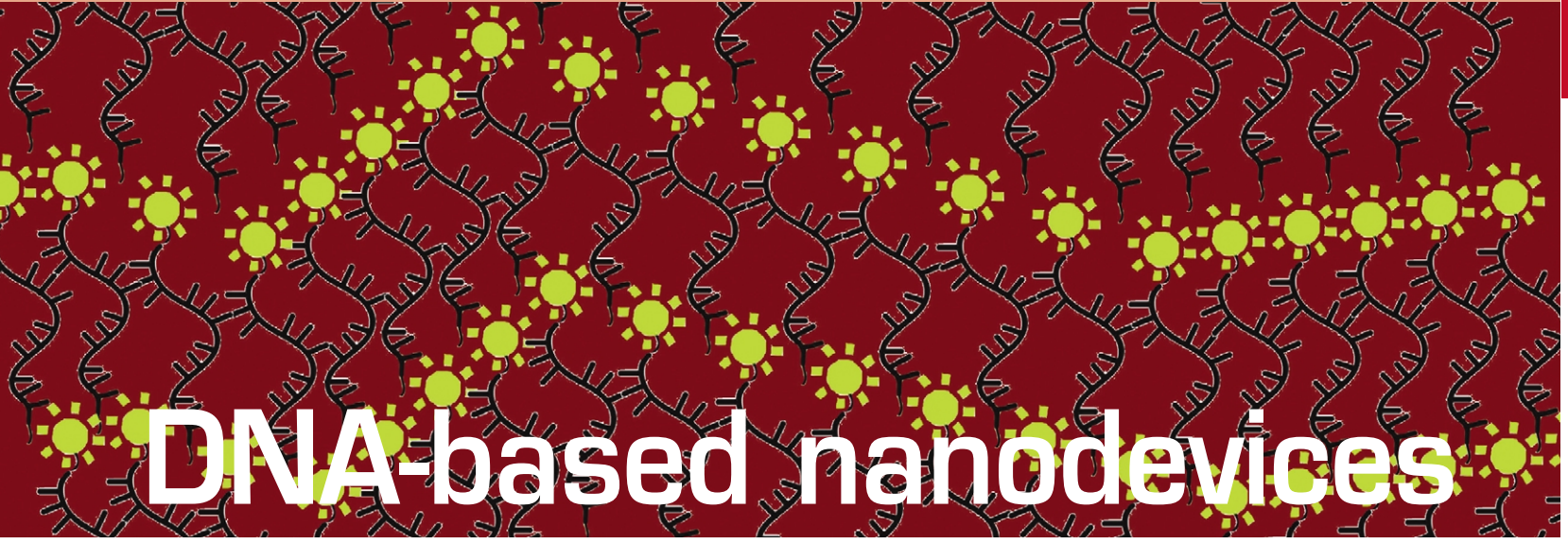

DNA-based nanodevices

DNA is not only in the focus of modern molecular biology, but also plays an increasingly important role as a building block for nanoscale materials and devices. In recent years, many researchers in nanoscience have used the unique, programmable molecular recognition properties of DNA to build nanostructures by self-assembly and to realize artificial, machine-like devices. We here give a brief survey of this field and discuss the possible applications of DNA-based nanodevices either as nanoscale motors and actuators, or as novel biosensors with built-in information-processing capability.

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Current research on molecular devices and machines is extremely diverse[1]. Biophysicists and biochemists gain increasing insight into the nanomechanical principles of operation of the many machine-like macromolecular complexes found in living cells. There is a huge amount of research on the 'obvious candidates' such as the myosin[2] family or kinesin[3] – molecules that have the function of molecular motors[4]. However, it has become clear in recent years that many other protein complexes display intimate coupling of nanomechanical switching and, for example, enzymatic function[5]. On the other end of the complexity scale, organic chemists are trying to synthesize machine-like devices 'from scratch' using the enormous recent progress in supramolecular chemistry.

As a comparatively new 'class' of molecular devices, DNA-based nanodevices[6] assume an intermediate position between the biological and synthetic worlds. On the one hand, DNA nanodevices use the highly specific molecular recognition properties of DNA bases, which are at the heart of DNA's biological role as an information storage molecule. On the other hand, DNA devices are 'designed' and synthesized chemically. Usually, there is no biological counterpart of these artificially constructed DNA-based nanodevices, although in some cases they bear a remarkable resemblance to naturally occurring RNA structures such as ribozymes or riboswitches[7]. In the following, a short survey is given of the basic principles of DNA nanodevices and the methods used for their experimental characterization. Two of the major research directions in the field are discussed: (i) progress towards realization of artificial molecular motors based on DNA; and (ii) the use of DNA-based nanodevices as novel biosensors and their coupling to biological phenomena.

Coupling information processing and mechanical action with DNA

What makes DNA so attractive for molecular self-assembly and the realization of molecular nanodevices is the intimate relationship

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between DNA sequence and structure. Two DNA strands bind together in a double helix when their sequences are complementary. However, if there is a large fraction of base mismatches between the two strands, they will not bind but remain single stranded. As double-stranded DNA (dsDNA) is much more rigid than single-stranded DNA (ssDNA), control of the degree of hybridization allows the 'programming' of molecular structures composed of rigid and flexible elements. DNA sequences can also be regarded as molecular 'addresses' – the number of distinct sequences of length N is 4^N (there are four different DNA bases), and becomes extremely large for large N. Conformational changes induced by DNA duplex formation can therefore be designed in such a fashion that they are brought about by a specific input sequence. This allows, for example, the specific addressing of one type of device in a pool containing several species of DNA-based nanodevices. It also allows coupling of the action of DNA-based nanodevices to naturally occurring nucleic acids, e.g. mRNA, or to the output of artificial DNA-based information processing cascades as found in DNA computing.

Experimental methods for the characterization of DNA-based nanodevices

Experimental methods applied to the study of DNA-based nanodevices are, not surprisingly, very similar to the characterization methods of naturally occurring molecular structures and machines used in biochemistry and biophysics. One of the most commonly applied biochemical techniques to study DNA hybridization is gel electrophoresis. This technique is also extensively used to study hybridization events that induce conformational changes in DNA-based nanodevices. However, electrophoretic studies do not allow for more detailed biophysical and structural studies of the operation cycles of devices. Here, fluorescence resonance energy transfer (FRET) experiments have become the method of choice. FRET uses the nonradiative transfer of excitation energy between two fluorophores with overlapping emission and excitation spectra, which occurs via dipole-dipole coupling of the dyes. Energy transfer is distance dependent and efficiently occurs for dye-to-dye separations below 10 nm. FRET is therefore well suited to the characterization of conformational changes occurring in this range. As one turn of a DNA double helix is constructed from approximately ten base-pairs, corresponding to a length of approximately 3.4 nm, FRET is particularly useful for those devices composed of mechanical elements with lengths between 10 bp and 30 bp. Current advanced fluorescence spectroscopic methods allow for the characterization of single molecules, and have recently been applied to the study of DNA-based nanodevices[8,9]. Since molecular devices act as individual units and display considerable variability because of their 'statistical' nature, single molecule studies are expected to become increasingly important in this field. Apart from FRET between fluorophores, other distance-dependent energy transfer phenomena have also been used, e.g. quenching of fluorescence by metal surfaces[10,11], and coupling between surface plasmons in metal nanoparticles[12]. These additional energy-transfer events may allow one to investigate conformational changes on length scales other than those amenable to FRET studies. In some cases, the structure of DNA-based nanodevices in their various conformational states can be directly monitored using atomic force microscopy (AFM)[13,14]. This is only possible if the devices and their conformational changes are large enough to be observed by the AFM – e.g. by adding large supramolecular 'pointers' to the DNA structures, or by incorporating the switching elements into ordered, two-dimensional DNA lattices. Finally, the forces exerted by DNA-based actuators have actually been measured using the DNA-induced bending of microcantilevers[15]. This represents the first experimental proof that DNA-based nanodevices could eventually act as 'molecular motors'.

Toward molecular motors based on DNA

DNA-based nanodevices that can move or change conformation have been developed rapidly since the first example was reported in 1998[16]. However, none have reached the point of being of practical use and thus 'toward' is to be emphasized in the section title. So far, a number of different prototypes have been developed. These include devices that are driven by DNA hybridization and branch migration, walk and rotate, use Hoogsteen bonding to form multiplex (rather than double helix) DNA structures, and respond to environmental conditions. At least some of these concepts will need to be combined and/or new ones developed before such devices will be of practical use.

Three major features arising from Watson-Crick base pairing have been put into use: the different mechanical properties of ssDNA and dsDNA; single-stranded extensions ('toeholds'); and strand displacement ('branch migration'). For branch migration, single-stranded extensions at the end of dsDNA are used as points of attachment for 'effector strands' that specifically remove single DNA strands from the duplex.

In 2000, Yurke *et al.*[17] collated these ideas to build 'DNA tweezers' in which two DNA duplexes are connected by a short single strand acting as a flexible hinge (Fig. 1). The resulting structure is similar in form to a pair of open tweezers. By adding a 'set' strand to which the tweezers' ends hybridize, the tweezers can be closed. A 'reset' strand that attaches to a toehold on the set strand is then added. This reset strand displaces the set strand from the tweezers through branch migration, freeing them to open again. The tweezers' ends are labeled with a donor/acceptor fluorophore pair, and their relative movement is measured using FRET techniques.

Using similar concepts, Simmel and Yurke[18] have developed a nanoactuator that switches from a relaxed, circular form to a stretched conformation. 'DNA scissors' have been demonstrated by Mitchell and Yurke[19] in which two sets of tweezer structures are joined at their hinges with short carbon linkers. The motion of one set of tweezers is transduced to the other part, resulting in a scissor movement. Recently, a DNA-based nanodevice resembling tweezers has been applied as a

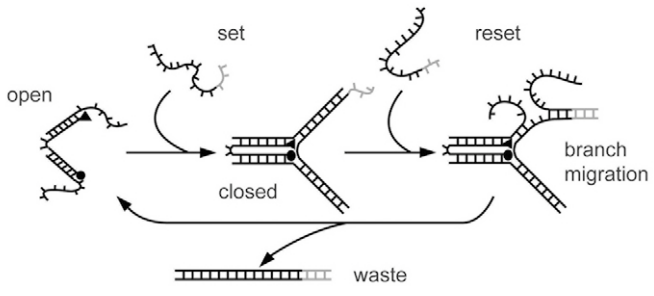

Fig. 1 In the open state DNA tweezers are composed of three strands of DNA forming two double-helical arms connected by a short single-stranded hinge. Hybridization with a 'set' strand closes the two arms. The set strand can be removed via branch migration using the fully complementary 'reset' strand, thus restoring the open state under formation of a 'waste' product. (Reprinted with permission from[65]. © 2006 Wiley-VCH Verlag.)

'force gauge' to establish how much work a DNA-distorting protein can do when it binds to DNA[20].

The tweezers have been refined by Chen *et al.*[21] who used the action of a DNAzyme (DNA molecule with enzymatic activity) to drive the device autonomously. Single molecule analysis of tweezers developed by Müller *et al.*[9] has led to the conclusion that addition of the closing strand does not always lead to perfect closure of the tweezers. Instead, other structures can occur, such as bonding of multiple closing strands per tweezer device, without the device closing. These unwanted forms need to be addressed with improved sequence-design of the device. Chhabra *et al.*[22] have now developed fully addressable DNA-based molecular tweezers along a DNA track to actuate coupling reactions in a programmable fashion.

A more complicated device structure has been developed by Yan *et al.*[13] based on 'paranemic-crossover' DNA. This is a DNA structure that can be formed by reciprocal exchange between strands of the same polarity on two DNA double helices at every possible position[23]. When parts of this structure are removed and replaced by DNA sections without crossovers, molecules in a 'juxtaposed' structure are produced in which two helices are rotated by 180° with respect to the previous structure.

There have also been considerable efforts to realize 'walking devices' that attach to 'track' strands, inspired by naturally occurring biological molecular motors such as kinesin. To achieve motion, enzymes are added in some cases to assist by providing new 'joining' and 'cutting' capabilities.

Shin and Pierce[24] introduced a simple walker (Fig. 2) that is a double-strand with two single-stranded extensions (acting as 'legs'). Specific 'fuel' attachment strands bind the legs to single-stranded extensions ('footholds') periodically placed along a double-strand track. Specific 'removal' strands unbind the legs using branch migration. Brownian motion provides movement, and the order of the adding attachment and removal strands directionality. A similar, more complex device has been developed by Sherman and Seeman[25]. Tian and Mao[26] have demonstrated molecular gears using essentially the same principles.

An autonomous device using ligase to bind connections, and restriction enzymes to cleave connections, has been developed by Yin *et al.*[27]. In contrast, Tian *et al.*[28] have produced DNAzyme walkers that move along a track consisting of RNA. Notably, the enzymatic properties of the walker provide cleaving as required, doing away with the need for extra restriction enzymes and ligases. A similar concept was recently used to construct 'molecular spiders'[29].

Under certain conditions DNA can form triplexes and quadruplexes. A shape-changing device similar in principle to DNA tweezers has been developed by Li and Tan[30] and Makita *et al.*[31]. The device is based on a structure known as the G-quartet, and functions using an added strand and branch migration. By using a protection strand as discussed previously, Wang *et al.*[32] have managed to improve the speed of such devices.

Structures other than G-quartets are possible; these are generally operated by changing the environmental conditions. Changing the buffer environment by addition and removal of intercalators, proteins, salt, and ion species have so far been used to activate devices, along with changes in pH levels and temperature. In 1998, Yang *et al.*[16] developed a system consisting of a circular duplex in the shape of a cruciform. By adding and removing ethidium, double-stranded branch migration extrudes the cruciform under negative super-coiling conditions. Niemeyer *et al.*[33] changed the concentration of Mg ions to induce a structural transition between two states in superhelical DNA.

The very first well-structured device involved a transition from right-handed B-DNA to left handed Z-DNA. Using a double-crossover (DX) structure with a poly-CG sequence, Mao *et al.*[34] switched the device by adding $[Co(NH_3)_6]^{3+}$.

As already mentioned, DNA can also form triplexes. In a DNA triplex, a third DNA strand binds along the major groove of a regular 'B'-form double helix by Hoogsteen bonding, a base-pairing mode alternative to the Watson-Crick scheme. Chen *et al.*[35] varied the pH to form an ordered triplex helix from a duplex and a disordered strand, resulting in nanomechanical motion. This has been used to control chemical reactivity[36].

A further structure is the 'i-motif', in which four DNA strands are held together by an unconventional base pair between a protonated

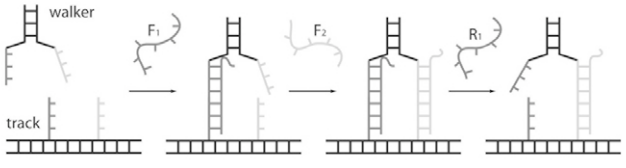

Fig. 2 A simple molecular walker based on DNA. The walker can be connected to single-stranded protrusions of a dsDNA 'track' using 'fuel' strands. The 'feet' can be selectively unlinked from the track using 'removal' strands and reconnected to other anchor points using new fuel strands. (Reprinted with permission from[65]. © 2006 Wiley-VCH Verlag.)

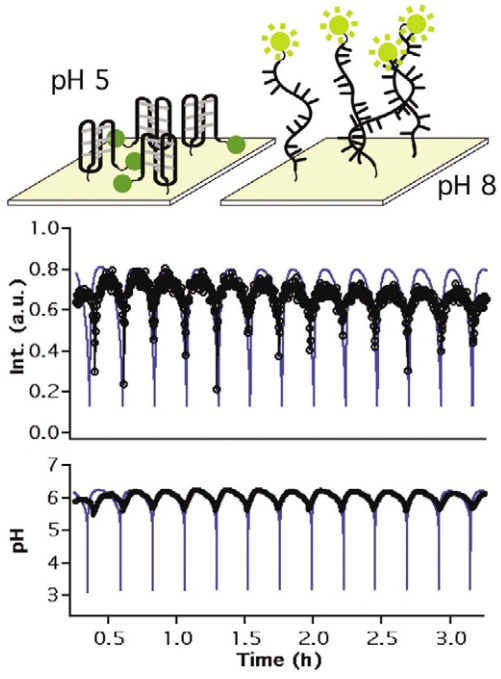

Fig. 3 At low pH values, the surface-tethered i-motif adopts its closed conformation, which forces the pH independent dye into close proximity with a Au surface. The resulting quenching of the dye (upper trace) can be monitored over multiple cycles of a pH-oscillation (lower trace) driven by a chemical oscillator. (Adapted from[11].)

and an unprotonated cytosine base. This pH-dependent structure has been studied intensively by Liu *et al.*[10,15,37]. Liedl *et al.*[11,38] used a chemical oscillator to drive proton concentration changes that generate a pH-based conformational transition between a random conformation and the i-motif. The system has been improved by attaching DNA to a solid substrate (Fig. 3) in a continuous flow stirred tank reactor, in principle allowing an infinite number of autonomous conformational switching events. Xiao *et al.*[39] have developed a thrombin-binding 'aptamer' (see next section) on a surface based on the G-quartet, while Fahlman *et al.*[40] used the same structure for a shape-changing device dependent on Sr ions and ethylenediaminetetraacetic acid (EDTA).

Recently, the pH-dependent self-assembly of Au nanoparticles based on the i-motif has been achieved by Seela and Budow[41]. The solution containing nanoparticles modified with the i-motif is observed to change from red to blue within a narrow pH range and is reversible.

Novel biosensors involving DNA-based nanodevices

Because of the properties described above and its inherent biocompatibility, DNA is perfectly suited for sensing tasks and information processing in a biological environment. In nature, DNA strands interact with a variety of proteins, e.g. during transcription or during repair of single- and double-strand ruptures. If a particular DNA strand does not natively interact with a protein, then it may be possible to link the protein synthetically to the DNA[42]. Another approach toward DNA (or RNA)-protein-hybrids is the use of 'aptamers': strands of nucleic acid that fold into structures that bind strongly to a protein. Aptamers have been isolated successfully in *in vitro* selection experiments (SELEX) for a large variety of proteins and for other small ligands like ATP or cocaine[43-47]. In many cases, aptamers have an affinity to their targets comparable to that of antibodies towards their antigens[48]. Thus, aptamers may participate in two different kinds of molecular recognition events: (i) binding to their aptamer target; and (ii) binding to their Watson-Crick complementary strand. Competition between these two types of recognition can be used to construct sensors and switches. Nutiu and Li[49-51] monitored the binding of biomolecules to an aptamer and the subsequent release of its complementary strand using FRET. Dittmer *et al.*[52] used a similar concept to bind and release the human blood-clotting factor α-thrombin cyclically. The thrombin aptamer consists of a G-rich 15-mer that, in the presence of K ions, folds into two stacked intramolecular G-quadruplexes connected by three loops (Fig. 4). In this conformation, the aptamer binds to α-thrombin. A single-stranded 'toehold' on one end of the aptamer facilitates the binding of a partly complementary effector strand. The resulting duplex is not able to bind to the α-thrombin, which is consequently released. When a third strand fully complementary to the effector strand is added, the aptamer is displaced from the effector by branch migration. Now the aptamer can fold into the thrombin-binding conformation again and the cycle can be repeated. In recent experiments, Yan and coworkers[53,54] incorporated the thrombin-aptamer into DNA-scaffolds, which can form micron-sized DNA arrays. With these arrays, they

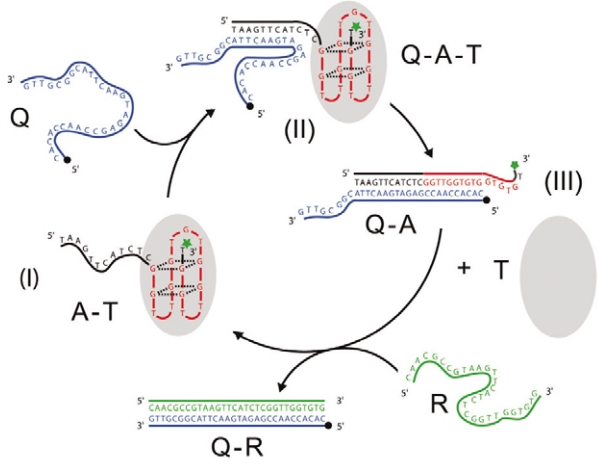

Fig. 4 Cyclical release and binding of human α-thrombin (T). The thrombin aptamer-thrombin complex (A-T) dissociates after the addition of an oligonucleotide Q, that replaces thrombin (I-III) via branch migration. A removal strand R forms a fully complementary double helix (Q-R) with the strand R, thus setting the aptamer strand (A) free, which can bind the thrombin again. (Reprinted with permission from[52]. © 2004 Wiley-VCH Verlag.)

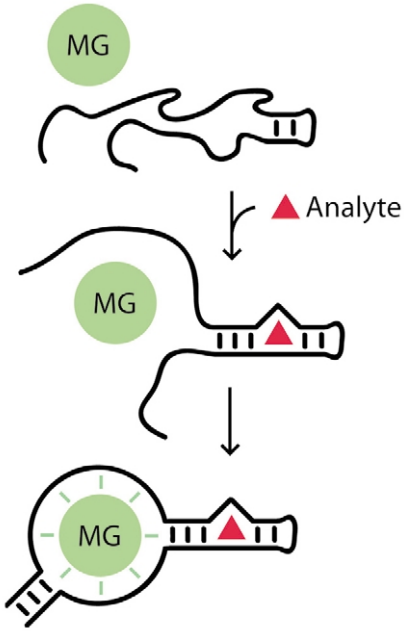

Fig. 5 The MGA as a reporter system for analyte binding. The RNA strand comprises an aptamer sequence for an analyte in its middle section and aptamer sequences for the malachite green molecule at its two end regions. In the absence of the analyte, neither the analyte aptamer nor – as a consequence – the MGA can bind. Upon introduction and binding of the analyte, the MGA can form and bind the reporter molecule malachite green, which is accompanied by a strong fluorescence increase. (Adapted from[55].)

were able to detect thrombin in nanomolar concentrations using fluorescence microscopy.

Stojanovic and Kolpashchikov[55] recently presented a versatile concept for the specific sensing of three different molecules: (i) adenosine triphosphate (ATP); (ii) flavin mononucleotide; and (iii) theophylline. The recognition of an analyte to a DNA or RNA aptamer is transduced via a short RNA communication module to a reporting domain, in this case the malachite green aptamer (MGA) (Fig. 5). Upon binding of the dye malachite green to the aptamer, its quantum yield increases up to 2000-fold. The two stems of MGA are reduced to only a few base pairs each, thus significantly lowering the probability for MGA folding into its malachite green binding conformation. In order to act as a reporter for a biomolecule, one of the two MGA stems should be elongated with the sequence of a second aptamer that binds to the target analyte. This second aptamer folds only in the presence of its analyte, which in turn enables the MGA to adopt its binding conformation. The strong increase of the fluorescence intensity of the bound malachite green dye can be monitored with standard fluorescence techniques.

There are a number of recent studies that use the action of DNA modifying enzymes for elaborate sensing processes. With the help of the restriction enzyme FokI, Beyer and Simmel[56] have been able to translate an arbitrary DNA sequence into a DNA oligonucleotide triggering the release of thrombin by an aptamer. Stojanovic *et al.*[57] have used the catalytic properties of a hammerhead-type deoxyribozyme to discriminate between 15-mers with single base pair resolution. Recently, Weizmann *et al.*[58] detected single-stranded viral DNA with a detection limit of 10 fM using the autocatalytic synthesis of a DNAzyme. All these examples impressively demonstrate the outstanding potential of DNA-based nanodevices as an easy-to-handle, cost-efficient, and reliable sensor molecule for complex sensing tasks in the life sciences.

Using genetic mechanisms to control DNA-based nanodevices

The complex interaction of activation, inhibition, transcription, and translation of genes involving proteins, DNA, and RNA ensures the survival and proliferation of cells and organisms in a variable environment. By adapting parts of these mechanisms, we can not only learn more about the underlying biochemical networks, but also engineer novel molecular machinery *in vitro* and potentially *in vivo*.

Pioneering work has been performed by the Libchaber group[59,60], in which they demonstrate the 'principles of cell-free genetic circuit assembly' . Along similar lines, Dittmer *et al.*[61] realized a simple gene regulatory 'switch' to actuate DNA tweezers (see above) using short RNA strands transcribed from an artificial DNA template (Fig. 6). The dsDNA template includes a promoter sequence for RNA polymerase from phage T7, directly followed by a sequence encoding the LacI

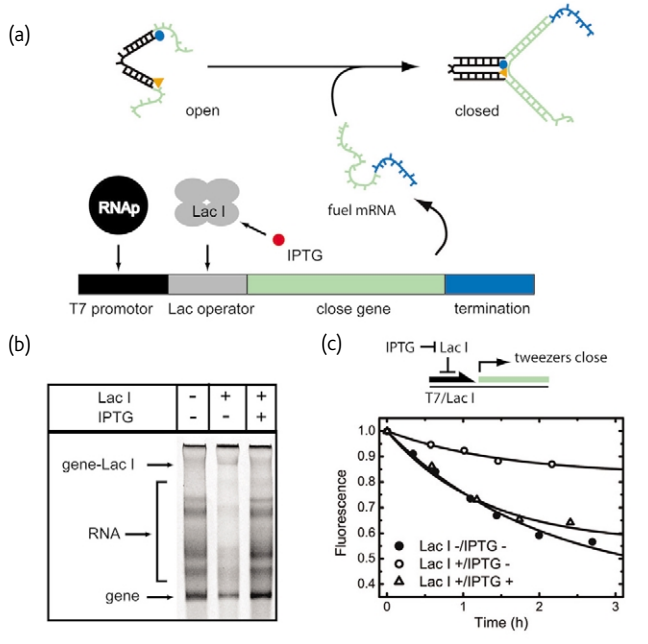

Fig. 6(a) Working principle of a regulated gene encoding the closing sequence for DNA tweezers (see text for explanation). (b) Gel electrophoresis and (c) FRET experiments prove the regulatory behavior and functionality of the network. (Reprinted with permission from[61]. © 2005 Wiley-VCH Verlag.)

operator. In the absence of LacI, T7 RNA polymerase can transcribe the 'close gene', which is located downstream of the Lac operator. The RNA synthesized from the template closes the tweezers, which can be monitored by a decrease in fluorescence. In the presence of LacI protein, the transcription is hindered, unless the small molecule isopropyl β–D-thiogalactoside (IPTG), which inhibits the binding of LacI to the operator, is added. These experiments show that artificially synthesized DNA machines can be controlled by genetic mechanisms *in vitro*. Kim *et al.*[62,63] have proposed artificial transcriptional circuits composed of mutually inhibiting and activating genes that can provide controlled amounts of various actuator RNA strands for networks of DNA-based nanodevices. From these initial experiments, it is expected that autonomous molecular systems will be developed that are able to react to various environmental stimuli and could potentially find application as intelligent sensing devices or in drug delivery systems.

Conclusions

Much has happened during the last decade in the emerging field of DNA nanotechnology. Starting from relatively simple conformational changes, DNA-based nanodevices can already accomplish complex movements like unidirectional walking and fulfill highly sensitive sensory tasks. The successful combination of DNA-based nanodevices with genetic machinery, and implementation of concepts developed in DNA computing[64], promise the ultimate realization of intelligent molecular-sized devices that can sense environmental information and react according to their implemented molecular programs. nt

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