Chapter 18: DNA Nanorobotics

Harish Chandran
Department of Computer Science, Duke University
harish@cs.duke.edu

Nikhil Gopalkrishnan
Department of Computer Science, Duke University
nikhil@cs.duke.edu

John Reif
Department of Computer Science, Duke University
Adjunct, Computing and Information Technology, King Abdulaziz University, Saudi Arabia
reif@cs.duke.edu

This chapter overviews the current state of the emerging discipline of DNA nanorobotics that make use of synthetic DNA to self-assemble operational molecular-scale devices. Recently there have been a series of quite astonishing experimental results - which have taken the technology from a state of intriguing possibilities into demonstrated capabilities of quickly increasing scale and complexity. We first state the challenges in molecular robotics and discuss why DNA as a nanoconstruction material is ideally suited to overcome these. We then review the design and demonstration of a wide range of molecular-scale devices; from DNA nanomachines that change conformation in response to their environment to DNA walkers that can be programmed to walk along predefined paths on nanostructures while carrying cargo or performing computations, to tweezers that can repeatedly switch states. We conclude by listing major challenges in the field along with some possible future directions.

Keywords: self-assembly, DNA motors, nanosystems, DNA walkers, nanomachines, DNA computing, DNAzymes
1 Introduction

DNA self-assembly is an emerging scientific discipline that seeks to engineer nanoscale systems created out of DNA strands. The underlying principle of DNA self-assembly is the programmability of DNA strands based on the specific Watson-Crick binding of DNA bases, adenine (abbreviated A), cytosine (C), guanine (G) and thymine (T). Typically, A prefers to pair up with T while C with G. Hence the sequence ATATC would hybridize to its reverse complement (complement of the molecule written in reverse) GATAT. The sequences for a set of DNA molecules can be designed such that they interact among themselves in specific ways. For example, three molecules A, B and C can be designed such that first part of A is reverse complementary to the last part of C, last part of A is reverse complementary to first part of B and last part of B is reverse complementary to first part of C. In the right chemical soup, these molecules can assemble into a 3 way DNA junction (see Figure 1).

This basic principle allows us to program nanoscale DNA objects of required geometry and has resulted in a myriad of nanostructures (see [1, 2, 3, 4, 5, 6, 7, 8, 9, 10] for some illustrative examples). But more importantly, dynamic behavior of these objects can be controlled via the action of DNA enzymes that act upon specific sequences of DNA strands, competitive DNA hybridization or environmental changes such as pH or temperature. This chapter reviews some of the recent advances made in this emerging field of DNA nanorobotics. We begin by listing some of the challenges of DNA nanorobotics.

1.1 Challenge of DNA Nanorobotics

The aim of DNA nanorobotics is the design and fabrication of dynamic DNA nanostructures that perform specific tasks via a series of state changes. We limit ourselves to task that involve some form of robotic motion, such as locomotion or conformational changes. State changes can be viewed at different granularities, from the hybridization/denaturing of a single base to hybridization/denaturing of entire strands. These state changes can be effected autonomously, in which case the system switches state without external intervention while in other cases precise amounts of specific species, such as DNA strands or enzymes, are introduced to enforce state changes. It should be noted that different copies of the nanostructures

![Figure 1: A 3 way junction involving 3 DNA strands.](image-url)
might be in different states at the same time and we are generally interested in the overall average behavior system.

Various challenges arise in attempting to create a DNA nanorobot. Design of the DNA robot begins at the domain level where the overall mechanism of action of the robot is developed without actually assigning DNA sequence to the strands. Instead the different interacting segments of the DNA strands that constitute the robot are assigned a domain name which in the next step is assigned to specific DNA sequences. The mapping of domains to DNA sequences is done with care to avoid spurious interaction among the various domains.

Another important consideration at this stage is the fuel that powers the robot. Typically, robots are powered either by enzymes that act upon specific DNA strands of the robot or by the energy of hybridization of freely floating single stranded fuel DNA with specific strands of the DNA robot. Sometimes entropic effects can be used to power a state change. For example, two DNA strands that are held together by the hybridization of a small domain might denature spontaneously leading to an increase in the entropy of the system.

Other challenges include actual assembly of the DNA nanorobot and its purification, setting up initial operating conditions and finally designing experiments that validate the proposed mechanism of action of the DNA nanorobot. Since it is very hard to directly observe the operation of the robot, other means of real time detection, such as FRET (Froster Resonance Energy Transfer), are typically used. Each step in the process of creating DNA nanorobots is quite challenging.

We first briefly describe the working of two naturally occurring protein motors that have served as inspirations for various DNA nanorobots and then discuss the properties of DNA that make it an ideal material for mimicking such motors.

### 1.2 Natural Examples: Myosin and Kinesin

Kinesin is a motor protein that moves directionally along a microtubule powered by hydrolysis of ATP to ADP while transporting large cargo. It is a dimer, each component of which has a globular head at one end connected to a tail region via a long, thin stalk (see [11]). The head has binding domains to the microtubule while the tail binds to the cargo. The common mechanism that accounts for its movement is the hand over hand mechanism where one of the kinesin heads remains anchored to the microtubule while the other swings over and beyond it.

Myosins (see [12]) are a class of motor proteins similar to Kinesins. They bind to actin filaments via their head domains and push along them using the energy generated by ATP hydrolysis. Many myosin molecules bind to different locations on the actin filament and combine to push it. The power stroke occurs while the myosin is bound to actin. The myosin is detached from the actin at the end of the power stroke. ATP hydrolysis causes rebinding and the cycle repeats.
1.3 DNA: An Ideal Material for Molecular Robotics

Below we list some reasons why DNA is a material uniquely suited for building and manipulation at the molecular-scale. From the perspective of design, the advantages are:

1. A variety of predictable geometries can be achieved by carefully programming the interaction of DNA sequences.
2. The structure of most complex DNA nanostructures can be reduced to determining the structure of short segments of dsDNA (double-stranded DNA). The basic geometric and thermodynamic properties of dsDNA are well understood and can be predicted by available software systems from key relevant parameters like sequence composition, temperature and solution composition.
3. Design of DNA nanostructures can be assisted by software. To design a DNA nanostructure or device, one needs to design a library of ssDNA strands with specific segments that hybridize to (and only to) specific complementary segments on other ssDNA. There are a number of software systems (developed at NYU, Caltech, Harvard, Arizona State, and Duke University) for design of the DNA sequences composing DNA tiles and for optimizing their stability, which employ heuristic optimization procedures for this combinatorial sequence design task.

From the perspective of experiments, the advantages are:

1. The solid-phase chemical synthesis of custom ssDNA (single-stranded DNA) is now routine and inexpensive; a test tube of ssDNA consisting of any specified short sequence of bases (<150) can be obtained from commercial sources for modest cost (about half a US dollar per base at this time); it will contain a very large number (typically at least $10^{12}$) of identical ssDNA molecules. The synthesized ssDNA can have errors (premature termination of the synthesis is the most frequent error), but can be easily purified by well-known techniques (e.g., electrophoresis as mentioned below).
2. The assembly of DNA nanostructures and devices is a very simple experimental process: in many cases, one simply combines the various component ssDNA into a single test tube with an appropriate buffer solution at an initial temperature above the melting temperature of the target nanostructure, and then slowly cools the test tube below the melting temperature. Various devices can be implemented by simple strand displacement processes.
3. The assembled DNA nanostructures and devices can be characterized by a variety of techniques. Gel electrophoresis provides information about the relative molecular mass of DNA molecules, as well as some information regarding their assembled structures. Other techniques like Atomic Force Microscopy (AFM), Transmission Electron Microscopy (TEM) and Cryo-Electron Microscopy (cryoEM) provide images of the actual assembled DNA nanostructures on 2D surfaces and in 3D. These can be used to study snapshots of the device in its
various stages of operation. DNA strands can be coupled to fluorescent molecules and their corresponding quencher molecules. The fluorescent signal from a fluorescent emitter-quencher pair is sensitive (at nanometer resolution) to the distance between them. These markers can be attached to different moving parts of the nanorobot and a fluorescence spectrophotometer be used to infer the configuration of the DNA robot.

1.4 Outline of the Chapter

In this chapter, we classify various efforts in building DNA based molecular motors and devices under four broad categories. In section 2, we describe devices that respond to changes in their environment. The environmental changes can be used to actuate these devices. In section 3, we describe DNA based motors that use enzymes that act upon DNA strands to effect the desired change in state. These enzymes are both protein enzyme and deoxyribozymes (DNAzymes). In section 4, we describe DNA devices and motors that are operated only by the competitive hybridization and denaturation of DNA strands. The key to these devices is the process of strand displacement (defined later). Finally in section 5, we describe programmable molecular devices that compute specific functions when undergoing state changes. These devices in theory can undergo complex motions based on the program they are executing. In section 6 we make concluding remarks and state some open problems.

2 DNA Nanomachines that Switch Conformation Based on their Environment

The earliest demonstration of conformational changes of synthetic DNA induced by changes in salt concentration (sodium and magnesium ions) was achieved by Pohl and Jovin [13]. Mao et al. [14] connected two DX [1] tiles by a short piece of dsDNA and used conformational change between Z and B forms of this DNA segment to reposition the two DX tiles (Figure 2). When the connecting segment is in the B form (right-handed double helix), the DX tiles are on the same side of the connecting segment, while in the Z form (left-handed double helix) they switch to opposite sides. This confor-
mational switching behavior is demonstrated using FRET (Froster Resonance Energy Transfer) experiments. The branching point of two homologous double strands that form a Holliday junction [15] can migrate by exchange of their hybridized bases, lengthening one of the double strands while correspondingly shortening the other. This process is called branch migration. Yang et al. [16] showed that one can effect branch migration by applying torsional forces to one of the dsDNA of the Holliday junction. The torque is applied by the use of Ethidium, which intercalates between the strands of a dsDNA, unwinding it. This induces branch migration.

The i-motif [17] is a single strand of DNA which at pH 5 folds up into a compact three-dimensional single-stranded DNA structure in which two DNA double helices have their base pairs fully intercalated by C-C base pairs. The relative orientation of the double helices is antiparallel, so that each base pair faces its neighbors. A conformational switch in the i-motif can be achieved in the presence of a complementary DNA strand by increasing the pH to 8 [18, 19]. A light sensitive dye can be used [20] to induce reversible pH changes which lead to repeated conformational change of the i-motif. A similar autonomous switching effect can be achieved by using chemical oscillators that regulate pH [21, 22]. Cao et al. [23] demonstrate that a solution of gold nanoparticles conjugated to DNA strands changes color when the pH changes color when the gold particles aggregate. The pH dependent conformational change of the i-motif has been converted into a visual signal by using it to aggregate gold particles [24]. Alternatively, the conformational switch of the i-motif can be detected using a polythiophene derivative (PMNT) which forms an interpolyelectrolyte complex with the i-motif through electrostatic interactions exhibiting a relatively red-shifted absorption wavelength [25]. The i-motif was used to bend and straighten an array of microfabricated silicon cantilevers via electrostatic repulsion [26]. The process, controlled by pH changes, was found to be highly reversible and exerted single motor forces of about 11 pN/m.

Chen et al. [27] (Figure 3) and Brucale et al. [28] show alternate DNA motifs that demonstrate pH sensitive reversible conformation switching based on a DNA duplex-triplex transition.

The first ever in vivo pH sensitive conformation switching DNA nanomachine was demonstrated by Modi et al. [29]. The device switches from an open state to a closed triangular state in acidic conditions. The change in state is observed via FRET. The nanomachine is delivered inside a fruitfly cell by attaching it to transferrin, a protein absorbed through endocytosis.
3 DNA Nanomachines Powered by Enzymatic Actions

Many initial demonstrations of DNA based nanomachines make extensive use of protein enzymes. Typically, ligase and various restriction enzymes are used. We first give a brief introduction of how these enzymes operate and then survey DNA nanomachines that use them.

3.1 Introduction to Enzymes that Manipulate DNA

There are a wide variety of known enzymes and other proteins used for manipulation of DNA nanostructures that have predictable effects. Interestingly, these proteins were discovered in natural bacterial cells and tailored for laboratory use.

DNA restriction (Figure 4) is the cleaving of phosphodiester bonds between the nucleotide subunits at specific locations determined by short (4-8 base) sequences by a class of enzymes called nucleases. Endonucleases cleave the phosphodiester bond within a polynucleotide chain while exonucleases cleave the phosphodiester bond at the end of a polynucleotide chain. Some nucleases have both these abilities. Some restriction enzymes cut both the strands of a DNA double helix while others cut only one of the strands (called nicking).

DNA ligation (Figure 5) is the rejoining of nicked double stranded DNA by repairing the phosphodiester bond between nucleotides by the class of enzymes known as ligases.

DNA polymerases (Figure 6) are a class of enzymes that catalyze the polymerization of nucleoside triphosphates into a DNA strand. The polymerase "reads" an intact DNA strand as a template and uses it to synthesize the new strand. The newly polymerized molecule is complementary to the template strand. DNA polymerases can only add a nucleotide onto a pre-existing 3-prime hydroxyl group. Therefore it needs a primer, a DNA strand attached to the template strand, to which it can add the
first nucleotide. Certain polymerase enzymes (e.g., φ-29) can, as a side effect of their polymerization reaction, efficiently displace previously hybridized strands.

In addition, Deoxyribozymes (DNAzymes) are a class of nucleic acid molecules that possess enzymatic activity - they can, for example, cleave specific target nucleic acids. Typically, they are discovered by in-vivo evolution search and have had some use in DNA computations.

Besides their extensive use in other biotechnology, the above reactions, together with hybridization, are often used to execute and control DNA computations and DNA robotic operations. The restriction enzyme reactions are programmable in the sense that they are site specific, only executed as determined by the appropriate DNA base sequence. Ligation and polymerization require the expenditure of energy via consumption of ATP molecules, and thus can be controlled by ATP concentration.

3.2 DNA Motors Based on Enzymatic Actions

Yin et al. [30] demonstrate a molecular motor that transports two short DNA segments along a linear track. The two segments are indicated in red (Figure 7) and are passed from A to B to C in a sequence of steps mediated by different enzymes. The double stranded segments A, B and C are attached to a linear double stranded track by flexible single stranded regions. The ends of A and B can therefore be in proximity, whence they may bind via their complementary sticky ends. Now the DNA ligase T4 seals the nick joining segments A and B into a single double stranded segment. The restriction enzyme PflM I now cuts at its two recognition sites, allowing the segments A and B to separate, with the red segments now transported to B. Note that the restriction enzyme cuts asymmetrically and hence this step is irreversible. The same process
can now take place between B and C, with a different restriction enzyme, BstAP I, recognizing two distinct sites between B and C. Again, the restriction step is asymmetric and prevents the red segment from being passed back along the linear track. The whole process is autonomous, the track setup and the three enzymes are mixed together in one pot and the reaction is allowed to proceed to completion without external mediation.

A similar motor was suggested by Sekiguchi et al. [31], with an aim to allow the walker to walk along 2D and 3D paths (Figure 8). They suggest through simulations and initial experimental demonstrations that their design can feasibly walk along pre-programmed paths but full experimental demonstrations are not achieved.

One of the weaknesses of the Yin et al. [30] motor is that the cargo is transported only two steps. Bath et al. [32] demonstrate a very similar DNA motor which walks constantly along longer distances. Their design is also autonomous, with the help of the catalytic action of the restriction enzyme N.BbvC II (Figure 9). The track is a single strand of DNA, with periodically spaced single stranded DNA stators hybridized along its length. The cargo is passed unidirectionally along neighboring stators. When the cargo is attached along its full length to a stator, the restriction enzyme cuts the stator at a recognition site. The short segment of the stator attached along the top of the cargo can now float away (driven by entropic effects). The next stator along the track can now bind to the cargo and detach it from the previous stator, thus moving the cargo by one step. The process is repeated till the fuel is exhausted or the cargo reaches the end of the track. The cargo cannot move back since the bridges have been burnt behind the toehold by which it
binds to the stator is no longer present.

A modification of the Bath et al. [32] motor uses DNAzymes (DNA strands with enzymatic RNA restriction activity) instead of using restriction enzymes [33]. The track, as before, is a single strand of DNA. The stators are however identical RNA sequences positioned periodically along the track (Figure 10). The cargo is a DNAzyme that cleaves the RNA stators at a sequence specific site when hybridized to them. The mechanism of the motor is otherwise identical to the motor of Bath et al. [32] described above.

Chen et al. [34] developed an autonomous version of the DNA tweezers of Yurke et al. [35] (see section 4) that opens and closes a DNA nanostructure by the catalytic activity of a DNAzyme (Figure 11). The flexible linear DNA nanostructure can either be in an open state where its two ends are held apart by a short double stranded region or in a compact closed state where the ends are close together because the single stranded DNAzyme collapses into a coil as a result of entropic forces. The switch from the closed to the open state occurs via the binding of a RNA sequence $S$ while the reverse switch occurs when the DNAzyme cleaves $S$ and its short sub-strings float away due to entropic effects. Note that if we throw in an excess of strand $S$, this cycle would execute many times in an autonomous manner.

Bishop and Klavins [36] describe a chemical reaction network model of the autonomous tweezers of Chen et al. [34] and suggest that certain waste products build up over time that inhibit the switching of the tweezers between its two states. They suggest a modification of the autonomous tweezers in which the waste products are selectively digested using the enzyme ribonuclease H.

Sahu et al. [37] demonstrated transport of a cargo along a circular track powered by a strand displacing DNA polymerase, phi-29. Figure 12 shows the circular single stranded wheel $W$ mounted on the circular track $T$. The wheel is driven forward by the polymerization of the primer sequence $BP$, while the protection sequence $BQ$ prevents spontaneous displacement of the wheel in the absence of the polymerase.
4 DNA Motors Based on Hybridization Reactions

While protein enzymes are powerful and efficient, they are difficult to predictively modify. Also, the range of environmental conditions in which enzyme mediated DNA nanomachines operate is restricted as compared to pure nucleic acid systems. There have been attempts to replace enzymes and achieve the same functionality by programming purely nucleic acid systems, with a fair degree of success. In particular, there have been some ingenious enzyme-free nanomachines driven by the energy of hybridization of DNA strands. The key to these devices is the process of strand displacement. We first describe the strand displacement process and then briefly review these devices.

4.1 Introduction to DNA Strand Displacement

Strand displacement is the displacement of a single strand of DNA from a double helix by an incoming strand with a longer complementary region to the template strand. The incoming strand has a toehold, an empty single stranded region on the template strand complementary to a subsequence of the incoming strand, to which it binds initially. It eventually displaces the outgoing strand via a kinetic process modeled as a one dimensional random walk. Strand displacement is a key process in many of the DNA protocols for running DNA autonomous devices. Figure 13 illustrates DNA strand displacement via branch migration.
4.2 DNA Motors Based on Hybridization Reactions

The first application of strand displacement processes to a DNA nanomachine is the molecular tweezers of Yurke et al. [35]. The tweezers nanostructure consists of two double stranded DNA arms linked via a flexible single stranded region (Figure 14). Single stranded sticky regions extend beyond the ends of the arms. The tweezers can exist in two states, the open state when the sticky ends are unbound and the closed state when the sticky regions are hybridized to a fuel strand $F$. The tweezers transitions from the open to the closed state via the hybridization of the fuel strand $F$.

It transitions from the closed to the open state when the strand $F$ binds to $F$ via a toehold and strand displaces it, freeing the sticky regions of the tweezers. The complex $FF$ is produced as a waste product of this cycle.

Sherman and Seeman [38] demonstrate a bipedal walker that moves along a linear track, evocative of kinesin and myosin. However, their biped walker moves forward in an inchworm fashion where the relative positions of the leading and trailing leg do not change. The walker system has the following parts...

Figure 14: The tweezer cycling between open and closed states. Reprinted by permission from Macmillan Publishers Ltd: Nature [35], © 2000.

Figure 15: Single step of the inchworm walker. Reprinted with permission from Sherman and Seeman [38], © 2004 American Chemical Society.

Figure 16: Single step of the foot over foot walker. Reprinted with permission from Shin and Pierce [39], © 2004 American Chemical Society.
Figure 15: a track (blue), two legs (brown), two feet (pink and orange) and two footholds (green and turquoise). The walker progresses along the track by the binding and unbinding of the feet on the footholds. The binding occurs simply when a single stranded set strand binds a foot to its foothold by forming a bridge across them. The unbinding occurs when this bridge is stripped away via a toehold due to the strand displacement action of unset strands.

Shin and Pierce [39] demonstrate another similar bipedal walker but with the difference that their walker moves in a foot over foot manner (like kinesin) where each step the trailing foot swings past the leading foot. Their walker W (Figure 16) consists of two single stranded legs partially hybridized together, leaving single stranded attachment regions on each. The track is a double stranded helix with single strand stators jutting out at periodic intervals. Locomotion is achieved by hybridizing and denaturing the legs of W to the stators in a precise sequence. First, the legs of W are anchored to the first two stators by the use of bridging DNA strands. The trailing leg is then pried loose by using a detachment strand to strand displace away its bridging strand via a toehold. The single stranded leg then swings over and binds to the next stator, representing a step of the walker. The new trailing leg is now also pried loose in the same manner. Note that the walker may move backwards if the sequence of attachments and detachments is reversed.

The DNA motor of Tian and Mao [40] (Figure 17) operates on the same principle as the walker of Shin and Pierce [39] with the exception that the cargo walks along a circular track and returns to its original position after three steps. Due to the symmetry of the design, the cargo and the track have the same geometric circular structure.

Another similar approach is taken by Yin et al. [41] where a biped walker walks hand over hand along stators attached to a double stranded linear track. The key difference is that the stators are in the form of hairpins and the process is autonomous because the stators have identical sequence and the two legs of the walkers have the identical...
complementary sequence (Figure 18). The walker is driven forward when its trailing leg is detached from the stator by the fuel strand B via a toehold-mediated strand displacement process and the leg swings over to the next stator in line. However, there is about a 50% chance at each step that the leading foot is detached from the stator, in which case the walker halts. There is also a slight probability that both the legs of the walker detach from the track.

The walkers we have seen so far are not autonomous and thus it is difficult to run them for many steps. Green et al. [42] have designed an autonomous biped walker that functions as a Brownian ratchet. The walker moves along a linear track with an asymmetric bias towards one end of the track, with the help of fuel supplied by DNA hairpins. The walker moves using the foot over foot mode of transport. The trailing foot is much more likely to detach from the track as compared to the leading foot. Once detached, the trailing foot may swing forward ahead of the leading foot or may reattach back at its original position, with about equal probability. The net result of this is that the walker is biased towards stepping forward rather than back, and behaves like a Brownian ratchet. The walker assembly is illustrated in Figure 19. Note that the trailing and leading feet are in competition for the same subsequence on the track. When the trailing foot loses, it exposes a toehold by which the fuel strand H1 invades and detaches it. This is the asymmetry that makes the detachment of the trailing foot much more likely. Once detached, a further fuel strand H2 takes away H1 and allows the foot to attach back to the track, either at the same location or further along the track, in which case a forward step is taken.

Venkataraman et al. [43] constructed a DNA motor inspired by bacterial pathogens like *Rickettsia rickettsii*. The motor transports a single stranded cargo by polymerization, with the cargo always located at the growing end of the polymer. The system consists of two meta-stable hairpins H1 and H2 and an initiator strand (A) which carries the cargo (R) (Figure 20). The hairpins are relatively unreactive in the absence of the initiator, but in its presence a chain reaction occurs which builds a linear double stranded polymer, with each hairpin unfolding to attach as a bridge between two hairpins of the other type. The byproduct of the polymerization is the transport of the cargo relative to the initiator strand. The whole process is autonomous.
Programmable DNA Nanomachines

So far we have reviewed DNA nanomachines that are highly specialized; they are designed for executing a particular task: transporting cargo, walking along a linear track or changing the state of a nanostructure. The behavior of these nanomachines cannot be significantly affected without a major redesign. In this section we examine programmable DNA nanomachines which are capable of a range of programmed behavior.

Figure 20: Motor that transports cargo by polymerization. Reprinted by permission from Macmillan Publishers Ltd: Nature [43], © 2007.

Figure 21: An input RNA nanostructure routed through a DNAzyme network. Reprinted from Reif and Sahu [44], © (2009), with permission from Elsevier.
Reif and Sahu [44] propose designs for a RNA nanostructure that walks on a 2D addressable DNA surface. A network of DNAzymes is embedded on a 2D plane, and the input nanostructure is routed over it. The path the input nanostructure takes can be programmed by modifying its sequence. The transport of the walker across the surface can be understood as a finite state machine that switches states based on input. The input is encoded as a set of hairpins on the walker which are successively digested by various DNAzymes of the automaton. The DNAzyme that currently binds the walker indicates the state of the automaton. At each stage the sequence on the walker that corresponds to the next input symbol is consumed by restriction action of the DNAzyme, the walker is transported to the next DNAzyme on the surface and the next input to be consumed is exposed. Figure 21 shows a couple of example automata transitions. Such a machine can be used to route a walker on a 2D lattice grid, for example an input of 1 causes the walker to move down while 0 causes it to move to the right (Figure 22). The key advantage of this design is that the surface is not destroyed as the walker is transported over it and thus can be reused. Several walkers can, in theory, independently walk the surface at the same time.

Pei et al. [45] demonstrate diffusion of a multi-pedal DNA walker (henceforth referred to as a spider) on a 2D substrate. In simple terms, their spider crawls a 2D surface in a biased random walk. The body of the spider is a molecule of streptavidin, and its four legs are DNAzyme molecules attached to the body. The spiders crawl a surface by attaching and detaching from RNA substrates (Figure 23). The attachment occurs via DNA-RNA hybridization while the detachment is via the catalytic restriction of the RNA stator by the DNAzyme followed by spontaneous denaturation from short strands due to entropic effects. Once a leg detaches from a substrate it binds (with high probability) to a new substrate and the process continues. Thus, the spider is biased towards binding unvisited substrates.
This work was recently extended in Lund et al. [46] to allow the spider to crawl along a specific programmed pathway on a fully addressable DNA origami substrate [6]. As before the spider has a streptavidin body, three DNAzyme legs and one DNA leg used to anchor it to its start point (Figure 24). The path the spider must follow is specified by precisely placed RNA substrate strands sticking out of the origami structure. This programmed track can be assembled with high yield in a simple one pot reaction. The spider first attaches to the start site using its anchor leg. The anchor is strand displaced from the start site by an incoming trigger strand, which allows the spider to start crawling. The DNAzyme legs now attach and cleave RNA substrates along their path. The DNAzyme legs have longer complementary sequences to unvisited substrates than to visited ones and hence stay attached to the former for longer durations, eventually cleaving them. This introduces a motion bias for the spider towards unvisited substrates and results in a biased random crawl. The spider stops when it reaches the end of its path where it encounters special stop substrates which are DNA strands that cannot be cleaved by the DNAzyme legs. The spider operates autonomously once it is displaced from its anchor.

A similar multi-pedal walker was demonstrated in Gu et al. [47]. In addition to walking on a DNA origami substrate, the walker picks up cargo in a programma-
ble manner. This is akin to an assembly line where each component may be added to the already built assembly, or omitted if so desired. The operation of the walker is illustrated in figure 25. The walker is a triangular DNA nanostructure with single stranded sticky ends for its three hands (which pick up cargo) and four feet (which help it move along stators on the origami surface). The cargoes are distinguishable gold nanoparticles. Each cargo station can be either in an ON state, donating cargo, or in an OFF state, not donating cargo. As the walker moves by the stations, it picks up cargo from ON stations in one of its hands. Each station corresponds to a specific hand with which it may interact. The details of the walking and cargo pick up are illustrated in figure 26. The techniques for driving the walker forward and for picking up cargo are similar to the many DNA strand displacement based walkers we have seen previously. Note that the process is not autonomous and requires addition of appropriate fuel strands at specific time instants.

6 Conclusion

The ultimate goal of nanorobotics is the creation of autonomous nanosystems that are capable of carrying out complex tasks. Additionally, we would like these systems to be programmable in the sense that one should not resort to complete redesign of the system to achieve simple changes in target behavior.

The programmability of DNA makes it an ideal construction material at the nanoscale. DNA self-assembly offers a massively parallel method for low cost manufacturing of complex nanosystems. In the past two decades, several ingenious DNA nanostructures of increasing complexity have been demonstrated. Encour-
aged by these results, attempts were made to control the dynamic behavior of DNA nanostructures. This chapter reviewed some of these preliminary efforts.

Early attempts of DNA nanorobotics enforced state changes of complex DNA nanostructures using environmental parameters such as salt and pH levels while other efforts utilized protein enzymes and DNAzymes to effect state changes. More recently, enzyme free systems driven by hybridization of fuel DNA strands and entropic effects were achieved. Some of the systems reviewed were autonomous, while others required manual addition of precise amount of various reagents to enforce state change. Some of the systems were programmable, where one can easily modify the behavior of the nanorobots while others were not programmable and require comprehensive redesign of the nanosystem. Several theoretic designs for complex programmable autonomous DNA nanorobots have been proposed. Simplified versions of these have been demonstrated in the laboratory.

These pioneering efforts have provided a glimpse into the future of DNA nanorobotics and have demonstrated its enormous potential. Many challenges remain and provide exciting opportunities for research. We have barely begun a long and fascinating journey.

References


