DNA Nanodevices

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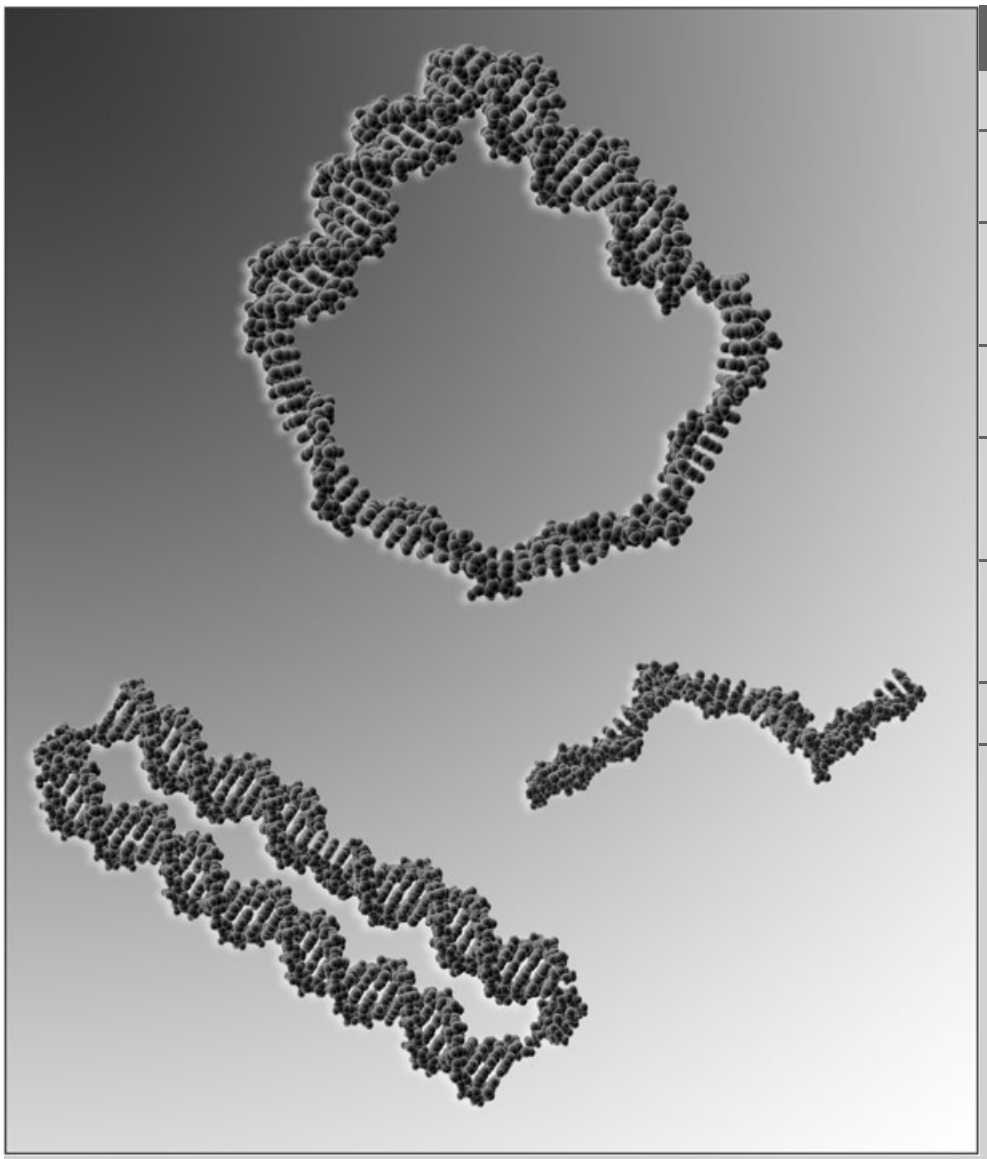

A DNA actuator, which switches from a relaxed, circular form to a stretched conformation.

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From the Contents

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The molecular recognition properties of DNA molecules combined with the distinct mechanical properties of single and double strands of DNA can be utilized for the construction of nanodevices, which can perform ever more tasks with possible applications ranging from nanoconstruction to intelligent drug delivery. With the help of DNA it is possible to construct machinelike devices that are capable of rotational motion, pulling and stretching, or even unidirectional motion. It is possible to devise autonomous nanodevices, to grab and release molecules, and also to perform simple information-processing tasks.

1. Introduction

Biological information is stored in the base sequences of DNA molecules. Highly specific base-pairing interactions allow for replication of DNA and transcription of its information into RNA.[1] In other words, the specific interactions between two nucleic acids are "programmed" into their sequences. Taken together with the polymer mechanical properties of DNA molecules, the notion of sequence-programmability has led researchers in nanoscience to think about a utilization of DNA molecules for the construction of artificial nanosystems. DNA can be used to build supramolecular devices or as a template for materials synthesis. Quite recently, it has been shown that DNA can not only be utilized to build such static nanostructures, but also to construct simple, machinelike nanomechanical devices. This Review provides an overview of the DNA nanodevices realized so far and the major current research themes in this field.

In Section 2., a brief overview of DNA nanotechnology as a whole is given. The most important properties of DNA molecules are introduced and examples are given in which DNA has been utilized for nanoconstruction and materials synthesis. Section 3. surveys the prototypical DNA nanomachines that have been realized so far. These are simple DNA constructs, which are capable of primitive movements such as rotation or stretching. Section 4. discusses the important problem of locomotion and recent advances in the construction of DNA motors. Section 5. introduces new developments in which functional nucleic acids such as aptamers or ribozymes have been incorporated into DNA nanodevices. Section 6. gives an overview of attempts to process molecular information using DNA structures and thereby control the motion of DNA nanodevices. Finally, the outlook in Section 7. briefly discusses future directions and possible applications for DNA nanodevices.

2. Overview: DNA Nanotechnology

2.1. Important Properties of Nucleic Acids

Several biophysical and biochemical aspects of DNA are particularly important for DNA-based nanodevices and nanostructures: First and foremost, the unique base-pairing interactions between complementary bases,[1,2] second, the distinct polymer mechanical properties of single- and double-stranded DNA,[3] and finally, the electrostatic properties of DNA and RNA as highly charged polyelectrolytes.[4]

Single-stranded DNA is a heteropolymer that consists of nucleotide units linked together via phosphodiester bonds (Figure 1). The nucleotide units themselves consist of a deoxyribose sugar unit to which one of four so-called "bases" are attached at the 1′ carbon site. Two of the four bases—adenine and guanine—are purines, and the other two—thymine and cytosine—are pyrimidines. In the famous Watson–Crick (WC) base-pairing scheme, adenine can bind to thymine through two hydrogen bonds, and guanine can bind to cytosine via three such bonds. In RNA, uracil takes the role of thymine and the sugar unit is ribose. If the base sequences of two DNA strands are exactly complementary, that is, if for each base on one strand the corresponding WC partner is found on the other strand, the strands may bind together to form a double helix. Under typical buffer conditions (at least 100 mM salt concentration; neutral pH) a DNA duplex assumes its native "B" conformation. In this form, the distance between two bases is 0.34 nm and the helix completes one turn for each 10.5 base pairs (bp). The diameter of the B-form duplex is 2 nm. For single-stranded DNA, the mean distance between two bases is 0.43 nm, slightly greater than in the duplex. In RNA duplexes or in DNA–RNA hybrids, the "A" conformation is found with a diameter of 2.6 nm and 11 bp per turn. Under certain buffer conditions, and for special sequences, DNA may also undergo a transition to a left-handed helical conformation known as Z-form DNA, which has a diameter of 1.8 nm and 12 bp per turn.[2]

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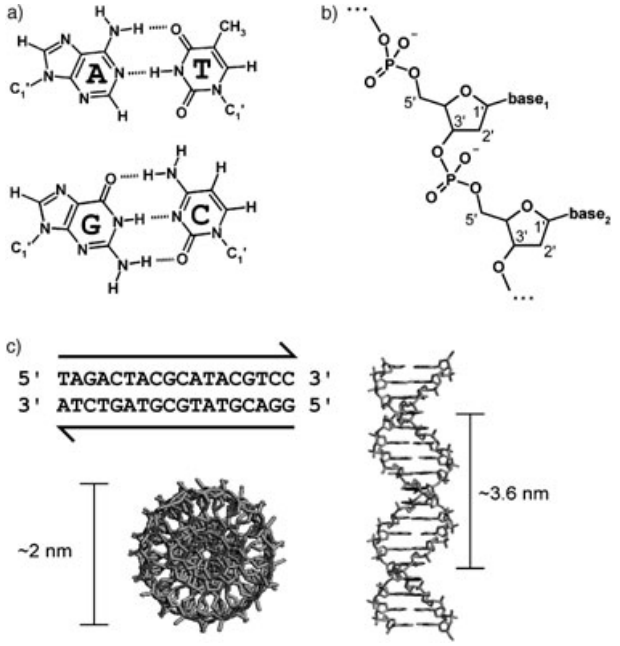

Figure 1. Important features of DNA: a) Watson–Crick base pairs formed between adenine (A) and thymine (T), and guanine (G) and cytosine (C); b) in single-stranded DNA, nucleoside units (deoxyribose + base) are linked by phosphodiester bonds; c) two complementary strands of DNA form a double helix. Top-left: A symbolic representation of a DNA duplex of 21 bp. Bottom-left: Looking down the axis of the double helix. Right: Two helical turns of a duplex made from 21 bp.

The stability of the double helix is determined by a variety of factors: the binding energy due to hydrogen bonds, stacking interactions between neighboring base pairs, entropic contributions, and many others. The stability can be controlled by a variety of external parameters, such as salt concentration or temperature. At $T = 298$ K and a monovalent salt concentration of 1 M, the mean value of the binding free energy between two bases in a double-helical context (i.e., containing stacking interactions) is $\Delta G^0_{298} \approx -75$ meV (calculated from the thermodynamic parameters given elsewhere[5,6]). Due to their three hydrogen bonds, G–C pairs are more stable than A–T pairs. The thermodynamic properties of a given sequence can be calculated quite reliably using the wealth of thermodynamic data collected for DNA over the last decades,[6] for example, using the computer program HYTHER.[7,8]

Many of the DNA nanodevices discussed in the following Sections are driven by this hybridization energy. It is expected that the maximum force which may be generated by hybridization is on the order of 15 pN, a force which has also been measured by atomic force microscopy (AFM) in a variety of DNA-unzipping experiments.[9–11]

If two strands of DNA do not match perfectly, they may bind to each other more weakly, or remain single-stranded, depending on the number of mismatches. DNA may also fold back upon itself to form "hairpin loops". From a nanoconstruction point of view, this means that structure is determined by sequence, which opens up the possibility of programmable nanoassembly. By choosing the appropriate DNA base sequences, one can design arbitrary networks consisting of single- and double-stranded sections.

Due to the differing mechanical properties of single- and double-stranded DNA, self-assembled DNA structures can be thought of as networks consisting of relatively stiff elements connected by flexible joints: The stiffness of a polymer can be characterized by its persistence length l_p, which is the correlation length for the tangent vector along the polymer.[12,13] On a length scale comparable to l_p, a polymer can be regarded as a rigid rod, whereas for lengths much larger than l_p, the polymer is flexible. It has been found that the mechanical properties of double-stranded DNA can be well described by the wormlike chain (WLC) model, with a persistence length of typically 50 nm or 150 bp.[14–16] By contrast, single-stranded DNA has a much shorter persistence length, on the order of only 1 nm.[15,17,18] Thus, for the DNA structures of interest here with a length on the order of 10–100 bases, duplex DNA really is a stiff molecule, while single-stranded DNA is flexible. The elastic properties of DNA are sequence-dependent, which in some cases can be used to make fine adjustments. For instance, it has be shown that poly(dT) is much more flexible than poly(dA).[19]

Under neutral buffer conditions the negative charges on the phosphate groups in the backbone render DNA a highly

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charged polymer.[4] This is quantified by the Oosawa–Manning parameter ξ,[20,21] which expresses the inverse line charge density of a polyelectrolyte in units of the Bjerrum length $l_B = e^2/4\pi\varepsilon_r\varepsilon_0 k_B T$, where ε_r is the permittivity of the solvent and k_B is the Boltzmann constant. For DNA, $\xi = 4.2$, which is well above the critical value of $\xi = 1$ above which counterion condensation is expected to set in.[4] This has a variety of important consequences: For templated materials synthesis, DNA may be used to localize positively charged precursor molecules along its backbone. Furthermore, depending on the nature and concentration of the counterions, two DNA duplexes may interact strongly with each other electrostatically. The interaction is mediated by the counterion cloud and is usually repulsive but sometimes also attractive.[22,23] Under certain conditions, these effects can strongly influence the performance of DNA nanodevices, for example when a conformational change brings two DNA duplex units into close proximity.

Apart from the advantageous properties of DNA, the many biochemical techniques available for its manipulation and characterization make supramolecular construction with DNA particularly convenient. Many DNA-modifying enzymes are available that can be used to cut DNA at specific sites or to ligate two pieces of DNA together. Techniques such as polymerase chain reaction (PCR) or cloning can be utilized to make large numbers of copies of given DNA sequences. But maybe the most important factor for DNA nanoconstruction is the capability of producing oligonucleotides of arbitrary sequence using automated synthesis methods. Therefore, the sequences necessary for a computer-designed model can be readily translated into a physical real-world structure.

2.2. Supramolecular Construction with DNA

Since the early 1990s, the unique molecular recognition properties of complementary strands of DNA and the mechanical properties of single-stranded and duplex DNA mentioned in the previous Section have been utilized for the construction of a variety of geometric objects and two-dimensional lattices. There are a number of excellent reviews on these structures,[24–26] and we just mention a few of the hallmarks in this field. The potential of DNA was first utilized by Seeman's group in the realization of a supramolecular structure with the topology of a cube[27] and later of a truncated octahedron,[28] or of DNA catenanes such as Borromean rings.[29] The construction of two-dimensional networks of DNA from four-way junctions was initially compromised by the relatively large angular flexibility of the branch points of the junctions.[30–32] This problem was resolved with the introduction of the "double-crossover" constructs in which two four-way junctions were joined to form an inherently more rigid object.[33] From these "DX tiles", Winfree et al. constructed the first two-dimensional DNA lattices.[34] The persistence length of DX structures was later shown to be twice that of duplex DNA.[35] Recently, other complex structures have been realized, such as triple-crossover constructs,[36] DNA crossbar lattices,[37] or DNA nanotubes.[38]

An interesting approach is the utilization of triangular building blocks for DNA nanoconstruction, [39,40] since these are intrinsically rigid objects. One of the first demonstrations of three-dimensional objects from DNA—the octahedra—is also based on triangular subunits.[41]

Another innovative approach towards supramolecular construction is the utilization of RNA rather than DNA. Leontis and co-workers[42,43] have devised a number of "RNA tectons" that specifically interact with each other using non-WC interactions. In these RNA structures, loop elements are recognized by specific binding pockets, which are very similar to key–lock interactions between proteins.

2.3. DNA Templating and Scaffolding

To utilize DNA for the assembly of non-biological nanostructures such as nanoelectronic circuits, it is necessary to attach functional nanostructures to DNA or to chemically modify DNA itself. The conductive properties of DNA itself are not sufficient for any application in a conventional electronic circuit. For this reason, a number of groups have modified DNA with electronically functional materials such as metals,[44–49] semiconductor nanoparticles,[50,51] conductive polymers,[52–54] or carbon nanotubes.[55,56] In most of these cases, an ionic precursor such as a metal ion or metal complex was attached electrostatically to the DNA backbone or was directly bound to the DNA bases. Subsequent chemical steps such as reduction or polymerization led to the template-directed deposition of materials along the DNA molecule. In the case of carbon nanotubes, DNA was attached either covalently[55] or by utilization of antibody recognition events.[56] There is also a large body of work in which DNA has been used to organize colloidal metallic and semiconductor nanoparticles[57–60] or proteins[61] into crystals and networks.

A different approach is the utilization of DNA recognition for organic synthesis. Here reactive chemical compounds are attached to complementary strands of DNA. Duplex formation between the complementary strands brings the compounds into close proximity, so that they can react with each other. A thorough review of applications of this concept has been published recently.[62]

3. Prototypes of Nanomechanical DNA Devices

DNA can not only be used to build supramolecular structures or act as a template for materials synthesis; it can also be utilized to produce nanoscale movements. These movements are usually based on conformational changes, which are triggered by changes in buffer composition or by hybridization between complementary strands of DNA. The conformational changes are transitions between single-stranded DNA and duplex DNA or between unusual and conventional DNA conformations. In the following, we differentiate between DNA devices driven by small molecules

and DNA devices driven exclusively by hybridization and branch migration.

3.1. DNA Devices Fuelled by Changes in Buffer Composition

The first DNA nanomechanical device demonstrated was based on the B–Z transition of DNA.[63] In the B–Z device, two double crossover structures are connected by a DNA strand containing the sequence $d(CG)_{10}$ (where cytosine residues are methylated at the C5 position). This sequence is particularly prone to undergoing a B–Z transition. The transition can be triggered by a change in the concentration of cobalt hexammine ($[Co(NH_3)_6]^{3+}$) from 0 to 0.25 mM. The $d(CG)_{10}$ section changes its helicity from right-handed to left-handed; this corresponds to a change in twist of −3.5 helix turns. From this, an overall change in the conformation of the device results in which the two DX sections are rotated with respect to each other by one half-turn (see Figure 2). The motion of the B–Z device can be monitored in fluorescence resonance energy transfer (FRET) experiments.[64–66] FRET occurs between a fluorescent dye (the "donor") and another chromophore (the "acceptor") if the absorption band of the acceptor overlaps with the emission band of the donor fluorophore. The efficiency E_T of this process varies as

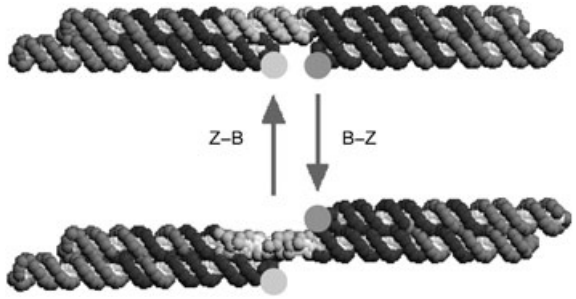

Figure 2. The B–Z device is made from three strands of DNA. One strand (dark and light gray) forms two double-crossover (DX) arms with two other strands (gray). The hinge (light gray) connecting the arms is a $d(CG)_{10}$ sequence which undergoes a B–Z transition upon addition of cobalt hexammine. This transition rotates the DX arms with respect to each other (reproduced with permission from Ref. [63])

$$E_T = \frac{F(D)-F(DA)}{F(D)} = \frac{1}{1+(R/R_0)^6} \quad (1)$$

where $F(DA)$ and $F(D)$ denote the fluorescence intensities of the donor in the presence or absence of the acceptor. The distance R_0 at which FRET efficiency is 50% is called the Förster distance; R_0 usually lies between 2 and 6 nm. For this reason, FRET is a convenient tool to characterize nanoscale movements and is a frequently applied technique in the field of DNA nanodevices. The B–Z device was characterized using fluorescein as the FRET donor and Cy3 as the acceptor. For this FRET pair, the Förster distance is approximately 5.6 nm. The transition from the B-form to the Z-form leads to a change in FRET efficiency from 20% to 5% due to the larger distance between the dyes in the Z conformation of the device. Using the FRET technique, it could be shown that the B–Z device indeed can be cyclically switched between its two conformations and thus represents a very simple example of a molecular machine based on DNA.

A different approach towards nanoscale motion was taken by Niemeyer et al.:[67] In their device, magnesium-ion-induced DNA supercoiling was utilized to produce nanoscale movements that could be characterized using AFM. In networks of dsDNA connected by biotin–streptavidin linkers, two neighboring DNA duplexes may condense into a single supercoiled structure in the presence of multivalent ions such as Mg^{2+}, which results in a change of network connectivity. In principle, it should be possible to reversibly switch between the conformations of such a network as the supercoiling transition itself is reversible.

A concept termed "duplex pinching" by Fahlman et al.[68] is based on the guanine quadruplex conformation of DNA (Figure 3a).[69] In the G quadruplex, four guanine residues can form a total of eight hydrogen bonds with each other in a cyclic arrangement. Under certain conditions, the strength of this interaction is comparable to conventional WC base-pairing. G-quadruplex formation is often found in G-rich sequences and can occur not only intramolecularly, but also intermolecularly, where two or four single strands of DNA are held together by G quartets. For their devices, Fahlman et al. made use of the fact that the G-quadruplex conformation is stabilized by the presence of potassium or strontium ions. They constructed a DNA duplex with six internal G–G mismatches connected by a short hinge section consisting of three A–T base pairs. Upon addition of Sr^{2+} ions, which strongly stabilize G-quartet formation, the duplex assumes a "pinched" conformation in which the G–G mismatches form intramolecular G quartets. This transition can be reversed by the addition of the chelator ethylenediamine tetraacetic acid (EDTA) which binds the strontium ions. The authors proposed that duplex pinching may be used to introduce contractile elements into DNA supramolecular structures.

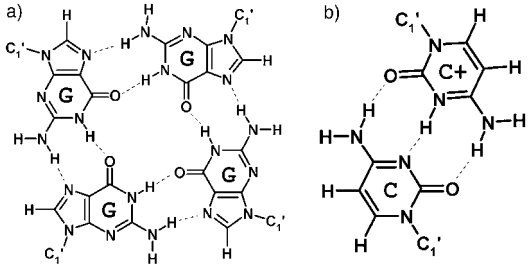

Figure 3. Nonstandard interactions between DNA bases: a) A guanine quartet formed by cyclic hydrogen bonding between four guanine bases; b) a base pair between a protonated (C+) and an unprotonated (C) cytosine.

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A variety of other DNA devices have been constructed that are fuelled by protons rather than by metal ions.[70,71] Proton-fuelled devices utilize the fact that nucleic acid bases can be protonated at low pH values. For instance, adenosine can be protonated at the N1 nitrogen position ($pK_a = 3.5$) and cytidine can be protonated at the N3 position ($pK_a = 4.2$).[2] These protonated bases can form additional non-WC base pairs, for example, C–C+ (Figure 3b). The DNA device realized by Liu and Balasubramanian[70] is based on an unusual DNA conformation called the "i-motif", which occurs in long stretches of cytosine residues with a transition at around pH 6.5.[72–74] Below this value, a C-rich DNA strand X assumes an intramolecular quadruplex conformation in which C–C+ base pairs occur in a staggered arrangement (see Figure 4). At higher pH values, strand X

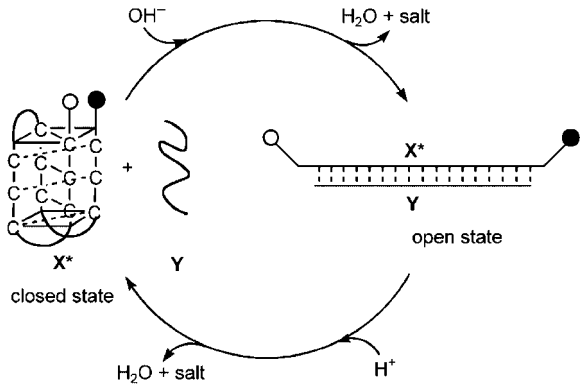

Figure 4. A proton-fuelled device based on the i-motif. In the closed state at low pH values the cytosine residues in strand X form C–C+ base-pairs. These stack up on top of each other to form the "i-motif". At higher pH values, cytosine is deprotonated and X can base-pair with the complementary strand Y to form the open state. Reproduced with permission from Ref. [70].

can hybridize with the complementary strand Y. Therefore the device can be cycled between an extended duplex state and a closed quadruplex state by the subsequent addition of hydrogen and hydroxide ions. By attaching a fluorescent dye at the 5′ end and a quencher group at the 3′ end, the conformational changes of the device can be monitored by changes in fluorescence intensity as the quenching efficiency is strongly dependent on the distance between the chromophores, similar to the FRET effect introduced above. From the free-energy difference of the two states and the change in distance between the endpoints of strand X, the authors inferred an average closing force of 11.2 pN. As "waste products" only NaCl and H_2O are produced.

Another device driven by protons utilizes DNA-triplex formation. Triplex formation can occur when an oligonucleotide binds to the major groove of a homopurine–homopyrimidine duplex.[69] There are two possible orientations for the third strand, namely, parallel or antiparallel to the homopurine stretch. One of the common triplex motifs involves C–G:C+ triplets, where the third strand is oriented in parallel with the homopurine sequence. As this triplex can only form when the cytosine residues in the third strand are protonated, a duplex–triplex transition can be induced by changing the pH value. Mao and co-workers recently inserted an appropriate sequence into a DNA nanomechanical device, the conformation of which could be switched by inducing a duplex–triplex transition in this "motor section".[71] A simpler DNA construct utilizing the duplex–triplex transition has already been described elsewhere.[75]

3.2. Devices Driven by DNA Hybridization and Branch Migration

A different operational principle for DNA devices was introduced by Yurke et al. with the "DNA tweezers".[76] In this concept, a 40-base-long strand Q is hybridized with two 42-nucleotide (42 nt) strands S1 and S2 over a length of 18 bp each. This results in a supramolecular structure, as depicted in Figure 5, in which two 18-bp-long duplex "arms" are connected by a 4-nt-long single-stranded flexible hinge.

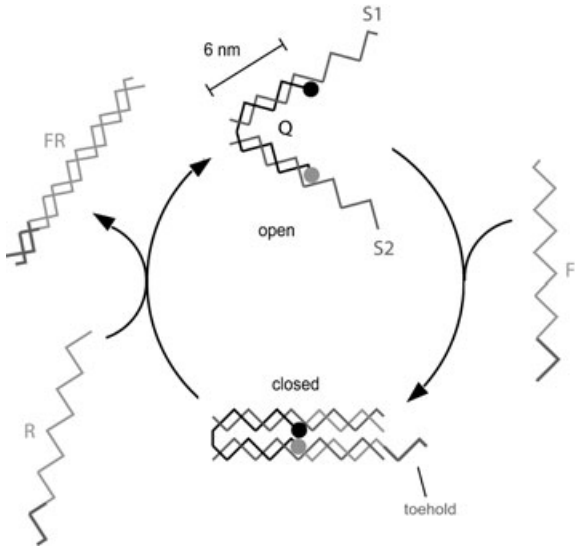

Figure 5. DNA tweezers. In the "open" state the tweezers structure is formed by three strands S1, S2, and Q. As indicated, the "fuel" strand F can hybridize partly with the single-stranded section of S1 and partly with S2. This closes the tweezers structure. In the "closed" state, F still has an unhybridized single-stranded section (the "toehold"), where the complementary "removal" strand R can attach. F is removed from the tweezers by R via branch migration (see Figure 6) and restores the open state. Two fluorescent dyes are indicated (black and gray dots), which are used for FRET characterization of the device (adapted from Ref. [76]).

Two 24-nt-long sections of S1 and S2 remain unhybridized and form single-stranded extensions of the arms. To drive the machine into a closed conformation a fourth, 56-nt-long DNA strand termed the "fuel strand" F is added of which 24 bases are complementary to the unhybridized section of S1 and another 24 bases are complementary to the extension of S2. The hybridization of F with these device sections pulls the arms of the tweezers structure together. The remaining eight unhybridized bases of F serve as a point of attachment for another DNA strand R (the "removal

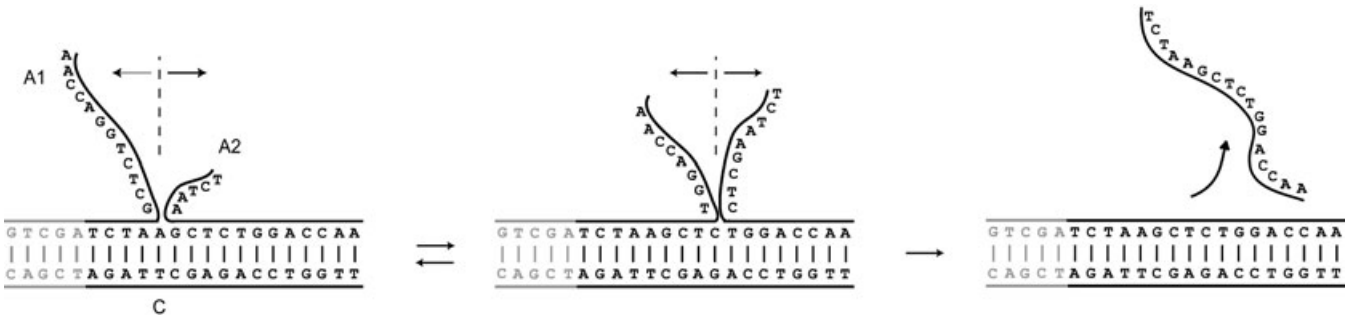

Figure 6. Branch migration: Apart from the gray "toehold" section, strands A1 and A2 are both complementary to strand C. The branch point can perform a random walk along strand C. Due to the toehold section, A1 cannot be completely displaced from C by A2 and finally "wins" the competition.

strand"), which can displace F from the device by a process called "branch migration".

In three-stranded branch migration, two homologous DNA strands A1 and A2 try to bind to one complementary strand C (see Figure 6). At the branch point where the two competing strands meet, thermal fluctuations may break a base pair, for example, between A1 and C, which gives A2 the opportunity to make a base pair with C. Such processes lead to a random motion of the branch point along C. In the case of short sequences C, one of the competing strands A1 or A2 will finally bind with its full length and completely displace the other strand from C. The four-stranded version of this process is well known from genetic recombination in which two homologous DNA duplexes "cross over" and exchange strands.[1] Three-stranded branch migration with an average step time of approximately 10–20 μs[77,78] is considerably faster than its four-stranded counterpart (step times of one to several hundreds of ms[78]). For short stretches of DNA, strand removal occurs relatively fast. The removal of a 40-base-long DNA molecule takes about 20–80 ms.[79,80]

In the case of the tweezers, branch migration leads to the formation of a "waste" duplex F–R and returns the S1-Q-S2 structure of the tweezers to their starting configuration. By the alternate addition of fuel and removal strands, the device can be cycled through its "open" and "closed" states many times. As described for the DNA devices in the previous Section, the operation of the tweezers can be monitored by FRET between fluorophores attached to the ends of the arms of the device.

Quite generally, strand displacement by branch migration provides a tool by which DNA hybridization can be employed for DNA devices in a reversible manner. Duplex formation can be used to pull molecules together or stiffen the connection between two nodes in a network. These structural transitions can be reversed by adding recognition tags to the DNA effector strands, which are recognized by removal strands and from where branch migration can begin. This principle has been utilized recently in a variety of other DNA nanodevices.

In a DNA "actuator", the single-stranded extensions of the arms of the DNA tweezers are joined to form a closed "motor section".[81,82] This allows the device to perform both pulling and stretching movements (see Figure 7).

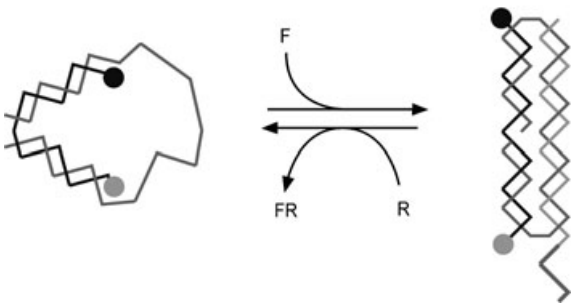

Figure 7. Stretching motion of the DNA actuator: The actuator is similar to the DNA tweezers, but with the two arms connected by a single-stranded loop. Hybridization of the loop with fuel strand F stretches the device. The relaxed state can be restored by branch migration using the removal strand R. A different kind of fuel strand can be used to close the structure analogously to the DNA tweezers (adapted from F. C. Simmel, B. Yurke, *Phys. Rev. E* **2000**, *63*, 035320).

Another variation of the tweezers was termed "DNA scissors".[83] In this case, two sets of tweezers structures are joined at their hinges by short carbon linkers. The motion of one set of tweezers is transduced to the other part, resulting in a scissors-like movement.

A more complex device was constructed by Seeman and co-workers based on multiple crossover motifs.[84] Using branch migration, a DNA structure can be switched between a "paranemic crossover" conformation and a "juxtaposed" configuration. The paranemic crossover motif is a four-stranded DNA structure which can be thought of as consisting of two adjacent double helices of which strands of the same polarity are exchanged at every possible site (Figure 8a).[85] If parts of this motif are removed and replaced by DNA sections without crossover, molecules such as the "juxtaposed" JX_2 structure (Figure 8a) result in which two helices are rotated by 180° with respect to the corresponding section in the "paranemic crossover" PX structure. In the PX–JX_2 device, the central section of a construct based on the PX motif is defined with DNA molecules, which can be removed by branch migration (Figure 8b). After removal, these molecules can be replaced by DNA strands that switch the device to the JX_2 configuration. Again using branch migration, the device can be switched back to the original PX structure. As shown by

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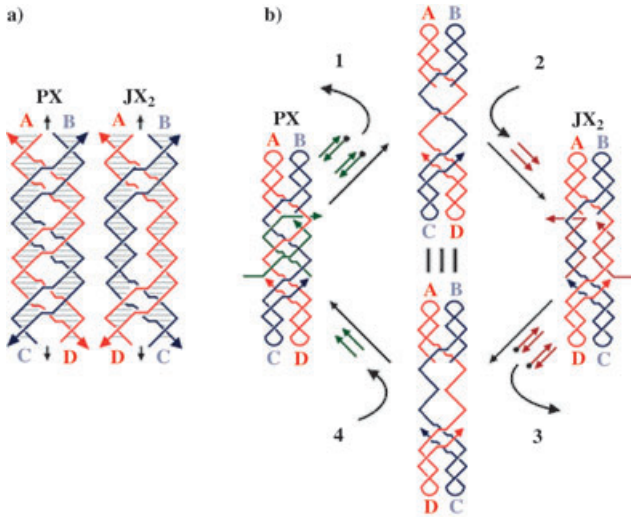

Figure 8. The paranemic crossover device: a) Two double strands of DNA cross over at every possible site to form a paranemic crossover (PX) structure. Removing the inner two crossovers juxtaposes the helices D and C with respect to each other (JX_2); b) from this PX–JX_2 transition, a reversibly switchable device can be constructed. The innermost double crossover of the PX structure can be resolved by removing the green strands by branch migration (1). Insertion of the violet strands leads to the formation of the JX_2 structure (2). JX_2 can be switched back to PX by another branch-migration and strand-insertion process (3,4). Reproduced with permission from Ref. [84].

Seeman and co-workers,[84] the switching between the two states of the device could be impressively visualized with AFM. Yan and co-workers recently showed that even the topology of large supramolecular DNA networks could be switched employing a similar mechanism.[86]

Other devices utilizing branch migration are based on unusual DNA tertiary structures such as the G quartets already encountered in Section 3.1. In the experiments described by Li and Tan[87] and by Alberti and Mergny,[88] a single-stranded DNA structure formed by stacked intramolecular G quadruplexes is switched to a stretched duplex structure by the addition of the complementary DNA fuel strand. This transition can be reversed by removing the fuel strand using branch migration as described above.

Among the great advantages of using DNA as a fuel rather than a small molecule is its sequence specificity—DNA not only drives the motion, it also serves as an address label for a device. In a situation with several distinct DNA devices, specific devices can be operated with specific fuel molecules. Recently this notion was particularly emphasized by Shin and Pierce,[89] who have specifically addressed single-stranded DNA labels attached to a double-stranded DNA scaffold. DNA or RNA strands can also be seen as a specific molecular signal to which a DNA device can respond by mechanical action (examples for this will be discussed in Section 6.).

3.3. The Problem of Free-Running Devices and Waste Products

The devices described so far are all driven by a similar principle: An external operator adds some sort of chemical fuel (ions, protons, DNA strands) that drives the system out of equilibrium, that is, under the new conditions the devices are not in a thermodynamically stable state. The system then decays to a "new" equilibrium, which results in a conformational change accompanied by some sort of motion. This process can be reversed by adding a different kind of fuel (buffer exchange, chelators, hydroxide ions, complementary DNA strands), which neutralizes the effect of the first fuel chemical. Again, the system decays to the new equilibrium state which is, almost, the same as the old one. There are a variety of problems with this approach. First of all, the devices described so far are operated in closed systems, that is, at constant temperature and pressure with no material exchange with the environment except when the fuel is added. This means that the waste products resulting from the reaction between fuel chemicals remain in solution. Therefore, of course, the system after one operation cycle is not exactly the same as the starting system. In the case of proton-driven devices, the waste products typically are NaCl and H_2O. This leads to a continuous dilution of the devices and also demands a continuous increase in the amount of fuel added.

For DNA-driven devices, the problem arises that fuel and removal strands have to be chosen in a complementary fashion. When working with stoichiometric quantities, each reaction cycle produces an amount of waste duplex DNA equal to the total amount of device strands. A practical problem related to this is to add the strands in exact stoichiometry. If the fuel and removal strands are not added in exactly equal amounts, in each cycle some of the strands will be left over and immediately react with fuel or removal strands in the next cycle. This quickly deteriorates the device performance and ultimately brings it to a halt. If one tries to overcompensate stoichiometric errors in each cycle one faces the problem of an exponential build-up of waste strands and an exponentially growing need for fuel. A more fundamental problem, of course, is that the accumulation of waste will ultimately poison the system. Increasing amounts of waste products make it more difficult to drive the system out of equilibrium as their presence favors the "backward" reaction. For most practical purposes so far, where devices have only been cycled on the order of 5–10 times, this is not a severe problem. Waste duplexes with a length of 20–40 bp are extremely stable (the half life for the decay of an 18 bp duplex at 20 °C and 1 M NaCl is on the order of 10^9 years!) and therefore a back-reaction is not expected under normal experimental conditions. In the experiments by Yan and coworkers,[84,86] the waste problem has been tackled by using biotinylated fuel strands, which can be removed with streptavidin-coated magnetic beads after each reaction cycle.

The concept of alternating the addition of fuel and neutralizing chemicals also makes it difficult to construct continuously running devices that operate for several cycles without interference by an external operator. In the case of

the DNA tweezers and similar devices, fuel and removal strands cannot be added simultaneously as they would immediately react with each other rather than driving the device through its states. One possible solution for this problem is based on the control of hybridization kinetics between complementary strands.[90] As indicated in Figure 9a,

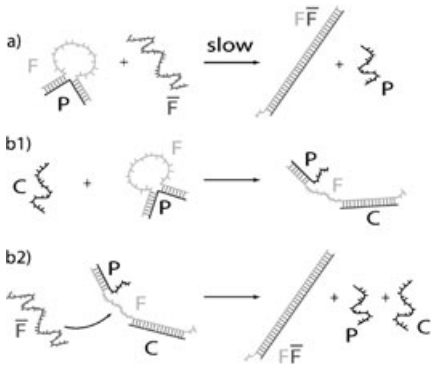

Figure 9. a) "Protection" strand P forces F into a loop conformation. In this conformation hybridization with the complementary strand $\bar{F}$ is slowed down considerably; b) in contrast, the short catalyst strand C can open the loop structure (b1). $\bar{F}$ can attach to the opened loop structure and displace strands P and C from F (b2). C continuously switches between a single-stranded conformation and a stretched duplex conformation (in F-P-C), until all of the fuel FP + $\bar{F}$ is used up (adapted from Ref. [90]).

the hybridization between two complementary strands F and $\bar{F}$ can be slowed down considerably by forcing the strand F into a loop conformation using a protection strand P. Hybridization can be accelerated by using a "catalyst" strand C, which can open the loop structure (Figure 9b). The intermediate structure F-P-C can be attacked by $\bar{F}$, which leads to the formation of the waste products F–$\bar{F}$ and P. Strand C is then available to open another F–P structure. Such a catalyst strand C could be incorporated into a DNA device, which would then be continuously driven through its states by the consumption of the metastable "fuel pair" F–P and $\bar{F}$. The fuel molecules could be added simultaneously as a reaction with the DNA device would be much faster than a direct reaction. This approach is analogous to the enzymatic action of biological molecular motors and machines. In biological systems, energy is stored in metastable fuel molecules such as ATP. The breakdown of ATP to ADP and phosphate is catalyzed by molecular motors, and the energy gained from the reaction is used to produce motion. Other approaches towards free-running systems based on enzymes or on ribozyme action are discussed in Sections 4.1. and 5.3.

It is clear that the realization of self-running devices will also require the adaptation of new experimental techniques for research on DNA nanodevices. So far, the "clocked" operation of DNA devices greatly simplifies their characterization as their function can be assessed in bulk experiments. All the devices are driven out of equilibrium at the same time and collectively (but incoherently) change to the conformation of their next state. Unsynchronized free-running devices will require techniques for single-molecule observation as they are common for the characterization of biological molecular motors.[13]

The problems discussed in this Section also point towards an important difference between the artificial devices realized so far and biological motor proteins: Biological systems are open systems that operate under non-equilibrium conditions. The fuel consumed by molecular motors (such as ATP) is a metastable chemical, which is continuously supplied as a result of energy-producing catabolic reactions to which motor action (in general, in the form of energy-consuming reactions), is ultimately coupled.

4. Unidirectional Motion Driven by DNA

One of the most challenging goals in the field of artificial molecular devices is the construction of a molecular machine with similar capabilities to a biological molecular motor. In biology, forces are generated in a variety of ways, for example, by polymerization of cytoskeletal filaments or by conformational changes of motor proteins such as myosin or kinesin.[13,91–93] These motor molecules are responsible for most of the motion exhibited by living organisms. They drive cilia and flagella, are responsible for muscle contraction and also actively transport substances within cells.[13,94] A large variety of other processes in living cells are carried out by complexes of proteins that can be viewed as sophisticated assembly machines.[1] Many of the features exhibited by these biological examples would also be desirable in an artificial context, for instance, to aid in nanoscale assembly processes or to provide micro- and nanostructures with the capability of movement.

Biological motors like the myosins, kinesins, or dyneins are complex proteins that walk on supramolecular tracks such as actin filaments or microtubuli.[13,94] Even though it is hard to imagine how such a sophisticated machinery could be built on the basis of DNA alone, the general features of these motors can serve as a guideline for the construction of artificial DNA motors. In analogy to the biological motors, the recent DNA "walker" systems (which will be discussed later) consist of tracks made of DNA that have single-stranded "binding sites" for a DNA walker. The walker can be just a single DNA strand or a slightly more complex DNA structure, which can be translocated unidirectionally along the DNA track using various techniques. Directionality is introduced by using unique sequences for the binding sites, that is, essentially by making an asymmetric track. So far, this has resulted only in very simple devices capable of a rudimentary form of locomotion. However, these are important "first steps" in the right direction.

4.1. Walking with the Help of Enzymes

An ingenious approach to achieve autonomous unidirectional motion using DNA and three DNA-modifying enzymes was employed by Yin et al.,[95] who created a double-stranded DNA "track" with three anchorages A, B, and C,

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which are connected to the track with a 4 nt hinge (see Figure 10). Each anchorage has a 3 nt unhybridized section at its end. The DNA walker itself consists of a total of six nucleotides that reside on the ends of the two strands of anchorage A in the starting configuration (denoted A*). Anchorage A* can hybridize with the sticky end of anchorage B, then T4 ligase is used to covalently link the anchorages to form the structure A*B. The base sequences of A and B

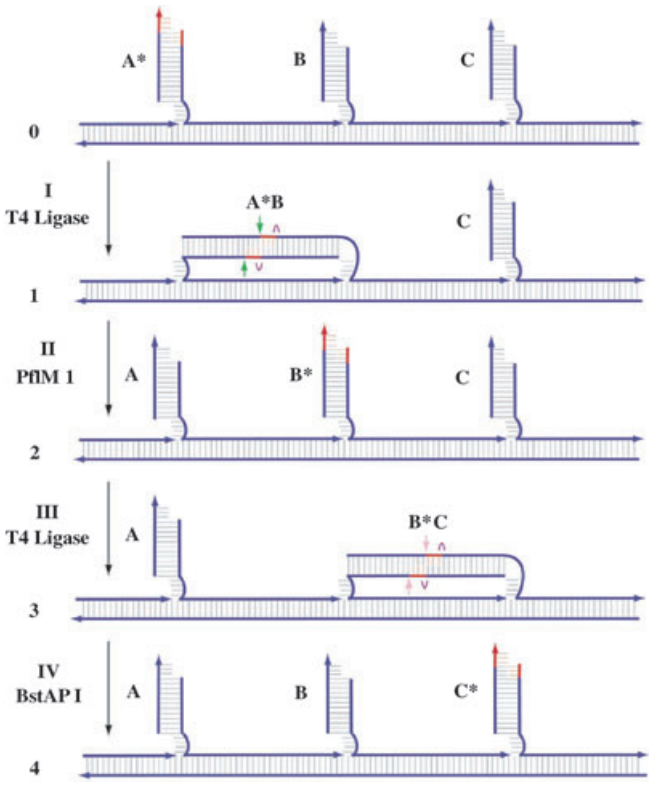

Figure 10. An enzyme-assisted DNA walker: The six nucleotides marked in red are transported from anchorage A to anchorage C in steps I–IV. This is achieved by joining neighboring anchorages with T4 ligase and cleaving with two different restriction endonucleases (PflM I and BstAP I). The operation principle of the device is described in more detail in the text (reproduced with permission from Ref. [95]).

are chosen in such a way that A*B contains a site that is recognized by the restriction enzyme PflM I. After the cutting of A*B by the enzyme, the six walker nucleotides reside on anchorage B (i.e., B*). The new sticky end can now base-pair with anchorage C, and then B* and C are again covalently linked by T4 ligase. The duplex B*C now contains the recognition site of the endonuclease BstAP I. After cutting by the enzyme the six nucleotides of the walker are attached to anchorage C. Overall, the six nucleotides originally residing on the end of anchorage A have been translated to C by the action of T4 ligase, PflM I, and BstAP I without external interference. Ligation consumes ATP as fuel and constitutes the irreversible step in the device operation. The direction of the motion is determined by the restriction sites and the walker cannot step back.

4.2. DNA Walkers and Gears

In contrast to the device discussed in the previous Section, the following devices do not need enzymes for their operation. However, they are not autonomous and have to be driven by the addition of fuel strands.

A very simple walker related to the rewritable DNA memory mentioned in Section 3.2. was recently demonstrated by Shin and Pierce.[96] A double-stranded DNA scaffold with single-stranded DNA labels attached to it served as a track for the walker—similar to the scaffold the authors described elsewhere.[89] The walker itself is a DNA duplex with two single-stranded extensions. As described in Figure 11, specific DNA strands are used to connect the single-stranded extensions to the labels on the track. The connector strands are equipped with single-stranded toehold sections and can be displaced from the device by their complementary strand via branch migration. The free-DNA "foot" can then be connected to the next free-label strand on the track. This can be repeated several times with the appropriate connector and removal strands to move the walker to arbitrary addresses on the track.

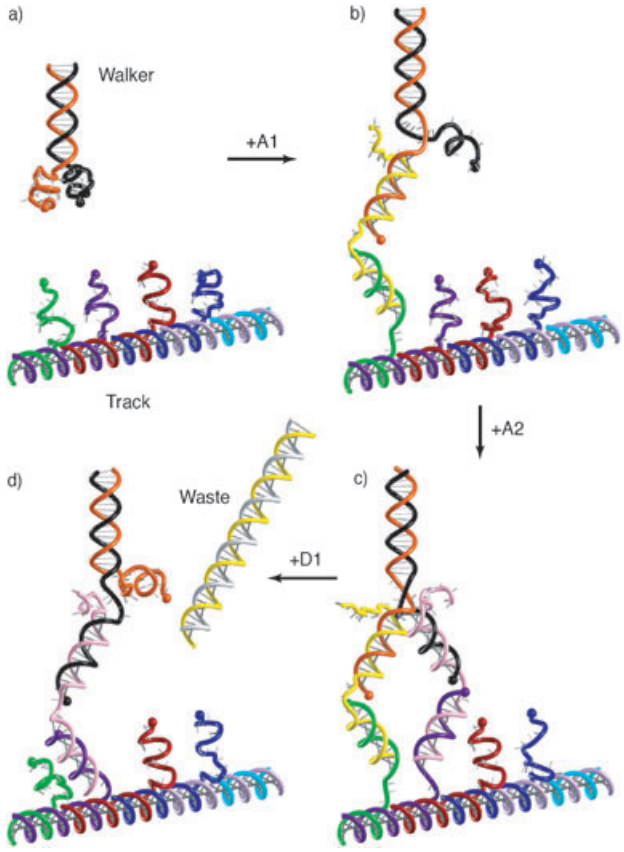

Figure 11. A DNA walker based on hybridization and branch migration: a) The walker consists of duplex DNA with two single-stranded "feet". The track is duplex DNA with single-stranded extensions as binding sites for the walker; b and c) the feet of the walker can be attached to the track using linker molecules A1 and A2; d) a linker can be displaced from the track by a removal strand (D1). The foot is then free to bind to the next binding site on the track using another linker molecule (reproduced with permission from Ref. [96]).

A very similar principle, but with a more complex arrangement, was adopted by Sherman and Seeman.[97] Here the walker (a "biped") consists of two DNA duplexes joined by flexible single-stranded linkers. This biped device can be translocated along a track that consists of a triple crossover (TX) molecule containing single-stranded address labels. The duplexes of the biped also contain single-stranded extensions and can be attached to the addresses on the track via DNA linker molecules. Again, these connections can be removed by branch migration and the free foot can be connected to the next "foothold".

A different kind of unidirectional motion was recently realized in the "molecular gears" system by Tian and Mao.[98] In this system, two DNA "circles" roll against each other driven by a mechanism based on hybridization and branch migration. The two circles consist of a closed single strand C, to which three other strands are hybridized. These strands contain flexible hinges made of thymine residues and single-stranded extensions (termed "teeth"). The two circles can be connected by DNA linker strands, which are partly complementary to one "tooth" of one circle and another tooth of the other circle. Due to the flexible hinges in the construction, two circles can be actually connected by two linker strands simultaneously. As in the case of the walkers, the linker strands contain single-stranded overhang sections to which removal strands can attach and displace the linker strands from the device, thus leaving the circles with only one connection. By the alternate addition of linker and removal strands in the correct order, the two circles can be made to roll against each other in one direction.

It is important to note that only the enzyme-driven devices can work autonomously so far. All the other walkers have to be driven from outside by a rather awkward procedure in which they are forced to take one step after another and each binding site has to be specifically addressed. In these cases, an external operator actually has to keep track of where the walker currently stands in order to move it in one direction. To displace a device over a longer distance, many cycles of binding and unbinding have to be externally controlled.

With the exception of the triple-crossover track in the device described by Sherman and Seeman,[97] the tracks used for the walkers are rather floppy. This is not a problem at the moment as all the prototype walkers only make one step or two. To achieve locomotion over longer distances, however, the track would need to be more stiff. Floppy tracks also would lead to extensive crosslinking within a track or between several tracks, as a DNA walker could easily bind to a distant binding site. The DNA tracks used so far are basically double-stranded DNA with nicks at the positions of the labels, that is, their persistence length is expected to be less than that of duplex DNA. In comparison, biological "tracks" such as F-actin or microtubuli have persistence lengths of 15 μm and 6 mm.[13] Possible solutions to this problem are to use stiffer DNA structures as tracks (such as the TX molecules) or to immobilize the DNA track in an appropriate way.

Finally, none of the devices are based on a conformational transition comparable to the "power stroke" that gives kinesin and myosin proteins their directionality.[13] The DNA walkers simply detach from the track by some mechanism and find their next binding site diffusively. A power stroke may not be necessary for many applications, but it is unclear how well diffusive DNA motors will work under a load.

5. DNA Devices Incorporating Functional Nucleic Acids

5.1. Aptamers and Ribozymes

The role of DNA in the previously mentioned machines is to provide a structural basis and the chosen base sequence determines the switchable elements that enable reversible motion. Thus, the DNA is relatively passive. To progress to the next stage of complexity, beyond just movements resulting from configurational changes, DNA machines should be able to perform functional tasks. This requires the incorporation of functional and active units within these devices. Functional nucleic acids are found in aptamers and ribozymes.[99–101] These are specific sequences of generally single-stranded RNA and DNA, which adopt a tertiary structure capable of a variety of activities including ligand recognition[102–104] and the catalysis of a number of chemical reactions, among them cleavage,[105–107] ligation,[108,109] and peptide-bond formation.[110]

Although natural ribozymes can be found, the vast majority have been artificially created through in vitro evolutionary selection techniques. In the systematic evolution of ligands by exponential amplification (SELEX),[99] DNA or RNA sequences capable of specific and high-affinity binding to proteins or small molecules are isolated from a pool of over 10^{15} chemically synthesized sequences, which have been partitioned and amplified based on their ability to bind to the target. After 5–10 rounds of selection, the pool is highly enriched with sequences that have the desired ligand-binding property. Modifications of this process also allow for the selection of sequences capable of catalyzing reactions such as cleavage and the ligation of nucleic acids.[101]

As was anticipated in a review by Niemeyer and Adler,[111] one of the next logical steps in the development of DNA-based molecular machines was to include functional nucleic acids into DNA nanodevices. By combining aptamer and ribozyme sequences with the structural and switchable elements in DNA nanomachines, devices capable of performing useful tasks can be constructed. One such functional nanomachine containing an aptamer sequence was developed to bind and release a specific protein upon instruction. The catalytic property of ribozymes has also been taken advantage of to allow a DNA machine to continuously switch between two states.

5.2. Aptamer-Based DNA Devices

Although nucleic acids have only four different subunits and thus, compared to proteins, a limited number of chemi-

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cal moieties for binding, aptamers can bind a surprisingly broad range of proteins and small molecules with affinity comparable to that of antibodies to their antigen. This functionality of aptamers has been used in the construction of a machine that can controllably bind and release a single protein molecule. A "nanohand" was created by combining a DNA aptamer selected to bind the human blood-clotting protein, thrombin, with the operating principles of DNA-based nanomachines.[112] The 15 nt aptamer sequence 5′-GGTTGGTGTGGTTGG-3′ folds into a secondary structure consisting of two G-quartets, in the presence of potassium ions (Figure 12). The machine in its standard state binds thrombin. It can be instructed to release the protein by the addition of single-stranded DNA containing a sequence that

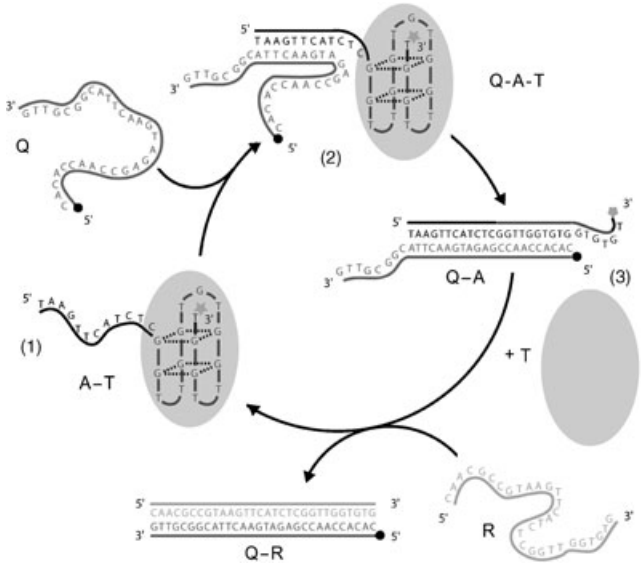

Figure 12. A switchable aptamer device: In the folded state (1) the aptamer device A can bind to the protein thrombin (T). In (2) the opening strand Q attaches to the 12 nt long toehold section 5′-TAAGTTCATCTC-3′of the device and displaces the protein. In the stretched duplex conformation (3) the device does not bind to thrombin. The removal strand R can attach to a second toehold section in strand Q and displace Q from A by branch migration. Strand A then refolds and binds the protein thrombin again (reproduced from Ref. [112]).

is complementary to a portion of the aptamer sequence; this results in an unfolding of the aptamer to form a duplex, which is unable to bind the protein. The protein can be bound once again by the addition of another DNA strand, fully complementary to the previously added single-strand DNA, which permits the aptamer machine to be released and return to its protein-binding quadruplex form. The operation of this machine can be monitored using FRET with dyes positioned as shown in Figure 12. The release of protein by the machine can also be directly followed with fluorescence anisotropy experiments. In principle, such a nanohand can be constructed to bind any protein and ligand for which an aptamer exists.

5.3. Autonomous Machines based on Nucleic Acid Enzymes

Hybridization-driven machines rely on the external addition of single-strand DNA at well-defined stages to bring about motion. One major aim for nanomachines is the development of strategies for autonomous operation in which the machines function free of human intervention and ultimately independently act based on decisions made in response to stimuli (see also Sections 3.3. and 6.). One possible realization of autonomy was demonstrated by Chen et al. in a machine consisting of a DNA actuator[81] modified with an RNA-cleaving DNA enzyme.[113] In its standard state, the DNA enzyme is in a compact form and results in a closed-state for the actuator (Figure 13). In the presence of an RNA substrate (actually, a DNA/RNA chimera), the

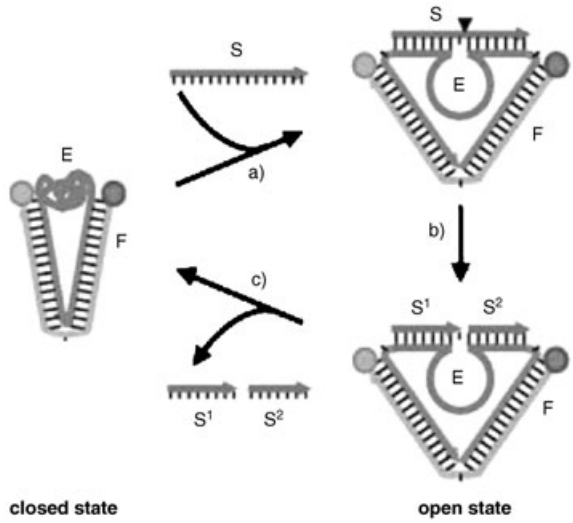

Figure 13. An autonomous device incorporating a DNA enzyme: a) The device consists of two duplex arms formed by the strands F and E. Strand E contains the sequence of an RNA-cleaving DNA enzyme. When the chimeric RNA/DNA substrate S binds to strand E, the arms of the device are opened; b) substrate S is cleaved by the enzyme into shorter strands S1 and S2; c) the duplexes with S1 and S2 are less stable and the strands S1 and S2 dissociate from strand E. The device then returns to its collapsed initial state. This operation cycle is repeated autonomously until all substrate is use up (reproduced with permission from Ref. [113]).

enzyme extends to its catalytically active structure as it forms a duplex with the substrate and the actuator assumes its open configuration. Cleavage of the substrate causes a weakening of the hybridization forces and the cleaved substrate is released, which leads to the collapse of the enzyme and thus an automatic closure of the actuator. As long as the substrate is present, the machine can undergo several cycles of operation, in this case 20 in 30 min, independent of external influence. When using a non-cleavable DNA substrate, the device can also be deliberately forced into a stalled configuration.[114]

6. DNA Devices and Information Processing

The seminal paper by Adleman[115] on the solution of the Hamiltonian path problem using molecular biology techniques marks the starting point for the fascinating research area of DNA computing (see, for example, Refs. [34,116–121]). An overview of this field will not be attempted here, however, it should be stressed that it is important to also consider the information-carrying nature of DNA in combination with the information-processing abilities of many enzymes as important parameters for the construction of future DNA-based nanodevices.

6.1. Autonomous Computing with DNA

An ingenious approach towards autonomous information processing with DNA-based devices was taken by Benenson and co-workers.[122–124] They utilized the unique DNA-cleaving properties of the class IIS restriction endonuclease *Fok*I in combination with clever DNA-sequence design to build the molecular realization of "finite automata". Finite automata are computing machines with a finite number of internal states, which can undergo transitions between these states according to certain rules. A computer program for such an automaton prescribes a series of such transitions. The hardware for the automata originally consisted of the restriction enzyme *Fok*I, T4 ligase, and ATP (later it was shown that ligase and ATP were not essential for the computation[123]). The software was a mixture of specially designed DNA molecules. The operation of the automaton is based on the fact that *Fok*I cleaves its double-stranded substrate 9 and 13 nt offset from its recognition site, thereby creating a 4 nt sticky end. The various states and input symbols for the automaton are therefore represented by different 4 nt long sequences. Benenson et al. use *Fok*I to sequentially cleave off pieces of a double-stranded program and thereby create a series of transitions between the various states of the automaton.[122] Finite automata are related to Turing machines but have limited computing power. The DNA automata were used to make simple decisions, such as to determine whether a given input symbol occurs more than once. In a more recent paper, Benenson et al. were able to demonstrate how such DNA automata could actually find applications in autonomous diagnosis and drug-delivery systems.[125] To this end, the transition molecules for a *Fok*I-based DNA automaton similar to the one described in Ref. [122] were designed to be active only in the presence of specific mRNA molecules. With these transition molecules, a molecular computer could be realized that could decide whether the levels of certain mRNA molecules were high or low. Depending on the decision, a short DNA strand was administered as a "drug". The authors chose a realistic scenario: The mRNA levels sensed by their automaton indicate the underexpression or overexpression of genes involved in prostate cancer. The DNA drug released after the computation could inhibit the synthesis of a cancer-related protein by binding to its corresponding mRNA.

A different approach towards autonomous computing with DNA is based on DNA enzymes (deoxyribozymes, see Section 5.). Stojanovic and co-workers[126] designed a variety of logic gates based on the action of the RNA-cleaving deoxyribozymes E6[127] and 8–17.[106,128] A chimeric DNA/RNA hybrid structure with an RNA base at the appropriate position is cleaved by the DNA enzymes when bound to their substrate recognition sections. These recognition sequences can be protected by self-hybridized stem–loop modules. By base-pairing with a DNA strand complementary to the loop sequence, the stem–loop modules can be opened. This makes the substrate recognition sequence accessible for the substrate to be cleaved. By combining several such controlling and catalytic modules, it was possible to realize simple logic gates such as AND or XOR,[126] a DNA half-adder,[129] and a molecular computer that could autonomously play (and win) the game "tic-tac-toe".[130]

The significance of these results for the field of DNA nanodevices is the fact that DNA molecules here serve as an input for an autonomous (molecular) computation process and also as an output. This opens up a fascinating possibility: Coupling a computational module to a DNA nanomechanical device would allow the control of the action of the device in response to an external molecular signal. This could also be made conditional, that is, depending on the nature of the signal, a variety of actions could be taken. Similarly, the temporal/logical order of the action of a variety of devices could be controlled by computational processes. It is also conceivable that DNA-based devices could interact with each other by exchanging signaling molecules.

6.2. Genetic Control Circuits

It has long been recognized that biochemical reaction networks have information-processing properties.[131,132] This is particularly obvious in the case of genetic networks in which the expression level of proteins is regulated by complex interactions between regulatory molecules (such as transcription factors) and genes. The best-studied example of a genetic switch is the *lac* operon in *E. coli* bacteria, in which the expression of the protein β-galactosidase is regulated by the relative abundance of lactose and glucose. Since the discovery of the *lac* operon many gene regulatory motifs have been found, which usually rely on feedback mechanisms and nonlinear cooperative phenomena. Quite recently, knowledge about these motifs has also been used to construct artificial gene networks. By transferring circuit designs known from electrical engineering to artificial regulatory networks, such functions as a bistable genetic switch,[133] a genetic oscillator,[134] or a sender–receiver system[135] could be synthetically implemented in bacteria and similarly in vitro.[136] An overview of the developments in "synthetic biology" can be read elsewhere.[137]

It is quite natural to think about a possible utilization of gene regulatory mechanisms to control the behavior of DNA-based nanodevices as conformational changes of these devices can be brought about by DNA or RNA strands. A first step in this direction was taken when DNA

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tweezers (see Section 3.2.) were operated by an mRNA fuel strand that was transcribed from an artificial "fuel gene" (see Figure 14).[138] By regulating the transcription of fuel genes by using regulatory motifs known from natural and artificial genetic networks, it should be possible to program complex behavior into DNA devices or to intimately couple their operation to genetic mechanisms.

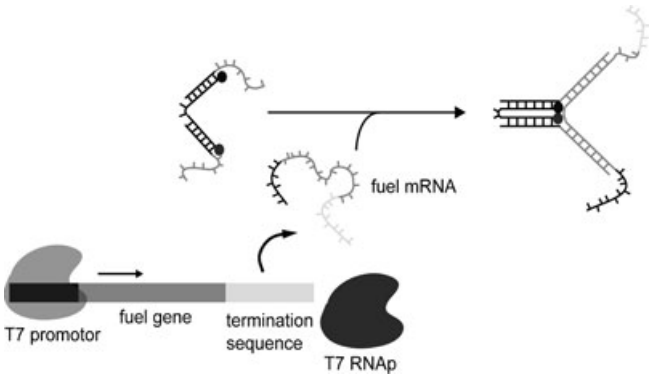

Figure 14. Transcriptional control of DNA tweezers: Instead of using a DNA fuel strand, the fuel for the tweezers is encoded in an "artificial gene" with a promoter and a termination sequence for the T7 RNA polymerase. The polymerase translates the fuel gene into "fuel mRNA" which closes the tweezers (adapted from Ref. [138]).

7. Outlook

In this Review, we have presented an overview of the current state of research into DNA-based devices. In addition to the impressive accomplishments in DNA nanoconstruction, DNA has now proven to be also an interesting material for the realization of artificial molecular machines. DNA has been used to induce simple nanoscale movements, to grab or release molecules, and to process information; it has been further shown that these tasks can be performed in an autonomous way. In the future, the various capabilities of DNA devices will be integrated into more complex molecular machines. One could think of a combination of a DNA molecular computer, a DNA motor, and a DNA aptamer in which the computer decides where a molecule attached to a DNA aptamer is to be brought by the motor and when it has to be released. As shown above, all of the components for such a device have been demonstrated already. Such systems could be important in the context of nanoassembly or intelligent drug delivery—depending on the application in an artificial or in a biomedical context.

It will also be extremely interesting to see whether one can couple these "semi-biological" devices to inorganic nanostructures or, at the other extreme, to utilize them in a biological context. Nano–bio hybrids could find applications as novel biosensors or biomolecular actuators; DNA devices could assist in molecular self-assembly or be part of biomolecular diagnosis and drug-delivery systems.

Of course, there are also many fundamental issues to be addressed: What forces can be effectively generated by DNA-based motors? What is the stalling force when under load? How fast can a DNA motor run? To answer these questions, advanced biophysical techniques will have to be applied to these artificial molecular devices, in particular techniques for single-molecule characterization.

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