ACCOUNTS of chemical research

Article

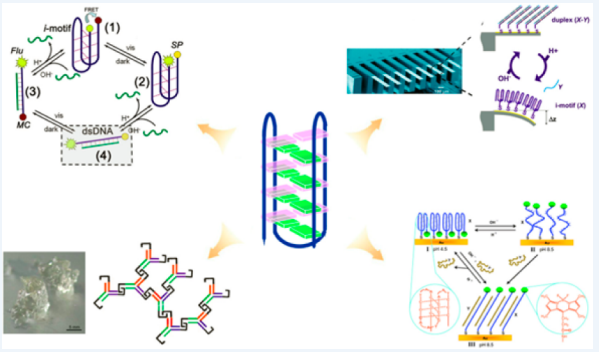
pubs.acs.org/accounts

DNA Nanotechnology Based on i-Motif Structures

Yuanchen Dong, Zhongqiang Yang, and Dongsheng Liu*

Key Laboratory of Organic Optoelectronics & Molecular Engineering of the Ministry of Education, Department of Chemistry, Tsinghua University, Beijing 100084, China

CONSPECTUS: Most biological processes happen at the nanometer scale, and understanding the energy transformations and material transportation mechanisms within living organisms has proved challenging. To better understand the secrets of life, researchers have investigated artificial molecular motors and devices over the past decade because such systems can mimic certain biological processes. DNA nanotechnology based on i-motif structures is one system that has played an important role in these investigations.



In this Account, we summarize recent advances in functional DNA nanotechnology based on i-motif structures. The i-motif is a DNA quadruplex that occurs as four stretches of cytosine repeat sequences form C·CH$^+$ base pairs, and their stabilization requires slightly acidic conditions. This unique property has produced the first DNA molecular motor driven by pH changes. The motor is reliable, and studies show that it is capable of millisecond running speeds, comparable to the speed of natural protein motors. With careful design, the output of these types of motors was combined to drive micrometer-sized cantilevers bend. Using established DNA nanostructure assembly and functionalization methods, researchers can easily integrate the motor within other DNA assembled structures and functional units, producing DNA molecular devices with new functions such as suprahydrophobic/suprahydrophilic smart surfaces that switch, intelligent nanopores triggered by pH changes, molecular logic gates, and DNA nanosprings. Recently, researchers have produced motors driven by light and electricity, which have allowed DNA motors to be integrated within silicon-based nanodevices. Moreover, some devices based on i-motif structures have proven useful for investigating processes within living cells.

The pH-responsiveness of the i-motif structure also provides a way to control the stepwise assembly of DNA nanostructures. In addition, because of the stability of the i-motif, this structure can serve as the stem of one-dimensional nanowires, and a four-strand stem can provide a new basis for three-dimensional DNA structures such as pillars. By sacrificing some accuracy in assembly, we used these properties to prepare the first fast-responding pure DNA supramolecular hydrogel. This hydrogel does not swell and cannot encapsulate small molecules. These unique properties could lead to new developments in smart materials based on DNA assembly and support important applications in fields such as tissue engineering.

We expect that DNA nanotechnology will continue to develop rapidly. At a fundamental level, further studies should lead to greater understanding of the energy transformation and material transportation mechanisms at the nanometer scale. In terms of applications, we expect that many of these elegant molecular devices will soon be used in vivo. These further studies could demonstrate the power of DNA nanotechnology in biology, material science, chemistry, and physics.

1. INTRODUCTION

Cytosine-rich DNA domains are widely found in genomes, for example, in telomeres and the promoters of oncogenes.[1,2] In 1993, Gueron and co-workers found that the DNA sequence d(C_5T) can form a special tetraplex structure in slightly acidic conditions consisting of two parallel duplexes maintained by C·CH$^+$ pairs (Figure 1) intercalated with each other in an antiparallel orientation; it was named the "i-motif".[3,4] For the last two decades, i-motif structures have been intensively investigated. Studies have proved that (i) if there is more than one stretch of cytosines in one DNA strand, bimolecular and intramolecular i-motif structures are possible,[5,6] (ii) i-motif structures can usually be stabilized in the pH range of 3−7 depending on sequences and environment,[4,7] and (iii) i-motif structures have specific absorptions in the circular dichroism (CD) spectrum (a strong positive band near 285 nm, a smaller negative band near 260 nm, and a crossover at approximately 270 nm).[8] This technology is commonly used to characterize the formation of i-motif structures because it is much more convenient than X-ray diffraction and nuclear magnetic resonance methods.[3,9]

2. STATIC ASSEMBLIES AND THEIR APPLICATIONS

As illustrated in Figure 1, i-motif structures are possible with one, two, or four DNA strands; the loop-region DNA

Special Issue: Nucleic Acid Nanotechnology

Received: February 19, 2014
Published: May 20, 2014

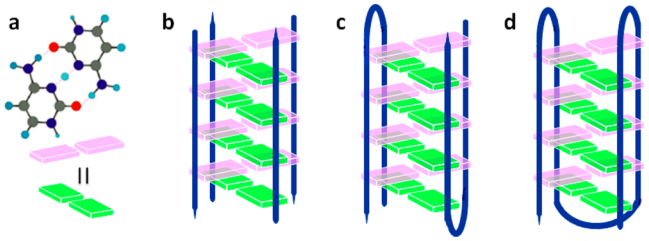


Figure 1. Schematic illustration of i-motif conformations: (a) C·CH$^+$ pairs; (b) tetramer i-motif structure; (c) dimer i-motif structure; (d) intramolecular i-motif structure.

sequences that connect the adjacent cytosine stretches play an important role in determining the conformations and stability of the final assemblies.[10] Recently, i-motif structures have become an important scaffold in DNA nanotechnology because (i) i-motifs have four DNA strands in their stem structures, which provides more choices in the design and fabrication of DNA nanostructures especially in three dimensions, (ii) i-motif structures formed by three to five bases have comparable stability to ten-base-pair-long duplexes, which can simplify design and synthesis, and (iii) its pH responsiveness could provide additional choices in controlling the assembly processes. The following are some examples of i-motif structures that have been used in static nanostructure assemblies.

2.1. Controllable Assembly Based on i-Motif Structures

For the past three decades, constructing well-defined static structures has been the main focus of DNA nanotechnology. Several delicate strategies have been developed, such as tile-by-tile and DNA origami, which are primarily based on DNA duplexes. In the meantime, i-motifs have also been used to fabricate one-dimensional nanowires and three-dimensional nanostructures.[11−14] For example, d(C7) can be assembled into micrometer DNA wires because of the outstanding stability provided by i-motifs.[11] Different from duplex DNA, i-motifs can accommodate four free ends at each side, which can spread in four directions in the *XY* plane perpendicular to the direction of the i-motif stem. As shown in Figure 2, we have prepared two i-motif structures possessing single-stranded ends that were complementary to each other; they further assembled into DNA pillars with an i-motif stem and duplex branches.[12] By leaving thiol groups on the duplex branches to catch gold nanoparticles (AuNPs), the DNA pillars could direct the assembly of AuNPs into two parallel lines or helical structures. These pillars can further assemble into more complicated DNA frameworks by introducing proper sticky ends to the duplex branches.

The assembled i-motif structures have been used as a template to prepare circular single-stranded DNA. By rational design, the formation of intramolecular i-motif structures can align their 5′-phosphate and 3′-hydroxy groups in the proper position to be ligated into a normal phosphodiester bond in the presence of *N*-cyanoimidazole.[15] Because i-motif structures are only stabilized by acidic pH, the circular DNA has no strong internal base-pairing effects under physiological conditions. These circular DNA could be excellent antisense strands because they are resistant to exonuclease and can bind to the target DNA strands efficiently. Because of the outstanding stability of i-motif structures, this strategy could extend to bimolecular i-motif structures and can minimize the circle to nine nucleotide residues.[16] It is noteworthy that the 9-mer

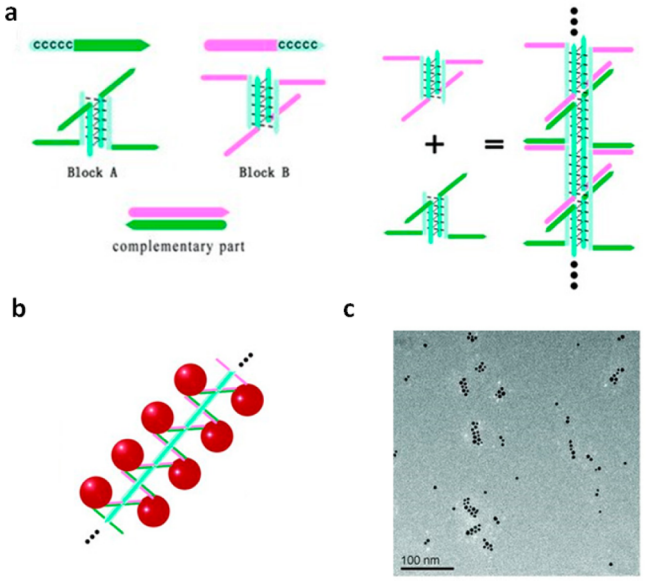


Figure 2. DNA pillars constructed from an i-motif stem and duplex branches. (a) Scheme of the two building blocks and the DNA self-assembly process. (b) Schematic of DNA pillar-directed AuNP assemblies. (c) TEM image of assembled structures with 5 nm AuNPs attached.

DNA circle migrates faster than its linear form in polyacrylamide gel electrophoresis. This result hints that the nucleotide residues in the circle might be packed tighter than those in the linear format and that the circle has strong internal tension. In addition, this strategy could be applied to G-quadruplexes to prepare G-rich circular DNA.[17,18] These results not only provide a new strategy to prepare circular DNA but also profoundly impact our understanding of the physical properties of cyclic DNA and the function of CG-rich regions in a genome.

2.2. Hydrogels Based on i-Motif Structures

Other than preparing precisely addressable units at nanometer scale, DNA assembly could also enable macro-sized material preparation by sacrificing some accuracy in assembly. In 2009, Liu and co-workers reported the first pure DNA supramolecular hydrogel that was formed by only three 37-mer DNA strands.[19] As shown in Figure 3, three partially complementary sequences assembled together to form a Y-shaped DNA nanostructure at pH 8. This Y-scaffold has a rigid double-stranded central domain and three half i-motif sequences at its ends as interlocking domains. When pH is lowered to 5 with the formation of intermolecular i-motif structures between scaffolds, the Y-scaffolds are connected to form a three-dimensional network in solution, namely, the hydrogel. Because of the outstanding stability of i-motifs, this hydrogel is very strong compared with other supramolecular hydrogels. In addition, the formation of an i-motif structure is reversible, and its stabilizing mechanism is distinct from that of the duplex; thus, the hydrogel/solution transition can be quickly switched by adjusting the pH. Based on a similar strategy, hybrid hydrogels that switch by adjusting the pH could also been produced.[20]

It is worth noting that this hydrogel does not swell and cannot encapsulate small molecules, possibly because of the materials that form the hydrogel network. In a traditional hydrogel, there are "soft" (longer than their persistence length)

dx.doi.org/10.1021/ar500073a | *Acc. Chem. Res.* 2014, 47, 1853−1860

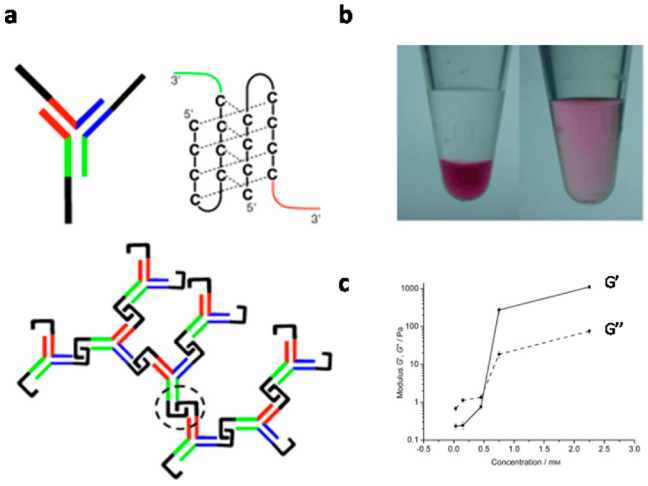


Figure 3. pH-triggered, fast-responding DNA hydrogel based on i-motifs: (a) principal structure of the pH-responsive DNA gel with the three-dimensional assembly of DNA Y units; (b) gel transition switched by pH change with AuNPs trapped in a DNA hydrogel; (c) rheology tests for DNA hydrogels with different Y-unit concentrations (2.25, 0.75, 0.45, 0.15, and 0.03 mM) at pH 5.0. When the shear storage modulus (G') is much greater than the shear loss modulus (G''), the material behaves like a solid; in contrast, when G' is smaller than G'', the material behaves like a liquid.

polymer chains between cross-linking points; during the swelling process, the coiled chains are stretched and the distance between cross-linking points increases. Thus, the whole volume of the network increases (swelling). Between cross-link points of the DNA hydrogel, there are only duplex and i-motif structures; they are all "rigid" (shorter than their persistence length) structures that cannot provide the flexibility for changes in distance, which means no swelling. Moreover, random coiling of polymer chains in traditional hydrogel network makes the mesh size of the network a normal distribution, which enables the hydrogel to encapsulate molecules of any size. The rigid structures of the DNA hydrogels eliminate very small mesh sizes in the distribution, which means less encapsulation of small molecules. These findings have inspired the design and preparation of a series of hydrogels based on DNA assembly. Their properties, for example, small molecule and green fluorescent protein (GFP) permeability, designable responsiveness, and good mechanical strength, have made them promising in potential applications in cell culture and tissue engineering.[21,22]

2.3. Other Assemblies Based on i-Motif Structures

With established modification methods, DNA sequences containing cytosine stretches can be attached to gold nanoparticles, carbon nanotubes, graphene, etc., and confer to them pH triggered assembly via the formation of intermolecular i-motif structures.[23−28] These studies enrich the field of DNA nanotechnology and provide a quick, reliable, and reversible method to control the assembly of entities at the nanometer scale.

3. DYNAMIC ASSEMBLY BASED ON i-MOTIF STRUCTURES: DNA MOLECULAR MOTORS DRIVEN BY pH CHANGES

Molecular motors are molecules or molecular assemblies that can perform movements upon stimulation and translate energy into mechanical movements or other energy formations.[29] Most enzymes are natural protein molecular motors. Recently, artificial molecular motors have attracted more and more attention because they are structurally clear and simple, durable, and easily functionalized. Among them, DNA is distinct because it is a natural product that has been used to fabricate artificial molecular motors.

Around the millennium, making the assembly "move" became an important direction in DNA nanotechnology, and several prototype DNA molecular motors have been proposed.[29,30] They are driven by ions or chain-exchange reactions, where the reliability of the motors' cycling is always the biggest challenge. Additionally, the running speed (normally in the range of minutes or hours) and working ability are issues frequently questioned. In 2003, Liu and Balasubramanian reported a novel DNA molecular motor driven by pH changes and based on an i-motif structure that can run at subsecond levels in a reliable and clean manner.[31] This example breaks the speed and reliability limitations in this field and enables further studies on the working ability and driving mechanism of the DNA molecular motors.[32] In addition, pH change, as an excellent bridge, empowers the development of new methods for driving DNA motors, including light, electrical signals, and so on, which broaden the applications of DNA molecular motors.[33−36]

3.1. The pH Driven DNA Molecular Motors Based on i-Motif Structures

As illustrated in Figure 4, the DNA molecular motor is composed of a 21-mer single-stranded X containing four CCC

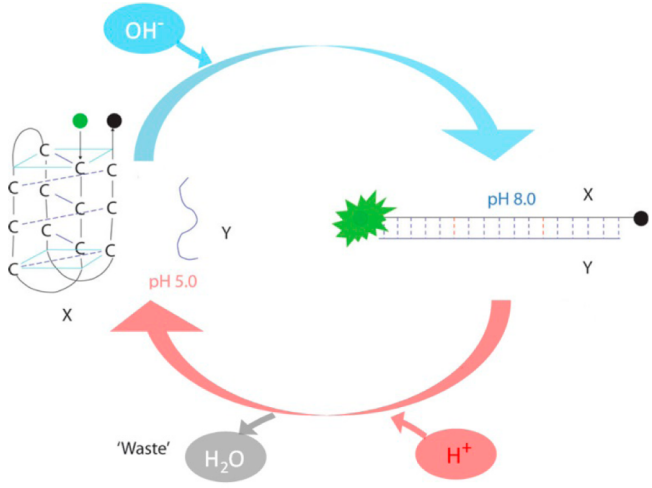


Figure 4. Schematic figure of the working cycle of i-motif DNA motors.

stretches and a 17-mer single-stranded Y, which is partially complementary to X. At slightly acidic pH, the X sequence folds into a compact intramolecular i-motif structure with its 3′ and 5′ ends close to each other, which corresponds to the motor's "closed" state; changing the pH to slightly basic conditions will result in unfolding of the i-motif and formation of the duplex XY, representing the motor's "open" state. Because the neutralization reaction is an irreversible reaction that could be finished in a very short time, the pH changing speed is only limited by diffusion of the proton and hydroxyl groups, which theoretically could be much lower than milliseconds; thus, the operating speed of this DNA molecular motor is only limited by the intramolecular chain movement,

dx.doi.org/10.1021/ar500073a | Acc. Chem. Res. 2014, 47, 1853−1860

which can occur at milliseconds to seconds. The pH change is highly reversible, and the byproducts of the cycling are only salt and water, which do not affect the DNA secondary structures.[31] Crystal diffraction data showed that the difference in distance between the open and closed states is nearly 5 nm; the energy difference in each half cycle is nearly balanced, and both are at the 10 kcal/mol level. Thus, the motor could produce a similar force output upon both the opening and closing processes, which would benefit its potential applications.

3.2. Demonstration of the Working Ability of the DNA Molecular Motors

Many characteristics of the DNA molecular motors suggest that they might produce a force output; however, direct evidence has been difficult to obtain. Because the motor can only generate nanometer motion and piconewton (pN) force outputs, providing direct evidence for the output is a significant challenge in physics. In 2005, Shu et al. developed a delicate system that showed the working ability of this motor.[32] As shown in Figure 5, the DNA motor was immobilized onto a microcantilever in its open state and formed a compact monolayer under basic conditions; the cantilever was in a relaxed position. When the pH was shifted to acidic, the DNA motor contracted and caused a compressive surface stress on the cantilever, which bent the cantilever. This was the first demonstration of an artificial DNA molecular motor that could perform micromechanical work.

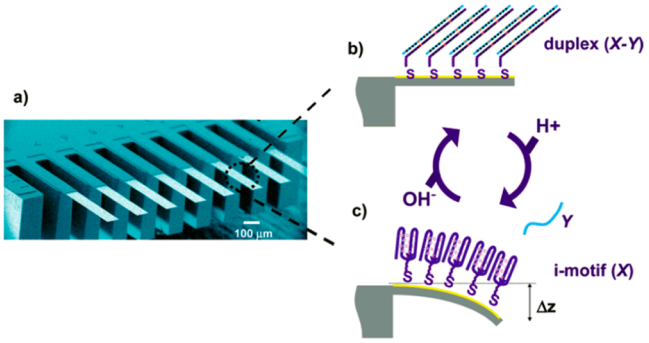


Figure 5. Conformation changes on a micromechanical cantilever array: (a) scanning electron microscope image of an array of the cantilevers; (b) schematic diagram of a cantilever functionalized on one-side with a thin film of gold and a monolayer duplex structure; (c) i-motif induced repulsive in-plane surface forces that cause the cantilever to bend downward.

3.3. Variations of the Methods for Driving i-Motif Motors

In addition to adding acids or bases, many chemical processes can also cause pH changes. Thus, new types of driving methods for DNA motors are realized via the bridge of "pH change". For example, in 2005, Liedl and Simmel reported a strategy to power the DNA motor with a chemical pH oscillator.[34] The oscillator worked by alternative oxidation of sulfite and thiosulfate by iodate, which is accompanied by periodic production or consumption of protons. The DNA motor could be cycled several times before the chemicals were consumed. To match the reliability of this DNA motor, we successfully coupled it with a light-induced pH change reaction and produced the first noncontact driven DNA motors in 2007.[35] Limited by the pH cycling mechanism, its speed is very slow, and the UV light may damage the DNA structure. Years later, with the help of Fan's group, we made a three-electrode device; it has a pH-sensitive IrO_2 reference electrode and can reach a target final pH precisely and quickly when the potential between the reference and working electrodes is provided.[36] The performance of this device perfectly matches the DNA motor and can drive the motor cycling in a reliable and swift manner. These results enrich the methods for driving DNA motors and also provide a solid support for future applications.

4. APPLICATION OF THE MOTOR: FUNCTIONAL MOLECULAR DEVICES BASED ON i-MOTIF STRUCTURES

With well-established DNA nanostructure assembly technology and functionalization methods, the DNA motor could be easily integrated with other functional units and permit the fabrication of assemblies with new functions, namely, DNA molecular devices. In these devices, the DNA motor exerts spatial changes upon stimulation, which can change the topology of the functional units and can lead to the desired property change. The motor must be robust and reliable, and less crosstalk should occur with other DNA units. Thus, a DNA motor based on the i-motif structure is an excellent candidate that has been widely used in the past decade for fabricating functional molecular devices, such as intelligent surfaces and

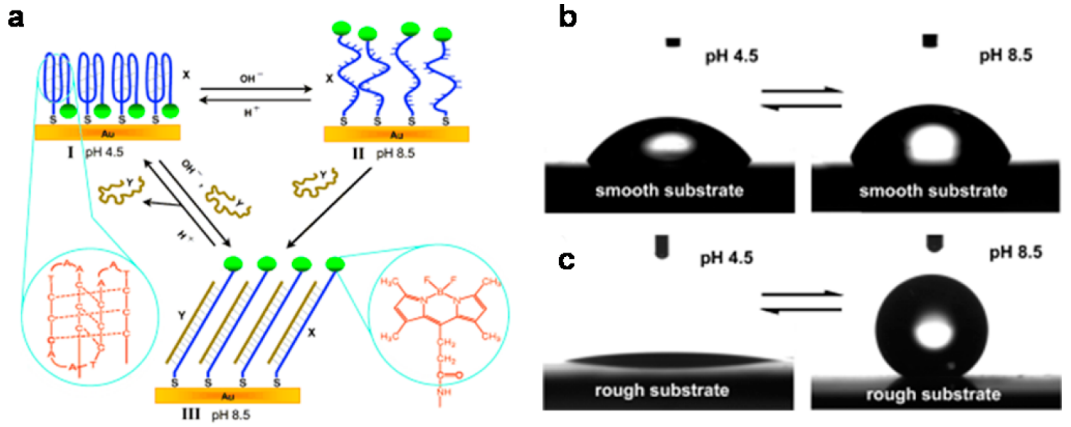


Figure 6. Intelligent reversibly switchable surfaces driven by DNA motors: (a) scheme of the three-state smart surface with tunable wettability with changes in pH; profiles of water droplets at pH 4.5 and 8.5 on a smooth (b) and on a rough substrate (c) showing the difference in wettability of states I and II.

dx.doi.org/10.1021/ar500073a | Acc. Chem. Res. 2014, 47, 1853−1860

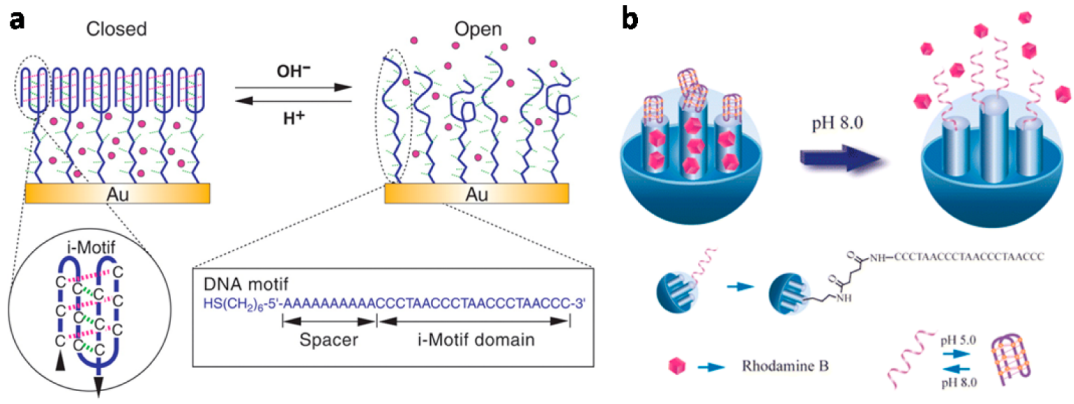


Figure 7. Controlled release based on i-motif motors: (a) DNA nanocontainer system immobilized on a planar surface; (b) DNA nanocontainer system applied to a mesoporous silica nanoparticle system.

nanopores, controllable release devices, and molecular logic gates, among others.

4.1. Intelligent Surfaces and Nanopores

With one end immobilized onto the surface, the motion of the motor can result in chain density changes, accompanied by spatial position changes of the free end. By attaching a fluorophore onto the free end, the assembled motor monolayer on the gold surface became a smart fluorescent array.[37] At pH 8, the open state motor keeps the fluorophore away from the gold surface and a strong fluorescent signal could be obtained; when the pH is adjusted to 5, the closing motion of the motor drags the fluorophore to the surface and the fluorescent signal is quenched. Meng et al. demonstrated that this DNA motor is strong enough to move quantum dots up and down in the solid/solution interface and produced a switchable photoelectric conversion device.[38] Inspired by the switchable fluorescent array, Wang et al. reported an intelligent surface that can change its wettability with pH triggers.[39] As shown in Figure 6, a large hydrophobic molecule was attached to the motor's free end, and the surface was hydrophobic when the motor was in its open state because the hydrophobic molecule layer was presented. When the motor changed to the closed state, the DNA hydrophilic backbone was presented, and the surface wettability changed toward the hydrophilic direction. In these examples, we can find that DNA motors can provide switchable scaffolds and the modified units can provide functionality. Because the functions of the devices are normally produced by the cooperative motion of numerous motors, the immobilization density is always the most challenging technical topic to ensure the motor's motion is effective. In a similar strategy, using arginine-glycine-aspartic acid (RGD) with a photolabile caging group as the functional units, Qu and coworkers reported a near-infrared and pH responsive surface for reversible cell adhesion.[40]

DNA motors have also been immobilized on the inside walls of nanopores, providing pore gating properties. In the nanopore, different DNA conformations have a distinctive hindrance to ions that carry currents. When the pore and DNA sizes match, obvious current changes will occur when the conformation is changed. In 2008, Xia et al. reported the first pH gating intelligent nanopore based on i-motif molecular motors.[41] To explain the gating mechanism, Hou et al. fabricated a potassium-responsive nanopore and investigated the gating process in depth.[42] In addition to fabricating an artificial nanopore that can mimic the function of a natural protein potassium channel, this study also proved that densely packed quadruplexes and duplexes can both hinder the transportation of potassium ions and that the height of these structures is directly related to the gating efficiency. Under the same immobilization density, the hindrance of a single-stranded DNA is much lower and could be considered as the open state. From these results, we can also propose that the effective nanopore size could be finely tuned by designing a stepwise DNA assembly.

4.2. Nanocontainers and Other Devices for Controlled Release

The cooperative motion of the DNA motors has also been used to fabricate nanodevices that can load small molecules for controlled release. As shown in Figure 7, i-motif motors are anchored onto ultraflat gold surfaces via a single-stranded poly-A connector.[43] After a careful annealing process, the i-motif domain formed a densely packed monolayer; however, the poly-A domain is still loosely packed. The space left between the single strands is considered a nanocontainer, which can carry ions and small molecules; the folded i-motif monolayer is the "lid", which could be opened by changing the pH. This strategy has been successfully transplanted onto gold and mesoporous silica nanoparticles and led to several types of intelligent drug delivery systems.[44,45] In these reports, the loading capacity and circulating properties are greatly improved.

4.3. Manipulating Intermolecular Interactions in Solution

As described above, each stroke of the motor can approximately produce a 5 nm linear movement. This output can be used to control the spatial position of the different objects in solution, which helps with the investigation of distance-dependent nonspecific or specific interactions at the molecular level. In 2008, Liu et al. reported a photo-pH dual-modulated fluorescence switch based on the i-motif structure, in which the FRET process between the fluorescein and photochromic moieties was modulated by the DNA motor and the resulting device can mimic the function of a Boolean logic operation in solution.[46] Other than small objects, similar strategies have been employed to investigate macromolecule interactions.[47] Two third-generation hydrophobic dendrimers with amphiphilic peripheral layers have been attached to the ends of the DNA motor. Along with the cycling of the motor, dendrimers were reversibly merged and disassociated. From this study, we also found that the interactions between dendrimers are very strong and can heavily influence the dynamics of attached DNA structures; in this case, the melting point of the folded i-motif

dx.doi.org/10.1021/ar500073a | Acc. Chem. Res. 2014, 47, 1853−1860

structures was increased by more than 10 °C. Systematic studies also revealed that this phenomenon is general; first-generation dendritic and even small hydrophobic molecules can significantly change the melting points of their attached assembled DNA structures. Considering that most fluorophores contain certain sized hydrophobic groups, the interactions between fluorophores should be taken into account in the future when using FRET methods to study kinetics of DNA or protein secondary structural changes. In 2012, Chen and co-workers employed a stopped-flow circular dichroism (SFCD) technique to study the kinetics of the i-motif because it can provide millisecond time resolution and does not require modifications on the DNA.[48] The results reveal that (i) both folding and unfolding processes could be completed at a time scale of 100 ms, which is very similar to natural ATP motors, and (ii) the folding and unfolding processes are pH-dependent. Based on these results, Chen et al. proposed that (i) both processes could comprise many steps, (ii) there is only one rate limiting step in the unfolding process, where two protons are cooperatively neutralized, and (iii) in the folding process, there must be at least two steps, one of which involves three protons cooperatively binding to the DNA molecule.

These studies also inspired the development of a series of DNA nanodevices that can be used to study the cooperative DNA aptamer−protein and protein−protein interactions at the nanometer scale.[49−51] The i-motif structures have also been incorporated into self-assembled DNA dendritic systems. The conformation switch of the i-motif motors can change the size and the functional group density on the surface of the system.[52,53]

4.4. Other Nanodevices Based on i-Motif Structures

In addition to the above-mentioned examples, i-motif structures have also been widely used to fabricate smart devices, such as DNA nanosprings,[54] a unidirectional DNA walker,[55] and a pH indicator,[56] which have been applied to cell systems to investigate intracellular processes.

5. CONCLUSION AND PROSPECTS

In summary, the distinct properties of i-motif structures have greatly advanced the development of DNA nanotechnology. For static structure assembly, the i-motif is a great choice for process control in a stepwise assembly other than varying the analogue of sticky ends. Because of its outstanding stability, self-complementary analogue, and quick formation properties, the i-motif structure has inspired the preparation of the first fast-responding pure DNA supramolecular hydrogel; the unique mechanical and encapsulation properties of this hydrogel stimulated recent rapid development of smart materials based on DNA assemblies and their applications in tissue engineering. For molecular motors and devices, the DNA motor based on i-motif structures improved the running speed limit of artificial molecular motors to the millisecond-level, which is comparable to natural protein motors. The motor has been demonstrated to be reliable and robust and could be easily integrated with other DNA assembled structures and functional units to enable other kinds of DNA molecular devices. In the meantime, light- and electrically driven DNA motors based on the i-motif could be realized via the "pH-change" bridge, which means that this molecular motor can be easily adopted into current nanotechnology based on silicon.

Thus, DNA nanotechnology based on i-motif structures is ready for in-depth fundamental and applied studies. Currently, more efforts are needed to study the biological activity of i-motifs *in vivo*, and small molecules and proteins that can specifically interact with i-motifs. In addition, rather than circuitously demonstrating its working ability via collective effects, the ability to directly measure the output of a single DNA molecular motor is needed. With the rapid development of experimental physics, measuring force at the piconewton level with nanometer spatial resolution in solution should become feasible. These studies will also advance our understanding of the energy translating mechanism at the nanometer scale. In addition, more delicate DNA devices are necessary, and the focus of such studies will move from individual devices to hybrid systems and ultimately "assembly lines", which can be used to conduct much more complicated functions and mimic biological systems. In addition to these *in vitro* studies, their *in vivo* applications will be the driving force to push the field to the next level. In the past few years, Yamuna et al. has used DNA nanodevices based on i-motif structures as a pH sensor to map spatial and temporal pH changes inside living cells and whole living organisms; with proper modifications, this technique can also be used to explore different cellular endocytic pathways.[56−58] These studies are important for the future of the DNA nanotechnology. We can expect more and more materials and molecular devices based on i-motif structures to be used in diagnostics, drug delivery, and molecular operations in living systems; these devices can produce a significant impact on the sciences and human life.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: liudongsheng@tsinghua.edu.cn.

Funding

We thank the National Basic Research Program of China (973 program, No. 2013CB932803), the National Natural Science Foundation of China (No. 91027046, 21121004), the NSFC-DFG joint project TRR61, and the Beijing Municipal Science & Technology Commission for their financial support.

Notes

The authors declare no competing financial interest.

Biographies

Yuanchen Dong received his B.S. degrees in chemistry and biology from Jilin University, China, in 2010. He then went to Tsinghua University in China to pursue his doctoral degree. His Ph.D. thesis focused on organic synthesis, DNA nanotechnology, and assembly of amphiphilic molecules.

Zhongqiang Yang received her B.Engr. (2000) and M.Sc. (2003) degrees from Heilongjiang University and Jilin University in China, respectively. In 2003, she obtained a Gates Cambridge Trust and Overseas Research Studentship and pursued her Ph.D. at the University of Cambridge, U.K. She worked with Prof. Wilhelm T. S. Huck at the Melville Laboratory for Polymer Synthesis. In 2007, she went to the University of Wisconsin—Madison, USA, performing postdoctoral work with Prof. Nicholas L. Abbott. In 2010, she joined the Chemistry Department at Tsinghua University as an associate professor. Her research interests include DNA-related materials and their applications.

Dongsheng Liu graduated from the University of Science and Technology of China with a B.S. degree in 1993. After working in the Institute of Chemistry at the Chinese Academy of Sciences for six years, he attended Hong Kong Polytechnic University and finished his

Ph.D. there in 2002. He then worked as a postdoctoral research associate at the NanoIRC and the Department of Chemistry at Cambridge University, U.K. In 2005, he became a principal investigator at the National Centre for NanoScience and Technology in China. Since June 2009, he has been a full professor of the Department of Chemistry at Tsinghua University. His current research is primarily focused on using biomolecules to fabricate nanostructures and nanodevices.

REFERENCES

(1) Shafer, R. H.; Smirnov, I. Biological aspects of DNA/RNA quadruplexes. *Biopolymers* **2000**, *56*, 209−227.

(2) Brooks, T. A.; Kendrick, S.; Hurley, L. Making sense of G-quadruplex and i-motif functions in oncogene promoters. *FEBS J.* **2010**, *277*, 3459−3469.

(3) Gehring, K.; Leroy, J. L.; Gueron, M. A tetrameric DNA structure with protonated cytosine.cytosine base pairs. *Nature* **1993**, *363*, 561−565.

(4) Leroy, J. L.; Gehring, K.; Kettani, A.; Gueron, M. Acid multimers of oligodeoxycytidine strands: Stoichiometry, base-pair characterization, and proton exchange properties. *Biochemistry* **1993**, *32*, 6019−6031.

(5) Rohozinski, J.; Hancock, J. M.; Keniry, M. A. Polycytosine regions contained in DNA hairpin loops interact via a four-stranded, parallel structure similar to the i-motif. *Nucleic Acids Res.* **1994**, *22*, 4653−4659.

(6) Leroy, J. L.; Gueron, M.; Mergny, J. L.; Helene, C. Intramolecular folding of a fragment of the cytosine-rich strand of telomeric DNA into an i-motif. *Nucleic Acids Res.* **1994**, *22*, 1600−1606.

(7) Rajendran, A.; Nakano, S.; Sugimoto, N. Molecular crowding of the cosolutes induces an intramolecular i-motif structure of triplet repeat DNA oligomers at neutral pH. *Chem. Commun.* **2010**, *46*, 1299−1301.

(8) Manzini, G.; Yathindra, N.; Xodo, L. Evidence for intramolecularly folded i-DNA structures in biologically relevant CCC-repeat sequences. *Nucleic Acids Res.* **1994**, *22*, 4634−4640.

(9) Kang, C.; Berger, I.; Lockshin, C.; Ratliff, R.; Moyzis, R.; Rich, A. Crystal structure of intercalated four-stranded d (C3T) at 1.4 A resolution. *Proc. Natl. Acad. Sci. U. S. A.* **1994**, *91*, 11636−11640.

(10) Yang, Y.; Sun, Y.; Yang, Y.; Xing, Y.; Zhang, T.; Wang, Z.; Yang, Z.; Liu, D. Influence of tetra(ethylene glycol) (EG_4) substitution at the loop region on the intramolecular DNA i-motif. *Macromolecules* **2012**, *45*, 2643−2647.

(11) Ghodke, H. B.; Krishnan, R.; Vignesh, K.; Kumar, G.; Narayana, C.; Krishnan, Y. The i-tetraplex building block: Rational design and controlled fabrication of robust 1D DNA scaffolds through non-Watson-Crick interactions. *Angew. Chem., Int. Ed.* **2007**, *119*, 2700−2703.

(12) Yang, Y.; Zhou, C.; Zhang, T.; Cheng, E.; Yang, Z.; Liu, D. DNA pillars constructed from an i-motif stem and duplex branches. *Small* **2012**, *8*, 552−556.

(13) Mei, H.; Budow, S.; Seela, F. Construction and assembly of chimeric DNA: Oligonucleotide hybrid molecules composed of parallel or antiparallel duplexes and tetrameric i-motifs. *Biomacromolecules* **2012**, *13*, 4196−4204.

(14) Li, T.; Famulok, M. i-Motif-programmed functionalization of DNA nanocircles. *J. Am. Chem. Soc.* **2013**, *135*, 1593−1599.

(15) Li, T.; Liu, D.; Chen, J.; Lee, A. H. F.; Qi, J.; Chan, A. S. C. Construction of circular oligodeoxyribonucleotides on the new structural basis of i-motif. *J. Am. Chem. Soc.* **2001**, *123*, 12901−12902.

(16) Liu, D.; Chen, J.; Lee, A. H. F.; Chow, L. M. C.; Chan, A. S. C.; Li, T. Small circular oligodeoxynucleotides achieved from self-assembling entities. *Angew. Chem., Int. Ed.* **2003**, *42*, 797−799.

(17) Chen, J.; Liu, D.; Alex, H.; Qi, J.; Albert, S.; Li, T. G-quadruplex as a new class of structural entities for directing the formation of circular oligodeoxyribonucleotides. *Chem. Commun.* **2002**, 2686−2687.

(18) Zhou, T.; Chen, G.; Wang, Y.; Zhang, Q.; Yang, M.; Li, T. Synthesis of unimolecularly circular G-quadruplexes as prospective molecular probes. *Nucleic Acids Res.* **2004**, *32*, e173−e173.

(19) Cheng, E.; Xing, Y.; Chen, P.; Yang, Y.; Sun, Y.; Zhou, D.; Xu, L.; Fan, Q.; Liu, D. A pH-triggered, fast-responding DNA hydrogel. *Angew. Chem., Int. Ed.* **2009**, *48*, 7660−7663.

(20) Cheng, E.; Li, Y.; Yang, Z.; Deng, Z.; Liu, D. DNA-SWNT hybrid hydrogel. *Chem. Commun.* **2011**, *47*, 5545−5547.

(21) Jin, J.; Xing, Y.; Xi, Y.; Liu, X.; Zhou, T.; Ma, X.; Yang, Z.; Wang, S.; Liu, D. A triggered DNA hydrogel cover to envelop and release single cells. *Adv. Mater.* **2013**, *25*, 4714−4717.

(22) Xing, Y.; Cheng, E.; Yang, Y.; Chen, P.; Zhang, T.; Sun, Y.; Yang, Z.; Liu, D. Self-assembled DNA hydrogels with designable thermal and enzymatic responsiveness. *Adv. Mater.* **2011**, *23*, 1117−1121.

(23) Seela, F.; Budow, S. pH-dependent assembly of DNA-gold nanoparticles based on the i-motif: A switchable device with the potential of a nanomachine. *Helv. Chim. Acta* **2006**, *89*, 1978−1985.

(24) Wang, W.; Liu, H.; Liu, D.; Xu, Y.; Yang, Y.; Zhou, D. Use of the interparticle i-motif for the controlled assembly of gold nanoparticles. *Langmuir* **2007**, *23*, 11956−11959.

(25) Sharma, J.; Chhabra, R.; Yan, H.; Liu, Y. pH-driven conformational switch of "i-motif" DNA for the reversible assembly of gold nanoparticles. *Chem. Commun.* **2007**, 477−479.

(26) Cheng, E.; Yang, Y.; Liu, D. pH-controlled carbon nanotube aggregation/dispersion based on intermolecular i-motif DNA formation. *J. Nanosci. Nanotechnol.* **2010**, *10*, 7282−7286.

(27) Qu, K.; Ren, J.; Qu, X. pH-responsive, DNA-directed reversible assembly of graphene oxide. *Mol. BioSyst.* **2011**, *7*, 2681−2687.

(28) Zhao, Z.; Wang, L.; Liu, Y.; Yang, Z.; He, Y.-M.; Li, Z.; Fan, Q.-H.; Liu, D. pH-induced morphology-shifting of DNA-b-poly (propylene oxide) assemblies. *Chem. Commun.* **2012**, *48*, 9753−9755.

(29) Liu, H.; Liu, D. DNA nanomachines and their functional evolution. *Chem. Commun.* **2009**, 2625−2636.

(30) Liu, D.; Cheng, E.; Yang, Z. DNA-based switchable devices and materials. *NPG Asia Mater.* **2011**, *3*, 109−114.

(31) Liu, D.; Balasubramanian, S. A proton-fueled DNA nanomachine. *Angew. Chem., Int. Ed.* **2003**, *42*, 5734−5736.

(32) Shu, W.; Liu, D.; Watari, M.; Riener, C. K.; Strunz, T.; Welland, M. E.; Balasubramanian, S.; McKendry, R. A. DNA molecular motor driven micromechanical cantilever arrays. *J. Am. Chem. Soc.* **2005**, *127*, 17054−17060.

(33) Liedl, T.; Olapinski, M.; Simmel, F. C. A surface-bound DNA switch driven by a chemical oscillator. *Angew. Chem., Int. Ed.* **2006**, *45*, 5007−5010.

(34) Liedl, T.; Simmel, F. C. Switching the conformation of a DNA molecule with a chemical oscillator. *Nano Lett.* **2005**, *5*, 1894−1898.

(35) Liu, H.; Xu, Y.; Li, F.; Yang, Y.; Wang, W.; Song, Y.; Liu, D. Light-driven conformational switch of i-motif DNA. *Angew. Chem., Int. Ed.* **2007**, *46*, 2515−2517.

(36) Yang, Y.; Liu, G.; Liu, H.; Li, D.; Fan, C.; Liu, D. An electrochemically actuated reversible DNA switch. *Nano Lett.* **2010**, *10*, 1393−1397.

(37) Liu, D.; Bruckbauer, A.; Abell, C.; Balasubramanian, S.; Kang, D.-J.; Klenerman, D.; Zhou, D. A reversible pH-driven DNA nanoswitch array. *J. Am. Chem. Soc.* **2006**, *128*, 2067−2071.

(38) Meng, H.; Yang, Y.; Chen, Y.; Zhou, Y.; Liu, Y.; Chen, X. a.; Ma, H.; Tang, Z.; Liu, D.; Jiang, L. Photoelectric conversion switch based on quantum dots with i-motif DNA scaffolds. *Chem. Commun.* **2009**, 2293−2295.

(39) Wang, S.; Liu, H.; Liu, D.; Ma, X.; Fang, X.; Jiang, L. Enthalpy-driven three-state switching of a superhydrophilic/superhydrophobic surface. *Angew. Chem., Int. Ed.* **2007**, *46*, 3915−3917.

(40) Li, W.; Wang, J.; Ren, J.; Qu, X. Near-infrared-and pH-responsive system for reversible cell adhesion using graphene/gold nanorods functionalized with i-motif DNA. *Angew. Chem., Int. Ed.* **2013**, *125*, 6726−6730.

(41) Xia, F.; Guo, W.; Mao, Y.; Hou, X.; Xue, J.; Xia, H.; Wang, L.; Song, Y.; Ji, H.; Ouyang, Q. Gating of single synthetic nanopores by

proton-driven DNA molecular motors. *J. Am. Chem. Soc.* **2008**, *130*, 8345−8350.
(42) Hou, X.; Guo, W.; Xia, F.; Nie, F.-Q.; Dong, H.; Tian, Y.; Wen, L.; Wang, L.; Cao, L.; Yang, Y. A biomimetic potassium responsive nanochannel: G-Quadruplex DNA conformational switching in a synthetic nanopore. *J. Am. Chem. Soc.* **2009**, *131*, 7800−7805.
(43) Mao, Y.; Liu, D.; Wang, S.; Luo, S.; Wang, W.; Yang, Y.; Ouyang, Q.; Jiang, L. Alternating-electric-field-enhanced reversible switching of DNA nanocontainers with pH. *Nucleic Acids Res.* **2007**, *35*, No. e33.
(44) Song, L.; Ho, V. H.; Chen, C.; Yang, Z.; Liu, D.; Chen, R.; Zhou, D. Efficient, pH-triggered drug delivery using a pH-responsive DNA-conjugated gold nanoparticle. *Adv. Healthcare Mater.* **2013**, *2*, 275−280.
(45) Chen, C.; Pu, F.; Huang, Z.; Liu, Z.; Ren, J.; Qu, X. Stimuli-responsive controlled-release system using quadruplex DNA-capped silica nanocontainers. *Nucleic Acids Res.* **2011**, *39*, 1638−1644.
(46) Liu, H.; Zhou, Y.; Yang, Y.; Wang, W.; Qu, L.; Chen, C.; Liu, D.; Zhang, D.; Zhu, D. Photo-pH dually modulated fluorescence switch based on DNA spatial nanodevice. *J. Phys. Chem. B* **2008**, *112*, 6893−6896.
(47) Sun, Y.; Liu, H.; Xu, L.; Wang, L.; Fan, Q.-H.; Liu, D. DNA-molecular-motor-controlled dendron association. *Langmuir* **2010**, *26*, 12496−12499.
(48) Chen, C.; Li, M.; Xing, Y.; Li, Y.; Joedecke, C.-C.; Jin, J.; Yang, Z.; Liu, D. Study of pH-induced folding and unfolding kinetics of the DNA i-motif by stopped-flow circular dichroism. *Langmuir* **2012**, *28*, 17743−17748.
(49) Zhou, C.; Yang, Z.; Liu, D. Reversible regulation of protein binding affinity by a DNA machine. *J. Am. Chem. Soc.* **2012**, *134*, 1416−1418.
(50) Xin, L.; Zhou, C.; Yang, Z.; Liu, D. Regulation of an enzyme cascade reaction by a DNA machine. *Small* **2013**, *9*, 3088−3091.
(51) Wang, W.; Yang, Y.; Cheng, E.; Zhao, M.; Meng, H.; Liu, D.; Zhou, D. A pH-driven, reconfigurable DNA nanotriangle. *Chem. Commun.* **2009**, 824−826.
(52) Chen, P.; Sun, Y.; Liu, H.; Xu, L.; Fan, Q.; Liu, D. A pH responsive dendron-DNA-protein hybrid supramolecular system. *Soft Matter* **2010**, *6*, 2143−2145.
(53) Zhou, T.; Chen, P.; Niu, L.; Jin, J.; Liang, D.; Li, Z.; Yang, Z.; Liu, D. pH-responsive size-tunable self-assembled DNA dendrimers. *Angew. Chem., Int. Ed.* **2012**, *124*, 11433−11436.
(54) Wang, C.; Huang, Z.; Lin, Y.; Ren, J.; Qu, X. Artificial DNA nano-spring powered by protons. *Adv. Mater.* **2010**, *22*, 2792−2798.
(55) Wang, Z.-G.; Elbaz, J.; Willner, I. DNA machines: Bipedal walker and stepper. *Nano Lett.* **2010**, *11*, 304−309.
(56) Modi, S.; Swetha, M.; Goswami, D.; Gupta, G. D.; Mayor, S.; Krishnan, Y. A DNA nanomachine that maps spatial and temporal pH changes inside living cells. *Nat. Nanotechnol.* **2009**, *4*, 325−330.
(57) Surana, S.; Bhat, J. M.; Koushika, S. P.; Krishnan, Y. An autonomous DNA nanomachine maps spatiotemporal pH changes in a multicellular living organism. *Nat. Commun.* **2011**, *2*, 340.
(58) Modi, S.; Nizak, C.; Surana, S.; Halder, S.; Krishnan, Y. Two DNA nanomachines map pH changes along intersecting endocytic pathways inside the same cell. *Nat. Nanotechnol.* **2013**, *8*, 459−467.