a quencher (**Q**) at both ends of its stem, while the 3′ end of the hairpin is connected to a regular PCR primer *via* a polymerization stopper (**S**, a non-DNA molecule, *e.g.* hexaethylene glycol, that prevents readthrough of the polymerization through the hairpin). The primer part of the hairpin anneals to the template and is elongated to yield a hairpin-modified PCR product. During the heating cycle, the new product dissociates from the template, and the hairpin structure is opened thermally. Upon cooling, the stem of the hairpin can hybridize intramolecularly with the PCR product, separating the fluorophore and the quencher, and thus allowing a quantitative readout of the PCR process.

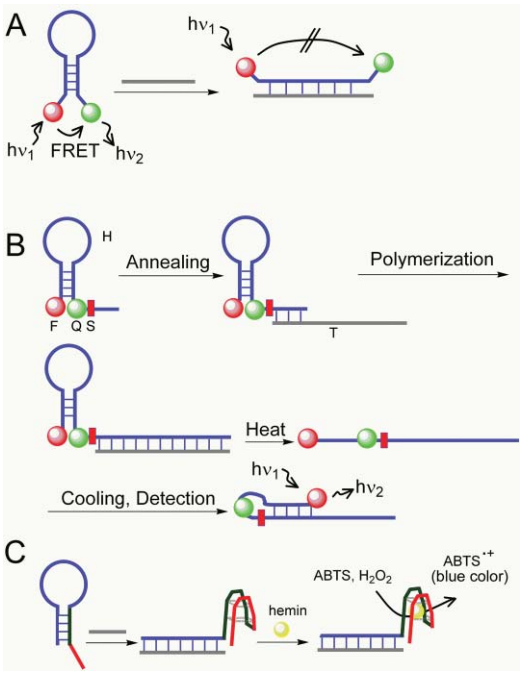

Fig. 11 Mechanical transitions of hairpin DNA upon hybridization with complementary nucleic acids.

The hybridization-induced change in hairpin fluorescence has proven to be a particularly valuable tool for the real-time imaging of RNA and DNA content in living cells. Fluorescent beacons have been employed to visualize viral infections,[37] cancer markers,[38] and expression profiles[39] in cell cultures.

The access to catalytic nucleic acids (DNA or RNA) by the SELEX selection procedure[40,41] from a library of randomized nucleic acid sequences, adds a further dimension to mechanically active DNA hairpins by the integration of catalytic nucleic acids within the structures. This has been demonstrated with the incorporation of a DNAzyme that mimics peroxidase function into a DNA hairpin,[42] Fig. 11(C). The DNAzyme consists of a G-quadruplex structure that binds hemin in between the quadruplex layers.[43] The resulting hemin–G-quadruplex was found to catalyze the oxidation of ABTS (2,2′-azinobis(3-ethylbenzothiozoline)-6-sulfonic acid), by H_2O_2, as well as to catalyze the generation of chemiluminescence in the presence of H_2O_2–luminol.[44] A hairpin structure that includes the two segments **A** (red) and **B** (green) in a single-stranded nucleic acid structure that self-assembles into the G-quadruplex–hemin peroxidase-mimicking DNAzyme was employed to analyze DNA. The single-stranded loop **C** (blue) is complementary to the analyte DNA, while the segment **B** hybridizes within the hairpin stem. The stability of the double-stranded stem prevents the formation of the DNAzyme structure. Hybridization of the analyte with the loop region opens the hairpin, and the G-quadruplex–hemin complex self-assembles into the active DNAzyme structure. The DNAzyme then catalyzes the oxidation of ABTS, and allows the colorimetric readout of the mechanical opening of the hairpin by the analyte.

The mechanical opening of hairpin structures of nucleic acids by DNA or proteins was employed to develop bioelectronic sensor devices.[45] A nucleic acid in a hairpin structure was linked to an electrode surface through one end, while the other end was tethered to a redox-active ferrocene unit, Fig. 12(A). In the hairpin configuration the redox-active unit was placed in close proximity to the electrode surface, resulting in electrical contacting and effective oxidation of the ferrocene groups. Hybridization with the analyte DNA opened the hairpin and resulted in a rigid double-stranded conformation accompanied by the retraction of the redox-active group to a remote position that lacked electrical contact with the electrode. The blocking of the electrical communication between the redox-active site and the electrode was used as a reporting signal for the mechanical opening of the hairpin structure and the sensing of DNA. A related process was used to analyze proteins by a redox-active aptamer associated with an electrode.[46] A nucleic acid functionalized with a redox-active methylene blue unit was linked to an electrode, Fig. 12(B). The electroactive group revealed electrical communication with the electrode in the flexible single-stranded conformation. The nucleic acid included, however, the G-base sequence triggered by thrombin to self-assemble into a G-quadruplex aptamer structure that binds thrombin. The formation of the G-quadruplex–thrombin complex removed the methylene blue from the proximity of the electrode surface, and this mechanical translocation interrupted the electron transfer between the redox-active group and the electrode. This process was utilized for the development of an amperometric thrombin-sensing electrode based on the controlled assembly of the active aptamer structure. Several related methods to analyze DNA or proteins by the mechanical control of nucleic acid structures were described in recent years.[47,48]

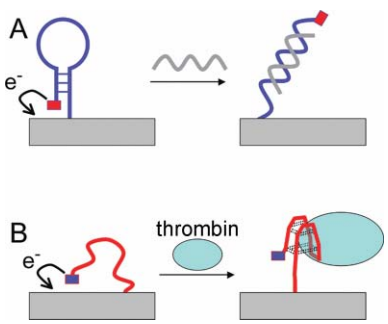

Fig. 12 Sensing applications of hairpin DNA for the electrochemical detection of DNA (A) and the protein thrombin (B). Redox labels are depicted as rectangles.

Dirks and Pierce have introduced the hybridization chain reaction concept,[49] in which the consecutive opening of hairpin DNAs by an initiator oligonucleotide causes a cascade of opening and joining of the hairpins, leading to the polymerization-like formation of multimeric DNA structures.

A lead (Pb^{2+}) biosensor was developed based on the DNAzyme-catalyzed scission of gold nanoparticle-aggregates.[50] A DNA template (**12**) that includes repetitive sequence units for the hybridization with complementary DNA-functionalized Au nanoparticles (**13**) causes their aggregation. The DNAzyme (**14**) consists of a hairpin structure, and its two ends hybridize with the single-stranded domain in between the nanoparticles, Fig. 13. In the

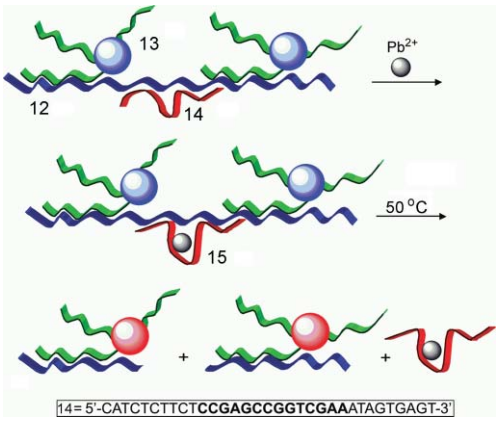

Fig. 13 The analysis of Pb^{2+} ions *via* the activation of a Pb^{2+}-dependent DNAzyme that triggers the cleavage of a DNA strand and leads to the de-aggregation of Au nanoparticle clusters. The active site is highlighted in the DNAzyme sequence.

presence of Pb^{2+} the DNAzyme attains its active configuration (**15**) and cleaves the template upon annealing, and at 50 °C the shortened double-stranded DNA units are separated. Thus, while the original aggregate is stable at 50 °C, the cleaved DNA assembly is separated to the individual nucleic acid-functionalized nanoparticles, a process that leads to the release of the active DNAzyme cutting unit. The Au nanoparticle aggregate exhibits a blue color originating from an interparticle coupled plasmon, while the separated Au nanoparticles display a red color that corresponds to the plasmon absorbance of the separated nanoparticles. Thus, the separation of the nanoparticle aggregation was followed spectrophotometrically, and a colorimetric Pb^{2+} ion sensor based on the mechanical cleavage of the nanoparticle aggregate was developed.

A DNA-based machine of enhanced complexity for the detection of DNA was accomplished by the development of a DNA–protein (FokI) cutting machine that replicates itself upon analysis of the target DNA.[16] The system mimics several elements of macroscopic machines: (i) It is activated by an external signal. (ii) The system performs a mechanical scission function. (iii) The machine is fed with a fuel that permits its continuous operation. (iv) The cutting of the fuel yields a waste product that spotlights the operation of the machine and the sensing process through the generation of fluorescence. The process, Fig. 14, was exemplified by the analysis of one of the Tay–Sachs genetic disorder mutants. A nucleic acid hairpin structure (**a**) is employed as the analyzing probe. The hybridization of the mutant DNA (**b**) with the probe opens the hairpin structure, and yields the double-stranded structure (**I**). This process generates the Fok I binding domain, and the association of the endonuclease to the duplex leads to the scission of the double-stranded recognition sequence, and to the formation of a DNA–Fok I cutter, **II**. The hairpin nucleic acid (**c**) acts as the fuel and includes sticky ends that hybridize with the cutter unit. It also includes in its structure a fluorophore **F** (fluorescein) and a quencher unit **Q** (tetramethylrhodamine), which are positioned at distances that lead to the total quenching of the fluorescence. The hybridization of (**c**) with the cutter **II** leads to a duplex that is cleaved by the cutter, yielding waste products (**d**) and (**e**) and the complex **III** that is a cutter unit by itself and is fuelled by the original substrate (**c**). Thus, the

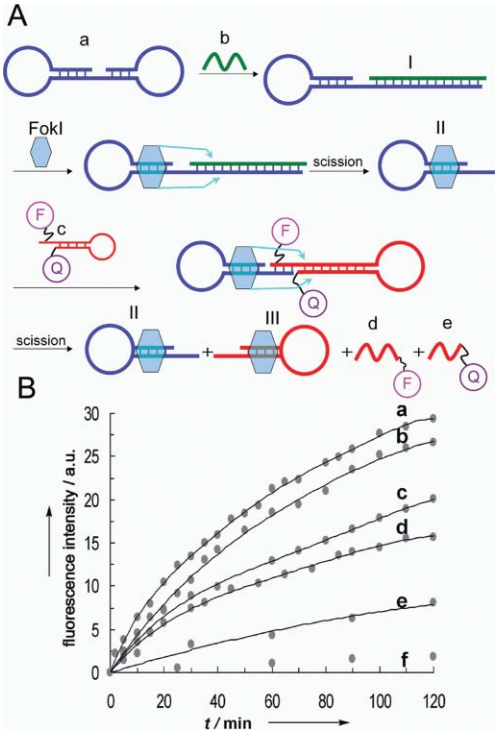

Fig. 14 (A) A self-replicating FokI–DNA machine generation upon the analysis of strand (**b**). The FokI–DNA machine is activated by the fuel (**c**) and yields a fluorescent waste product as a reporter for the operation of the machine. (B) The time-dependent fluorescence intensities generated by the machine upon analysing different concentrations of target mutant DNA from 10^{-6} M to 10^{-14} M (a–d), wild type DNA (10^{-6}, e), and a foreign DNA (10^{-6}, f). (Part B reproduced from ref. 16 with permission from Wiley-VCH.)

primary detection of the analyte (**b**) triggers the formation of the DNA scission machine, and in the presence of fuel (**c**), the autonomous replication of the cutter unit is activated. The newly formed waste product (**d**) is fluorescent due to the separation from the quencher unit, and thus spotlights the operation of the machine and the sensing process. Fig. 14(B) shows the time-dependent increase in fluorescence intensity generated by the system analysing different concentrations of (**b**). The presence of the mutant sequence could be detected by the DNA–FokI machine with a sensitivity that corresponds to 1×10^{-14} M.

A DNA–FokI-based machine was employed as a DNA signal translator that triggers the release of a protein,[17] Fig. 15(A). The system consists of a hairpin structure **A**, the enzyme FokI, and a double-stranded DNA comprising a nucleic acid (**g**) that is hybridized to a protecting nucleic acid (**h**). The nucleic acid (**g**) includes two complementary single-stranded ends (**X**) and (**Y**). The hybridization of the protecting nucleic acid (**h**) to (**g**) yields a stable duplex that prevents the hybridization of the units (**X**) and (**Y**). Introduction of the "input" nucleic acid (**k**) activates the machine by separating the duplex between (**h**) and (**g**), a process that yields a stable double-stranded "waste" (**l**). The single-stranded ends of the separated nucleic acid (**g**) self-assemble into the hairpin structure (**ga**), and the sticky ends of (**ga**) and the hairpin (**A**) generate the complex (**P**), which includes the binding site for FokI. Scission of the complex P regenerates (**ga**) and FokI, and the component (**A**) is cleaved into fragments that lack

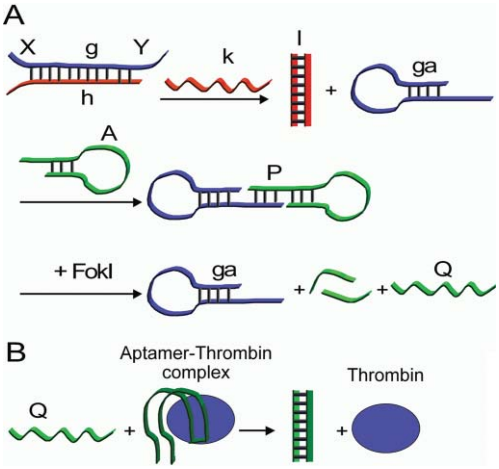

Fig. 15 A FokI–DNA machine for the controlled release of an aptamer-bound protein.

duplex stabilization because of a low contact of complementary bases. The released single-stranded nucleic acid (**Q**) may be employed as an effector unit that triggers a secondary device, for example, a G-quadruplex self-assembled from a single-stranded DNA that acts as an aptamer for thrombin.[51] Previous studies have demonstrated that hybridization of a nucleic acid to the aptamer perturbs the G-quadruplex and separates the aptamer–thrombin complex.[52] Accordingly, an effector nucleic acid **Q** that hybridizes with the thrombin aptamer was designed, Fig. 15(B). The hybridization event led to the separation of the thrombin molecule from the aptamer and to the disassembly of the G-quadruplex structure. The latter effect was followed by labeling the aptamer with a donor–acceptor pair of dyes. In the G-quadruplex the aptamer reveals FRET emission, due to the proximity of donor and acceptor. The dissociation of thrombin and the dismantlement of the G-quadruplex block the FRET process. This DNA–FokI machine mimics several fundamental biological functions operating in nature. The "allosteric regulation" is a key principle of many biocatalytic transformations, and inter-protein communication is often triggered by a conformational change, induced by an effector–cofactor molecule. Similarly, in the present system, the aptamer–protein complex is separated by the conformational perturbation of the aptamer caused by the effector nucleic acid generated by the DNA–FokI machine. The system highlights the versatility of future applications of DNA machines. The nucleic acid sequence in the strand (**g**), which forms the (**g**)–(**h**) duplex, is of arbitrary composition and does not participate in the functional operation of the machine. As a result, this sequence may be considered as the probe nucleic acid that upon sensing of (**h**) activates the machine. Furthermore, the system reveals a chain process where the presence of a certain DNA sequence translates into an effector molecule that triggers the release of a protein. Such systems may be valuable for controlled drug-delivery.

Conclusions and perspectives

Substantial progress has been achieved with the design of DNA-based machines that perform mechanical functions such as scission, rolling or directional motion. Albeit at first glance these concepts seem to only satisfy scientific curiosity, the emerging

systems highlight extremely valuable and promising applications of DNA-based machines. Ultrasensitive biosensing schemes may be envisaged, and promising amplification routes for bioanalysis have already been described. The autonomous synthesis of DNAzymes and the replication of catalytic DNAs represent the "seeds" of analytical processes that might replace the legendary PCR protocol. The DNA-based machines are of value not only for DNA analysis, but might be coupled to other biosensing events such as antigen–antibody studies. The coupling of DNA machines to antibody labels and their use as immunological amplicons is a natural extension.

Other future applications of DNA-based machines would include controlled transport and release of drugs and the activation of nucleic acids at cellular targets by external triggers. The recent advances in the application of siRNA highlight the potential of short single-stranded oligonucleotides for the *in vivo* manipulation of gene expression.[53] The use of DNA machines might have an even broader impact in the rapidly developing field of nanobiotechnology. The autonomous synthesis of nucleic acid sequences of pre-designed composition might provide useful templates for the synthesis of metallic or semi-conductor nanocircuitry. Alternatively, the machine-synthesized nucleic acid hairpins may act as templates for the secondary self-assembly of ordered metal and semi-conductor nanoparticles, thus leading to the fabrication of nanoscale devices.

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