F. C. Simmel and Y. Krishnan

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Molecular Functional Units

Nucleic Acid Based Molecular Devices

*Yamuna Krishnan and Friedrich C. Simmel**

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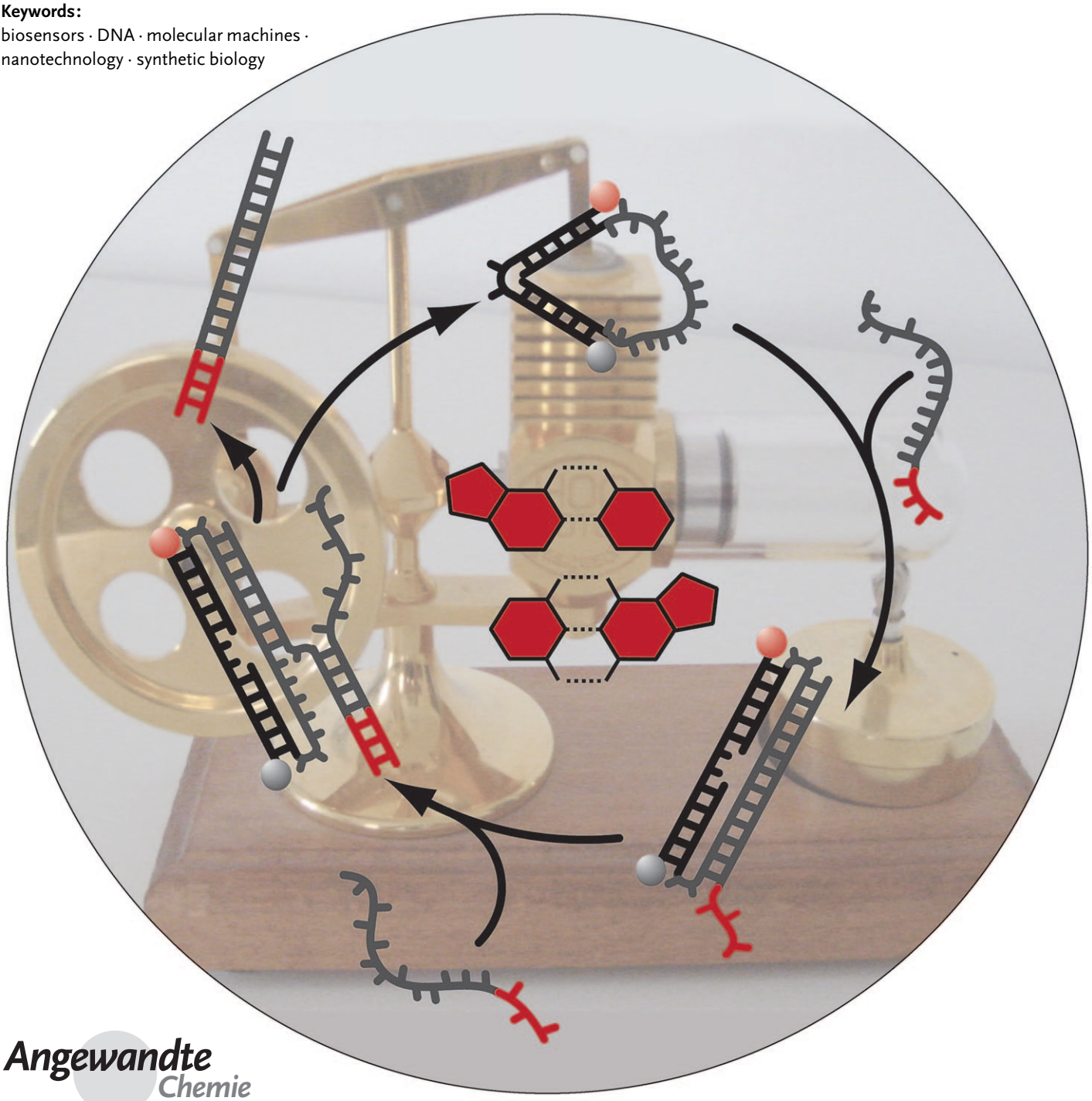

In biology, nucleic acids are carriers of molecular information: DNA's base sequence stores and imparts genetic instructions, while RNA's sequence plays the role of a messenger and a regulator of gene expression. As biopolymers, nucleic acids also have exciting physicochemical properties, which can be rationally influenced by the base sequence in myriad ways. Consequently, in recent years nucleic acids have also become important building blocks for bottom-up nanotechnology: as molecules for the self-assembly of molecular nanostructures and also as a material for building machinelike nanodevices. In this Review we will cover the most important developments in this growing field of nucleic acid nanodevices. We also provide an overview of the biochemical and biophysical background of this field and the major "historical" influences that shaped its development. Particular emphasis is laid on DNA molecular motors, molecular robotics, molecular information processing, and applications of nucleic acid nanodevices in biology.

From the Contents

1. Introduction

The idea that the unique molecular recognition properties of DNA molecules might also be used in a completely nonbiological context originated in the early 1980s, when Seeman proposed the building of supramolecular crystals from them[1]—a proposal that was only recently realized by Seeman's group with the synthesis of millimeter-sized DNA crystals.[2] In 1994, another "artificial" application was described for DNA in computing. In this year, Adleman published the "wet-lab" solution for a computational problem by using DNA and standard molecular biology techniques.[3] About ten years ago, the field of "DNA nanotechnology" was further extended by the first experimental demonstrations of switchable molecular structures made from DNA, often called DNA "nanomachines" or DNA "nanodevices".[4,5]

An independent line of research had already started in the early 1990s with the development of functional nucleic acids such as aptamers or ribozymes.[6] These were also utilized as molecular switches, that is, as allosteric aptamers or aptazymes. Finally, in 1996, the extremely fruitful biosensing concept of "molecular beacons" (MBs) was introduced; these may also be regarded as simple molecular devices based on DNA.[7,8]

The independent development of functional nucleic acids and molecular beacons provided important tools and components for the field of DNA nanodevices, and today there are many interdependencies between all subfields—computational functions and conformational switching often go hand in hand, and elaborate supramolecular constructions frequently form the basis of a DNA device. The timeline shown in Figure 1 gives a rough overview of major developments in the different areas, and some of the "cross-fertilizations" are indicated.

As can be seen from the studies presented in this Review, molecular devices made from nucleic acids now come in a large variety of types and sizes—some devices are composed of only one DNA strand, others of more than 200! The main common element is probably the fact that these devices are designed structures and have a designed function. The more complex devices, at least, often combine several functionalities—submodules—to achieve a given task. In most cases, the sequence-programmability of DNA or RNA molecules is used both to define a molecular structure—that is, to "build" it from single strands—and to switch the structure between different conformations with distinct functionality.

In this Review we aim to cover the most important developments in this field over the last several years, and group them into subsections that represent the major directions of research. On a more fundamental level—motivated largely by nanotechnology and biophysics—researchers would like to learn how to construct artificial "molecular machines".[9] As a consequence of their predictable interactions and ready availability, DNA molecules are an outstanding material for the design and synthesis of structures with machinelike properties. One of the most challenging tasks here is the generation of forces and motion, and many researchers have developed DNA-based molecular motors and walkers. These more fundamental issues are

[*] Dr. Y. Krishnan
National Centre for Biological Sciences (NCBS)
Tata Institute of Fundamental Research, GKVK
Bellary Road, Bangalore, 560065 (India)
Prof. Dr. F. C. Simmel
Lehrstuhl für Bioelektronik E14, Physikdepartment
Technische Universität München
Am Coulombwall 4a, 85748 Garching (Deutschland)
Fax: (+49) 89-289-11612
E-mail: simmel@ph.tum.de

presented in Sections 3 and 4. Also driven by materials science and nanotechnology is the attempt to synthesize switchable and intelligent materials, containers, and delivery devices. Studies in this direction are covered in Section 5. Recent developments concerning the strong interplay between DNA computing and DNA devices are presented in Section 6. Finally, Section 7 is devoted to the large body of present and future applications in biology, which ranges from biosensors to in vivo imaging and therapeutics. In this section, there is inevitable overlap with many other research areas, and sometimes the distinction between a "DNA nanodevice" and a "biosensor based on DNA" is somewhat fuzzy. Sensing modules will certainly play an important role as components of more complex molecular devices in the future—for example, in the context of controlled delivery units. Biosensors based on nucleic acids are interesting components for molecular devices, but—as detailed in Sections 4–6—not every DNA nanodevice is simply a complicated sensor or has sensing function at all.

Many reviews on this topic are highly recommended to the reader, particularly those by Seeman, Willner, Simmel, and Turberfield,[10] and these provide complementary perspectives on specific aspects of nucleic acid architectures and related devices. This current Review seeks to present a comprehensive overview of this emerging area and its broad impact on the molecular sciences.

2. Biophysical and Biochemical Background

In this section we highlight a few key aspects of DNA biophysics and biochemistry that form the basis of the design, construction, and operation of nucleic acid nanodevices. These encompass the prediction of secondary structure, mechanical and thermodynamic stability, and also the extensively used unconventional conformations of DNA such as G quadruplexes and i motifs. This relatively comprehensive overview is intended for the uninitiated reader or newcomers to the field. Specialists may want to skip directly to Section 3. For a more rigorous understanding of nucleic acid structure, the reader is directed to Ref. [11].

2.1. Biophysics of DNA Duplex Formation

Most nucleic acid nanodevices rely—in one way or another—on the formation of stable double-stranded complexes between sequence-complementary (single) strands. Many devices consist of both single- and double-stranded parts, which are used as flexible or rigid molecular segments, respectively. Skillful combination of these elements conveys distinct mechanical and chemical properties to the resultant devices. Single strands may be used simply as flexible joints, and also as addressable molecular tags to which complementary strands can attach. Duplexes are typically used as rigid building blocks, but may also contribute to the chemical function of the devices through incorporation of binding sites or chemical modification.

Duplex formation occurs during assembly of the structures from their single-stranded component molecules, but is also utilized for the "mechanochemical" operation of many devices. The thermodynamics and kinetics of duplex formation as well as the mechanical properties of double-stranded and single-stranded nucleic acids are, therefore, central to the construction as well as the function of nucleic acid nanodevices.

2.1.1. Thermodynamic Stability of Nucleic Acid Structures

Two strands of DNA or RNA with completely complementary sequences can bind to each other and form a fully base-paired duplex structure. The stability of this structure is governed by stacking interactions between neighboring base pairs. The free energy of a fully matched duplex can be calculated quite accurately within the "nearest neighbor model", which makes use of extensive tables of thermodynamic data derived from experiments on model sequences.[12] A variety of computer programs and web interfaces are available today that allow the calculation of thermodynamic properties of DNA or RNA molecules under different experimental conditions, such as monovalent or divalent salt concentration, for example, the well-known mfold algorithm,[13] the Vienna package,[14] HYTHER,[15] and NUPACK.[16] Several programs already support more advanced design goals that are of interest in the context of the assembly of DNA nanomoieties. The Vienna package, for example, contains an inverse folding algorithm for single-

Yamuna Krishnan received her BSc in chemistry from Madras University in 1994 and her PhD from the Indian Indian Institute of Science, Bangalore in 2002. She worked on G quadruplexes with Shankar Balasubramanian at the University of Cambridge as an 1851 Research Fellow and returned to the National Centre for Biological Sciences, TIFR, Bangalore, India, in 2005. She is a recipient of the Indian National Science Academy's Young Scientist Medal. Her research focuses on self-assembled molecular devices based on nucleic acids for sensing and delivery applications in biological systems.

Friedrich Simmel received his PhD in experimental physics from the Ludwig-Maximilians-Universität (LMU) in Munich, Germany, in 1999 with Jörg Kotthaus. He then carried out postdoctoral studies with Bernard Yurke at the Bell Laboratories (Murray Hill, USA) before returning in 2002 to LMU Munich as leader of an Emmy Noether junior research group devoted to bionanotechnology. Since 2007, he has been a full professor of physics at the Technical University in Munich. His research focuses on DNA-based self-assembly, nanopore biosensors, biomolecular nanodevices, and synthetic gene regulatory networks.

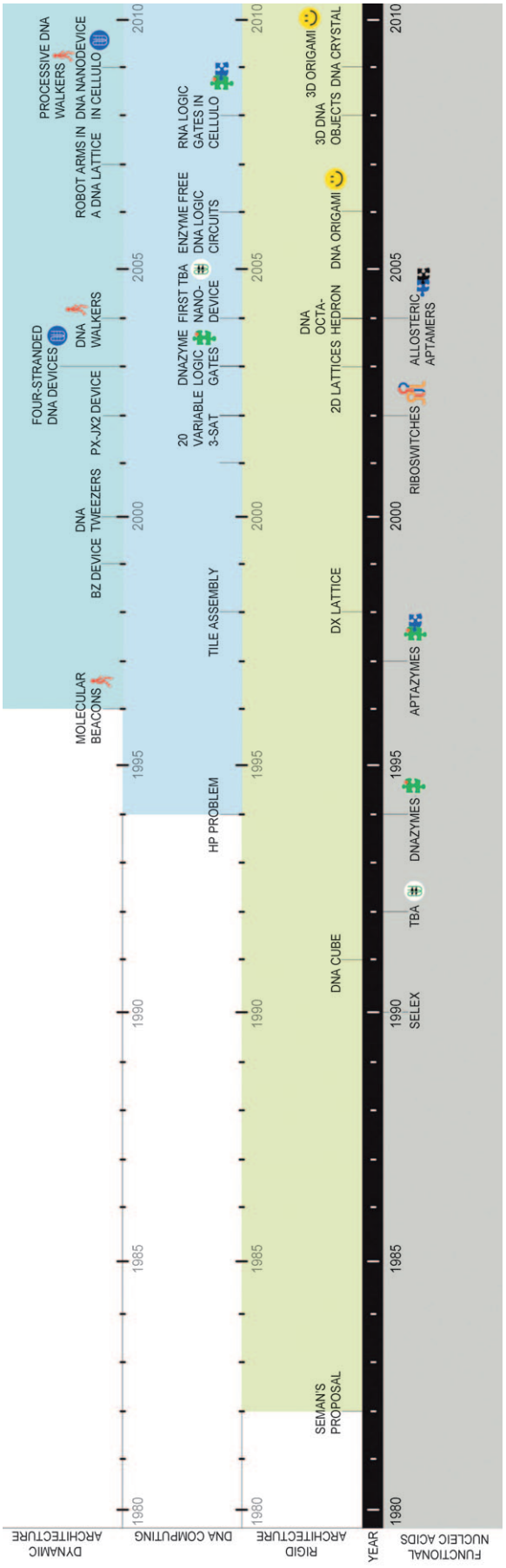

Figure 1. Timeline of the key developments related to molecular devices based on nucleic acids. For clarity, the devices from structural DNA nanotechnology are grouped into three main classes: rigid architectures, dynamic or movable architectures, and DNA computing. The independent evolution of functional nucleic acids is indicated below in gray. Here, key modules from functional nucleic acids—such as the thrombin-binding aptamer (TBA)—that have been integrated into devices in the former field are indicated. Developments in structural DNA nanotechnology and related functional modules or concepts are indicated by similar symbols.

stranded RNA structures, while NUPACK also allows for predictions of multistrand folding.

The availability of advanced computational tools that allow the accurate prediction of folding and thermodynamic properties is a major advantage of DNA-based nanotechnology—it facilitates a more rational design approach than what is possible with other technologies or chemical approaches available today. However, there are limitations, and in many cases one has to adopt a semiheuristic design strategy. When designing bistable molecular switches from aptamers, one may want to shift the equilibrium from one structure to the other by the addition of a small molecule. The influence of the small molecule binder has to be evaluated empirically in binding assays and the switching properties have to be optimized by "manual" adjustment of the sequence. Similar problems arise when working with modified nucleic acids containing unnatural bases or intercalators. Here, the melting transition of the duplexes has to be studied experimentally by using, for example, temperature-dependent absorbance measurements.

For in vivo applications, another important issue is the stability and kinetics of nucleic acid nanodevices in a cellular context. In the crowded cellular environment, the effective concentrations differ from those used in standard in vitro experiments that are performed in well-mixed buffer solutions. This results in excluded volume and osmotic pressure effects, which are known to have a pronounced influence on nucleic acid structures.[17] For example, it was shown that three-way junctions[18] or G-quadruplex structures in telomeres[19] can be stabilized under such conditions of molecular crowding.

2.1.2. *Kinetics of Duplex Formation, Hybridization Catalysts, and Strand Displacement*

The kinetics of strand association and dissociation determines the dynamic behavior of nucleic acid nanodevices. At high Na^+ concentrations or in the presence of magnesium ions, typical rates for DNA hybridization between complementary strands are on the order of $10^6\,\text{M}^{-1}\text{s}^{-1}$.[11] Consequently, the motion of nucleic acid nanodevices—typically operated at nanomolar (nM) to micromolar (μM) concentrations—that are driven by hybridization reactions is on the timescale of seconds to minutes. Within living cells, the presence of a large number of binding partners may alter the kinetics considerably.[20]

The hybridization rate can be drastically reduced in the presence of secondary structures. For example, hairpin DNA molecules with complementary sequences will only hybridize extremely slowly when their double-stranded stem is sufficiently long and their loop section is sufficiently

short. In fact, the control of hybridization rates by the formation and breaking of secondary structure has become increasingly important in the design of reaction networks from DNA, which find application in molecular computation and robotics (Sections 4 and 6). In these applications, hybridization between two strands is deliberately inhibited by the formation of secondary structure. During operation, this structure is controllably broken by appropriately chosen "helper" strands—so-called hybridization catalysts.[21,22] An example of hybridization catalysis is shown in Figure 2: two

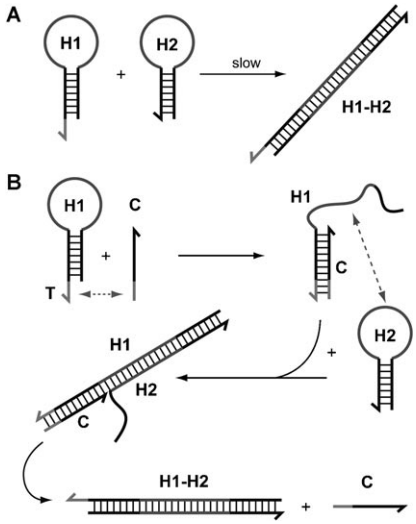

Figure 2. The principle of hybridization catalysis. A) Two hairpin molecules H1 and H2 have complementary sequences except for the single-stranded extension of H1 called the "toehold". H1 and H2 only hybridize very slowly with each other because of steric restrictions and the stability of the hairpin stems. B) Catalyst strand C is added, which is complementary to the stem of H1. It can attach to H1 at the toehold, open the hairpin, and make the loop sequence more accessible for hybridization. H2 can now hybridize with H1 much more efficiently and displace catalyst C in the final step.

DNA hairpins with complementary sequences hybridize with each other only slowly. A DNA catalyst complementary to the stem and part of the loop of one sequence facilitates the opening of the hairpin. This makes the nucleotides within the loop available for hybridization with the complementary hairpin. In this process, the catalyst strand is displaced from the hairpin again, thus making it available for another catalysis cycle. In this way, the rate of hybridization can be easily increased by several orders of magnitude.

The mechanism of hybridization catalysis involves several strand-displacement reactions, in which the catalyst strand first invades the stem of the hairpin and is later removed from it by the complementary hairpin. Strand displacement proceeds through a process called "branch migration". Branch migration is often used for the operation of nucleic acid nanodevices when it becomes necessary to remove a DNA or RNA already hybridized to a nucleic acid structure. It can, therefore, be utilized to drive nanodevices through a work cycle that involves molecular stretching (by hybridization of two strands) and relaxing (by removal of a strand from a duplex).

In principle, strand displacement by branch migration can always occur between a single-stranded (ss)DNA and a double-stranded (ds)DNA molecule when the single strand has a base sequence homologous to one of the duplex strands. Driven by thermal fluctuations, a DNA duplex can partially open at its ends (a process referred to as "fraying") and a homologous free single strand in the solution may take its chance and attach to the complementary sequence within the duplex. The result is a three-stranded structure, in which two strands with the same sequence compete for binding with their complement. The branch point—the position where both homologous strands meet—then performs a thermally driven random walk along the length of the complementary strand until one of the competing strands dissociates.[23]

The process of strand displacement can be sped up considerably by using a "trick" introduced by Yurke et al. (Figure 3).[5,24] When one of the strands of a duplex is extended by a short sequence, this single-stranded overhang may serve as the nucleation site (or "toehold") for the attachment of a complementary strand. This results in a three-stranded branch structure, from which a branch migration process can start. In this case, the overall process is biased: whereas the long strand can displace the shorter strand completely, the opposite cannot occur, as the long strand is attached to the toehold. Toehold-initiated strand displacement typically works well, when the rate of dissociation from the toehold is much smaller than the rate of strand displacement. For practical applications, toehold lengths of 5–8 nucleotides are utilized.

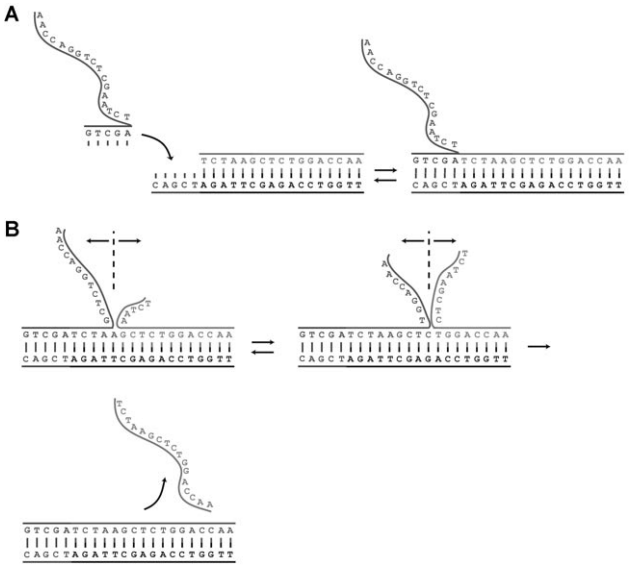

Figure 3. Strand displacement by branch migration. A) A DNA duplex can be extended by a short single-stranded "toehold" to speed up a strand-displacement process. The DNA strand coming in from the left can attach at the toehold and start a branch migration process from there. B) During the branch migration process, two DNA strands with identical sequence compete for binding to a complementary strand. When the left sequence is attached at a toehold, the displacement process is biased and favors dissociation of the right strand.

It has to be noted that three-stranded branch migration occurs much faster than in four-stranded Holliday structures.[25] Branch migration in Holliday junctions is also strongly dependent on the magnesium concentration, as Mg^{2+} ions stabilize the stacked conformation of these structures and strand migration cannot occur efficiently.[26] The rate of strand displacement reactions can also be influenced by the presence of certain cationic polymers, which has already been utilized for the operation of DNA nanodevices (see Section 3.3).[27]

2.1.3. Mechanical Properties of Single and Double Strands

In its B form, double-stranded DNA is a helical molecule with a diameter of 2 nm and a distance of 0.34 nm between adjacent base pairs. The rise of the helix is around 10.5 base pairs (bp) per turn. Cyclization assays[28] as well as direct mechanical measurements with magnetic traps,[29] optical tweezers,[30] and hydrodynamic stretching[31] have determined the "persistence length" L_p of dsDNA to be 50 nm, or 150 bp. The persistence length is a polymer parameter, which indicates how fast a polymer changes its tangential orientation when followed along its contour. L_p is directly related to the bending rigidity of the polymer. Duplex DNA, on the nanometer length scale below L_p, can therefore often be regarded as a rigid "rodlike" molecule. As DNA nanodevices are typically composed of strands with computer-generated, random sequences, the "rigid rod" assumption should typically hold true. One has to bear in mind, however, that the mechanical properties of dsDNA can be dramatically altered for special sequences such as in "A tracts". Furthermore, when the electrostatic screening length at low salt concentrations becomes of the order of the distance between charges on the backbone, the stiffness of the DNA additionally increases because of their mutual repulsion.[32,33] On the other hand, multivalent ions can decrease the persistence length.[33]

The persistence length of dsRNA has been less studied, but recent experiments with magnetic tweezers and AFM indicate a slightly higher persistence length of about 60 nm.[34] Duplex RNA and DNA/RNA hybrid molecules assume the A form double helix, which has a larger diameter (2.6 nm) than the B form, but rises only 0.24 nm per bp. The different dimensions and mechanical properties have to be considered, for example, when hybrid devices containing both DNA and RNA molecules are constructed.

Single-stranded DNA is considerably more flexible than dsDNA, but its mechanical properties depend much more strongly on environmental conditions and sequence. Values between 0.75 nm at high ionic strength and up to 10 nm at low salt conditions have been reported.[35] Some sequences—for example, poly(dA)—tend to be more rigid than others as a result of stronger single strand stacking interactions.

In the buffer conditions, under which nucleic acid nanodevices are operated, however, it is usually safe to assume that single-stranded molecules are relatively flexible, while double-stranded molecules are stiff. Correspondingly, flexible joints and hinges are made from ssDNA or ssRNA, whereas stiff "arms" or "limbs" are made from double-stranded nucleic acids.

2.2. Unusual Nucleic Acid "Motifs"

2.2.1. DNA Structures

Synthetic homopolymeric DNA and RNA were used in early studies on understanding the structure, base pairing, and base-stacking properties of DNA and RNA duplexes as they were considered to be simplified model systems. Eventually it was found that these synthetic homopolymers actually formed different unusual conformations involving non-Watson–Crick base pairing. A-Rich RNA and DNA have been shown to form parallel duplexes called A motifs (Figure 4C),[36,37] C-rich RNA and DNA sequences formed i tetraplexes—i motifs—(Figure 4B),[38] while G-rich RNA[39] and DNA sequences form G quadruplexes (Figure 4A, for an excellent review see Ref. [40]). Considered by many as a potential anticancer target,[41] G quadruplexes have proved to be one of the most desirable targets of small-molecule binders[42] and protein engineering.[43] Quadruplexes and i motifs are also formed by nucleic acid mimics[44] and as hybrids with DNA or RNA.[45] Some unusual structural variations inspired by these four-stranded motifs that present untapped potential as structure-directing elements and for functional molecular display are also shown in Figure 4.

A GU-rich sequence forms an octameric structure where G tetrads and U tetrads are intercalated, as seen in i motifs that result in eight "Us" being displayed in an ordered spatial orientation (Figure 4D).[41] Pentaplexes based on isoguanine (iG) have also been engineered by using iG and narrowing the angle at which the Watson–Crick and Hoogsteen hydrogen-bonding sites are displayed (Figure 4E).[47] Triplexes are three-stranded nucleic acid structures discovered by Felsenfeld et al.,[48] where the third DNA or RNA strand is accommodated in the major groove of a DNA, RNA, or RNA–DNA duplex by hydrogen bonding with the Hoogsteen face of the nucleobase.[49] Excellent reviews on triplex structures can be found in Refs. [50].

Naturally occurring DNA sequences afford a fund of unusual structures. Many genomic sequences consisting of expandable repeats end up forming a myriad of unusual motifs (Figure 5), such as imperfect hairpins composed of $(CNG)_n$ repeats (Figure 5A), G quartets composed of $(CGG)_n$ repeats (Figure 5B), slip-stranded DNA[51] (Figure 5C), and different triplexes formed by $(GAA)_n$ repeats (Figure 5D,E). Within the cell, triplexes are present as the unusual H-DNA motifs, whose formation possibly modulates or is modulated by DNA supercoiling[52] or potentially even as nodule–DNA motifs.

2.2.2. Unusual RNA Structures

The crystal structure of tRNAs transformed our perception of RNA structure. Suddenly RNA seemed capable of not just Watson–Crick base pairing that leads to the formation of an A helix, but also noncanonical base paring, tertiary interactions, intercalation, coaxial stacking, base triples, and metal-ion binding. The crystal structures of many large RNAs[53] expanded on these basic structural motifs and showed how these unusual modes of nucleobase association were responsible for the overall three-dimensional architec-

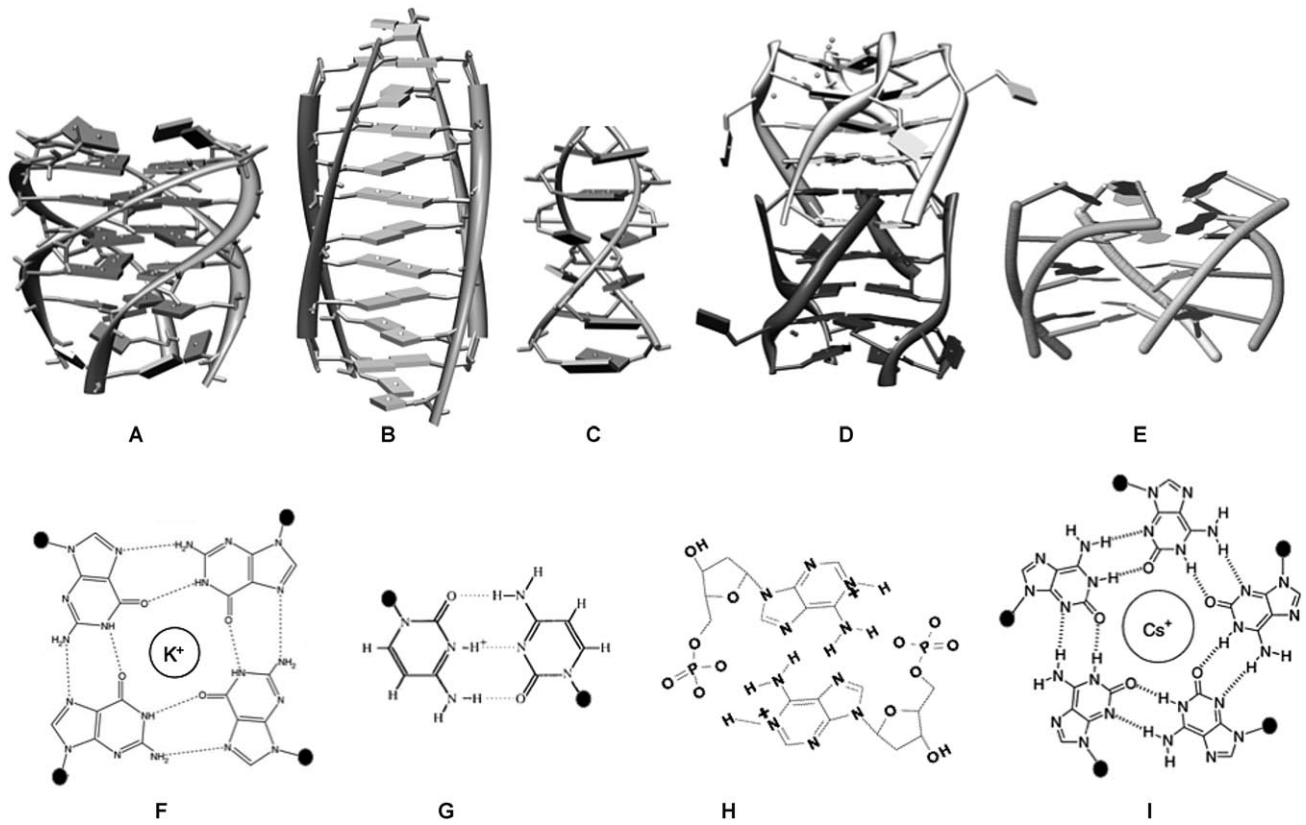

Figure 4. Unusual structures inspired by four-stranded DNA motifs. A) Tetramolecular G quadruplex;[46] B) I motif;[38] C) bimolecular A motif;[37] D) an octaplex formed from r(UGUGGU) comprising intercalated G tetrads and U tetrads that displays eight U bases;[41] E) a pentaplex formed from isoguanine-containing strands;[42] F–I) corresponding base-pairing schemes: F) G tetrad, G) C-C^+ base pair, H) AH^+-H^+A base pair, I) isoG pentad.

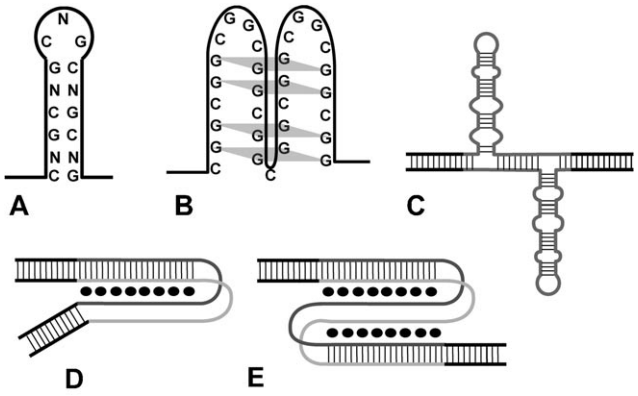

Figure 5. Repetitive DNA sequences in the genome can also form unusual motifs such as A) imperfect hairpins, B) G-quartet-based structures, C) slip-stranded DNA, D) triplex-containing H-DNA, where the light gray and dark gray regions indicate either purine-rich or pyrimidine-rich sequences, E) nodule DNA, where the single strand thrown out of one triplex in (D), becomes the third strand of an adjacent triplex and vice versa.

ture of the RNA strand. RNA secondary structure may simply be described as double helices joined by various types of loop topologies. These give rise to hairpins, internal loops, and junction loops.[54,55] Recurrent secondary structures have been classified in the Structural Classifications of RNA (SCOR) database.[56] Sequence comparisons of rRNA molecules revealed three classes of hyperabundant, terminal loop motifs comprised of four nucleotides, or tetraloops—the UNCG, GNRA, and CUYG classes.[57] Internal loop motifs include cross-strand purine stacks, bulged G motifs, A platforms, bulge-helix-bulge motifs, and metal-binding motifs.

2.2.3. *Tertiary Structural Motifs in RNA*

Protein chains that are incorporated into α helices and β sheets tend to be globular upon folding, while RNA chains incorporated into helices tend to fold to form flat pancake-like structures as a result of tertiary interactions.[54] Two major structural motifs that contribute to this are coaxial stacking[58] and the formation of pseudoknots (described by Burkhard, Turner, and Tinoco in Ref. [59]). Tertiary interactions with unusual base pairing are generally mediated through loop–loop interactions. External or hairpin loops are often involved in tertiary interactions such as GNRA-tetraloop–receptor,[60] kissing hairpin,[61] D-loop–T-loop,[62] and lone pair–triloop.[63] Many internal loop motifs effectively distort the orientation of the helices that they are embedded in by unwinding of the helix or by introduction of an angle between the helical axes. Examples include the kink-turn,[64] hook-turn,[65] and adenosine platforms.[66] Other examples of tertiary interactions include the ribose zipper,[67] A-minor,[68] and the G-ribo

motifs.[69] The paucity of RNA crystal structures has so far impeded the identification and classification of unusual motifs, but they are key to arriving at generalized RNA architectural principles and identifying common folds.

2.3. Functional Nucleic Acids

2.3.1. Aptamers

An aptamer is a nucleic acid sequence (DNA or RNA) that is typically 15–40 nucleotides or longer and binds specifically to a given molecular target.[6,70] In solution, the nucleic acid sequence folds up in three dimensions to form a specific molecular shape. The shape adopted by a given aptamer allows it to form a binding site into which the target molecule may then fit. Alternatively, isolated aptamers may merely be preorganized in shape and bind to their target through an induced-fit mechanism. Nucleic acid aptamers are selected against molecular targets, and they can already be identified after a few repeated rounds of in vitro selection. In vitro selection allows the identification of rare, functional RNA or DNA molecules from a pool of typically 10^{15} different sequences. Subsequent to selection, a given pool of nucleic acids may be amplified by using the molecular biology approaches of reverse transcription and the polymerase chain reaction (PCR). The molecular target is immobilized on a solid support, and the pool of 10^{15} different sequences is passed through. The retained RNAs are eluted, reverse transcribed, amplified by PCR, transcribed, and then the entire cycle is repeated with progressively higher stringencies. This process is referred to as SELEX (systematic evolution of ligands by exponential amplification)[71] and makes it possible to identify only those sequences which bind the target with high affinity.

Given the huge numbers of permutations possible in nucleic acid sequences, these scaffolds are capable of adopting extraordinarily diverse molecular shapes. Thus, aptamers have been obtained against myriad molecular targets, including small molecules, toxins, reaction intermediates, literally any class of protein, and even whole cells. In addition to exhibiting exquisite specificity, aptamers also generally bind their targets with high affinities. The majority show a dissociation constant K_d in the nanomolar regime for proteins and in the micromolar regime for small molecules. Since aptamers are made of short lengths of nucleic acids, they have several advantages over antibodies, which are large proteins. Unlike antibodies, aptamers are amenable to in vitro synthesis unlike antibodies. This results in a low batch-to-batch variability. They may be easily labeled without compromising target affinity,[72] have a greater ability to sustain temperature and environmental insults, and so have a longer shelf-life. There are many methods to reduce or even abolish cross-reactivity, and aptamers may even also be selected under nonphysiological conditions. For these reasons aptamers are replacing antibodies in a number of biological assays (see Section 7.1).

The low-molecular weights of aptamers endow them with excellent pharmacological properties, such as short circulation times, better target accessibility, and rapid clearance. Thus, aptamers are finding increasing importance in molecular therapeutics, where they are also beginning to replace protein antibodies (see Section 7.3). Despite having a tenfold lower molecular weight, aptamer analogues of antibodies bind their targets with comparable affinities and specificities, as well as having much lower immunogenicity. Their accessibility by chemical synthesis and their uniform quality make them more amenable to commercial production. Advents in chemical analogues of nucleic acids and the adaptability of solid-phase synthesis have ensured that aptamers can incorporate several chemical modifications that finely modulate their stability and circulation times; an overview of this has been given by Pestourie et al.[73]

2.3.2. Ribozymes and DNAzymes

Developing metal-ion-specific aptamers has been a challenge, primarily because of the lack of appropriate immobilization tools. However, several nucleic acid based enzymes show metal-ion-dependent catalytic activity. Such catalytic RNA molecules, called ribozymes, are either naturally occurring (excellently reviewed recently in Ref. [74]), or have been evolved by SELEX-type[6] approaches. DNA equivalents called DNAzymes[75] have thus far only been selected by artificial methods. RNA, in particular, can fold into complex three-dimensional shapes, and hence it presents a malleable scaffold to engineer catalytic centers and binding pockets. Many selections of novel RNAzymes and DNAzymes are centered around phosphoester transfer reactions and have been reviewed extensively.[6] The substrates of most naturally evolved ribozymes are other RNA strands. In contrast, test-tube-evolved DNAzymes and RNAzymes have been shown to be capable of catalyzing a variety of chemical reactions,[76] such as Diels–Alder reactions,[77] aldol reactions,[78] Michael reactions,[79] N-glycosidic bond formation,[80] and acylation reactions.[81] Many enzymatic reactions have also been recapitulated by RNAzymes and DNAzymes including cholesterol esterase,[82] N-glycosylase,[83] capping with AMP,[84] and guanylyl transferase.[85] Functional nucleic acids are also finding increasing use in sensing,[86,87] molecular computation,[88] targeted delivery, and therapeutics.[89]

There are many examples where several functional units from aptamers and ribozymes are combined to give allosteric aptamers or "aptazymes" (Section 7.1). In proteins, allostery involves spatially separated binding sites that communicate with each other through a conformational change triggered by the binding of an effector to one of the binding sites. The same principle has been utilized for the construction of allosteric ribozymes and aptazymes. Such structures are of considerable interest in the context of information processing, signal transduction, and also in biosensing (Sections 6 and 7).[90,91]

2.4. DNA Sequence Design

A variety of strategies have been developed for the design of artificial structures from nucleic acids.[92] According to Dirks et al.,[93] one can differentiate between a "positive" and "negative" design approach. The positive approach is the

optimization of the affinity of a nucleic acid sequence to fold into a given target structure. In the negative approach, the goal is to avoid folding into unwanted structures, that is, to optimize specificity for the target. The latter approach is often taken in heuristic methods, such as sequence symmetry minimization, where repetition of subsequences of a certain length is precluded. However, both methods have their trade-offs: For example, a sequence chosen following the positive design strategy can have the highest affinity for the target structure, but it may assume an alternative structure with an even lower free energy. From extensive kinetic folding simulations, Dirks et al. found that negative design generally leads to better results than positive design strategies, but the best option is to supplement negative design with a positive component. For example, this is achieved when sequence design is based on the evaluation of the partition function of the nucleic acid structure to maximize the thermodynamic probability to fold into the target structure. A heuristic strategy was developed by Jaeger et al.[94] for RNA nanostructures, which utilizes structural data of naturally occurring RNAs for the design of artificial constructs.

2.5. DNA Synthesis

Molecular devices have been constructed from many types of organic and inorganic molecules, as well as from supramolecular complexes of those.[9] One of the major advantages of devices based on nucleic acids over all other approaches is their ready availability. Driven by the increasing demand for artificial oligonucleotides in the life sciences, DNA and RNA synthesis has been automated in recent decades, which has led to continually decreasing synthesis costs. DNA nanotechnology is clearly a beneficiary of this development: Cheap and automated synthesis allows researchers without synthesis capabilities to participate in DNA nanotechnology research. Hence, the development of novel devices is already more of a "design" task than a "synthesis" task. In principle, a complete automation of the manufacturing process is already conceivable today—nanostructures and nanodevices designed and tested on a computer can be readily translated into DNA sequences, which in turn can be synthesized and assembled automatically.

3. Molecular Switches Made from DNA

DNA-based molecular switches are DNA assemblies that can flip reversibly between two or more states in a controllable manner. External stimuli that trigger the change of the state can be photons, temperature, pressure, magnetic or electric fields, or altered chemical environments. Thus, DNA assemblies have been induced to change their states in response to temperature,[95] photoisomerization,[96–98] presence or depletion of various ions,[99] and protein binding.[100,101] A special role is played here by conformational changes driven by sequence-dependent hybridization reactions—these are highly specific and allow one to precisely address a particular state change or a particular switch within a mixture. In many cases, multistability is manifested in different properties of the assembly such as fluorescence, electron transfer, isomerizations, mechanical properties, or chemical reactivity, which can be utilized for various applications, as discussed in Sections 4–7.

3.1. Molecular Beacons

Among the first and simplest, yet most successful DNA-based molecular devices constructed so far are the so-called "molecular beacons" (MBs).[7,8] Molecular beacons are single-stranded hairpin stem structures doubly labeled with a fluorophore and a quencher molecule. In the hairpin conformation, the fluorophore and quencher are in proximity and the fluorescence of the beacon is low. In the presence of a DNA or RNA strand complementary to the loop sequence, however, the hairpin will unfold to form a double-stranded structure. In this conformation, the fluorophore and quencher are spatially separated, which results in a strong increase in the fluorescence. The stability of molecular beacons can be optimized for sensitivity and fast response by the appropriate choice of loop size and stem length. This simple sensing strategy has found numerous applications and has been adopted for a large variety of different sensing tasks. For an overview of these, the reader is referred to the excellent review by Tan and co-workers[8] (see also Section 7.1). Several aspects of MBs are significant in the context of nucleic acid nanodevices. First of all, a similar fluorescence-based sensing strategy was employed for the characterization of many of the devices discussed below. Furthermore, hairpins play an important "minimal" structural element of many DNA devices. Finally, precise control over the stability and switching kinetics of hairpin stems—as already indicated in Section 2.1—lies at the heart of the operation principle of many DNA- or RNA-based switches, machines, and motors.

3.2. Buffer-Dependent Devices

The first example of an artificially constructed "nanomechanical" device—even though it was not termed as such at that time—was published by Seeman and co-workers in 1998.[102] Here the position of a DNA four-way junction embedded within a circular DNA molecule was moved by changing the degree of supercoiling of the DNA circle. It was already proposed in this study that the motion of the junction could be driven by transition from the B to the Z structure. The left-handed Z form of dsDNA is adopted by alternating purine and pyrimidine sequences in the presence of certain cations such as hexamminecobalt(III) ($[Co(NH_3)_6]^{3+}$). The conformational change from a right-handed to a left-handed DNA helix can be exploited to produce torque or a rotary motion. In 1999, Mao, Seeman et al. demonstrated the first rotary nanomechanical device that was based on this transition.[4] In the "B-Z device", two double cross-over DNA structures were connected by a double strand containing the sequence $d(CG)_{10}$ (with C5-methylated cytosine), which is particularly prone to undergo a B-Z transition.[103] The

transition was triggered by changing the concentration of $[Co(NH_3)_6]^{3+}$ from 0 to 0.25 mM. The B-Z transition of the $d(CG)_{10}$ section resulted in one double-crossover motif (DX) unit being rotated with respect to the other by 3.5 helix turns. The resulting change in the distance between different parts was monitored by fluorescence resonance energy transfer (FRET) experiments,[104] a technique that has since become a standard characterization tool in the field. FRET is the radiationless transfer of excitation energy from one fluorophore to another (or a nonfluorescent quencher as used for MBs), which occurs through dipole–dipole interactions. Energy transfer leads to a reduction in the fluorescence intensity of the "donor" fluorophore and an increase in the fluorescence of the "acceptor". As a function of distance R between donor and acceptor, the transfer efficiency is reduced as $1/(1+(R/R_0)^6)$, where the characteristic distance R_0—the Förster distance—is typically on the order of a few nanometers. In FRET experiments, donor and acceptor molecules are strategically attached to parts of the molecules under investigation, whose nanoscale motion is to be monitored. In more recent studies, the B-Z DNA structural switch has been used to modulate the fluorescence properties of pyrene-functionalized nucleobases.[105]

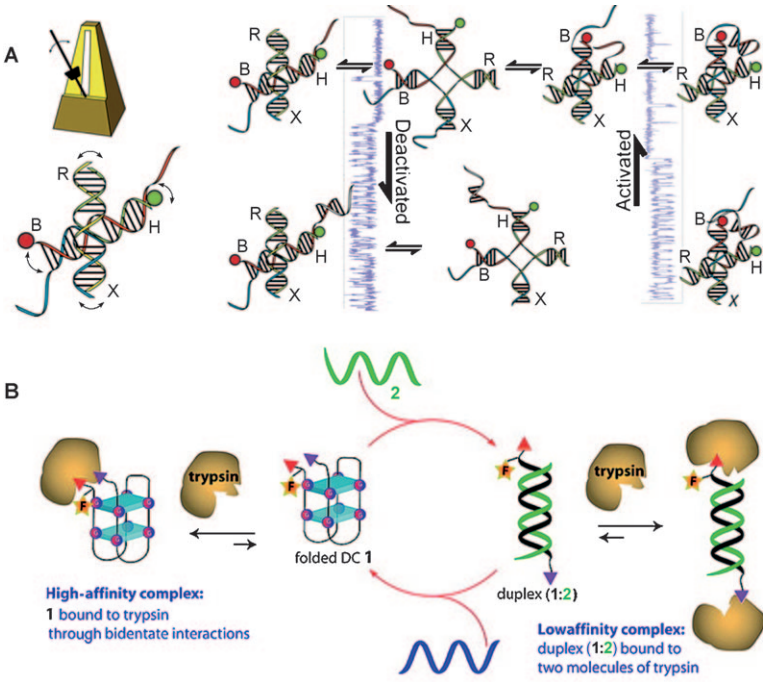

Figure 6. A) Manipulation of the conformation of a Holliday junction by addition of Mg^{2+} ions and an activator strand results in a device that functions like a nanoscale metronome (top left corner).[107] B) A G-quadruplex-forming oligonucleotide positions ligands in a bidentate manner that achieves cooperative binding to a target protein.[110] DC = DC = DNA–small molecule chimera. Reproduced with permission from the American Chemical Society.

3.2.1. *Switching with Magnesium*

A simple form of nanoscale motion was achieved by Niemeyer et al. by using magnesium-induced DNA supercoiling.[106] To this end, they synthesized networks of dsDNA connected by biotin–streptavidin linkers. In these networks, two neighboring DNA duplexes could condense into a supercoiled structure in the presence of Mg^{2+} ions and the resulting change of the network connectivity could be monitored by atomic force microscopy (AFM).

A more recent example of switch design involving the Holliday junction motif modulates the Holliday junction to create a nanoscale "metronome".[107] The Holliday junction folds into compact conformations called stacked X structures in the presence of divalent metal ions such as Mg^{2+} ($\geq$100 μM).[108] There are two alternative conformations, however, and the Holliday structure may flip between these two, roughly reminiscent of the motion of a metronome (Figure 6A). The ticking of the metronome can be influenced by an activator or deactivator strand that hybridizes to the assembly, and its speed can be controlled by manipulating the Mg^{2+} concentration. As the ticking transitions occur stochastically, the dynamics of the metronome had to be characterized by using single-molecule FRET rather than bulk techniques.

Magnesium ions are also crucial for the proper folding of large structural RNA molecules, and this property has been exploited to achieve Mg^{2+}-triggered folding and unfolding of the *Tetrahymena* ribozyme, which in turn toggles the formation and dissociation of a small DNA duplex.[99] Other divalent metal ions such as Zn^{2+} have also been used to switch a ternary DNA assembly between its M-DNA and B-DNA forms.[109]

3.2.2. *Triplex Switching*

Structures that transition between duplex and triplex forms under pH control have also been constructed (Figure 7). Mao and co-workers designed a ternary complex with a GC-rich duplex and a collapsed C-rich domain (shown in red in Figure 7).[111] Upon acidification, the C-rich domain is protonated, and is accommodated in the major groove of the GC-rich duplex domain. The formation of the C^+G-C triplex causes a pinching of the assembly, which positions two fluorophores close to each other, as shown in Figure 7A. A similar strategy was adopted by the Samori research group to create a simple duplex system with a C-rich overhang that could fold back into the duplex major groove at low pH values (Figure 7B).[112] This folding-back mechanism of a C-rich overhang has also been utilized to control chemical reactions at specific sites between reactive moieties within such DNA switches (see Section 5.4).[113] The pH-induced duplex to triplex transition has been exploited to assemble gold-nanoparticles into clusters reversibly.[114] A recent example of a single-helical to parallel-duplex switch, also toggled by pH, is exhibited by short poly d(A) segments that fold into A motifs with remarkable speed.[37]

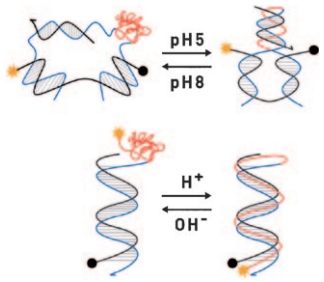

Figure 7. Two examples of molecular switches that utilize the triplex-forming ability of a C-rich sequence (red) that gets protonated under acidic conditions and forms a CG-C$^+$ triplex strand.[111,113] The conformational changes are monitored using FRET between a fluorophore (yellow star) and a quencher molecule (black).

3.2.3. *Devices Based on the i Motif*

An important finding in 2002 was the independent conceptualization and validation of the B-DNA to G-quadruplex transition as a nanoswitch by the research groups of Mergny and Tan.[115] B-DNA sequences where the G-rich strand has quadruplex-forming potential also have a C-rich strand with i-motif-forming potential.[116] Thus, shortly after, the Balasubramanian research group was able to validate a switch, whereby an i-motif-forming sequence at pH 5 was trapped as a duplex at near neutral pH (Figure 8A).[117] Here

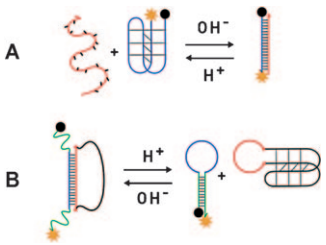

Figure 8. A) The first i-motif-based nanoswitch. The switch uses a pH toggle. At acidic pH values the C-rich strand (shown in blue) forms an i motif and at physiological pH values the blue strand is trapped as a duplex.[117] B) Transducing the molecular motion of an i switch to open and close a molecular beacon.[120]

the C-rich strand formed a scrunched i-motif conformation in one state and an extended duplex form in the other, similar to a molecular inchworm. Importantly, this study demonstrated the advantage of a toggle based on protons or hydroxy ions on the response times and cyclability of the DNA switch. A working cycle that generates by-products of water and salt, which are nontoxic to the system, results in the efficient reversibility observed in i-motif-based devices. Furthermore, the high speed associated with protonation and deprotonation in aqueous media ensures that the rate-limiting step is the conformational change in the DNA strand.

DNA sequences that form i motifs have been shown to switch between their single-stranded and i-motif states when triggered by light.[118] Irradiation of an acidic solution of Malachite Green carbinol base with UV light causes an increase in the pH value of the medium, which then induces the i motif to unfold. In the dark, recombination of the Malachite green cation with the hydroxy ions relaxes the system back to an acidic pH value and a folded i motif. I motifs can also be switched efficiently between their folded and unfolded states by using electrochemical stimuli.[119]

Newer designs of i-motif-based switches, where the strand motion is transduced to two coaxially stacked DNA duplexes andthereby resulting in second-order lever motions on the nanoscale, have been constructed recently (Figure 9).[121,122] A

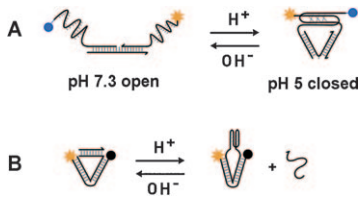

Figure 9. i-Motif-based switches that function as second-order nanolevers.[121,122]

key development was the first demonstration of i-motif folding and unfolding in response to a self-sustaining chemical reaction in situ that resulted in pH oscillations.[123] By using a variant of the oscillatory Landolt reaction, the environmental pH value was varied between pH 5 and 7. This device was shown to report on pH oscillations with high reversibility, even when immobilized on a 2D surface.[124] These two studies were important precursors to the validation that an i-motif-based DNA device could reversibly respond to environmental changes in the pH value within a living cell, while staying embedded in a biological 2D surface, namely the inner leaflet of an endosomal membrane (see Section 7).[121]

A number of i-motif-based switches have been used to transduce the chemical change of the pH value into other observable changes in assembly properties. The formation of i motifs has been used to reversibly cluster DNA-functionalized gold nanoparticles.[125] This results in a change in the optical properties of the clustered gold nanoparticles, which has been exploited as an efficient colorimetric assay to sense pH values in vitro with an impressive accuracy of 0.04 pH units.[126] An intriguing application of i-motif switching is the transduction of the structural change in the i motif onto another DNA device, namely the opening and closing of a molecular beacon (Figure 8B).[120] The nanomechanical motion of the opening and closing of the i motif has also been transduced to moving a fluorophore closer and farther from a gold surface,[127] as well as to bring about the mechanical motion of cantilevers coated with i-motif sequences on the basis of alterations in the surface stress.[128] These microcantilever experiments offered the first direct experimental proof that DNA-based nanodevices could actually be used to generate forces. Changes in surface properties induced by arrays of i-motif-forming sequences have been utilized to alter the nature of a surface comprised of immobilized i-motif DNA sequences between superhydrophilic and superhydrophobic states by the Liu and Jiang research groups.[129]

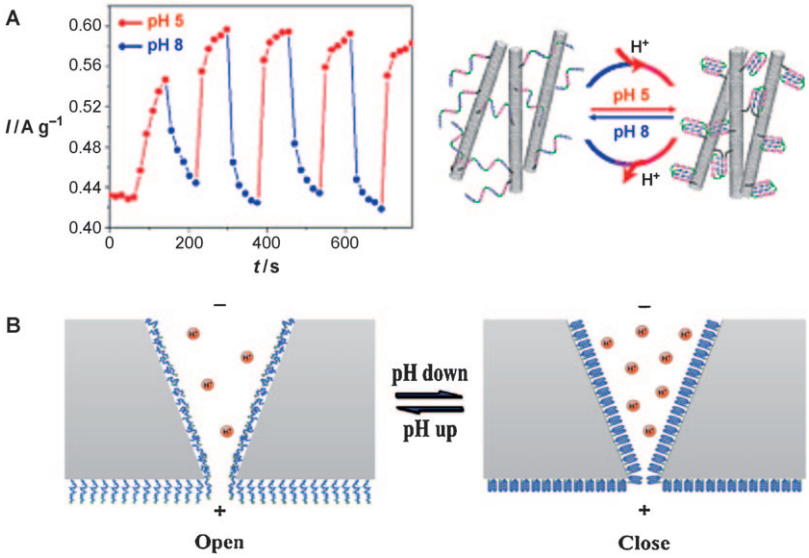

Figure 10. A) i Motifs used to align functionalized carbon nanotubes and modulate the electrochemical activity of a CNT-modified electrode.[130] B) i-Motif-functionalized pores are opened and closed with the aid of pH changes, which is reflected by an enhanced or reduced ion conductance of the pore; red spheres represent H^+.[131] Reproduced with permission from the Royal Society of Chemistry (A) and American Chemical Society (B).

The formation and dissociation of i motifs has been used to reversibly align collections of i-motif-functionalized CNTs, whose electrochemical properties switched between their aligned and monomeric forms (Figure 10 A).[130] In an exciting development, a solid-state device with a conical pore that was surface-functionalized by i-motif-forming sequences has been shown to mimic the opening and closing of ion channels (Figure 10 B). Here, when the DNA strands are in the unstructured state at a neutral pH value, the solid-state nanopore remains open and an ionic current can pass through it. At acidic pH values, the formation of i motifs by these sequences blocks the nanopore, and this is reflected in a reduction of the current.[131]

3.2.4. *G-quadruplex-Based Switches*

A doubly labeled G-quadruplex-forming oligonucleotide (GFO) was used to elucidate the duplex to quadruplex transition as the basis of a nanoswitch.[115] In the presence of a complementary C-rich strand with a toehold, the G-quadruplex conformation of the GFO is opened up and trapped as a Watson–Crick base-paired duplex with an overhang on the C-rich strand of the duplex (Figure 11 A). In the reverse step, the addition of an unlabeled G-rich strand competes with the doubly labeled GFO from the duplex, thereby forming duplex waste, and leaving the GFO in its folded quadruplex form.[115] The conformational changes were, again, monitored by FRET between the two labels.

In contrast to switches based on i motifs, the generation of duplex waste and the complex nature of the fuel resulted in lower cyclability and slower response times. The rate-limiting barrier in this device was the tendency for the G-rich reset ("antifuel") strand to also be folded into a quadruplex, which impedes effective strand invasion of the open state. However, the utilization of a catalytic strand that prevents the folding of the G-rich fuel strand was shown to speed up the restoration reaction.[132] Shortly after, Sen and co-workers demonstrated that a duplex with intervening G-rich domains in the center could fold into G quadruplexes, and bend the overall duplex into a closed or "pinched" state (Figure 11 B).[133] Duplex pinching was triggered by the addition of Sr^{2+} ions, which are potent positive regulators of quadruplex formation, and relaxed by sequestering the divalent metal cations with an effective chelator such as ethylenediaminetetraacetate (EDTA). Exquisite control over the quadruplex structure has recently been demonstrated by the Balasubramanian research group, who showed a G-rich oligonucleotide could be switched between its parallel and antiparallel quadruplex topologies depending on the kind of small-molecular binder that is made available to it in solution. Again, these solution studies were also associated with limited cyclability because of poisoning

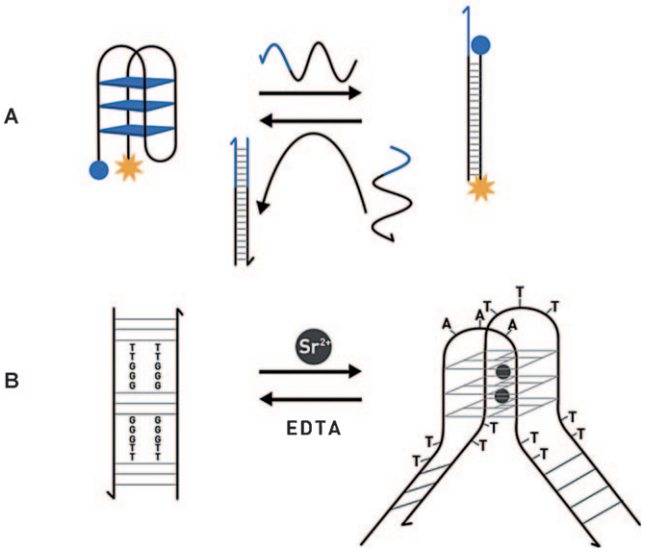

Figure 11. G-quadruplex-based devices A) Stretching out of a G quadruplex into a duplex with a "fuel" strand and reversal of the process with a complementary "antifuel" strand.[115] B) Duplex pinching by intervening G-quadruplex-forming sequences in the presence of divalent cations.[133]

of the system by additives.[134] Nevertheless, they still remain promising for surface-immobilized applications where solution replacement is feasible.

The duplex to quadruplex structural change has been transduced into a variety of outputs. Quadruplexes have been shown to function as molecular beacons, where the duplex stem is replaced by G-rich oligonucleotide segments.[135] Rather than fluorophores, the G-quadruplex scaffold can also be used to position two functional groups proximally at

the 5′- and 3′-termini of the structured quadruplex. Thus, Harris et al. have functionalized the corresponding ends of a GFO with ligands that bind to two alternate sites of trypsin. When the GFO is in the open, duplexed form, the trypsin is bound by an individual ligand. However, when switched to the quadruplex form, this scaffold shows enhanced binding of trypsin as a result of cooperative two-site binding (see Figure 6B).[110] The GFO is reversibly toggled between its quadruplexed and duplexed states by the addition of the standard fuel and antifuel strands.

Several G-quadruplex devices have exploited the reversibility associated with divalent metal ion induced formation and chelator-induced resetting of the system. The reversible chelation of a Ni^{2+} ion by a 2,2′-bipyridyl unit connecting G-rich segments in a DNA strand gave rise to a one-dimensional "G wire" that could be disrupted, in a reversible manner, into a disordered structure upon sequestering the Ni^{2+} ions with EDTA.[136] In an unusual example, the binding of ligand 360 A—a G-quadruplex-specific ligand—could be switched off in the presence of Cu^{2+} ions and regained when the system was reset with EDTA.[137] Since G-quadruplex formation is greatly facilitated by the presence of K^+ ions, surface-immobilized GFOs have been used to position ferrocene moieties closer to the surface in the presence of K^+ ions. This forms the basis of a reagentless detection platform for K^+ ions.[138] In other studies, FRET between two fluorophores[139] or between a surface functionalized with a cationic charged polymer and a fluorescently labeled GFO have also been used for the detection of K^+ ions.[140]

Many aptamers also contain G quadruplexes, and one of the best-known examples of this is the thrombin-binding aptamer (TBA).[141] Ferrocene-labeled TBAs can be utilized for the electrochemical sensing of thrombin, similar to the K^+ sensors mentioned above.[142]

In a different context, the TBA has been a powerful model system in which the G-quadruplex–duplex or G-quadruplex–single-stranded structural switches can transduce reaction cascades by thrombin binding and release. A pioneering example of this was the binding and release of thrombin by the TBA carrying an overhang, where thrombin release was triggered by the addition of a release strand R, complementary to the TBA overhang while partially overlapping the TBA sequence. The system was reset by the addition of an antifuel strand that was completely complementary to R.[143]

By using a conjugate of TBA attached to thrombin through a DNA linker, the addition of a complementary DNA strand causes a conformational rigidification of the linker, which in turn pulls the TBA out from its binding site on thrombin. This binding site is now free to catalyze a biochemical reaction, thereby resulting in fluorescence. The use of this cascade enabled a DNA sequence to be detected at concentrations of 10 nM.[144]

Both sense and antisense strands of telomeric DNA sequences can exist in a tetraplexed form. The G-rich strand can switch into a quadruplex conformation when triggered with metal ions, while the C-rich strand can switch into the i motif in the presence of hydrogen ions. Thus, Sugimoto and co-workers have used this system to build "logic gates" that combine the quadruplex and i-motif-forming capacity of both strands. By varying the environmental conditions, namely, the pH value and concentration of metal ions, they demonstrated its existence in four distinct states.[145]

3.3. Hybridization-Driven Devices

In the devices of the previous section, reversible nanoscale motion was induced by a repeated change in the buffer conditions. One of the major drawbacks of buffer-driven devices is the lack of specificity of the "effector signal". Changes in the buffer affect all the molecular species present and usually do not allow one particular type of "device" to be addressed. Buffer-based systems, therefore, only utilize the structural and mechanical properties of DNA and do not make full use of the "programmability" of DNA molecules. The higher specificity is accompanied, however, by a slower response of these devices.

The first example of a nanomechanical device that was not only made from, but also driven by, DNA molecules were the "DNA tweezers" reported by Yurke et al. in 2000.[5] Their operation principle is shown in Figure 12A. The original DNA tweezers were assembled from three strands of DNA. One central 40 nucleotide long strand and two 42 nucleotide long peripheral strands together form a structure, in which two 18 base pair long double-stranded "arms" are connected by a 4 nucleotide single-stranded "hinge". In the "open" state of the tweezers, 24 bases of each of the peripheral strands are unpaired. The tweezers can be brought into a "closed" configuration by hybridization to a 56 base "fuel" or "set" strand. Of these 56 bases, 48 are complementary to the single-stranded extensions of the arms of the tweezers. The remaining 8 bases are used as a "toehold" for a "reset" strand, which is complementary to the initial fuel strand. The reset strand can attach to this region and initiate a branch migration process, which displaces the fuel from the tweezers and, therefore, opens them again (see Section 2.1). The alternate addition of set and reset strands allows the tweezers to be cycled through their open and closed states. The motion of such a nanodevice can be monitored by FRET between two fluorescent labels or by monitoring the different conformational states by gel electrophoresis.

Even though the original tweezers had no specific function, several conceptual aspects demonstrated with this device were important. First of all, the device was driven in a sequence-specific manner—only set strands with the correct sequence actuate the DNA tweezers, which makes the device addressable. Importantly, while there were previous examples of hybridization-driven conformational switching—as in molecular beacons—the utilization of the branch migration process allowed, for the first time, the reversal of this process—that is, double-stranded sections of a structure could be made single-stranded again without thermal denaturation. Furthermore, in contrast to many other molecular switches, the tweezers system could, in principle, perform work—closing and opening of the tweezers proceeds by thermodynamically distinct paths, and during each operation cycle of the tweezers, a waste duplex is produced. The hybridization free energy of the waste duplex represents the

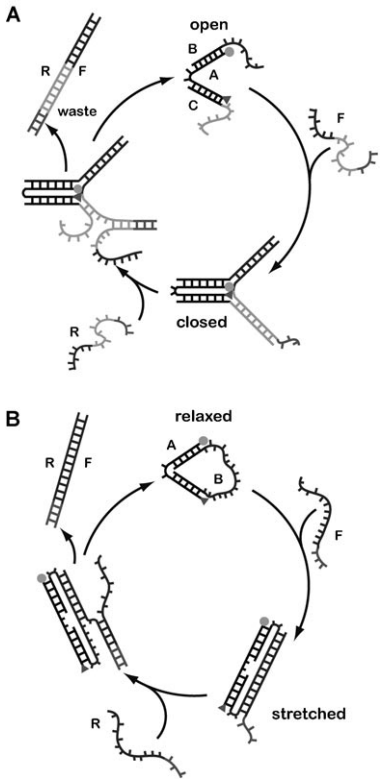

Figure 12. Two prototype DNA nanodevices that utilize strand exchange by branch migration. A) DNA tweezers[5] in the open state are formed by three DNA strands (A–C). Hybridization with a "fuel strand" F brings the tweezers into a closed conformation. Strand F can be removed from the closed tweezers with strand R in a branch migration process. This returns the tweezers to the open conformation and results in the production of a waste duplex R-F. B) A DNA actuator in the relaxed state is composed of two strands that form two rigid arms connected by a single-stranded ring.[146,147] Hybridization to a fuel strand F stretches the device, while removal of F by strand R brings the device back into the relaxed conformation.

maximum chemical energy available for one cycle of the device—in the case of a 56 base pair duplex, this is roughly 300 kJ mol^{-1}!

Many variations of the tweezers system have since been developed. By connecting the arms of the tweezers with a single-stranded loop, an "actuator" device was realized that could both stretch[146] and contract,[148] depending on the type of set strand used. The contraction of the actuator is analogous to the closing of the tweezers (Figure 12 A); its stretching motion is shown in Figure 12 B. A variation of this device incorporated an RNA-cleaving deoxyribozyme (or DNAzyme, see Section 2.3) in the loop region. The binding of a hybrid fuel molecule containing an RNA base to the substrate-recognition sequence of the DNAzyme results first in a stretching motion of the DNA device. The fuel is then cleaved by the DNAzyme into two smaller fragments, which dissociate from the device because of their lower thermodynamic stability.[149] This operation cycle can be improved by controlled degradation of the RNA fuel by RNase H.[150]

As mentioned in Section 2, the motion of hybridization-driven devices is limited by the relatively slow progress of hybridization and strand-displacement reactions. Several attempts have been made to improve the kinetics of such devices. A simple approach is to operate DNA devices at higher temperatures or concentrations.[151] Another possibility is to use special buffer conditions or additives. For example, Choi et al. utilized a cationic polymer (poly(L-lysine)-*graft*-dextran), which had been shown previously to speed up hybridization and strand-exchange reactions.[152] By using the poly(L-lysine)-dextran they could significantly improve the response and performance of DNA tweezers as well as of other nucleic acid devices.[27]

An interesting approach to control the motion of DNA nanodevices utilizes DNA bases modified with the photoswitchable molecule azobenzene, as demonstrated by Asanuma and co-workers[96] and Ogura et al.[98] Azobenzene can be switched from its *trans* to the *cis* configuration by illumination with light with a wavelength of 330–350 nm, whereas illumination at 440–460 nm switches the molecule back to the *trans* form. It is only in the *trans* form that azobenzene intercalates efficiently into the DNA double helix. By contrast, *cis*-azobenzene destabilizes a DNA duplex, and results in a considerably reduced melting temperature.[96,153] Asanuma and co-workers synthesized azobenzene-modified fuel strands for DNA tweezers which were able to close the tweezers only in the *trans* form.[96] Ogura et al.[98] utilized a fuel strand which was modified with azobenzene only in one half of the molecule. The unmodified segment was attached to one of the tweezers arms permanently, whereas the modified segment could be photoswitched to repeatedly open and close the tweezers. The intramolecular interaction in this approach results in much faster closing kinetics than for conventional tweezers. The utilization of photoswitchable DNA hybridization could be of great interest for many other applications in DNA nanoscience. Photoswitching could be used to control the operation of DNA-binding proteins with light, or to trigger and synchronize DNA-based reaction cascades.

One problem associated with the model system of the DNA tweezers is the tendency to form dimers. Rather than closing a single pair of DNA tweezers, fuel strands can also cross-link two or more tweezers structures, thereby resulting in a heterogeneous "closed" state. This can be recognized, for example, in gel electrophoresis experiments. Thus far, many studies on DNA nanodevices have neglected a rigorous characterization of these structures. For example, cyclical operation of DNA nanodevices is often demonstrated in FRET studies, but these experiments only have limited value when performed on a mixture of monomers and multimers. Their quantitative significance is further reduced, given that DNA molecules are generally not quantitatively labeled.

These problems can be circumvented, at least in part, by single-molecule fluorescence studies, which allow incompletely labeled DNA structures and their labeling stoichiometry to be identified. Single-pair FRET (spFRET) studies conducted on DNA tweezers have shown that a closed tweezers sample contains several subpopulations with different FRET efficiencies. The use of only the FRET values for properly closed and open tweezers allowed a much more accurate determination of distances within the DNA device

than bulk experiments. It is expected that such single-molecule techniques will play an increasingly important role as a diagnostic tool for the construction and characterization of nucleic acid nanodevices. For example, spFRET was recently also applied to a switchable DNA nanocontainer (see Section 5.2),[154] and it was also used to characterize the "nanometronome" already mentioned in Section 3.2.[107]

A more complex hybridization-driven device than the tweezers-related structures discussed above was developed by Yan et al.[155] The so-called "PX-JX_2 device" is based on "paranemic-cross-over" DNA and has a reduced tendency to form dimers. A paranemic DNA structure can be formed by reciprocal exchange between strands of the same polarity on two DNA double helices at every possible position (examples of PX and JX_2 structures are shown in Figure 13).[156] When parts of this structure are removed and replaced by DNA sections without cross-overs, molecules in a "juxtaposed" structure result in which two helices are rotated by 180° with respect to the paranemic structure. This motion can be used to rotate molecular structures attached to the device, which can be characterized, for example, by atomic force microscopy (AFM).

Seeman and co-workers have demonstrated several increasingly complex molecular devices based on the PX-JX_2 motif. For example, the PX-JX_2 motif was recently extended to a three-state device[157]—called a PX-JX_2-BX device—in which, in addition to the rotation of the PX section, the central part of the device could be made to contract and extrude two double-cross-over sections, which results in an overall crosslike conformation (Figure 13). Furthermore, it was shown that a pair of PX-JX_2 devices could be operated in parallel by using the same set of effector strands.[158] In this approach, one device was switched from the PX to the JX_2 conformation, while the other was switched in the opposite direction, thereby resulting in a reciprocating motion of the two structures. This study also represented one of the first examples in which two distinct devices were actually operated in parallel within the same reaction volume.

In a different series of experiments, Ding and Seeman introduced a PX-JX_2 "cassette" into a supramolecular network made from triple cross-over (TX) DNA tiles.[159] By using double-stranded "pointer molecules" attached to the cassettes, the switching between the PX and JX_2 states could be impressively visualized for the whole supramolecular array, thus alluding to the concept of a future assembly line of "DNA robots". A similar approach was recently adopted for a system based on DNA origami.[160]

As will be discussed in Section 7, one of the goals of nucleic acid nanotechnology is the operation of DNA-based nanodevices in vivo. Unfortunately, DNA does not occur as a single-stranded molecule in living organisms—RNA, however, does. For this reason, several research groups have already attempted to utilize RNA strands rather than DNA as effector molecules to drive DNA devices. Both the PX-JX_2 device[161] and DNA tweezers[162] have been shown to be operable with RNA effector strands. These examples represent the first attempts to direct the

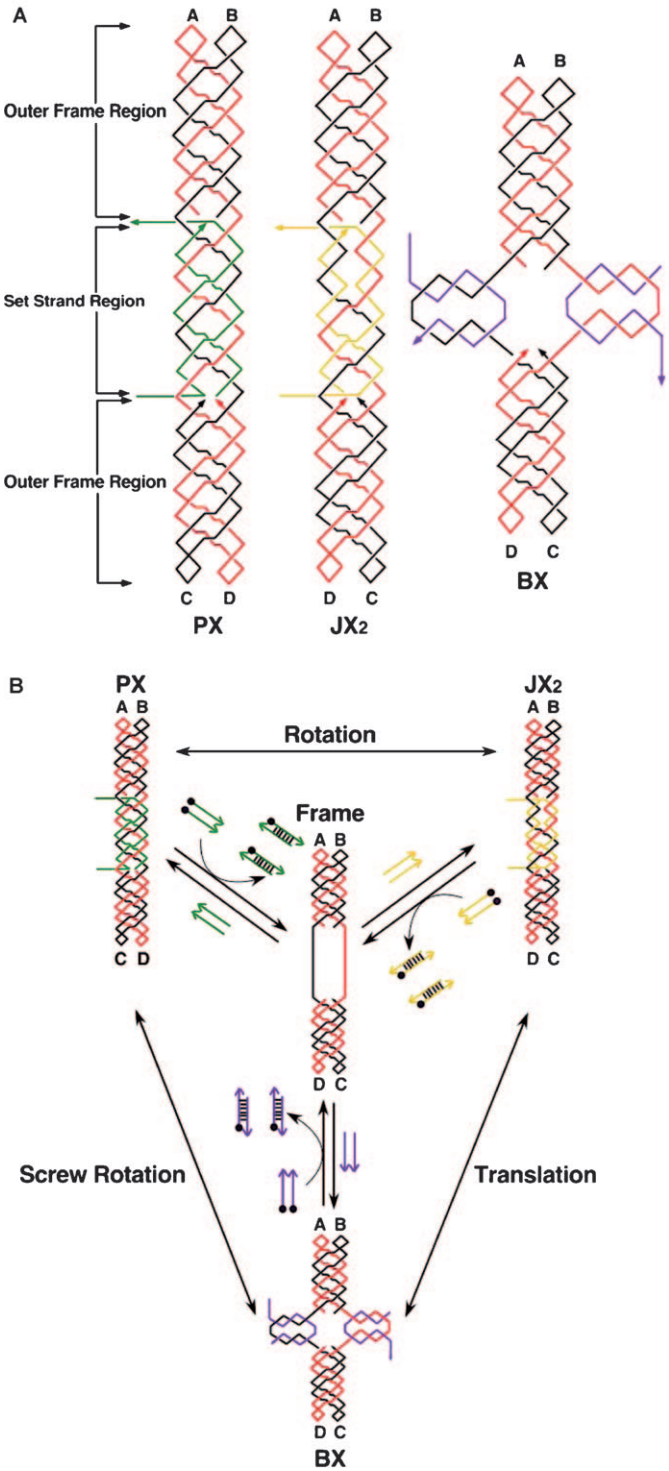

Figure 13. A three-state device based on strand exchange and the "PX", "JX_2", "BX" structures.[157] A) In the "paranemic" state PX, two double helices are connected with a maximum number of strand cross-overs. In the JX_2 state, two of them are removed, thereby resulting in a juxtaposition of the helices C and D. In addition, the inner region of the structure can be bulged out to form the BX state. B) The transitions between the different structures can be driven by the removal and addition of appropriate set strands. Reprinted with permission from The Proceedings of the National Academy of Sciences of the USA.

action of DNA devices with "genetic" information. Ref. [163] describes how the production of the RNA control sequences for DNA tweezers was actually put under the control of simple gene-regulatory elements in vitro.

3.4. Devices Incorporating Aptamers

Aptamers are extremely promising components for functional nanodevices, and many others, in addition to TBA, have already been utilized (see Section 3.2). An aptamer-based switch has used the AMP-binding aptamer in the first half of the working cycle and the addition of adenosine deaminase to reset the system.[164] This finding was then extended to create AMP/adenosine deaminase powered closing and opening of DNA tweezers with AMP aptamers at the tweezer termini conjoined by a short DNA strand that falls off when the aptamers are in their folded forms (Figure 14).[165] The binding of a protein to its aptamer has

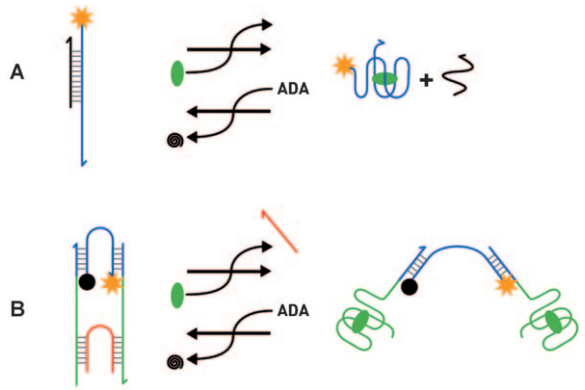

Figure 14. A,B) Molecular aptamer-based devices that transduce adenosine (green oval) binding into fluorescent read-outs, and the use of adenosine deaminase (ADA) to reset the system by degrading adenosine to inosine (black spiral).[164,165] The device in (B) is actually a combination of two aptamers with DNA "tweezers".

also been switched off in the presence of a partially complementary strand that unfolds the aptamer. The protein is converted from its inactive aptamer-bound form into its free active form. Thus, DNA-induced inactivation of a Taq pol aptamer is transduced into DNA polymerization activity by freed Taq polymerase, and this can be switched on and off reversibly.[166] In an elegant demonstration of the generic nature of switchability inherent to aptamer binding, Nutiu and Li designed assemblies that induce a change in fluorescence when complexed to the target molecule. In the absence of the target, the fluorescent assembly incorporating the aptamer binds to a segment of DNA labeled with a quencher. In the presence of the target, the formation of the aptamer–target module forces the quencher–DNA strand to dissociate from the assembly, which relieves the fluorescence quenching.[167] A few ways to achieve fluorescent read-outs of binding events by using aptamer modules is illustrated in Figure 22. Many more applications of functional nucleic acids are described in Sections 6 and 7.

4. Molecular Motors and Walkers

Molecular motors—molecules that generate forces and motion—are among the most impressive molecular machines found in nature. A large body of theoretical and experimental work has been devoted to the question of how molecular assemblies can transform chemical energy into directed movement in the presence of Brownian motion.[168] In recent years, researchers have begun to utilize the self-assembling properties of DNA and RNA molecules to construct experimentally the first nonbiological prototypes of such Brownian motors.[169]

4.1. Hybridization-Driven DNA walkers

The first walker systems made purely from DNA were based on a rather simple idea. Typically, a two-legged DNA walker—which contains two single-stranded "feet"—is initially connected to a DNA molecular track by joining the feet to single-stranded "footholds" that protrude from the track through "connector strands". The connector strands can be displaced from the track sequence-specifically by removal strands through branch migration. When a DNA "foot" is lifted from the track in such a way, it can be connected to the next free foothold strand on the track. This can be repeated several times with the appropriate connector and removal strands to move the walker to an arbitrary position on the track.

This principle was first used in the study by Shin and Pierce,[170] where the walker was simply a DNA duplex with two single-stranded extensions. A similar principle but a more complex arrangement was adopted by Sherman and Seeman,[171] who constructed a bipedal walker from two DNA duplexes joined by flexible single-stranded linkers. This walker could be translocated along a supramolecular track that consisted of a triple cross-over (TX) molecule equipped with single-stranded footholds.

Tian and Mao used essentially the same principle to generate a system involving a different kind of unidirectional motion which they termed "molecular gears".[172] In this system two circular DNA molecules were made to move with respect to each other, driven by the same mechanism of addition and removal of connector strands. The two circles consisted of a circular single strand to which three other strands were hybridized. These strands contained flexible hinges with single-stranded foothold extensions. The flexibility of the hinges enabled two circles to be linked with two connector strands simultaneously. By alternating the addition of linker and removal strands in the correct order, the two circles could then be made to roll against each other in one direction.

All these "first generation" systems have the severe drawback in that they are synchronized externally, that is, additional DNA strands have to be added manually for every single step of the walker. For this reason, a variety of concepts for the autonomous motion of DNA walkers were developed later on, which typically incorporated a catalytic reaction that either utilized enzymes or the principle of hybridization

catalysis. This principle is utilized in the second generation of DNA walkers, which was developed by Pierce, Turberfield, as well as Seeman and co-workers. In these systems, the DNA walker plays the role of a hybridization catalyst (see Section 2.1). It catalyzes the reaction between hairpin fuel strands or hairpin fuels and the track of the motors. The mechanistic details of this catalysis process are ingeniously designed to produce unidirectional motion of the walkers. As an example, the principle of the autonomous walker by Yin et al.[173] is shown in Figure 15.

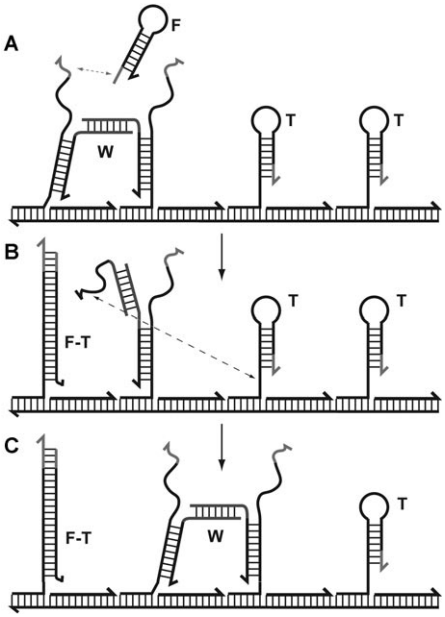

Figure 15. Autonomous, but nonprocessive DNA walker system. A) Initially, the walker W is attached to the track with its two "feet". Unoccupied footholds T on the track form "inert" hairpin structures. The fuel hairpins F can only be opened by occupied footholds. B) After hybridization of F with the leftmost foothold, the left foot of the walker is detached from the track. It can now occupy the next foothold hairpin to the right. C) After hybridization of W with T, the walker has effectively taken one step to the right. W acts as a hybridization catalyst for the reaction of fuel molecules F hairpins with foothold molecules T. Adapted from Ref. [169].

Based on a similar idea, a considerably more complex walker was recently developed by Omabegho et al. which could walk on a DX track rather than on a dsDNA track. By using two distinct footholds and an elaborate stepping scheme, autonomous and processive motion of the walker could be demonstrated over several steps.

For the two walkers developed by Yin et al.[173] and Omabegho et al.[174] the "burnt bridges" approach was used—as for many of the other walker systems realized so far. In this approach, the footholds on the track are either destroyed or made unusable after the walker has traversed. In this rather brute-force way, stepping back is prevented and directional motion is enforced. Unlike in natural systems, the supramolecular DNA tracks can, therefore, only be used once. Furthermore, it is not possible to operate several walkers on the same part of a track. This, however, would be highly desirable if DNA motor systems were really to be used as transport systems, such as kinesin motors in cells that carry organelles along microtubules. Turberfield and co-workers recently evolved an elegant operation scheme, where the track was not modified irreversibly.[175] The concept of this bipedal walker is shown in Figure 16. The walker has two feet

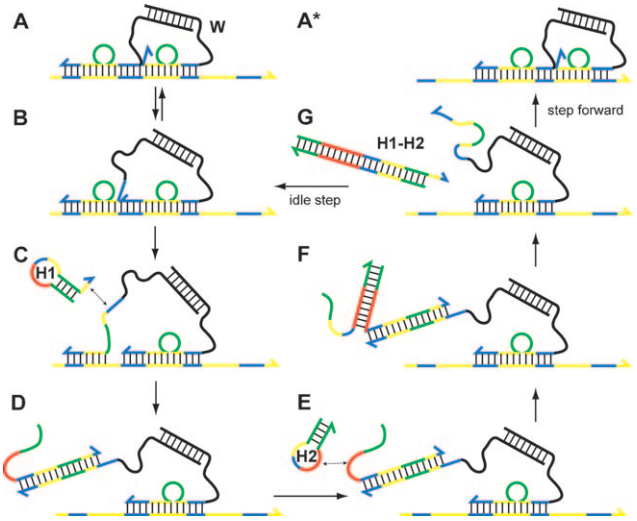

Figure 16. Autonomous walker by Green et al.[175] A,B) The two feet of walker W compete for binding to the single-stranded track, continuously switching between the states A and B. In position B, the left foot can be partially lifted and C,D) hybridizes to the hairpin H1 through an external toehold. E) Hairpin H1 is now activated and can hybridize to complementary fuel strand H2. F) A branch migration process removes waste duplex H1-H2 from the walker. G) The left foot is fully released from the track and can now diffusively take one step forward (A*) or rebind to its original binding site. Adapted from Ref. [169].

that can hybridize to a single-stranded DNA track. The binding sites for the feet, however, are designed to overlap slightly. The leading foot can induce unbinding of a DNA loop segment of the trailing foot, thereby making it accessible for hybridization with a fuel hairpin molecule. The opposite process—influencing the leading foot by the trailing foot—is not possible. As a consequence of this ingenious design, hybridization catalysis only occurs with the trailing foot, and therefore the motion of the walker is unidirectional and coordinated between the two feet.

4.2. DNA Walkers that Utilize Enzymes and Ribozymes

A variety of walker systems have been developed by using a hybrid approach, in which not only DNA hybridization, but also the action of enzymes or deoxyribozymes (DNAzymes) was used to generate motion. Yin et al.[177] demonstrated a first DNA device that utilized the action of a DNA ligase (covalently joining two DNA strands) and of restriction enzymes (cleaving connections) to translocate a DNA sequence along a one-dimensional scaffold. The device consisted of a double-stranded DNA track with regularly spaced DNA "anchors" that contained a "walker sequence"

with a sticky end that could be transferred from anchor to anchor by the action of the two enzymes. A very similar concept was later demonstrated by Turberfield and co-workers,[178] who utilized a DNA nicking enzyme (Figure 17). Again, the walker moved on a track of dsDNA with evenly spaced anchor sequences extending from it. In this case, the walker represented a single-stranded DNA molecule that could hybridize to one of the anchors, thereby creating the recognition sequence of the restriction enzyme N.BbvC IB. In the operation cycle of the walker, this enzyme introduces a single-stranded nick within the anchor strand to which the walker is attached. The disconnected section of the anchor dissociates, leaving a single-stranded toehold to which the neighboring anchor strand can attach. The walker strand is then displaced from the "old" anchor by branch migration and transferred to the new attachment point, after which the operation cycle resumes. Recently, Bath et al.[179] combined the concept of the processive DNA walker by Green et al.[175] with the action of the nicking enzyme N.BbvC IB, and demonstrated that their concept of "coordinated chemo-mechanical action" can be generalized to obtain energy for motion not only from hybridization, but also from other sources such as DNA or RNA hydrolysis.

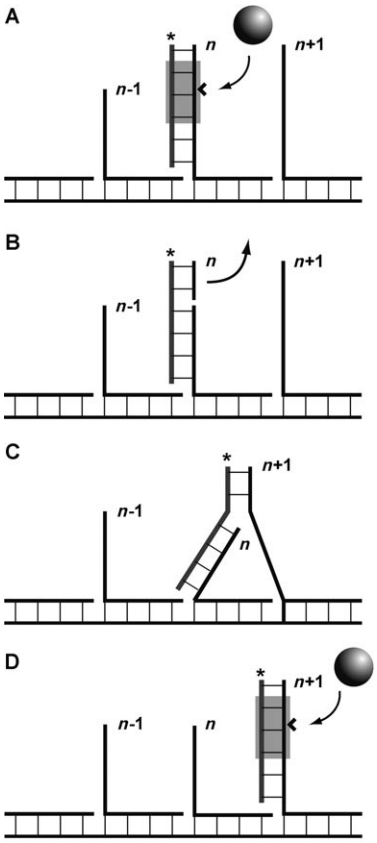

Figure 17. DNA walker based on a nicking enzyme.[178] A) The walker strand (marked *) is initially attached to anchor strand *n*. The anchor–walker duplex contains a recognition sequence (gray) for a nicking enzyme, which cleaves the anchor strand as indicated. B) This results in dissociation of the upper section of the anchor. C) Neighboring anchor strand ($n+1$) invades the anchor–walker duplex by branch migration, thus D) resulting in a complete transfer of the walker strand to the next binding site.

A different concept for DNA-based molecular motion was developed by Sahu et al.,[176] who utilized the highly processive polymerization and strand-displacement activity of the DNA polymerase from phage ϕ29. The system consisted of two interconnected rings: one circular "wheel" strand wound around a circular DNA track. A DNA primer was attached to the track molecule and extended by polymerization with ϕ29 DNA polymerase to induce motion of the wheel around the track. As this polymerase has a strong strand displacement activity, it could "push" the wheel away from its binding site and drive it along the track.

As mentioned in Section 2.3, certain biochemical reactions, such as phophodiester cleavage or ligation, can also be catalyzed by RNA or DNA molecules, so-called (deoxy)ribozymes. Some of the enzyme-driven DNA motor concepts described so far can, therefore, also be utilized in systems composed entirely of nucleic acids. For example, Tian et al.[180] have produced walkers from the RNA-cleaving "10-23" DNAzyme[181] that move along a double-stranded track with single-stranded footholds made from DNA/RNA hybrids containing a single RNA base. At each step, the DNAzyme attaches to one of the footholds and catalyzes its cleavage at the position of the RNA base. After dissociation of one of the cleavage products from the foothold, the DNAzyme is transferred to the next foothold by branch migration. This is essentially the same concept as that of the DNA walker by Bath et al.,[179] but with the nicking enzyme replaced by a DNAzyme.

A similar concept was recently used by Stojanovic and co-workers to construct "molecular spiders".[182] In this study four biotinylated 10-23 DNAzymes were attached to the four binding sites of the protein streptavidin to give a protein "body" with four catalytic "legs". This molecular assembly can be made to walk across a "lawn" of substrate molecules. Cleavage of the substrates means that the walker can never return to areas it has visited before. Even though the motion is essentially diffusive, it can be made directional by defining one-dimensional tracks of substrate molecules. This has recently been shown experimentally by using an "origami"-based track for the spiders. This has resulted in the first autonomous molecular walkers covering distances on the order of 100 nm.[183]

4.3. Polymerization Motors

In biological systems, forces are not only generated by molecular motors that show a "walking motion", but also by other processes such as polymerization of rigid molecular filaments. Cell crawling on surfaces is driven by cycles of extension and contraction, which are due to the continuous internal reorganization of the cytoskeleton.[184] The growth of filopodia, lamellipodia or microvilli, for example, is caused by polymerization of actin. The motion of certain pathogenic bacteria such as *Listeria monocytogenes* or *Rickettsia rickettsii* is also driven by the polymerization of actin. These bacteria

utilize the actin-based motility system of their host cells by nucleating actin filaments at one region of their surface to propel them through the cytosol at remarkable speeds of 10 μm min^{-1}. The moving bacteria leave behind a tail of actin filaments, which is also referred to as the "actin comet".[184,185]

Motivated by these biological examples, there have been attempts to also use DNA polymerization reactions to drive molecular motion. In Ref. [186], Venkataraman et al. demonstrated an artificial "DNA comet" which harnessed the "hybridization chain reaction" (HCR) previously introduced by Dirks and Pierce.[187] The HCR is based on catalysis of the hybridization between two hairpin structures by an initiator strand. Similar to the hybridization catalysis concept depicted in Figure 2, the initiator strand opens one of the hairpins and allows it to react with the second hairpin. This reveals a second catalytic region which opens another hairpin of the first type. The result is a chain reaction, in which the complementary hairpins hybridize with each other to form long filaments. By using fluorescently labeled DNA strands, the authors could demonstrate that the polymer grows between two initially neighboring strands in the filament, effectively separating them spatially during the course of polymerization. In analogy to the bacterial comet system, they then initiated the strand growth process on the edge of a DNA origami structure, thus resulting in long filamentous structures attached to a "DNA body" that could be visualized by atomic force microscopy.

5. Switchable Materials and Hybrid Devices

A variety of switchable and addressable molecular structures have been proposed in recent years for potential applications of DNA nanodevices. This comprises structures which change their geometry or their mechanical properties, and also devices which are able to capture or release nano-objects. In many cases, DNA hybrids were utilized, for example, DNA–protein conjugates, or branched structures involving organic linker molecules. In contrast to other strategies aimed at developing switchable materials, the main advantage of DNA as an effector molecular is, of course, its sequence-specificity, which allows the molecular switching process to be addressed precisely.

5.1. Gels and Molecular Networks

In the context of drug delivery and controlled release systems, there has been considerable interest in the development of switchable microgel systems, which can be used to trap pharmaceutical compounds and release them in response to an environmental trigger.[188] Another potential application of switchable gels is their use as "artificial muscles" as they may display pulsating mechanical behavior when subjected to periodically changing stimuli. Yurke and co-workers developed a DNA-switchable gel system, which was realized by copolymerization of acrylamide with DNA strands modified with a reactive group (acrydite).[189] The gel could be switched from the fluid state to the gel state by cross-linking the DNA–acrylamide strands with complementary DNA linker strands. The mechanical properties of the gel could be tuned by the amount of cross-linker strands, but it could also be switched back to the fluid state by removing the cross-linking strands by strand displacement with a removal strand. It was later shown that the system could be used to trap nanoparticles—in the form of fluorescent colloidal quantum dots—in the DNA–polyacrylamide gel and release them upon addition of an appropriate effector DNA (Figure 18).[190]

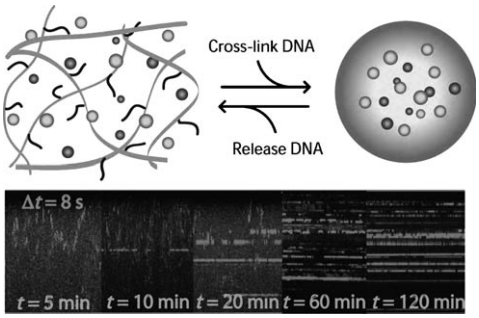

Figure 18. Top: DNA can be used as an addressable cross-linker for polymer hydrogels. By utilizing hybridization and strand removal by branch migration, the gelation process can be made sequence-dependent and reversible.[190] This has been used to reversibly trap and release fluorescent nanoparticles. Bottom: Fluorescence traces recorded from diffusing nanoparticles (kymographic representation) during various stages of the gelation process.

A variation of the DNA-cross-linked hydrogel by Yurke and co-workers was later realized by Mi and co-workers,[191] who used a cross-linking strand containing the sequence for the thrombin-binding aptamer. This was used to "load" the gel with the protein thrombin. In principle, it should be possible to also reverse this approach and make the resolution of an aptamer-cross-linked hydrogel dependent on the presence of the binding target in the gel. This could then be used to release drug carriers from the gel in response to a chemical signal.

There have also been several examples of switchable supramolecular networks that were made exclusively from DNA. For example, Luo and co-workers[266] produced dense hydrogels from branched DNA structures with a variety of branching topologies. These gels could be loaded with insulin, which was released over time upon degradation of the DNA. A switchable DNA polymer was demonstrated by Lubrich et al.,[192] who polymerized the DNA tweezers system[5] by using rolling-circle amplification. This resulted in a contractile nanostructure, which could be made to contract and extend in the same way as the monomeric tweezers. The corresponding changes in the length could be visualized by atomic force microscopy.

A few years earlier, Yan and co-workers had already realized a switchable DNA lattice, which could be switched between two different lattice spacings by the addition and removal of "stretching" strands.[193] The lattice consisted of cross-linked four-way junctions, which were connected with

partially self-complementary DNA sequences that could "bulge out" in a hairpin loop. A DNA strand complementary to the stem of the hairpins could hybridize to the hairpin and, therefore, stretch the lattice by a length of two turns of the double helix. The process could be reversed and the lattice be made to contract again by using the branch migration concept. One could also regard the array of movable "robotic arms" realized by Ding and Seeman, which was already mentioned above, as a more sophisticated example of a switchable DNA lattice.[159]

5.2. Switchable Containers

Potential applications in controlled delivery may also be expected from switchable DNA objects containing a cavity, in which proteins or other nanoscale objects can be trapped. In recent years, a variety of three-dimensional DNA objects have been realized,[194] among them polyhedra based on the assembly of a few DNA strands[195,196] and also three-dimensional origami structures composed of helix bundles.[197–199] In addition, a variety of structures have been realized, whose connectivity was determined by organic linker molecules.[200]

Turberfield and co-workers had already demonstrated that a protein (cytochrome *c*) could be incorporated into a DNA tetrahedron.[201] Furthermore, they could show that the area of the faces of the tetrahedron could be varied by changing the length of the edges.[154] This was achieved by using the switching principle employed by Yan and co-workers for the size-tunable lattice described above.[193] By using the same strategy, Aldaye and Sleiman switched the extension of DNA containers, whose edges were connected by organic vertex molecules.[202]

In a different approach, Gothelf and co-workers constructed a "box" by using the origami technique.[198] The box was constructed such that one side (the "lid") could be opened using DNA "keys". To this end, the lid was connected to one side of the box with a hinge, and to another side with "linker molecules". The linkers could be unzipped by the DNA keys by branch migration, thereby opening the box (Figure 19). So far, there has been no demonstration of a combination of "encapsulation" and "programmed delivery" using DNA-based containers. This will be a major challenge for future experiments.

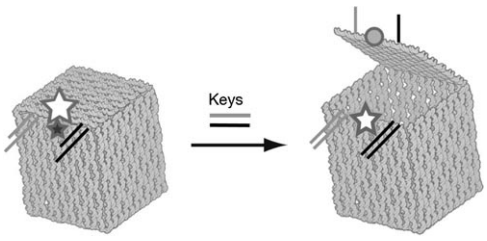

Figure 19. A molecular box made using the DNA origami technique.[198] The lid of the box is closed with two DNA duplexes. Strand displacement using DNA "key" strands can be used to open the box on demand. Reprinted with permission from the Nature Publishing Group.

5.3. DNA/Protein Chimeras

An interesting recent development in chemical biology is the synthesis of DNA–protein conjugates with potential applications as switchable and addressable "materials" as well as biochemical transducers.[203] We give here only a few examples. Choi et al. demonstrated that the activity of an "allosteric" enzyme–DNA conjugate could be changed through induction of a mechanical strain by DNA hybridization.[204] Using DNA–peptide conjugates, Seitz and co-workers were able to control the conformation of peptides through the formation of DNA duplexes, and hence control their biological activity.[205] This strategy was recently utilized to switch the activity of a protein kinase with a peptide nucleic acid (PNA) phosphopeptide hybrid. To this end, a peptide with an affinity for binding to the active domain of the kinase was initially forced into an inactive loop conformation by hybridization of the PNA conjugate with a complementary strand of DNA. The peptide could be released by an RNA molecule by strand displacement, which activated the kinase.[206] DNA conjugates with the photoswitchable fluorescent protein Dronpa and a fluorophore were used for live cell imaging applications, in which the fluorescence of the DNA constructs was switched and detected by using an optical lock-in detection method.[207] DNA–enzyme conjugates were recently also used to assemble artificial multienzyme complexes with enhanced catalytic efficiencies.[208]

5.4. DNA-Directed Synthesis

A challenging idea is the combination of switchable mechanical motion based on DNA nanodevices and DNA-directed synthesis—this would result in a "molecular assembly line" or an artificial "translation machinery".[209] DNA-directed synthesis is based on the idea that chemical reactants can be placed along a DNA scaffold in a sequence-programmable manner. These compounds, which are in proximity, are then made to react with each other with high efficiency. A large variety of compounds have already been synthesized in this way, as reviewed excellently in Ref. [210]. In principle, a DNA "code" could be translated into novel compounds or heteropolymers by this strategy.[210,211]

So far, DNA-directed synthesis has only been combined with DNA nanomechanical switching in a few cases. Chen and Mao demonstrated that mechanical switching of a DNA nanodevice can be used to "choose" between two alternative reactions. In this case, a DNA strand bearing a carboxy end group was brought close to either of two DNA strands modified with an amine function. Subsequent formation of a peptide bond consequently resulted in two distinct products.[113] Gu et al. showed that a PX-JX_2 device incorporated into a DNA origami structure could be used to assemble different patterns on the origami substrate.[160] The same group could recently even demonstrate the programmable arrangement of nanoparticles into patterns by a movable molecular "assembler".[212]

Even though there are severe practical problems involved, such as reaction turnover and scaling of the reactions, a

programmable molecular robotic line is certainly an outstanding scientific and conceptual achievement.

6. DNA Computation and Molecular Programming

6.1. DNA Computing

6.1.1. Traditional Approaches

Considering the capacity of DNA molecules for information storage, they seem to be a clear choice of substrate for a molecular computer. In fact, DNA-processing enzymes were already compared to Turing machines working on a DNA "tape" in 1973.[213] In 1994 computer scientist Leonard Adleman[3] demonstrated that a computational problem related to the famous "traveling salesman" problem could be solved experimentally by using DNA and the "toolkit" provided by molecular biology. In this problem, a route through a number of cities is sought in which each city is visited exactly once. As this problem belongs to the famous class of "NP-complete" problems—simply speaking, problems for which no efficient algorithm is known—Adleman's result fostered the hope that these computationally hard problems could be solved effectively using a DNA-based molecular computer. The general idea behind Adleman's approach was to generate DNA sequences combinatorially that encode all potential solutions to a computational problem. The correct answer to a problem could then be "fished" out from the pool of candidate solutions by using tools such as PCR and gel electrophoresis. A variety of related concepts were subsequently developed to solve other computational problems such as "satisfiability" problems (SAT),[214] game[215,216] or maximal clique problems,[217] and resolution theory proving.[218] In satisfiability problems, for example, the solution of a logical expression such as $S = (x_1 \text{ OR } x_2 \text{ OR } x_3)$ AND $(x_1 \text{ OR } x_2 \text{ OR } x_4)$ is sought, where x_i are Boolean variables. In a so-called 3-SAT problem, each of the clauses (the bracketed expressions) contains three variables. As a consequence of the combinatorially large number of potential solutions, these problems are computationally quite expensive, and this is where a highly parallel, DNA-based approach becomes interesting. One recent promising result is the DNA-based solution of a 3-SAT problem for 20 variables.[219] For the many elegant results generated in this more theoretical branch of DNA computation, the reader is advised to consult the lecture series accompanying the annual conference on DNA computing.

6.1.2. Autonomous Computing

In recent years, many concepts have been put forward that deviate strongly from Adleman's original algorithm. For example, type II-S restriction endonucleases have been utilized for a molecular realization of finite-state automata[220] or for the development of sensors and signal amplificiation schemes.[221] Simple algorithms have also been implemented in molecular self-assembly to produce supramolecular patterns.[222,223]

Stojanovic et al. have reported several DNA-based logic gates and circuits based on the catalytic properties of deoxyribozymes.[216,224] These were composed of DNA constructs containing deoxyribozymes, whose folding into a catalytically active conformation was inhibited in the absence of certain input molecules. Recognition of input effectors restored the catalytic activity of the deoxyribozymes, which could be used to generate a fluorescent output signal. With this principle, NOT, AND, XOR logic gates, and others were realized. A similar approach was taken by Penchovsky and Breaker to transform an allosteric ribozyme into a molecular logic gate.[225]

A promising application for such autonomous logic gates and automata lies in the development of "intelligent" biosensors that can integrate and evaluate a variety of environmental cues and trigger the release of a molecular signal or a therapeutic molecule. Following this line of reasoning, Stojanovic and co-workers demonstrated communication between bead-immobilized deoxyribozyme gates (Figure 20 A),[226] and recently also the release of a therapeutic peptide in response to an orally administrable drug.[227]

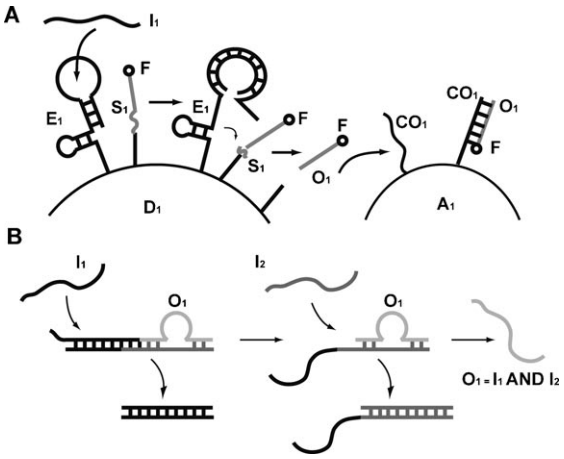

Figure 20. A) Chemical communication between "donor" and "acceptor" beads D_1 and A_1.[226] An input molecule I_1 activates deoxyribozyme E_1 by binding to its upper loop region. E_1 can then cleave neighboring substrate molecules S_1. This releases fluorescently labeled output molecules O_1 that can diffuse away and bind to complementary strands on the acceptor beads. F = fluorophore. B) Molecular logic with a hybridization cascade. Ouput molecule O_1 is only displaced from its complementary strand when both input molecules I_1 and I_2 are present.[228]

6.2. Molecular Programming

While there have been many experimental demonstrations of molecular "logic gates", only a few concepts so far have had potential for the construction of complex circuitry. This in part is due to the incompatibility of input and output signals (for example, small-molecule input, fluorescence output), and partly because of the lack of amplification and signal restoration stages, which would be required for "fan out".

Seelig et al. recently described a scalable approach to molecular information processing that is based on strand displacement by DNA branch migration and inhibition of hybridization by DNA hairpin formation (see Section 2.1).[228] DNA logical gates were constructed, in which hybridization of a DNA "output sequence" with another strand (for example, a downstream gate) was inhibited by hybridization with protecting strands. DNA "input strands" could remove the protecting strands by branch migration, thereby releasing the output strand. AND, OR, NOT, and threshold gates were constructed, as well as a signal restoration circuit by using this concept.[22] An example of an AND gate is shown in Figure 20B. To demonstrate the potential for "real world" applications, the presence or absence of a variety of microRNAs within a complex mixture of molecules was analyzed by a network of such DNA gates. Several other "hybridization cascades" were recently developed by the Pierce[173,187] and Winfree research groups,[229] and it was shown that such hybridization circuits can in principle be used to "emulate" the kinetics of arbitrary chemical reactions.[230]

A different approach towards molecular programming was recently taken by Kim et al.,[231] who introduced a method for transcriptional regulation in vitro that works without regulatory proteins. To this end, they split one strand of the double-stranded promoter region of a gene into two sections. Removal of one of these sections by branch migration results in an incomplete (partially single-stranded) promoter that is not recognized by RNA polymerase. In this state, transcription of the gene is switched "off". Adding the missing part of the promoter switches the gene on again. Kim et al. used this simple principle to develop an artificial gene regulatory "circuit" based on negative feedback loops between two genes, which displayed bistable behavior.

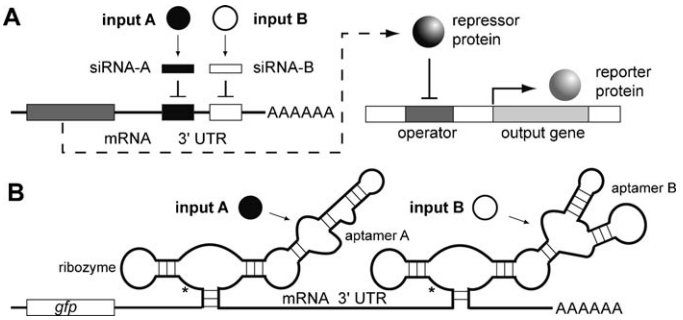

Figure 21. Two concepts for logic computation in vivo that rely on interference with the untranslated 3′-region (3′UTR) of mRNA molecules. A) Both molecular inputs A and B lead to the upregulation of small-interfering RNA molecules siRNA-A and siRNA-B. These siRNAs result in cleavage of the 3′-UTR of an mRNA by means of the RNAi machinery, thus preventing translation of a repressor protein that itself controls the production of a fluorescent reporter protein. Overall, this scheme represents the logical function (A OR B). Adapted from Ref. [236]. B) Cleavage of the mRNA for a reporter fluorescent protein (GFP) is achieved by two allosteric ribozymes included in the 3′-UTR. The cleavage site is indicated by (*). The ribozymes are allosterically controlled by two aptamer units. The binding of the molecular inputs A and B render their respective ribozymes inactive. GFP is thus only produced when both (A AND B) are present and the mRNA is not cleaved (adapted from Ref. [235]).

6.3. Computing In Vivo

There have recently been several attempts to implement DNA- or RNA-based computational modules also in vivo by combining diverse research directions such as DNA computing, RNA biology, and genetic engineering.[232] For example, Isaacs et al. engineered artificial RNA regulatory molecules to control gene expression in bacteria, and later Bayer and Smolke developed ligand-controlled allosteric riboregulators for the control of eukaryotic gene expression.[233] Win and Smolke recently used these principles to demonstrate the operation of a variety of logic gates in yeast (Figure 21B).[234,235] To this end, they implemented allosteric ribozyme switches in the untranslated 3'-region of a reporter gene (which codes for a fluorescent protein). Conformational changes induced by the binding of a combination of several ligands to their constructs rendered the RNA-cleaving ribozymes active (or inactive), and hence switched off (on) the synthesis of the fluorescent reporter. Rinaudo et al. were even able to implement logical functions in mammalian cells by using the RNAi machinery (Figure 21A).[236] An exciting experiment was recently performed by Topp and Gallivan, who developed artificial riboswitches in *E. coli* that controlled the synthesis of the Che Z proteins that are important for bacterial chemotaxis.[237] Che Z was only produced, when theophylline was bound to the aptamer section of the riboswitch. As Che Z switches the bacteria into the "running" state, this effectively reprogrammed their chemotactic machinery to follow a new chemical compound. As a result of their comparatively simple and programmable structures, RNA-based devices and control circuits should also be of considerable interest as components for artificial cells.[238]

As discussed here, DNA- or RNA-based computing devices could be used to control DNA assembly reactions[173,223] or to achieve biosensor tasks, in which not only binary information about the presence of a single molecule species is required, but also when a complex mixture of molecules has to be analyzed. An overview of applications of nucleic acid devices in biology follows in the next section.

7. Nucleic Acid Molecular Devices in Biology

Given the scope of the area, this section seeks to illustrate the diversity of biological applications of nucleic acid devices, with particular attention on the molecular basis of device function. For a more exhaustive coverage of the specific areas, the readers are directed to the latest specialized reviews which are mentioned in the relevant subsections.

7.1. Diagnostics and Sensors

Nucleic acid scaffolds have been used as in vitro and in vivo sensors for a range of biologically relevant targets such as ions, small molecules, proteins, and other nucleic acid sequences. The molecular basis of sensing for each of these

classes of targets is slightly different, and reveals a distinct molecular aspect of nucleic acid scaffolds in bringing about target recognition. The different methods for detection by DNA or RNA scaffolds have been dealt with in an excellent recent review by Liu et al.[87] Briefly, sensing by nucleic acid scaffolds may be optical (that is, turbidimetric, colorimetric, or fluorescent), electrochemical, quartz crystal microbalance (QCM), surface-plasmon resonance (SPR), acoustic, or cantilever-based methods.[239]

7.1.1. *Ion Sensing*

The most popular nucleic acid scaffolds to be used as sensors for metal ions are DNAzymes and RNAzymes. These generally utilize a change in the fluorescence or color observed when the DNAzyme/RNAzyme gets activated in the presence of the relevant metal ion and cleaves a labeled substrate. Several designs in the literature use a DNAzyme/RNAzyme–substrate complex labeled with a fluorophore (Figure 22 A–C, orange) and quencher (black) such that the complex exists in a fluorescently quenched form. The presence of a specific metal ion (M^{n+}) promotes cleavage in the DNAzyme/RNAzyme–substrate complex, thereby resulting in the dissociation of a shorter length of a cleaved fragment carrying one of the labels, which in turn results in an increase in the fluorescence (Figure 22 A). Such sensors function in solution or immobilized on surfaces. However, the former are not reusable, as they are irreversibly changed upon sensing. Despite this, they are quite advantageous since the detection is primarily based on kinetics and so they are highly selective and quite effective even in the presence of moderate background fluorescence in the system. In general, surface-immobilized sensors can achieve detection as low as 0.1–1 nM sensitivity, which is an order of magnitude better than solution-based sensors. Typical metal ions that have been sensed using DNAzymes include Cu^{2+},[240] Pb^{2+},[241] Zn^{2+},[242] and UO_2^{2+}.[243]

7.1.2. *Small-Molecule Sensing*

The resulting change in the fluorescence or other properties of labeled aptamers by the presence of small molecules has indeed revealed that aptamers are excellent sensors for their small-molecule targets, and these have been recently reviewed exhaustively.[87,239,246] Such individual aptamers can be envisaged as modules. The amenability of the nucleic acid scaffold to combine distinct modules has enabled the creation of functionally diverse devices.

For example, an allosteric aptamer is a scaffold that blends together two aptamer modules such that when one module is in the bound form, the binding at the other module is affected. As an illustration (Figure 22 B), Stojanovic and Kolpaschchikovn used the dramatic fluorescence enhancement of malachite green (MG) upon binding to its RNA aptamer to also sense many other bioactive small molecules such as adenosin-5′-triphosphate (ATP), flavin mononucleotide (FMN), and theophylline.[247] The RNA aptamer to MG was fused through a connecting module to an ATP aptamer, such that in the absence of ATP the MG aptamer domain was unstructured and unable to bind MG efficiently (Figure 22 B). In the presence of ATP, the structuring of the ATP aptamer module leads to a structuring of the MG aptamer domain so that it can now bind MG, which results in a dramatic enhancement in the fluorescence of MG. Willner and co-workers have shown with many examples that the combination of several DNA-based functional modules into "sensory cascades" can result in extremely enhanced sensitivity compared to "simple" sensors.[248]

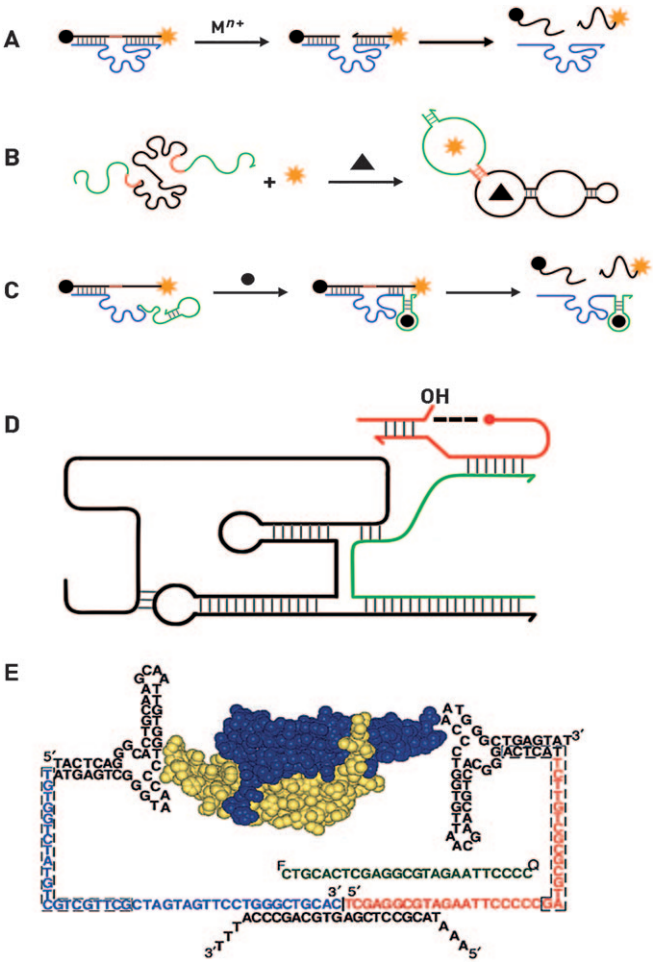

Figure 22. A) Nucleic acid enzymes (RNAzymes or DNAzymes) catalyze cleavage at a specific site (shown in red) upon binding a metal ion (M^{n+}), thereby resulting in relief of the fluorescently quenched state in the intact assembly. B) An allosteric aptamer-based device that combines a malachite green (MG) aptamer module (green) via a communication module (red) to another aptamer module that can bind another small molecule. Binding of this small molecule (▲), as shown on the right. C) Aptazymes similarly convert small-molecule sensing into cleavage events that result in fluorescent read-outs by coupling aptamer and DNAzyme/RNAzyme modules. D) An aptazyme device that amplifies viral RNA. The viral RNA is shown in green, ribozyme with ligation activity is shown in black, and the substrate RNAs for ligation are shown in red.[244] E) Aptamers provide enhanced detection sensitivity by coupling successful protein detection events to PCR. PDGF-BB (blue and gold) recognizes an aptamer with overhangs (red and blue) that are ligated with a splint (black), where the ligated termini are detected by PCR. Reproduced from Ref. [245] with permission from the Nature Publishing group.

When one of the modules of an allosteric aptamer is a DNAzyme/RNAzyme, such that small-molecule binding on one module results in the structuring and thereby catalytic activity of the DNAzyme/RNAzyme module, the resultant assembly is referred to as an aptazyme (Figure 22C).[249] The efficiency of small-molecule sensing by aptamers has been coupled with the convenience of detectability through cleavage associated with DNAzyme/RNAzyme activity to make aptazyme-based sensors. Based on the hammerhead ribozyme and an optimized communication module for a small-molecule aptamer module, Breaker and co-workers as well as others made a series of aptazyme sensors for cyclic adenosine monophosphate (cAMP), cyclic guanosine monophosphate (cGMP), and doxycycline among others.[91,250] Thus, the combination of and communication between distinct structural modules leads to exciting functional diversity in nucleic acid molecular devices. These properties could be exploited to create sensors that can perform logic operations (see Section 6) for sets of biologically related molecules and thereby function as diagnostic devices.

7.1.3. Proteins and Peptides

Given their favorable characteristics (Section 2.3), aptamers are being incorporated in many biological assays instead of antibodies.[246,251] These include analogues of enzyme-linked immunosorbent assays (ELISA), protein purification methods, Western blotting,[252] flow cytometry,[253] in vivo imaging,[254,255] and in microarrays.[256] Aptamer-functionalized stationary phases used for the purification of various bioactive small molecules and proteins by several types of chromatographic techniques have been extensively reviewed.[257] The first demonstration of a reporter-linked aptamer assay (RLAA) used a fluorescently labeled vascular endothelial growth factor (VEGF) aptamer to detect VEGF in serum with comparable sensitivity as standard ELISA.[258] Although this did not exploit the unique properties of aptamers, it emphasized the power of aptamer scaffolds compared to traditional antibodies. However, where aptamers have scored over antibodies is in the success of quantification through displacement assays. For example, since TBA has a lower affinity to labeled thrombin than native thrombin, complexes of TBA and labeled thrombin were coated on plates. The addition of native thrombin released the labeled thrombin into solution, and the remaining labeled thrombin on the plate could be easily quantified.[259]

One of the most powerful advantages of a nucleic acid scaffold is that PCR may be applied to amplify any detected signal. This is not possible with protein-based detection methods that use antibodies. In a key demonstration, homodimeric platelet-derived growth factor (PDGF-BB) was detected at zeptomole concentrations by using DNA aptamers carrying overhangs (Figure 22E).[245] When aptamer pairs bind PDGF-BB, the free ends of the overhangs are brought close enough to be circularized by the addition of a sequence that allows a splint ligation. The ligation product is amenable to detection by quantitative real-time PCR, while the unreacted probes are silent. This method was also used to detect human α-thrombin by using aptamer pairs directed to two distinct sites on thrombin.[245] Recently, an aptamer–protein complex was separated from unbound aptamer by using capillary electrophoresis, the complex was dissociated, and the amount of bound aptamer was detected by PCR. This method was used to detect less than 200 molecules of HIV-1 reverse transcriptase.[260]

Since aptamers may be fluorescently labeled without significantly affecting their recognition properties, optically tagged aptamers to cell-surface proteins have been used to tag cells expressing a specific surface protein from a population of cells.[261] As an illustration, Tan and co-workers used FRET nanoparticles (FRET-NPs), each with a specific fluorescent signature in response to a single excitation wavelength, as a tag on DNA aptamers. Aptamers against cell-surface proteins such as the sgc8 aptamer (specific for CEM cells), TDO5 aptamer (specific for Ramos cells), and T1 aptamer (specific for Toledo cells) were each tagged with a given FRET-NP. Each of these cell types could be identified and sorted from a complex mixture by using fluorescence activated cell sorting.[262] Aptamers against mesenchymal stem cells (MSCs) when tagged to magnetic nanoparticles could be used to label and enrich MSCs in a complex population.[263] Importantly, few aptamers have been identified with their cognate protein target, such as the pigpen protein in rat endothelial glioblastomas, postselection against whole cells.[264] Often, identified markers turn out to be biologically functional, for example, pigpen is associated with angiogenesis.[265]

Concepts from DNA nanotechnology can also be applied to develop sensors which do not only report on the presence or absence of a target molecule, but give access to physicochemical quantities that are otherwise difficult to obtain. For example, Seeman and co-workers have developed DNA nanodevices that allow the determination of binding forces and energies of DNA-binding proteins such as the integration host factor (IHF) or MutS.[101,266] Here, the bending and twisting of a supramolecular DNA structure is transduced to disrupt a section of dsDNA that acts as a force sensor.

7.1.4. DNA and RNA Sequences

The robust response of molecular beacons (MBs), its generalizability to detecting any type of sequence, and adaptability to virtually any fluorophore predisposes this rudimentary device to a variety of in vitro biological assays that have recently been reviewed in detail.[8] Here, we briefly discuss two major examples. The most widely used molecular biology assays for the detection of specific DNA sequences are RT-PCR and in single nucleotide polymorphism (SNP) detection. As a PCR progresses, a specific DNA sequence is amplified with time. A given amplified DNA sequence is detected in real-time by an MB, which reports on the progress of the PCR. The introduction of multiple MBs, each with different fluorophores capable of recognizing distinct DNA sequences, is a powerful tool for multiplex detection in a single reaction.[267] By using the thermodynamic properties of duplexes resulting from the detected sequences, it is possible to distinguish between the cognate DNA sequence and single mismatches. Single mismatches in key biological DNA sequences (SNPs) are molecular genetic markers in biomed-

ical research, diagnostics, and disease. MBs specific to a given DNA sequence can be designed such that a DNA sequence with an SNP forms a duplex with greatly reduced stability and can, therefore, be detected rapidly and sensitively.[268] In an interesting approach, Kolpashchikov combined the assembly of a DNA Holliday junction with MB sensing: the junction is composed of the analyte, two probe strands, and one MB strand. In this structure, the sequences cooperatively stabilize each other, thus resulting in a highly increased selectivity of this "binary DNA probe".[269]

In addition to small-molecule modulation as described earlier, allosteric aptamers or aptazyme activity can also be modulated by binding to a cognate DNA/RNA sequence. Such DNA and RNA sequences function as "effectors", where their binding onto a DNA/RNA scaffold "unmasks" a functional module such as a DNAzyme/RNAzyme present within the scaffold.[206] By integrating Kolpashchikov's "binary DNA probe" concept with DNAzymes, Mokany et al. developed modular "MNAzymes", in which an active ribozyme was cooperatively stabilized by an analyte strand and a doubly labeled substrate strand.[270]

In another elegant example, an aptazyme-based molecular device has been used to detect a viral RNA at attomolar concentrations.[244] Polisky, Seiwert, and co-workers used a ribozyme with RNA ligation activity. This ribozyme, upon binding with a specific region of viral RNA, becomes competent to ligate a substrate comprising two RNA strands (Figure 22 D). Without the viral RNA strand, the ribozyme is incapable of ligating the bimolecular RNA substrate. Upon recognition of the viral RNA region, the ligation activity of the ribozyme turns on, and the substrate is ligated with a three billion fold rate enhancement, thus leading to the exquisite detection sensitivity.

7.2. Biological Imaging

DNA and RNA scaffolds have been used as reporters of chemical entities intracellularly and in vivo. As mentioned in Section 3.2, devices based on i motifs and G quadruplexes have proved to be robust sensors of pH values and metal ions, respectively, both in solution and while immobilized. A key advance for DNA scaffolds as intracellular devices is a recent demonstration that an i-motif-based molecular assembly can sense pH values within endosomes of living cells. The Krishnan research group appended an i-motif-based device onto a protein such as transferrin through a biotin–streptavidin interaction.[121] This ternary complex was efficiently engulfed into endosomes by living cells via the transferrin receptor, thus resulting in the i-motif device being present only in endosomes positive for the transferrin receptor. As the endosomes matured, it was possible to capture the pH changes in real-time by using FRET between fluorescent labels appended on the i-motif device. However, the temporal resolution in these pH maps was rather low, and one must also be cautious that appending a large device–streptavidin complex onto any given protein might also alter the inherent trafficking properties of the protein.

Nucleic acid devices have been used to sense and image RNA molecules in living systems. Molecular beacons have been used to image mRNA molecules directly in real-time by target hybridization in living cells[271] and in oocytes of Drosophila embryos.[272] In another example, spatiotemporal mature microRNAs were sensed by a lacZ mRNA[273] or luciferase mRNA[274] with a microRNA responsive element in its 3′-UTR. Thus tissues where the microRNA was absent showed β-galactosidase (β-gal) or luciferase activity because of the efficient translation of the mRNA, while β-gal was absent in tissues where the microRNA was present. This method is now widely used to report on microRNA expression.

Nucleic acid scaffolds have also been used for in vivo imaging.[275] To this end, aptamers were chemically functionalized with optical imaging agents such as fluorophores,[276] quantum dots,[277] and magnetic nanoparticles.[278] In a key example, Smith and co-workers used an aptamer to elastase that binds the surface of activated neutrophils. Using this, the ^{99m}Tc-functionalized aptamer was shown to be capable of imaging inflammation in rats with gamma ray detectors.[254] The aptamer showed better performance than the antibody IgG, which is used clinically to image inflammation. This is attributed to faster clearance of the aptamer signal from the blood because of its low molecular weight.

7.3. Nucleic Acid Devices for Targeted Delivery and Therapeutics

Besides biosensing and bio-imaging, nucleic acid scaffolds have been used to construct a variety of molecular devices with much potential for in vivo applications.[279] DNA has been used to make a variety of 3D polyhedra[196,197,200,280] and Turberfield and co-workers have shown that small proteins may be positioned covalently within the hollow interior of a DNA tetrahedron (see Section 5.2).[201] Krishnan and co-workers have also shown that DNA polyhedra can be used to encapsulate free gold nanoparticles from solution with high efficiency.[281] Taken together these findings imply that DNA polyhedra could act as capsules for bioactive molecules larger than the pore size of the polyhedron. DNA polyhedra can function as nonleaky liposome analogues, as well as providing a protective and programmable casing for biodegradable molecular cargo. Recently it has also become evident that nanotubular structures might be better for delivery in some applications.[282] Yan and co-workers have been able to fabricate DNA nanotubes with exquisite precision over the dimensions and functionalization sites.[283] Indeed, DNA nanotubes derivatized with folic acid have been successfully delivered into cells.[284]

Just as functionalized liposomes have been used to deliver their entrapped cargo tissue-specifically,[285] molecular nucleic acid motifs have also been used as guiding modules in the tissue-specific delivery of molecular payloads in vivo. Cells belonging to different tissue types express different cell-surface receptors.[286] Tissue-specific delivery is most commonly achieved by the use of antibodies or ligands that recognize key cell-surface receptors of a given tissue, thus resulting in enriched concentrations around the tissue

target.[287] Antibodies to key receptors are being replaced by aptamer analogues in many such applications. The adaptability of the nucleic acid scaffold to chemical functionalization has enabled covalent attachment of a variety of molecular payloads to aptamers for their effective delivery in vivo. For example, prostate-specific membrane antigen (PSMA) is a protein that is overexpressed in many cancer cells. This protein continuously shuttles between the plasma membrane and the interior of the cell and, therefore, can be exploited to funnel molecules from the extracellular medium into the cell's interior. Thus, PSMA-binding aptamers covalently modified with a payload have been used to deliver moieties such as siRNAs,[288] small molecules (such as doxorubicin),[289] toxins,[290] as well as drug-loaded nanoparticles.[291] As a consequence of their capacity to get enriched around specific tissue types, together with their chemical functionalizability, aptamers are showing increasing potential to promote siRNA therapies for various diseases,[292] photodynamic therapy,[293] boron neutron-capture therapy,[294] and enzyme-replacement therapy[295] by enrichment of the relevant molecular agent at target sites.

Aptamers generated against drug targets such as coagulation factors, growth factors, hormones, inflammation markers, neuropathological targets, infectious disease associated proteins, and even whole organisms have shown great therapeutic potential. The reader is directed to excellent in-depth reviews by Nimjee et al. and Thiel and Giangrande on their development.[279,296] Here, we describe the general molecular principles by which these aptamer-based devices function in vivo. One of the ways is by the aptamer inhibiting the function of its molecular target, that is, the aptamer acts as a receptor antagonist (Figure 23A). For example, inhibiting vascularization is a key anticancer strategy and the aptamer pegaptanib inhibits vascularization by binding to and blocking the heparin-binding domain of VEGF-165.[297] Similarly, a class of G-quadruplex-forming oligonucleotides (antisoma) function as aptamers that bind to and inhibit nucleolin, thereby resulting in antiproliferative effects.[298] However, since nucleolin has many different cellular functions, the mode of action of antisoma has not been pinpointed to a specific function of nucleolin. Aptamers can also function as decoys by presenting a competing binding moiety to a molecular drug target (Figure 23B). The action of several RNA- or DNA-binding proteins that are drug targets may be inhibited by preventing their binding to their naturally occurring DNA/RNA sequences by presenting aptamers that incorporate these DNA/RNA sequences. Thus, HIV has been inhibited by targeting HIV-TAR,[299] tat,[300] or by mimicking the Rev response element.[301] Similarly, aptamers presenting competitor sequences for transcription factors such as the E2F family[302] or NFkB[303] could efficiently block transcription factor activity, thereby leading to possible treatments for eczema and dermatitis.

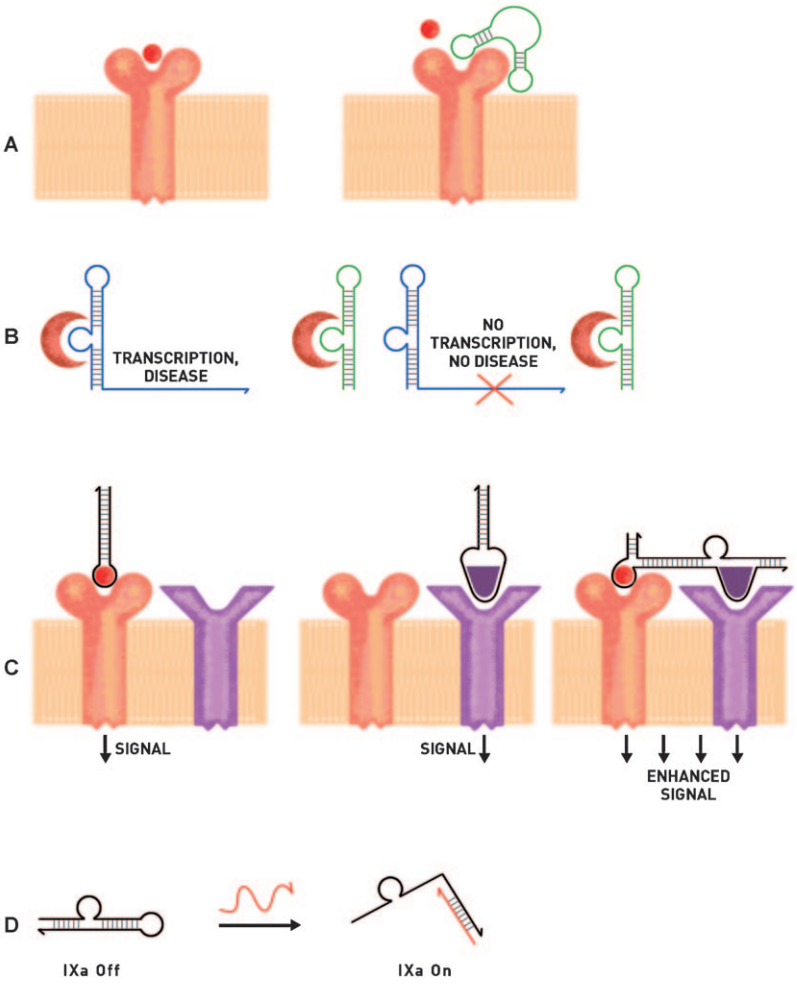

Figure 23. A) Aptamers functioning as receptor antagonists, where binding of the aptamer (green) prevents the binding of the natural receptor ligand (red). B) Aptamers functioning as decoys by mimicking the natural target and preventing a cellular response. C) Aptamers stimulate either the OX40 or the 4-1BB receptors. Combining both modules on a single aptamer scaffold achieves co-stimulation.[307] D) Aptamers function as switches in vivo. REG-1 aptamer (black) inhibits coagulation factor IXa. The introduction of an antidote strand (red) relieves the inhibition.[305,306]

An important aspect of nucleic acid scaffolds is their modularity. This biomolecular scaffold is predisposed to mixing and matching distinct modules to generate molecular devices of much greater functional diversity and efficiency. We have already described the realization of functional diversity, where a combination of modules gives rise to the aptazyme class of molecular devices (see Section 7.1). The case of greater functional efficiency is illustrated in the combination of multiple aptamer modules (Figure 23C). By combining aptamers to distinct motifs that are part of the same molecular target, one can generate bivalent aptamers that have increased affinity to the target compared to the individual aptamer-recognition modules.[304] Such a bivalent strategy can function by either target inhibition[305,306] or

target activation. This is beautifully illustrated in the case of the activation of an antitumor immune response, where aptamer modules to different targets were combined to achieve co-stimulation of T-cell receptors. Here, a bivalent aptamer targeting 4-1BB and OX40 receptors brought about far more efficient immune responses than either of the individual aptamers alone (Figure 23 C).[307]

Aptamer devices can also function as switches in vivo. A case in point exploits short aptamer half-lives. Anticoagulants are used during procedures such as in cardiovascular surgery. Under such conditions, aptamers bring about anticoagulative action for only a desired duration, following which they are efficiently degraded. For example, RNA aptamer REG1 (RB006) is used to recognize coagulation factor IXa and prevent coagulation (Figure 23 D). This inhibition of factor IXa can be relieved whenever desired by the introduction of an antidote RNA strand RB007, which hybridizes with RB006 and abolishes the binding between RB006 and coagulation factor IXa.[305,306]

Given the demonstrated potential of aptamer modules in targeted delivery and therapeutics, combining many of these structural motifs with evolving DNA architectures, DNA computation, and actuation could enable the realization of multifunctional or "smart" devices for delivery or therapeutic intervention.

8. Conclusion and Outlook

In recent years, DNA and RNA have proven to be exquisite molecules for the design and experimental realization of artificial molecular machines. The predictable, sequence-dependent structure formation by these molecules allow for the "programmable" assembly of supramolecular structures, which can be switched between a variety of distinct "states" or conformations. One of the key advantages of the nucleic acid scaffolds is their modularity and thereby their ability to couple multiple functional units on a single structure to give rise to multifunctional devices. The biochemistry and chemical technology required to produce these devices in appreciable quantities already exists and is improving continuously.

On a fundamental level, such DNA or RNA switches are currently used to study physical aspects of molecular machines in a nonbiological context. Theoretical concepts such as Brownian walkers and molecular computers are directly implemented by using DNA molecules. An exciting aspect of this approach is the possibility of a direct feedback between experiment and theory—many aspects of DNA devices such as mechanical stability or reaction kinetics can be easily "tuned".

However, researchers are clearly beginning to explore real-world applications of nucleic acid devices. Promising examples are found in switchable materials, molecular containers, and DNA-directed synthesis. There is a wealth of applications for nucleic acid devices in biology. Nucleic acids can be utilized to build biosensors, molecular computers, and diagnostic devices that even work in vivo.

Molecular switches based on nucleic acid scaffolds are, therefore, likely to have a wide-ranging impact in fields as diverse as materials science, the physics of biomolecular structure and function, and in biological systems. Nature already uses the structural plasticity inherent to both DNA and RNA scaffolds in response to specific molecular triggers to achieve specific outputs. Cellular function is a result of multiple computations involving nucleic acid structures that ultimately control gene expression. Thus, this scaffold is certainly also amenable to much more complex in vitro computation and actuation. One of the concerns here is that the speed of conformational changes associated with the nucleic acid scaffold will prove rate-limiting. Thus, there is a continuous need for devices that are ever faster and for the discovery of newer nucleic acid structural transitions that occur on millisecond or faster time-scales.[37,96,153]

In the future, the construction of more complex architectures will be key to understanding the mechanical and structural limitations of DNA or RNA as a material in construction on the nanoscale. These limitations will ultimately also dictate the scale of the complexity of switchable devices.

For some applications, nucleic acids may already be the "ideal" molecules—for example, when the coupling of molecular devices to genetic processes is intended, or when simple and robust biosensors are required. For other applications, substrates such as peptides or synthetic organic molecules may be more suitable, but they may not be sufficiently controllable at the moment. In these cases, nucleic acid devices are a ready alternative to explore the general principles underlying molecular self-assembly and molecular machinery.

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