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"Nano-oddities": Unusual Nucleic Acid Assemblies for DNA-Based Nanostructures and Nanodevices

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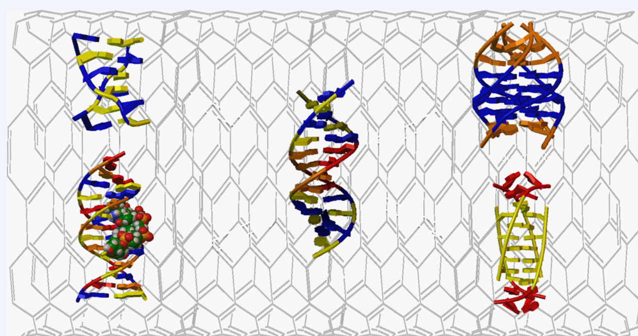

CONSPECTUS: DNA is an attractive polymer building material for nanodevices and nanostructures due to its ability for self-recognition and self-assembly. Assembly relies on the formation of base-specific interactions that allow strands to adopt structures in a controllable fashion. Most DNA-based higher order structures such as DNA cages, 2D and 3D DNA crystals, or origamis are based on DNA double helices stabilized by Watson–Crick complementarity. A number of nonclassical pairing patterns are possible between or among DNA strands; these interactions result in formation of unusual structures that include, but are not limited to, G-quadruplexes, i-motifs, triplexes, and parallel-stranded duplexes. These structures create greater diversity of DNA-based building blocks for nanomaterials and have certain advantages over conventional duplex DNA, such as enhanced thermal stability and sensitivity to chemical stimuli. In this Account, we briefly introduce these alternative DNA structures and describe in detail their utilization in a variety of nanomaterials and nanomachines. The field of DNA "nano-oddities" emerged in the late 1990s when for the first time a DNA nanomachine was designed based on equilibrium between B-DNA and noncanonical, left-handed Z-DNA. Soon after, "proof-of-principle" DNA nanomachines based on several DNA "oddities" were reported. These machines were set in motion by the addition of complementary strands (a principle used by many B-DNA-based nanodevices), by the addition of selected cations, small molecules, or proteins, or by a change in pH or temperature. Today, we have fair understanding of the mechanism of action of these devices, excellent control over their performance, and knowledge of basic principles of their design. pH sensors and pH-controlled devices occupy a central niche in the field. They are usually based on i-motifs or triplex DNA, are amazingly simple, robust, and reversible, and create no waste apart from salt and water. G-quadruplex based nanostructures have unusually high stability, resist DNase and temperature, and display high selectivity toward certain cations. The true power of using these "nano-oddities" comes from combining them with existing nanomaterials (e.g., DNA origami, gold nanoparticles, graphene oxide, or mesoporous silica) and integrating them into existing mechanical and optoelectronic devices. Creating well-structured junctions for these interfaces, finding appropriate applications for the vast numbers of reported "nano-oddities", and proving their biological innocence comprise major challenges in the field. Our Account is not meant to be an all-inclusive review of the field but should give a reader a firm grasp of the current state of DNA nanotechnology based on noncanonical DNA structures.

INTRODUCTION

DNA duplexes (dsDNA) have been used widely in the design of nanomaterials due to the ability of complementary strands to hybridize in a controllable fashion. Structures with Watson–Crick base pairing suffer from several intrinsic limitations, such as low resistance to heating and denaturing reagents, susceptibility to DNases, flexibility, deformability, and low sensitivity to chemical stimuli. In this Account, we will discuss alternative DNA structures and present examples of how these "oddities" have been used to construct DNA-based nanomaterials and nanodevices.

ALTERNATIVE DNA STRUCTURES

Alex Rich wrote 20 years ago: "DNA comes in many forms".[1] DNA can adopt structures involving unusual base pairs, triads, or quartets (Figure 1) with variable numbers of strands. Examples of these "oddities" are shown in Figure 2. Although a complete description of all unusual structures is impossible, those that have been incorporated into DNA higher order motifs are presented below.

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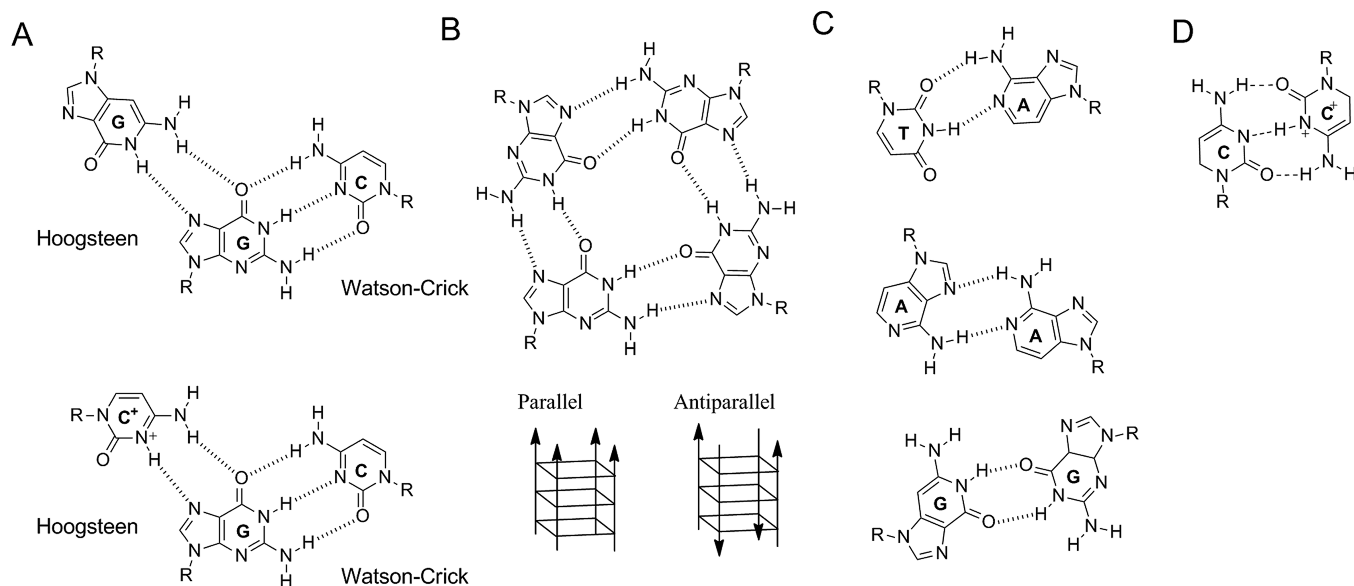

Figure 1. Schematic representation of (A) G−GC and C⁺−GC triplets, involved in purine and pyrimidine triplexes, respectively; (B) G-quartet and G-quadruplexes; (C) base-pairs in parallel stranded DNA, and (D) CC⁺ base pair. C⁺ in panels A and D implies cytosine protonation at the N3 position.

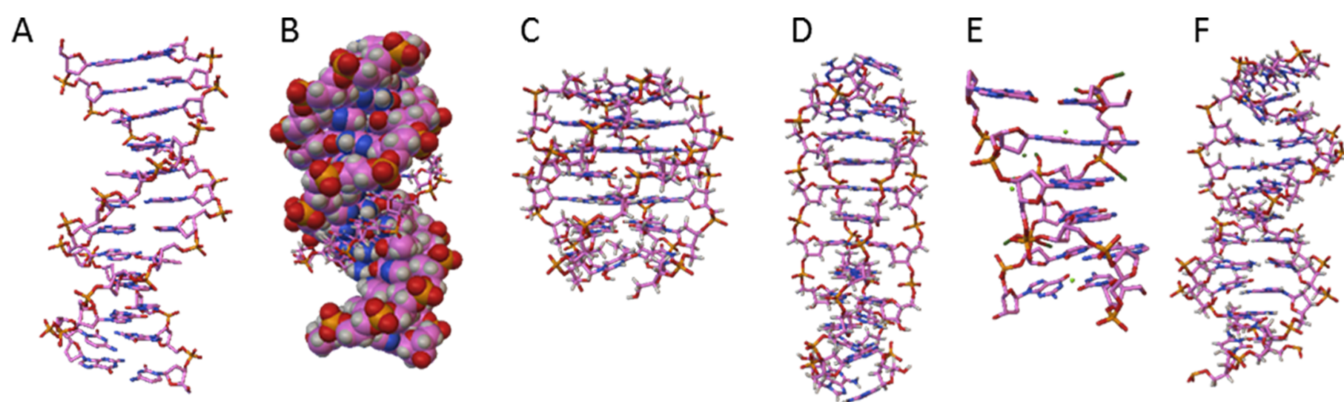

Figure 2. Reported structures of (A) B-DNA (X-ray, PDB 1BNA), (B) triplex DNA (NMR, PDB 1BWG), (C) four-stranded G-quadruplex (NMR, PDB 139D), (D) i-motif (NMR, PDB 1YBL), (E) Z-DNA (X-ray, PDB 4FS5), and (F) parallel-stranded duplex DNA (NMR, PDB 1JUU).

G-Quadruplexes

G-Quadruplexes (G4) are formed from at least two stacks of four guanines (called G-quartets) held together by Hoogsteen hydrogen bonding (Figure 1B). These structures have an ion core in the center that can host a variety of cations (usually Na^+ or K^+) and a negatively charged phosphate backbone.[2] G4 structures are highly polymorphic,[3] and are classified in terms of strand orientation (parallel, antiparallel, and hybrid "3 + 1") and stoichiometry (mono-, bi-, tri-,[4] and tetramolecular).[5] Parallel G4s with at least four G-quartets resist thermal heating and denaturing conditions. Unlike dsDNA, G4 structures are stable in ethanol,[6] in other dehydrating agents, and in eutectic solvents. Tetramolecular G4s are stable in the gas phase and are robust when imaged by atomic force microscopy (AFM).[7] Finally, chemical modification of quadruplexes is straightforward.[8,9]

Z-DNA

DNA sequences containing alternating purines and pyrimidines, such as poly(dG-dC)·poly(dG-dC), can adopt a left-handed double-helical structure, known as Z DNA. Z-DNA possesses a zigzag sugar phosphate backbone and an alternating *anti*/*syn* conformation of bases. Negative supercoiling, DNA methylation, cations at high concentration, and Z-DNA specific proteins and ligands facilitate B-to-Z transitions.[10]

The i-Motif

Cytosine-rich oligodeoxynucleotides or polynucleotides may adopt an i-motif structure (Figure 2D) at acidic pH.[11] The i-motif is a tetramer of two equivalent parallel-stranded right-handed duplexes "zipped" together in an antiparallel fashion. The structure is composed of intercalated hemiprotonated C·C⁺ base pairs (Figure 1D). Because this motif requires protonation of cytosine at the N3 position (with a pK_a below 7), formation can be controlled by pH.

Triplex DNA

Triplex DNA consists of dsDNA (with one purine-rich and one pyrimidine-rich strand) and a single-stranded triplex-forming oligonucleotide (TFO) that binds to the major groove of the duplex through Hoogsteen bonding with the purine-rich strand.[12] Pyrimidine triplexes rely on T−AT and C⁺−GC pairing (Figure 1A); the latter can be controlled with pH. Purine triplexes involve G−GC and A−AT quartets and are pH-insensitive.

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Parallel-Stranded DNA

The structure of a parallel-stranded DNA is maintained by reverse Watson–Crick base pairing between thymine and adenine[13] or alternating, symmetrical self-pairs of $G_{syn}{\cdot}G_{syn}$ and $A_{anti}{\cdot}A_{anti}$ (Figure 1C).[14] Parallel-stranded duplexes are reasonably stable under near-physiological conditions and have melting temperatures only 10–15 °C lower than their antiparallel counterparts.[15]

■ ASSEMBLY INTO HIGHER ORDER STRUCTURES

G4-Based 1D Superstructures and Homorecognition Problem

Sen's laboratory provided the first examples of G4-based wires (G-wires) based on a head-to-tail arrangement of tetrastranded dT_nG_3 units formed due to a slipped arrangement of the individual oligonucleotides.[16] They also prepared 1D G4-mediated synapsable duplex structures.[17] Similarly, Protozanova et al. demonstrated that $dA_{15}G_{15}$ formed so-called "frayed wires".[18] Another type of G-wire was constructed with the oligomer $dG_4T_2G_4$.[7,19] Kotlyar and colleagues reported efficient preparation of guanine-only nanowires from poly(dG).[20] These wires are long, stiff, heat resistant, mechanically stable, and insensitive to DNase I treatment.[21] Besenbacher and Mamdouh studied formation of wires of guanosine (guanine attached to ribose) as a function of time and mechanical shaking.[22] This G-wire formation occurred in the absence of any cation due to self-assembly of guanosines via $\pi-\pi$ stacking but was slower than in the presence of K^+. Shaking led to branching of the wires and eventually to thin film formation. The major drawback of all of these designs is the lack of control over the assembly process due to a homorecognition problem: because G-quartets are formed from four identical bases, a guanine from one G-rich strand can bond with its own strand or with another strand at *any* of the guanines. A similar homorecognition problem exists for i-motifs.

One way to exert control over G4 assembly is to combine addressable dsDNA with G4 DNA. Sugimoto's group prepared G-wires using a canonical Watson–Crick duplex in combination with antiparallel G4.[23] Recently, our laboratory used parallel-stranded DNA to direct the controlled assembly of parallel G4s.[24] The assembly process was rapid and structures formed were resistant to heat and denaturing conditions due to the exceptional stability of the G4 core.[24] We also used these structures as building blocks to prepare 1D G-wires. Szalai's group prepared G-wires using the design presented in Figure 3. Oligonucleotides with a central G-run were hybridized to form duplexes with a central G–G mismatch region. In the presence of potassium, the G-runs formed synapsable quadruplex building blocks that further assembled into 1D G-wires.[25]

DNA Origami Frame as a Visualization Tool

The specificity of complementary base pairing made possible the development of DNA materials such as 2D and 3D origami. Sugiyama and colleagues designed a 2D 100 × 80 nm^2 rectangular origami frame with an inner vacant space (40 × 40 nm^2) decorated with two or three pairs of connectors (Figure 4).[26] Attachment of desired DNA sequences to the connectors allows visualization of DNA conformational changes, DNA–protein interactions, and chemical reactions in real-time with single-molecule resolution using fast-scanning AFM. For example, G4 folding was visualized for the first time by attaching two duplexes containing contiguous G-G mismatches to the origami frame. Interstrand quadruplex

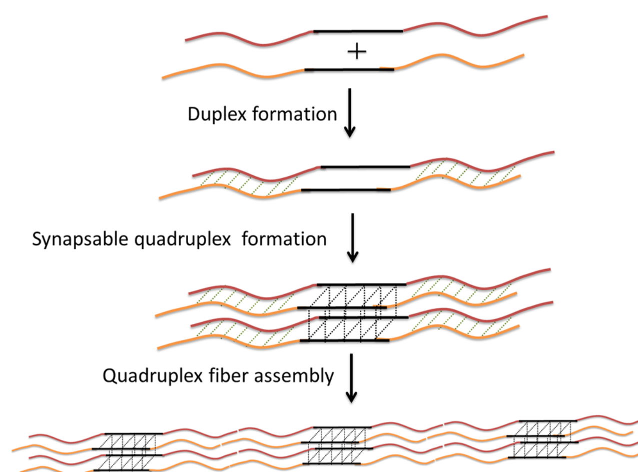

Figure 3. Assembly of synapsable G4-based nanowires (adapted from ref 25).

formation joined two duplexes in an X-shape visible by AFM (Figure 4).[27] Further, this system was adopted to study the G4-chaperone activity of the HIV-1 nucleocapsid protein[28] and to monitor the G4-stabilizing ability of selected ligands.[29] A two-switch logic system was designed by attaching three DNA strands that are either photoresponsive (containing azobenzene) or G-rich or both.[30] The DNA frame was also used to visualize the B–Z DNA transition (see below).[31] In an independent study, Fox and colleagues used the triplex approach to direct covalent interstrand cross-links to unique locations within a preassembled DNA nanostructure.[74]

DNA Junctions

To achieve desired complexity of DNA-based nanomaterials, a variety of DNA secondary structures need to be connected; most of these junctions have poorly understood structures. The exceptions are the B–Z DNA junction (where left- and right-handed DNA connect to each other by breaking of an A–T base pair and extrusion of bases)[32,33] and the recently characterized structurally diverse B–G4 junctions.[34] A "floppy" or "ill-defined" junction may compromise overall rigidity and precise positioning of a DNA device. Better understanding of these junctions may allow them to be used as hinges in nanodevices or as sites of modification for visualization.[33]

■ APPLICATIONS OF UNUSUAL DNA STRUCTURES

Construction of Nanodevices

Recent, unprecedented developments in DNA-centered nanotechnology have resulted in construction of functional nanodevices with potential in molecular sensing, computing, nanomedicine, transport, and smart materials. DNA nanodevices are artificial assemblies that can cycle between well-defined structural conformations under the influence of external stimuli. The conformational changes are accompanied by mechanical motions: extension/contraction, twisting, rotation, or translation of the device or its attached cargo. The operation of DNA nanodevices is usually monitored via Förster resonance energy transfer, FRET, which enables fast and sensitive detection. Advantages of DNA-based nanomachines are their simple design and synthesis, ease of modification, biocompatibility, programmability, and ability to function under mild aqueous conditions. The major challenge in utilization of DNA-

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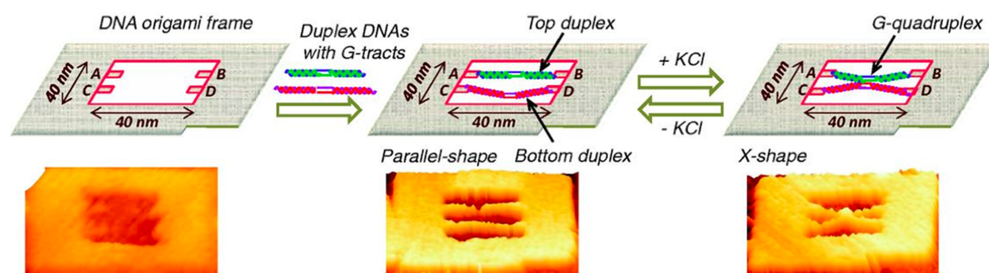

Figure 4. Design of an origami frame allowing the incorporation of two neighboring duplexes that can interact via quadruplex formation in the presence of K^+, leading to an X-shape structure observed by AFM (bottom right). Reprinted with permission from ref 27. Copyright 2013 Oxford University Press.

based systems is their integration with modern nano- and microscale technology.

The operation of a DNA nanomachine can be induced by the addition of fuel strands or cations or by a change in pH or temperature. Addition of complementary fuel strands converts a monomolecular motor DNA into a bimolecular duplex. Subsequent addition of antifuel strands removes the fuel strand via DNA branch migration (initiated by DNA sticky end pairing), freeing the motor strand and forming a waste duplex. Accumulation of double-stranded waste and slow kinetics of strand hybridization and invasion are the main disadvantages of such systems. Addition of cationic copolymer[35] or short DNA-strand catalyst[36] can significantly facilitate hybridization and speed up kinetics. An advantage is that an array of such machines can be powered simultaneously and individually by addition of sequence-specific fuel strands. For DNA nanomachines controlled by cations, pH, or temperature, conditions are chosen to favor one conformation over another. Cyclic operation of the machine is achieved by cycling between different buffers or temperatures. The operation is significantly simplified and mechanical energy can be harnessed when the nanomachine is attached to a solid support. Examples of nanomachines based on unusual DNA structures are detailed below.

DNA Nanodevices Based on B–Z Transition

The first successful DNA-based nanomechanical device was prepared by flanking a region switchable from B to Z DNA with two rigid double-crossover motifs.[37] High ionic strength induced the B–Z transition of the central proto-Z section, which was accompanied by ~3.5 turns of rotary motion and a 6-Å expansion in length (Figure 5). The structural rigidity of the double-crossover motifs prevented any distortion during B–Z transition. DNA machines based on rotary motion are largely unexplored, however. Recently, Sugiyama's laboratory reported an elegant rotor DNA design.[31] A proto-Z sequence was connected to a marker flag (15×8 nm^2), and the assembly was anchored into a DNA origami frame. Addition of Mg^{2+} induced the B–Z transition and rotation of the flag, which was monitored by AFM in real-time. The direction of motor rotation and number of turns were difficult to assess, however.

Operation of DNA nanodevice based on B–Z transition can be controlled not only by metal cations, but also by Z-DNA specific ligands and proteins. For example, anionic Ni(II) porphyrin binds selectively to Z-DNA inducing a CD signal in the porphyrin Soret band,[38] and photoluminescent cationic spermine-functionalized carbon dots induce B–Z transition even in low salt buffer.[39] Both Z-DNA binders have been used to construct logic gates with potential application in computing.

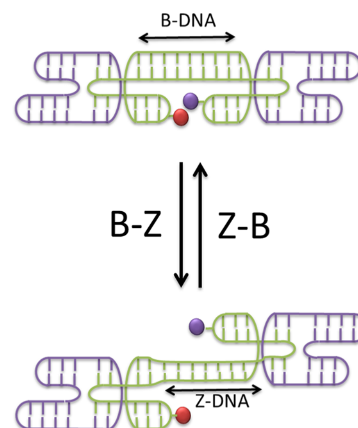

Figure 5. First nanodevice based on noncanonical DNA structure and driven by a B-to-Z transition. The device is made up of three cyclic strands of DNA, two blue strands and one central green strand with the proto-Z sequence d$(CG)_{10}$. Red and blue dots represent two fluorophores. Figure adapted from ref 37.

pH-Responsive Nanodevices

pH-responsive DNA devices are usually based on triplexes or i-motifs, which are stable only at acidic pH. At neutral or alkali pH, this DNA forms a duplex with a complementary strand. Cycling between these two states is usually done by addition of NaOH and HCl as fuels. Such fuels have low cost, have high ability to diffuse through any pore or channel, and produce waste products (water and NaCl) that do not affect device operation. Additional advantages include simple architecture, high reversibility, and stable cyclic operation. pH-driven devices are usually faster than DNA-fueled devices. A major drawback is that differential operation is not possible because all machines in the same sample will respond in the same way to changes in pH.

The first example of a pH-responsive DNA device relied on reversible C^+–GC triplex formation upon cycling of pH between 5.0 and 8.0. At pH 8.0 three DNA strands form three duplexes and a single-stranded region designed to bind to one of the duplexes (Figure 6A).[40] At pH 5.0, triplex formation results in a FRET signal. The operation of the device deteriorated over 16 cycles possibly due to photobleaching of fluorophores, inaccuracy in pH control, or dilution effects. Another nanodevice based on simpler architecture, but similar operation style, consisted of two partially complementary DNA strands; the longer stand had a linker followed by a TFO.[41] Under acidic condition protonation of TFO leads to its fast hybridization with the duplex that is predicted to result in a 6-nm movement. Recently Norden's group designed an elaborate

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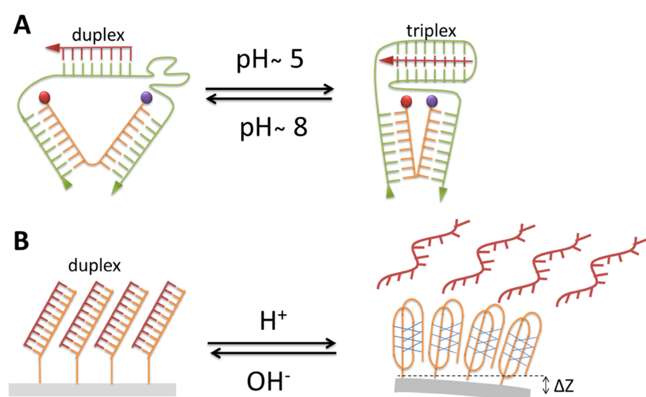

Figure 6. pH-driven DNA nanomachines based on duplex-to-triplex and duplex to i-motif transitions. (A) The device consists of three DNA strands and forms three duplexes under basic pH. A single-stranded region is designed to bind to one of the duplexes at acidic pH. (B) C-rich DNA is immobilized on a cantilever and bound to a complementary stand at high pH. Lowering pH leads formation of i-motif and increased repulsion among DNA strands, which creates compressive surface stress; bending the cantilever relieves the stress. Adapted from refs 40 and 45, respectively.

nonrepetitive structure built from 10 unique three-way branched oligonucleotides.[42] Uniquely designed TFOs were used to label this structure at eight individual addresses in an area of just 10 × 20 nm^2, leading to dense information content and high spatial precision. If pH is used as an input signal, information can be written and locked by triplex stability leading to FRET as an output; it can be erased by an increase in pH.

The i-motif-based nanodevices utilize C-rich sequences as motor DNA and a complementary sequence with a few mismatches to avoid G4 formation.[2] These machines can, in principle, undergo ∼5 nm mechanical motions and are characterized by ∼10−16 pN opening/closing forces. The first example of the i-motif-based pH sensor came from the Balasubramanian group.[43] Their machine did not degrade over at least 20 cycles and had a short response time of ∼5 s. It operated in solution, however, so its mechanical motion was random and could not be harnessed.

Attaching DNA to a solid support provides a significant advantage. Once anchored, DNA nanomachines can act cooperatively moving beyond nanoscale effects. In the simplest case, motor DNA labeled with rhodamine was attached a surface coated with thin optically transparent gold. At acidic pH, folding of DNA into the i-motif brought the fluorophore close to the gold surface resulting in ∼80% decrease in fluorescence. Alkali pH dissociated the i-motif and restored fluorescence with a relatively slow switching time of ∼5 min.[44]

When C-rich motor DNA was attached to an array of gold-coated micromechanical cantilevers,[45] nanoscale surface forces originating from pH-driven conformational change in DNA led to a macroscopic effect of bending the cantilever (Figure 6B). The device was robust and highly reversible. This design can be used in preparation of mechanical components such as switches, valves, or actuators in larger machines or as a "label-free" DNA biosensor. This work addressed the major challenge of integration of DNA nanomachines with mechanical devices.

By attaching C-rich DNA to a gold surface via a long linker, Jiang's laboratory built a nanocontainer that can be used for controlled drug delivery and release.[46] At low pH, i-motifs associate into a compact monolayer forming the nanocontainer's walls, and the linker remains single stranded and loosely packed, forming the nanocontainer itself. Molecules embedded into the container at low pH can be released in a controlled fashion at higher pH. The operation of the device was slow, but its closing was facilitated by application of alternating electric field. Another system capable of pH-controlled release of cargo is based on a rigid Y-shaped DNA nanostructure with three interlocking C-rich overhangs pointing away from each other (Figure 7). These overhangs hybridize into i-motifs to form a hydrogel capable of trapping the cargo and releasing it when pH is increased.[47]

A smart surface that changes its wettability in response to pH was constructed by modifying C-rich DNA with a highly hydrophobic fluoride-containing group and immobilizing it on a solid support.[49] Surface properties were cycled between superhydrophilic (exposed DNA backbone) and superhydrophobic (exposed hydrophobic group) by pH alterations. Cooperation between nanoscale motions of individual DNA motors leads to macroscopic changes in surface hydrophobicity.

To control access of a substance to a surface, C-rich DNA was attached inside pores within a membrane creating an artificial ion channel.[50] Formation of densely packed, rigid i-motifs constricted pore openings, effectively blocking the pores (OFF state). Unfolding of i-motifs to random coil allowed free diffusion of ions (ON state). The ON−OFF switch was controlled by pH. i-Motif-based DNA nanomachines have also found application in molecular computing. In one case, two DNA tweezers and a DNA substrate were combined to construct an enzyme-free pH-driven SET−RESET logic gate.[51]

An i-motif-based nanodevice was designed to act as a pH-dependent switch for a photosensitizer that produced singlet oxygen species, $^1O_2$, used to kill undesired cells.[48] The device consisted of a sensitizer, a quencher, and an i-motif prepared

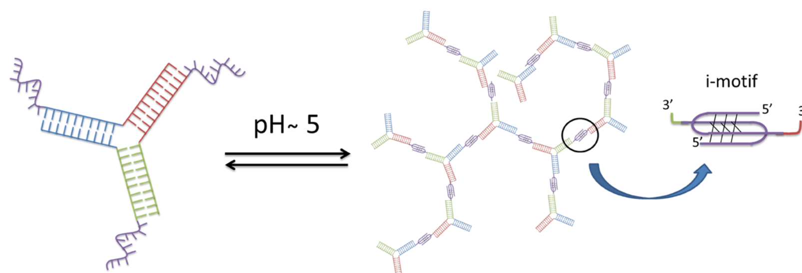

Figure 7. DNA hydrogel can trap and release a cargo upon pH-cycling. The basic motif is formed from three oligonucleotides linked by a rigid three-way junction. Adapted from ref 47.

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such that at low pH the sensitizer was held in close proximity to a quencher and no $^1O_2$ was produced. Unfolding the i-motif at higher pH moved the sensitizer and quencher apart allowing production of $^1O_2$. This system was prone to photobleaching.

Devices described above are controlled by an operator. The first *autonomous* DNA device was powered by the pH changes generated by oscillatory chemical reaction based on the iodate–sulfite–thiosulfate system (a variation of the Landolt reaction).[52] The nanomachine was significantly improved by immobilizing the DNA on a solid support and using an open flow-through reactor.[53] The DNA response time was fast, but the system was controlled by the oscillation reaction time period, which was rather slow at ∼20 min.

To meet the challenge of integrating DNA-based nanomachines into existing microelectronic devices for real-life applications, chemical stimuli need to be replaced with noncontact electrical or optical signals. The first UV-light controlled DNA nanomachine consisted of C-rich DNA mixed with a light-induced OH^- emitter, malachite green carbinol base (MGCB).[54] Device operation was controlled by light-induced dissociation of MGCB and its recombination with OH^-.

In Vivo Application of DNA-Based pH Probes

Many important biological processes are accompanied by pH changes. *In vivo* pH sensors could serve as quick diagnostic tools for human diseases. The Khrishnan laboratory pioneered methods for *in vivo* application of an i-motif-based nanodevice called the I-switch (Figure 8), which was used to monitor spatial and temporal pH changes associated with endosome maturation in *Drosophila* hemocytes[55] and in *Caenorhabditis elegans*.[56] The device entered endosomes via receptor-mediated endocytosis, maintained structural integrity for hours, and was nontoxic.[55,56] The I-switch is autonomous, reversible, and photostable and has high fidelity and a dynamic pH range of 5.3–6.6 in *C. elegans*[56] and 5.8–7.0 in *Drosophila*.[55] It was improved by addition of a tag that allowed its attachment to any biotinylated protein, and this system was used to measure pH changes associated with protein functions.[55] Recently, the Khrishnan laboratory addressed two other challenges, organelle-specific targeting of pH probes and simultaneous pH measurement.[57] Two DNA nanodevices modified with FRET pairs that display minimal crosstalk were designed and used simultaneously to measure pH along two different but intersecting endocytic pathways.

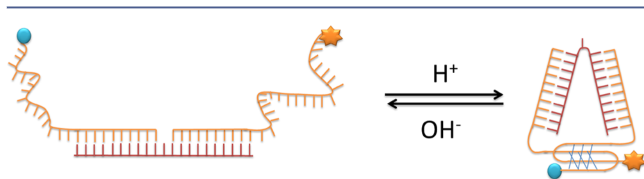

Figure 8. A pH-sensitive switch consisting of three oligonucleotides. The orange strands are partially complementary to the red strand and also bear C-rich DNA overhangs labeled with a fluorophore (yellow star) and a quencher (blue dot) that can form an i-motif at acidic pH. Figure adapted from ref 56.

Another biosensing platform composed of triplex and graphene oxide was used to monitor pH changes associated with apoptosis in living cells.[58] Graphene oxide has affinity for single-stranded but not triplex DNA; it can efficiently quench fluorescence of an adsorbed molecule and serve as a transporter of DNA into cells. The device efficiently translocates across cell membranes and does not interfere with cellular functions; it had low background fluorescence and was simple, efficient, and inexpensive.

Nanodevices Based on G4-Duplex Equilibrium

DNA devices based on G-rich DNA depend on the ability of this DNA to reversibly fold into G4 structures in the presence of selected cations. General designs are similar to those of i-motif-based machines. The devices are well-controlled, durable, highly reversible, and have high energy conversion efficiency. Applications of such devices include transport of nanoparticles, movement of microtubules, sensing of cations, and formation of selective nanochannels.

The first G4-based nanodevices were reported by our[59] and Tan's[60] laboratories. They consisted of a G-rich motor DNA and C- and G-fuels. Addition of C-fuel led to duplex formation, opening the device. Subsequent addition of G-fuel led to formation of a waste duplex that released the G-rich nanomotor and closed the device. The conformational switch between duplex and G4 resulted in ∼5 nm displacement with a calculated force of ∼8 pN.[59] The device required a precise amount of fuel for proper operation. Accumulation of GC waste and incomplete release of motor DNA lead to deterioration of device operation. The operation of the device

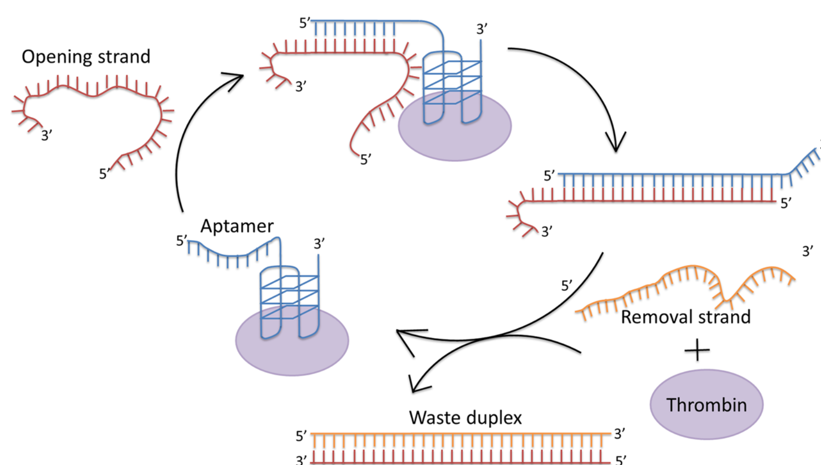

Figure 9. Operation cycle of the aptamer-based molecular machine in the presence of thrombin. Adapted from ref 62.

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is accelerated by increased temperature,[59] use of mutated antifuel strands, and addition of DNA catalyst.[36]

G4-based nanomachines can sense ions (K^+, Sr^{2+}, Pb^{2+}, Tb^{3+}), small molecules (cocaine, ATP), proteins (thrombin, lysozyme, adenosine deaminase), DNA, and cells. Readers are referred to a recent comprehensive review of these devices.[61] In many cases, application of G4-based nanomachines in sensing is based on the ability of the target to effectively induce and stabilize G4 structures; often such sensors are label free, which is their great advantage.

Aptamers that are G-rich can adopt G4 secondary structures. The well-known example is the thrombin binding aptamer (TBA) that interacts with human blood clotting factor, α-thrombin. A DNA nanodevice based on TBA and fueled by complementary strands serves as "molecular hand" that binds and releases thrombin in a controlled fashion (Figure 9).[62] Using different aptamers, "molecular hands" can be developed for controlled delivery of a variety of small molecules and proteins.

Willner and colleagues developed a general approach using a G4-based DNAzyme with horseradish-peroxidase-like activity for rapid, efficient, quantitative, and ultrasensitive detection of DNA, telomerase, Hg^{2+}, thrombin, cocaine, and other molecules, as described in detail in two recent reviews.[61,63] The G-rich DNAzyme adopts a quadruplex fold in the presence of hemin and catalyzes H_2O_2-mediated oxidation of 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) to produce a color change or luminol, which leads to chemiluminescence. The designs of these machines vary greatly, but in general, the presence of target leads to proportional or amplified formation of DNAzyme whose activity can be tracked colorimetrically or via fluorescence. Such DNA machines will find applications in diagnosis of genetic disorders, identification of pathogens, and forensics.

Gold Nanoparticle–DNA Conjugates

Gold nanoparticles (AuNPs) conjugated to DNA combine powerful molecular recognition properties of DNA with size-dependent optical properties of AuNPs. These conjugates display great stability and thus hold great promise. AuNP assemblies mediated by duplex, triplex, i-motif, and G4 DNA have been reported. The advantage of AuNPs assembled with noncanonical DNA structures is reversibility. *AuNP–triplex conjugates* are formed by attaching two different oligonucleotides to a AuNP: a hairpin duplex and the TFO sequence.[64] At neutral pH, nanoparticles are monomeric; at acidic pH, triplex formation leads to nanoparticle aggregation. The aggregation can be reversed by increasing pH. A colorimeteric assay was developed based on AuNP–triplex conjugates to screen for effective triplex binders.[65] These binders together with TFOs have potential therapeutic promise in controlling gene expression.

AuNP–i-motif conjugates rely on inter-[66,67] and intramolecular[68] i-motifs. The former were prepared by attaching a DNA oligonucleotide with one[67] or two[66] C-rich stretches to all nanoparticles. Nanoparticles are monomeric at neutral pH and aggregate at acidic pH. Interestingly, the switching rate was rather slow in one case (2 min)[66] but fast (<1 s) in another.[67] Intramolecular i-motif devices built from C-rich DNA and its complement attached to separate AuNPs operate in reverse: nanoparticles aggregate at basic pH due to duplex formation but dissociate at acidic pH due to i-motif folding. Switching is reversible and slow (~20 min).[68]

AuNP–G4 conjugates have been prepared based on *Oxytricha* telomeric DNA[69] or synthetic oligonucleotides with one G-run.[70] Nanoparticle assembly was induced by NaCl and was reversed by switching to low ionic strength buffer. Ion concentration and identity greatly affect the assembly process and stability of aggregates. When the G-rich sequence was terminated with thymine, aggregation of AuNP was not observed.[69,70]

Mesoporous Silica–DNA Conjugates

Mesoporous silica (MS) is an ideal material for controlled release because it is nontoxic and stable and has large load capacity and a tunable pore size. Current pore capping systems have multiple drawbacks and few are DNA-based. Two novel capping systems were reported recently. One consisted of C-rich DNA attached to MS; the folded i-motif capped the MS pores and released guest molecules at alkali pH.[71] A different cap was created by modifying MS with single-stranded DNA partially complementary to C-[72] or G-rich DNA[73] conjugated to AuNPs matched in size to the MS pores. At alkali pH or in the absence of K^+, respectively, complementary DNA sequences hybridize such that AuNPs cap the MS pores. Addition of K^+ or lowering the pH shifted equilibrium toward G4 or i-motif, respectively, leading to dissociation of nanoparticle cap and release of cargo.

■ CONCLUSION

Unusual nucleic acid structures offer unique opportunities for construction of DNA-based nanomaterials and nanodevices. Their peculiar sensitivities to pH, specific cations, or small molecules allow design of DNA-based materials responsive to these stimuli. There is still a relative paucity of studies taking full advantage of these "oddities", and we hope this Account will stimulate further interest in nanotechnologies based on noncanonical DNA.

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Notes

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