Social DNA Nanorobots

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Raluca Gordan

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in the Department of Computer Science in the Graduate School of Duke University

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ABSTRACT

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Abstract

DNA nanorobots are molecular-scale synthetic devices composed primarily of DNA, that can execute a variety of operations. In the last decades, there have been considerable advances in DNA nanorobots, which have been demonstrated to perform autonomous walking, maze traversal, and cargo delivery activities. A major challenge in the design of these DNA nanorobots is to increase the diversity of the types of activities they can perform, in spite of practical limitations on the complexity of each individual DNA-nanobot. This project takes inspiration from insects such as ants and honeybees, which perform a wide variety of relatively complex organized behaviors with very limited individual brains. Mobile DNA nanorobots (which we also term DNA walkers) are a class of DNA nanorobots which can move over a nanotrack composed of DNA stepping stones. The nanotrack may be 1D or 2D and may be either self-assembled DNA nanostructure or a set of DNA strands affixed to a surface. Autonomous mobile DNA nanorobots (also termed autonomous DNA walkers) are mobile DNA nanorobots that locomote autonomously. Here we propose social DNA nanorobots, which are autonomous mobile DNA nanorobots that execute a series of pair-wise interactions between pairs of DNA nanorobots that determine an over-all desired outcome behavior for the group of nanorobots. We present various designs for social DNA nanorobots that provide diverse behaviors including, Walking, Self-avoiding Walking, Flocking, Guarding, Attacking, Voting by Assassination, and Foraging.
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Chapter 1

Introduction

1.1 Motivation

Due to the simple base-pairing rule and predictable secondary structure of DNA, DNA has been considered as an ideal material to construct useful nanoscale structures, devices, and computers. In the past decades, researchers designed and experimentally realized molecular machines that can automatically conduct complex tasks such as autonomous walking, maze traversal, and cargo delivery activities. One of the greatest challenges in the field of Nanoscience is how to design molecular-scale systems with multiple mobile autonomous nanorobots which exhibit a group behavior that is significantly more complex than the behavior of individual nanorobots. We take inspiration from the field of Sociobiology pioneered by Wilson [51] for social insects such as ants and bees, which live in large colonies and exhibit complex collective behaviors, even though the individual insects have quite limited brains. Here we propose social DNA nanorobots, which are autonomous mobile DNA nanorobots that the interactions between simple individual DNA nanorobots determine an over-all desired outcome behavior for the group of nanorobots.

1.2 DNA Walkers

We use the term nanorobot for a molecular-scale device that can execute a variety of operations. A DNA (or RNA) nanorobot is a nanorobot composed of nucleic acid. The field of DNA nanorobotics has rapidly evolved from nonautonomous molecular devices (e.g., [58]) that each successive movement needed to be controlled externally, to subsequent autonomous molecular devices that operate without control from external environment. Autonomous DNA devices that executed in-place motions were demonstrated by [14, 5, 47,
A Mobile DNA nanorobot (which we also term a DNA walker) is a class of DNA nanorobots which can move over a nanotrack composed of DNA stepping stones. The nanotrack may be 1D or 2D and may be either self-assembled DNA nanostructure or a set of DNA strands affixed to a surface. An autonomous mobile DNA nanorobot (also termed an autonomous DNA walker) is a mobile DNA nanorobot that locomotes autonomously.

1.3 Prior Research

The concept of the DNA walker was first defined and named by Reif [34] in 2002, with two designs. Sherman [42] and Shin [43] then experimentally realized DNA walkers, and they were nonautonomous walkers that require external power for each step. In 2004, the first autonomous DNA walker was experimentally demonstrated [56, 55] by Reif’s group (in collaboration with Turberfield). This DNA walker and many subsequent DNA walkers [46, 48, 38, 18, 9, 44] made use of a series of enzymic reactions to power the locomotion. Sahu [38] demonstrated a DNA nanotransport device which is powered by polymerase φ29. The φ29 is a kind of strand-displacing polymerase, which can displace any DNA strand from its template when synthesizing new strand. The use of polymerase rapidly increases the speed of transportation process. Reviews of DNA based walkers are given in [12, 26, 31]. There are also many DNA walkers powered by hybridization reactions.

1.3.1 DNA Motors Based on Hybridization Reactions

In 2000, Yurke and Turberfield [58] demonstrated the first DNA walker that used DNA hybridization to power its movements. They constructed a DNA tweezer with two double-stranded DNA arms (which has a sticky end) linked by a flexible single-stranded region. The tweezer has an open state and a closed state, and the movements of the tweezer to and from the closed and open states is controlled by externally added ssDNA strands. The tweezer switches from the open state to the closed state when the fuel strand $F$ hybridizes...
with the sticky end of the arms. The tweezer switches from the closed state to the open state when the strand \( \bar{F} \) hybridizes with strand \( F \) and displaces the sticky end of the arms by toehold-mediated strand displacement. At the same time, the waste product, the double complex \( F \bar{F} \) will be produced.

Sherman and Seeman [42] experimentally demonstrated a bipedal walker that moves along a linear track by hybridization and dehybridization reactions. When the biped walker moves forward, the relative positions of its leading and trailing leg do not change.

Shin [43] demonstrated a similar bipedal walker that controlled by externally added ssDNA strands. Their walker moves in a foot-over-foot manner, which means, the leading foot switches to the trailing foot after each step. Firstly, the trailing foot will unbind with the track by hybridization and dehybridization reactions and move forward to bind with the next stator, at the same time, it switches to the leading foot. This process will repeat and the walker moves forward in a foot-over-foot manner.

Tian and Mao [47] also showed a foot-over-foot walker and it walks along a circular track and returns to its original position after three steps.

Yin et al. [57] showed a similar design of a biped walker that walks foot-over-foot along a linear track. The stators on the track have identical sequence and the two legs of the walkers have the identical sequence which is complementary to the sequence of stators. A fuel strand (Haripin B) can hybridize with the sticky end of stator which bind with the trailing leg. Due to the toehold-mediated strand displacement, the trailing leg detaches from the stator and moves forward to bind with next stator in line. However, at each step, the leading foot has the same chance to detach 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.

An autonomous biped walker moving along a linear track was designed by Green et al. [18] that functions as a Brownian ratchet.

Venkataraman et al. [48] inspired by bacterial pathogens, constructed an autonomous DNA motor that can transport a single stranded cargo by polymerization. The system
consists of two meta-stable hairpins H1 and H2, and an initiator strand (A) which carries the cargo (R). The two hairpins co-exist in the solution stably without the initiator. A chain reaction occurs after the introduction of the initiator. The complex AR hybridizes with hairpin H1. The polymerization process opens two hairpins and the cargo always located at the growing end of the polymer. After each polymerization process, the relative distance between the initiator and the cargo is further. The whole process is autonomous.

1.3.2 Prior Programmable DNA Nanomachines

The behavior of programmable DNA nanomachines can be significantly affected without a major redesign. Various schemes for programmable autonomous DNA nanorobots, that do computations as they walk, have been described; those of [54] use enzymes, and those of [35] use DNAzymes.

Reif and Sahu [35] proposed designs of autonomous programmable DNA robotic devices using DNAzymes. They showed an application as a DNAzyme router for programmable routing of nanostructures on a 2D DNA addressable lattice. The 2D DNA addressable lattice is embedded with a network of DNAzymes. The routed path for the input nanostructure 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. The various DNAzymes embedded on the 2D surface consumes the input in proper sequence. The state of the automaton is indicated by the DNAzyme that currently binds the walker. When the walker moves on the 2D surface, the surface will not be destroyed and can be reused.

Pei et al. [32] demonstrated a multipedal DNA walker (a spider) moves on a 2D substrate in a biased random walk. The body of the spider is a molecule of streptavidin, and its four legs are DNAzyme molecules. There are RNA stators on the 2D substrate that can hybridize with the legs of the spider. When the spider crawls on a surface, its legs attach and detach from the RNA stators. The attachment occurs via the 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 stator, it binds (with high probability) to a new stator and the process continues. Thus, the spider is biased towards binding unvisited substrates.

Lund [28] demonstrated DNA walkers that traversed paths on a 2D nanostructure guided by landmark molecules affixed to the 2D nanostructure. The spider with a streptavidin body and three DNAzyme legs can autonomously carry out sequences of actions. Firstly, the spider stands on the start point. After the introduction of the trigger strand, the spider crawls along the well-designed path. The DNAzyme legs cleave the RNA stators it visited, as a result, the unvisited stators have longer complementary sequences and are more likely to be hybridized with. These processes lead to a biased random crawl. The spider stops when it encounters special stop stators which are DNA strands that cannot be cleaved by the DNAzyme legs.

Programmable autonomous DNA nanorobots that transport a series of molecules to form a molecular-scale assembly line were demonstrated by the Seeman group [19] in 2010. Their walker can pick-up cargo in a programmable manner when they walk on the surface. This process was not autonomous and requires addition of appropriate fuel strands at specific time instants. The walker has three hands that pick up cargo and four feet that move on the surface. There are cargo stations on the surface. If the station can donate a cargo, it’s in an ON state, otherwise, it’s in an OFF state. When a walker encounters an ON station, it will pick up the cargo with its appropriate hand.

1.3.3 Autonomous DNA Walkers that Navigate Networks

Chao et al. [13] demonstrated a DNA navigator system that conducted single-molecule parallel depth-first search on a 2D DNA origami surface. They used DNA-PAINT to visualize the movement of the DNA navigator. The system consists of a physical implementation of a tree graph, fuel strands (hairpin $T_1$ and $T_2$) and an initiator strand $I$. The Initiator $I$ can open the $T_{1\text{ent}}$ hairpin on the trigger site. The opened $T_{1\text{ent}}$ hairpin then captures $T_2$ hairpin from the environment and opens $T_2$. Then the cascade reactions between $T_2$ and
Li et al. [27] explored the speed limit of toehold exchange reactions of a cartwheeling DNA walker. The walker W undergoes a head-over-heels movement between strand $F_1$ and $F_2$ by toehold-mediated strand displacement. They also designed a cartwheeling DNA walker with fluorescence reporter to characterize its translocation mechanism and kinetics.

Wickham et al. demonstrated a DNA-based molecular motor that can be routed through a network of tracks[50]. There is a network of branching tracks with 4 possible routes on the DNA origami surface. The design employed junction stators (a block strand and a stator strand) which stop the motor from moving forward. The motor is initially hybridized to the first stator (S1), and choose to turn left or right twice to reach the end of the path. After the motor turns to left/right at the first node, it will be blocked by a junction stator. The instruction of the unblock strand (which is complementary to the junction stator strand) removes the block strand and releases the stator strand. The motor can then step to the unblocked stator by toehold-mediated strand displacement. The resulting duplex (a motor strand and a stator strand) contains a new recognition site for the nicking enzyme. Enzyme cleaves the stator strand and generates a toehold that initiates migration of the motor onto the next stator. This process will repeat until the motor reaches the end of the path. They also showed the routes can be programmed by external instructions or the internal instructions carried by the motor itself.

1.3.4 Prior Autonomous DNA Walkers That Do Molecular Cargo-Sorting on a 2D Nanostructure

The work most similar to this proposal is the work of [45], which demonstrated an ingenious molecular-scale system with a group of autonomous DNA nanorobots executing a molecular cargo-sorting task on a 2D nanostructure.

The 2D nanostructure initially has various kinds of molecular cargo that needed to be transported to different targeted locations on a DNA nanostructure (a DNA origami surface). Each DNA nanorobot traversed a random walk over the 2D nanostructures and
when encountering a molecular cargo, they loaded and transported the cargo to the targeted location (the goal).

They used a simple algorithm to perform a complex cargo-sorting task. Their algorithm is: when the robot randomly walks on the surface, if it bumps into a cargo, the robot will pick it up and continue walking randomly; if it bumps into a goal that is the targeted location of the cargo, the robot will drop it off. The robot will repeat the above process until all cargos are sorted (Figure 1.1 (B)). They also developed three modular building blocks to implement the algorithm: a random walk block (Figure 1.1 (C)), a cargo pickup
block (Figure 1.1 (D)), and a cargo drop-off block (Figure 1.1 (E)). For the random walk block: the robot is a single-stranded DNA with one leg and two foot domains, randomly moves between the two types of track strands by reversible strand displacement reaction. For the cargo pickup block and drop-off block: the robot is a single-stranded DNA with one leg and two foot domains for walking, and one arm and one hand domain for picking up the cargo or dropping off cargos. The picking up and dropping off process are also via strand displacement reactions. To implement the cargo-sorting algorithm, the three modular building blocks should be combined as shown in Figure 1.1 (F). Each different type of cargo should have a unique cargo domain on their cargo strand and goal strand (Figure 1.1 (G)).

1.4 Sociobiology

The concept of Sociobiology was defined by Wilson [51] in 1975. It is a field that aims to investigate and explain the social behaviors of social animals, such as mating patterns, aggression, nurturance, pack hunting, and the hive society of social insects.

1.4.1 Sociobiology Studies of the Behavior of Social Insects

A high-level of social organization can be found in social insects that have the following three characteristics: cooperative brood care, overlapping generations, and a division of labor into reproductive and non-reproductive groups[6][30][52][16][53]. The social insects include ants and termites, and some social bees and wasps. The numerous insects all live together in the colonies, the collective structures are formed by individuals linking themselves to one another.[1] Social insects gain several advantages by living together. They work together to gain resources, share their findings with each other, defend their home when under attack, and attack other insects for territory and food. Social insect communities are divided into two castes by their function and behavior, a reproductive caste (e.g., the queen) and the sterile caste (workers and soldiers) [21]. The reproductive
caste carries out the basic function of reproduction, and the sterile caste takes care of the reproductive members. The workers are responsible for foraging, construction and repair of the nest and feeding the larvae and brood care, while the soldiers defend the colony against predators. The communication signals between the insects can be mechanical, optical, or chemical. Pheromone, a chemical factor that can trigger a social response in members of the same species, plays an important role in the development and maintenance of insect society [22]. Honey bee foragers can communicate information with a waggle dance (a particular figure-eight dance) [41] [17][49]; by performing this dance, the foragers can share information about the direction and distance to the food source. Honey bees of the species *Apis mellifera* also perform tremble dances, which recruit receiver bees to collect nectar from returning foragers[40].

1.4.2 Complex Collective Social Behaviors of Groups of Social Insects

We are inspired by the social behaviors of social insects such as ants and bees, which live in large colonies and exhibit complex collective behavior, even though the individual insects have quite limited brains. Notable diverse activities of social insects include:

- Random Walking: where insects of the colony make random walks.
- Flocking: a group of insects of the colony follow a selected leader individual insect.
- Guarding: a group of the insects of the colony follow, and guard from attack by another group, a particular individual insects of the colony.
- Attacking: a group of the insects of the colony attack another group.
- Communication: between insects of the colony.
- Democratic Group Decision Making (Voting): among groups of insects of the colony.
• Foraging: a select foraging group of the insects of the colony leave the colony and attempt to discover new sources of food, and then report back to the colony their discoveries.

• Harvesting: a harvesting group of insects of the colony travel (navigating by either (a) following successful foragers or (b) following their chemical trail, or (c) via instruction from successful foragers) to the new sources of food and harvest it.

Particularly complex collective behavior is found in honeybee colonies, where individual honeybees are specialized to perform specific functions (e.g., successful foragers use a flying dance language to communicate the direction of flowering plants [49] and other objects of interest). Seeley [39] demonstrated that honeybees also use a form of democratic voting (executed without input from the queen bee), to make important decisions, such as the best source of flowering plants and the best location for a new home for the honeybee colony. Ants and termites also are specialized to perform specific functions (e.g., attacking & foraging) and can communicate and lay down chemical tracks to specify the location of food sources.

Our idea is to adapt these collective behavior strategies above (1-7), for use for molecular-scale nanorobots, which will be specialized to perform specific functions.

1.4.3 Potential Fields for Generating Autonomous Group Social Activities

Another source of inspiration for collective behavior strategies by groups can be found in biology: for example, flocking of animals such as birds and schooling of amphibious animals. The behavior of these animals has been modeled by mathematical models and computer programs. In 1989, Beni [8, 7] developed one of the first such flocking model, which he called swarm intelligence and made applications to multi-robot motion planning systems. Subsequently the field of swarm intelligence [10, 23] and artificial flocking grew rapidly and found applications to many applied areas in addition to robotics, such as for
computer graphics. In 1994, Reif [36, 37] developed a general programmable scheme for multi-robot motion planning, termed Social Potential Fields, which made use of artificially defined potential fields that controlled the individual robots by weighted sum of decreasing functions of the distance and direction of other local robots; he demonstrated various autonomous group social activities, including flocking, attacking and guarding, using the Social Potential Fields technique. Unfortunately, the potential field models assume far-distance field effects that are not easy to implement using DNA nanorobots.
Chapter 2

Design and Simulation

Our approach is to adapt collective behavior strategies of social insects into molecular-scale nanorobots, which will be specialized to perform specific functions. We propose social DNA nanorobots, which are autonomous mobile DNA devices that execute a series of pairwise interactions (only between pairs of nearby nanorobots) on a 2D DNAnanotrack that determine an over-all desired outcome behavior as a group. We give detailed designs that program social behaviors via interactions between individual DNA molecules.

2.1 Social DNA Nanorobot Behaviors Designed

A basic behaviors of social DNA nanorobots is: Random Walking, where a group of DNA nanorobots make random traversals of a 2D nanotrack. For Random Walking, we will make use of a known design of [45].

The novel behaviors of social DNA nanorobots described here include:

- **Self-avoiding Walking**, where a group of DNA nanorobots walk on a 2D nanotrack and avoid the locations visited by themselves or any other DNA nanorobots
- **Flocking**, where a group of DNA nanorobots follow the movements of a designated leader DNA nanorobot.
- **Guarding**, where a group of DNA nanorobots follow and guard by a particular DNA nano-robot from attack by another group of DNA nanorobots
- **Attacking**, where a group of DNA nanorobots attack another group of DNA nanorobots.
- **Communication** between pairs of nearby DNA nanorobots: where a finite amount of information is transferred between a pair of nearby DNA nanorobots.
- **Voting by Assassination**, a process where there are originally two unequal size groups
of DNA nanorobots; when pairs of DNA nanorobots from distinct groups collide, one or the other will be assassinated (by getting detached from the nanotrack); eventually all members of the smaller groups of DNA nanorobots are assassinated with high likelihood.

- **Foraging and Harvesting**, where a group of designated foraging DNA nanorobots randomly walk on the 2D nanotrack, and can transform to a "discovery state" when they discover a target molecule (e.g., a group of gold nanoparticles attached to 2D surface), and a group of harvesting DNA nanorobots which follow the trail of foraging DNA nanorobots in discovery state and pick up the detected target molecules, and deliver the target molecules to a designated region of the 2D nanotrack.

### 2.2 Stochastic Simulations for the Behaviors of Social DNA Nanorobots

We also made stochastic simulations of the social DNA nanorobots behaviors listed above. Our simulation model is adapted from the Surface CRN Simulator of Clamons [33, 15]. A chemical reaction network (CRN) contains chemical reactions and their rates. In a 2D surface CRN model, each individual nanorobot to be simulated is a molecule attached at a specific position on a 2D surface, so that the nanorobot can only interact with neighbors and their attachment strands. The behaviors of DNA nanorobots moving on a 2D nanotrack can be modeled in this 2D surface CRN model as a set of chemical reactions between DNA walkers and DNA strands affixed to a surface. State transitions modeled chemical reactions (e.g., toehold-mediated strand displacements and dehybridizations) between DNA walkers and DNA strands affixed to a surface. We applied the Surface CRN Simulator specifically for optimized performance assessment of our social DNA nanorobot designs.
2.3 Detailed Design

Here are the detailed designs of the social DNA nanorobots that conduct walking, self-avoiding walking, flocking and voting by assassination in domain level.

2.3.1 A Prior DNA Nanorobot that Autonomously Walks

![Diagram of DNA nanorobot](image)

**Figure 2.1**: Design of a DNA Nanorobot that Executes a Random Walk.

There are many known designs for DNA nanorobots that make random traversals on a 2D DNA nanotrack. Ours uses a design similar to that of Thubagere [45] for random walks. A 2D nanotrack with a 2D array of attached ssDNA pads that are self-assembled on a 2D surface as illustrated in Figure 2.1.

There are two types of pads: (i) ssDNA $p_0 = B^* A^*$ attached at its 3’ end and (ii) ssDNA $p_1 = C^* B^*$ attached at its 5’ end. There is a single type of ssDNA nanorobot, the DNA Walker $W = ABC$ (see Figure 2.1), which operates as follows:

(a) A low concentration of $W$ walker strands are added to the buffer solution containing the 2D nanotrack, and a few of these $W$ strands hybridize to random pads of the nanotrack.
(b) The buffer solution is replaced, so as to remove the remaining non-hybridized $W$ walker strands from the solution surrounding the nanotrack.

(c) As described in Figure 2.1, at first, the $W$ strand hybridizes with a $p_0 = B^*A^*$ pad, which is the State 0. In State 1, the unpaired domain $C$ of $W$ hybridizes with domain $C^*$ of $p_1$. Then domain $B^*$ of pad $p_1$ can displace the domain $B^*$ of pad $p_0$, so domain $B$ of $W$ hybridizes with both $p_0$ and $p_1$, which is the intermediate State 1.5 and this process is reversible. If the strand displacement completes, it enters the State 2, in which $W$ detaches fully from the $p_1$ pad and hybridizes with a $p_1 = C^*B^*$ pad. From State 0 to State 2, $W$ walked from $p_0$ pad to $p_1$ pad due to hybridization and strand displacement. Similarly, $W$ walks from $p_1$ pad to $p_0$ pad when it processes from State 2 to State 1.5 to State 1 to State 0.

(d) $W$ walks successively from either (a) the $p_0$ to the $p_1$ pad of the nanotrack, or (b) the $p_1$ to the $p_0$ pad of the nanotrack, as described above. As result, $W$ walks randomly over nanotrack.

2.3.2 A Novel DNA Nanorobot that Executes a Self-Avoiding Walk

A self-avoiding walk (SAW) is a sequence of moves on a lattice (a lattice path) that does not visit the same point more than once. SAWs have a number of important applications, e.g., in the modeling of nucleic acids, peptides, and proteins. It is known [20] that a self-avoiding random walk on the 2D square lattice lasts an average of approximately 71 steps before the walker is trapped. (Note: While we could modify our design given the below to decrease the likelihood the walker gets trapped at a $p_0$ pad position, and so increase the average number of steps before the walker is trapped, but then the resulting system would not correspond to the classical self-avoiding random walk on the 2D square lattice.)

Here we described the design of a DNA nanorobot that makes a random self-avoiding traversal of a 2D DNA nanotrack (which is a 2D square lattice). Figure 2.2 illustrates a 2D DNA nanotrack with a 2D array of attached ssDNA pads and DNA hairpins pads. The
are two types of pads: (i) ssDNA $p_0 = B^* A^*$ attached at its 3’ end and (ii) DNA hairpin $p_1 = C^* B^* E_1^* E_2^* B$ attached at its 5’ end. The buffer solution also contains another DNA hairpin type $h_1 = E_2 E_1 B E_1^*$.

**Figure 2.2:** Design of a DNA Nanorobot that Executes a Self-Avoiding Walk.

The operation of a self-avoiding walker $W = ABC$ is as follows:

(a) A low concentration of $W$ walker strands are added to the buffer solution containing the nanotrack, and a few of these $W$ strands hybridize to random pads of the nanotrack.

(b) The buffer solution is replaced, so as to remove the remaining non-hybridized $W$ strands from the solution surrounding the nanotrack. Also, the buffer solution contains DNA hairpin $h_1$.

(c) As in Figure 2.2, the Walker $W$ first hybridizes with a $p_0 = B^* A^*$ pad, which is the State 0. In State 1, the unpaired domain $C$ of $W$ hybridizes with the domain $C^*$ of the pad $p_1$. Then the domain $B^*$ of $p_1$ can displace the domain $B^*$ of $p_0$, the domain $B$ of $W$ hybridizes with both $p_0$ and $p_1$ and also hairpin $p_1$ is opened, giving intermediate State 1.5. (Note this transition process is reversible.) If and when the strand displacement finishes, it enters State 2, in which $W$ hybridizes with a $p_1$ pad. In summary, transitioning from State
0 to State 2, the \( W \) nanorobot walks from \( p_0 \) pad to \( p_1 \) pad due to the hybridization and strand displacement.

(d) The ssDNA strand \( h_1 \) is in solution, with domain \( E_2 \) that could hybridize with the newly released \( E_2^* \) domain of \( p_1 \), so then \( h_1 \) is opened and displaces the domain \( B \) of \( W \). The unpaired domain \( AB \) of \( W \) can hybridize with the next \( p_0 \) and moves from \( p_1 \) to \( p_0 \), entering State 1. After some time, \( W \) detaches from \( p_1 \) and hybridizes with a \( p_0 \) pad, taking it back to State 0. Then the previously visited \( p_1 \) will hybridize with a hairpin \( h_1 \) from the buffer solution, which hinders the \( p_1 \)’s reformation of the hairpin, so \( W \) can not move back to the visited \( p_1 \).

(e) The \( W \) nanorobot walks from either (a) \( p_0 \) to a non-visited \( p_1 \) pad of the nanotrack, or (b) \( p_1 \) to a \( p_0 \) pad of the nanotrack, as described in part c, d. As a result, the \( W \) nanorobot avoids the locations visited by itself or any other DNA nanorobot when it moves on the nanotrack.

2.3.3 Flocking: Novel DNA Nanorobots that Follow a Leader DNA Nanorobot

This is a DNA nanorobot system with two types of DNA nanorobots (the designated leader and the followers): the leader makes a random traversal of a 2D nanotrack and the other DNA nanorobots follow the movements of the leader.

Figure 2.3 illustrates a 2D nanotrack with a 2D array of attached DNA hairpins is self-assembled on a 2D surface. There are two types of pads: (i) \( h_1 = B_1 B_2 A_2^* B_2^* B_1 A_1^* \) attached at its 3' end and (ii) \( h_2 = C_1^* B_1^* B_2 C_2 B_2^* B_1 \) attached at its 5' end. Let \( B_2 = B_{2a} B_{2b} \).

(Note: Observe that each of \( h_1 \) and \( h_2 \) self-assemble into a type of hairpin with two separate short loops: \( h_1 \) has two separate short loops, one with \( A_2^* \) and another with \( B_{2a}^* \), whereas \( h_2 \) has two separate short loops, one with \( C_2^* \) and another with \( B_{2b}^* \). This use of small hairpin loops is a deliberate design with the goal of inhibiting the hybridization follower \( W_2 \) with \( A_2^*, B_{2a}^*, C_2^*, B_{2b}^* \). This will make it more difficult for a follower \( W_2 \) to avoid following a leader \( W_1 \).)
There are two types of ssDNA nanorobots, the leader $W_1 = A_1B_1C_1$ and the follower $W_2 = A_2B_2C_2$, which operate as follows:

(a) $W_1$ (leader) and $W_2$ (follower) strands are added to buffer solution containing the nanotrack.

(b) The leader $W_1$ hybridizes with $h_1$ on the domain $A_1$ and opens the hairpin of $h_1$ by strand displacement; then a follower $W_2$ can hybridize with the newly opened $h_1$.

(c) The leader $W_1$ moves from pad $h_1$ to pad $h_2$ by hybridizing with $C_1^*$ of $h_2$ and opening $h_2$ by strand displacement; then follower $W_2$ can hybridize with the newly opened $h_2$.

(d) After $W_1$ and $W_2$ Walkers leave these pads, a limited number of further $W_2$ strands can walk nearby, and similarly follow leader $W_1$.

(e) The leader $W_1$ walks successively from either (a) a $h_0$ pad to a $h_1$ pad of the nanotrack, or (b) a $h_1$ pad to a $h_0$ pad of the nanotrack. Whenever a follower $W_2$ stand hybridizes with a $h_1$ pad, the hairpin $h_1$ needs to be open (where the loop of the hairpin was opened.

Figure 2.3: Design of DNA Nanorobots that Follow a Leader DNA Nanorobot.
by a leader $W_1$ and the loop closes after some time), so $W_2$ is forced to follow the leader $W_1$. Hence leader $W_1$ walks randomly over the nanotrack and is followed by a group of $W_2$ followers, as in Figure 2.3. (The max. size of group of $W_2$ followers is limited by time the hairpins $h_1, h_2$ remain open, and this parameter can be set by appropriate DNA strand design.)

2.3.4 Novel DNA Nanorobots that Vote by Assassination

Distributed voting is essential to many distributed computing and population protocols [29, 24, 4], where processors are restricted to pair-wise interactions. For example, distributed voting can be used for leader election which allows a process in a distributed system some special powers in the distributed system, often allowing for simplified protocols, reduced coordination and improved efficiency. The task of determining approximate majority in distributed computing can be reduced to the case where each processor has a binary value in \{0, 1\}; assuming an initial margin of disparity $\delta > 1$ between those processors with value 0 and value 1, then the problem is for the set of processors to settle on a majority value in \{0, 1\}. A fast randomized distributed protocol for approximate majority was given by Angluin et al [2, 3]. (Interestingly, Cardelli [11] observed that this approximate majority protocol was used in certain cell cycle switches.) Let an event with size parameter $n$ be high probability if it has likelihood $\geq 1 - \frac{1}{n^\alpha}$ for some constant $\alpha \geq 1$. Angluin et al [2, 3] proved that with high probability, their randomized distributed protocol $n$ processors reached consensus on a majority value after $O(n \log n)$ pair-wise interactions, assuming that the initial margin of disparity is $\geq \delta = c\sqrt{n} \log n$ for a constant $c \geq 1$. A slightly modified version of their protocol proceeds in $O(n \log n)$ stages, where in each stage a random pair of processors compare their values; if their values are the same they do nothing, and otherwise a random processor of the pair drops out from subsequent stages of the protocol. Afterwards, with high probability only processors with the same majority value remain. Then the other processors that previously dropped out are informed of that majority value.
We expect distributed voting to be also of central importance to programming complex behavior in social DNA nanorobots. We now describe on what may be viewed as a social DNA nanorobot implementation of the Angluin et al [2, 3] approximate majority protocol. Our design for distributed voting of DNA nanorobots has the nanorobots exhibit an antisocial behavior to achieve group decision making. The idea is the DNA nanorobots vote by assassination. There are originally two unequal size (with sufficiently large size difference) groups of DNA nanorobots; when pairs of DNA nanorobots from distinct groups collide, one or the other is assassinated (by getting detached from the nanotrack); eventually all members of the smaller of the two groups of DNA nanorobots are assassinated (detached from the nanotrack) with high likelihood.

**Figure 2.4**: Separate Walks of Assassinator Nanorobots $W_1$ and $W_2$.

Figure 2.4 illustrates a 2D nanotrack with a 2D array of attached ssDNA pads is self-assembled on a 2D surface.

Let $C = C_a C_b C_c$ where $|C_a| = |C_c|$ are between 3 to 5 bases pairs (sufficient to act as toeholds), and $|C_b| \geq 10$. There are three types of pads: (i) $p_0 = (B_2)^* C^* (B_1)^*$ attached at its 5’ end and with the ssDNA sequence $C_b$ hybridized to the complementary subsequence $C_b^*$ of $p_0$, (ii) $p_1 = (B_1)^* (A_1)^*$ attached at its 3’ end, and (iii) $p_2 = (A_2)^* (B_2)^*$ attached at...
its 3’ end. There are two types of ssDNA nanorobots: $W_1 = A_1B_1C$ and $W_2 = CB_2A_2$. Also, the ssDNA sequence $C_b$ is in sufficient concentration in the solution, allowing it to re-hybridize to $C^*_b$ of $p_0$ if strand-displaced.

Initially, a combination of an unequal concentration of $W_1$ and $W_2$ strands is added to the buffer solution containing the nanotrack; some $W_1$ and $W_2$ strands hybridize to random pads of nanotrack. The buffer solution is replaced, so as to remove the remaining non-hybridized $W_1$ and $W_2$ strands from the solution surrounding the nanotrack. Let $n_1$ and $n_2$ be the (unknown) numbers of $W_1$ and $W_2$ strands initially attached to the nanotrack and let $n = n_1 + n_2$. We assume $n > 0$ and the initial margin of disparity $|n_1 - n_2| \geq \delta$ with $\delta = c\sqrt{n}\log n$ and constant $c \geq 1$. Our goal is: to test if $n_1 > n_2$ or $n_1 < n_2$.

Randomized Assassination Protocol:

The nanorobots $W_1$ and $W_2$ operate as follows:

(a) As in Figure 2.4, $W_1$ and $W_2$, when separate, walk randomly over the nanotrack, The $W_1$ nanorobot walks only on the $p_0$ and $p_1$ pads of the nanotrack, whereas the $W_2$ Nanorobot walks only on the $p_0$ and $p_2$ pads of the nanotrack.

(b) As in Figure 2.5, whenever both a $W_1$ and a $W_2$ nanorobot collide at a common $p_0$ pad of the nanotrack, a random one of either $W_1$ or $W_2$ nanorobot is partially detached via strand-displacement at domain $C$, and then is melted off to enter the solution, and is not likely to reattach to the nanotrack.

**Note:** Our design includes a short ssDNA sequence $C_b$ initially hybridized to the complementary subsequence $C^*_b$ of $p_0$, which has purpose of substantially increasing the likelihood that $W_1$ and $W_2$ will simultaneously bind to a pad $p_0$: since once $W_1$ or $W_2$ is partly bound to $p_0$, it still has to engage in a relatively slow strand-displacement reaction to dislodge the $C_b$ (which was already hybridized to the complementary subsequence $C^*_b$ of $p_0$), increasing the likelihood that the second robot also attaches to $p_0$.

By this process, pairs of $W_1$, $W_2$ duel and randomly one of the nanorobots assassinates the other (which disassociates from the nanotrack).

Probabilistic Analysis of the Assassination Protocol:
Figure 2.5: Design of DNA Nanorobots that Vote by Assassination.

We assume:

- The initial margin of disparity is $|n_1 - n_2| \geq \delta$ with $\delta = c\sqrt{n} \log n$, and

- Whenever both a $W_1$ and a $W_2$ nanorobot collide at a common $p_0$ pad of the nanotrack, it is equally likely that the $W_1$ or $W_2$ nanorobot is detached from the nanotrack and enters the solution, and never re-attaches to the nanotrack.

Observe that ultimately, either:

- one or more $W_1$ remains attached to the nanotrack and all the $W_2$ are detached, or

- one or more $W_2$ remains attached to the nanotrack and all the $W_1$ are detached.

The following immediately follows from the prior probabilistic analysis of the Angluin et al [2, 3] approximate majority protocol: Ultimately, with high probability:

- if at least one $W_1$ remains attached to the nanotrack, then $n_1 > n_2$, and

- if at least one $W_2$ remains attached to the nanotrack, then $n_2 > n_1$.

There are originally two unequal size (with sufficiently large size difference; see (c)) groups of DNA nanorobots; when pairs of DNA nanorobots from distinct groups collide,
one or the other is assassinated (by getting detached from the nanotrack); eventually all members of the smaller of the two groups of DNA nanorobots are assassinated (detached from the nanotrack) with high likelihood, A 2D nanotrack with a 2D array of attached ssDNA pads is self-assembled on a 2D surface, as illustrated in Figure 2.4. There are three types of pads: (i) \( p_0 = (B_2)^*C^*(B_1)^* \) attached at its 5’ end, (ii) \( p_1 = (B_1)^*(A_1)^* \) attached at its 3’ end, and (iii) \( p_2 = (A_2)^*(B_2)^* \) attached at its 3’ end. There are two types of ssDNA nanorobots: \( W_1 = A_1B_1C \) and \( W_2 = CB_2A_2 \).

The nanorobots \( W_1 \) and \( W_2 \) operate as follows:

(a) A combination of an unequal concentration of \( W_1 \) and \( W_2 \) strands is added to the buffer solution containing the nanotrack; some \( W_1 \) and \( W_2 \) strands hybridize to random pads of nanotrack.

(b) The buffer solution is replaced, so as to remove the remaining non-hybridized \( W_1 \) and \( W_2 \) strands from the solution surrounding the nanotrack.

(c) Our goal is: Let \( n_1 \) and \( n_2 \) be the (unknown) numbers of \( W_1 \) and \( W_2 \) strands initially attached to the nanotrack. Assuming \( |n_1 - n_2| > c\sqrt{n} \) (with \( n = n_1 + n_2 \) and large enough constant \( c > 0 \)), then we wish to test if \( n_1 > n_2 \) or \( n_1 < n_2 \).

(d) As in Figure 2.4, the \( W_1 \) and \( W_2 \), when separate, walk randomly over the nanotrack. The \( W_1 \) Nanorobot walks only on the \( p_0 \) and \( p_1 \) pads of the nanotrack, whereas the \( W_2 \) Nanorobot walks only on the \( p_0 \) and \( p_2 \) pads of the nanotrack.

(e) But as in Figure 2.5, whenever both a \( W_1 \) and a \( W_2 \) nanorobot collide at a common \( p_0 \) pad of the nanotrack, a random one of either \( W_1 \) or \( W_2 \) nanorobot is partially detached via strand-displacement at domain \( C \), and then melted off, and so enters the solution, and is not likely to reattach to the nanotrack. By this process, pairs of \( W_1, W_2 \) duel; randomly one of the nanorobots assassinates the other (which disassociates from the 2D surface), with equal likelihood.

(f) Ultimately, either (a) one or more \( W_1 \) remains attached to the nanotrack and all the \( W_2 \) are detached, or (b) one or more \( W_2 \) remains attached to the nanotrack and all the \( W_1 \) are detached.
(g) Assuming $|n_1 - n_2| > c \sqrt{n}$ with $n = n_1 + n_2$, then for large enough $c > 0$,

- If $W_1$ remains attached to the nanotrack, then with high likelihood $n_1 > n_2$.
- If $W_2$ remains attached to the nanotrack, then with high likelihood $n_2 > n_1$.

(A similar design can also be used for Attacking Nanorobots.)

2.4 Simulation of Social DNA Nanorobots

Here we give initial simulation results of the social DNA nanorobots that conduct walking, self-avoiding walking and flocking. The simulation model is adapted from the Surface CRN Simulator written by Samuel Clamons[33, 15]. The surface CRN is a stochastic chemical reaction network where individual molecules are tethered to fixed positions on a surface such that they can only interact with neighbors, which is the same situation with our assumption. We will develop software extending the Surface CRN Simulator specifically for use for social DNA nanorobots, with a catalog of DNA nanorobot devices, improved visualization, and to allow automatic incrementally optimized performance assessment. This software should significantly improve our simulations of social DNA nanorobots.

2.4.1 A Prior DNA Nanorobot that Autonomously Walks

Figure 2.6 shows an example simulation run for the $W$ Nanorobot (in green) randomly walking on a 2D nanotrack. Where it randomly walks on the nanotrack, the grid turns to orange to show the trace of the $W$ nanorobot. The $W$ Walker traverses uniformly randomly over the nanotrack.

2.4.2 A Novel DNA Nanorobot that Executes a Self-Avoiding Walk

Figure 2.7 shows an example simulation run for a $W$ nanorobot (in green) that executes a self-avoiding random walk on a 2D nanotrack. Where it randomly walks on the nanotrack,
Figure 2.6: Simulation of a DNA Nanorobot that Executes a Random Walk.

The grid turns to orange to show the trace of the W nanorobot. The W walker firstly randomly moves over the 2D nanotrack and then eventually stops when all the pads around it are visited.

2.4.3 Flocking: Novel DNA Nanorobots that Follow a Leader DNA Nanorobot

Here is an example simulation run for a group of DNA Nanorobots that follow a leader DNA nanorobot randomly walk on a 2D nanotrack. The yellow and blue cells are the $W_1$ (leader) and $W_2$ (follower) nanorobots, respectively. The $W_1$ walker can randomly walk on the nanotrack, while the $W_2$ walker can only follow the $W_1$ walker or stay in place. If the $W_2$ walker follows the $W_1$ walker, the grid it visited turns to green to show the trace of the $W_2$ Nanorobot; if the $W_2$ walker doesn’t follow the $W_1$ walker, it will stay in place and
Figure 2.7: Simulation of a DNA Nanorobot that Executes a Self-Avoiding Walk. keep blue until the $W_1$ walker visited $W_2$’s neighbor again.

2.4.4 Novel DNA Nanorobots that Vote by Assassination

- Figure 2.9 gives an example simulation run for two groups of DNA Nanorobots with unequal sizes that vote by assassination on a 2D nanotrack; The original size of $W_1$ is large than $W_2(n_1 = 10, n_2 = 6)$ and eventually all members of $W_2$ DNA nanorobots are assassinated.

- Figure 2.10 gives an example simulation run for two groups of DNA Nanorobots with equal sizes that vote by assassination on a 2D nanotrack; The numbers of $W_1$ and $W_2$ strands initially attached to the nanotrack are equal ($n_1 = n_2 = 8$), so they have the same chance to win the game, and eventually all members of $W_2$ DNA nanorobots are assassinated.

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Figure 2.8: Simulation of DNA Nanorobots that Follow a Leader DNA Nanorobot.

Figure 2.9: Simulation of DNA nanorobots that vote by assassination where \( n_1 = 10 \) and \( n_2 = 6 \).
Figure 2.10: Simulation of DNA nanorobots that vote by assassination where $n_1 = 8$ and $n_2 = 8$.

- Figure 2.11 gives the results of a collection of simulation runs for DNA Nanorobots voting by assassination on a 2D nanotrack with different initial $n$, where the number of $W_1$ is larger or equal to the numbers of $W_2$ ($n_1 \geq n_2$) that initially attached to the nanotrack. The X-axis and Y-axis represent the initial $n_2/n_1$ and final $n_2/n_1$ respectively. Due to $n_1 \geq n_2$ initially, it is more likely that eventually $W_1$ remains attached to the nanotrack and all the $W_2$ are detached, and the final $n_2/n_1$ will convert to 0. With same $n$, when initial $n_2/n_1$ goes smaller, the final $n_2/n_1$ will approach 0 with higher likelihood. When $n$ goes larger, the final $n_2/n_1$ will approach 0 with higher likelihood.
Figure 2.11: Results of a collection of simulation runs for DNA Nanorobots voting by assassination on a 2D nanotrack with different initial $n$, where $n_1 \geq n_2$. 
Chapter 3

Conclusions

We proposed social DNA nanorobots: these are autonomous mobile DNA nanorobots that execute a series of pair-wise interactions that determine an over-all desired outcome behavior for the group of nanorobots. Our goal was to increase the complexity of the various tasks the nanorobots can execute and at the same time preserve a low design complexity for individual nanorobots. We presented detailed designs for social DNA nanorobots that perform novel behaviors of Self-avoiding Walking, Flocking, and Voting by Assassination, and their behaviors were simulated in the 2D surface CRN model.

Here are some possible future research work based on this project.

3.1 Further Development of Simulation Software for Social Nanorobots

Simulation software based on the Surface CRN Simulator of Clamons [33, 15] can be developed specifically for use with social DNA nanorobots. The software will allow high-level specification and visualizations of state transitions (modeled by chemical reactions such as toehold-mediated strand displacements and dehybridizations) between DNA walkers and DNA strands affixed to a 2D surface. The software could provide an editable catalog of DNA nanorobot devices, improved visualization, and allow automatic incrementally optimized performance assessment. This software should significantly improve performance assessments & design optimizations. Visual DSD [25] can be used to simulate the DNA hybridization and strand-displacement reactions of the individual DNA nanorobots and between pairs of DNA nanorobots.
3.2 Further Social DNA Nanorobot Behaviors

Our novel designs presented here for DNA nanorobots (Self-avoiding Walking, Flocking, and Voting by Assassinations) can be employed in designs for even more complex behavior. For example, other behaviors of interest for DNA nanorobots include:

- Guarding, where a group of DNA nanorobots follow and guard a particular DNA nanorobot from attack by another group of DNA nanorobots. Here we expect we can employ parts of our Flocking design.

- Attacking, where a group of DNA nanorobots attack another group of DNA nanorobots. Here we expect we can employ a simplification of the Assassination design.

- Foraging and Harvesting. In Foraging, a group of designated foraging DNA nanorobots randomly walk on the 2D nanotrack, and can transform to a "discovery state" when they discover a target molecule (e.g., a group of gold nanoparticles attached to 2D surface) (this makes use of our designs for Self-avoiding Random Walking). In Harvesting, a group of harvesting DNA nanorobots which follow the trail of foraging DNA nanorobots in discovery state and pick up the detected target molecules, and deliver the target molecules to a designated region of the 2D nanotrack. Designs for Foraging nanorobots may employ our designs for Self-avoiding Random Walking, and designs for Harvesting nanorobots may employ our design for Flocking.

3.3 Communication Between Distant Social Nanorobots

Prior Use of Potential Fields for Generating Autonomous Group Social Activities: Another source of inspiration for collective behavior strategies by groups can be found in biology: for example, flocking of animals such as birds and schooling of amphibious animals. The behavior of these animals has been modeled by mathematical models and computer programs. In 1989, Beni [8, 7] developed one of the first such flocking model,
which he called swarm intelligence and made applications to multi-robot motion planning systems. Subsequently the field of swarm intelligence [10, 23] and artificial flocking grew rapidly and found applications to many applied areas in addition to robotics, such as for computer graphics. In 1994, Reif [36, 37] developed a general programmable scheme for multi-robot motion planning, termed Social Potential Fields, which made use of artificially defined potential fields that controlled the individual robots by weighted sum of decreasing functions of the distance and direction of other local robots; he demonstrated various autonomous group social activities, including flocking, attacking and guarding, using the Social Potential Fields technique. Unfortunately, the potential field models assume far-distance field effects that are not easy to implement using local interactions between co-located DNA nanorobots.

Using Instead Diffusion of Pheromone-like DNA Molecules for Communication Between Social Nanorobots: However, recall that the communication signals between social insects include pheromones; these are chemical factors that can trigger a social response in members of the same species [22]. We are exploring the use of diffusion of DNA molecules for communication between social nanorobots in a manner similar to the use of pheromones in social insects. For example, this technique may be employed by Foraging nanorobots to report discoveries of target molecules.
Bibliography


