Toward In Vivo Disease Diagnosis and Treatment Using DNA

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***Abstract*— We propose a technique to diagnose and treat individual cells in the human body. A virus-like system delivers a copy of a diagnosis and treatment DNA complex to each cell. The complex determines whether the cell has a specific disease based on the presence or absence of indicator mRNA molecules and, if the diagnosis is positive, releases a proper drug for treatment. As a tool for the diagnosis and treatment system, we develop a DNA implementation of an arbitrary finite state machine.**

I. Introduction

Greater control over the world at the nanoscale holds exciting implications for medical science. Imagine having a nanoscaled intelligent "doctor" sitting inside every cell in your body waiting for things to go wrong. As soon as something goes wrong, the "doctor" diagnoses the problem and has the intelligence to take appropriate action, such as releasing a drug.

Many disease treatments affect all of the organisms' cells, whether they are healthy or not. Unfortunate side effects are most common in treatments for cancers, such as chemotherapy and radiation treatment. While fast-replicating cells, such as cancer-afflicted cells, are most affected, all cells in the body, especially those around the tumor, are irradiated and treated with chemotherapy. The approach discussed in this paper delivers a drug and a diagnosis unit together, to each cell. Within that cell, the diagnosis unit determines whether the cell is afflicted by the disease by identifying the presence of messenger RNA (mRNA) encoding for certain disease-specific proteins, and only delivers the drug to the cell if the cell is found to be afflicted, thus saving the healthy cells from unnecessary treatment. Since with cancer treatments, the treatment is often death of the cell, this approach would potentially save countless healthy cells.

Ehud Shapiro, at the Weizmann Institute of Science, Israel, has been one of the most vocal proponents of the "doctor in a cell" vision. The minimal requirements for such a "doctor" are that it be small enough to fit into living cells without disrupting homeostatic processes and that it be smart enough to compute a function mapping symptoms to drug release.

Recent advances in cellular biology allow the conception of drugs delivered to individual cells that can both diagnose and treat each cell independently. Benenson et al. [1], working with Shapiro, have demonstrated an approach to diagnose and treat diseases in vitro using DNA and restriction enzymes. Their approach, if modified to work in vivo and coupled with a delivery method, would allow a copy of a drug to travel to each cell, diagnose that cell independently of all other diagnoses, and treat each cell individually. Thus, such a drug would only affect sick cells and leave healthy cells untouched.

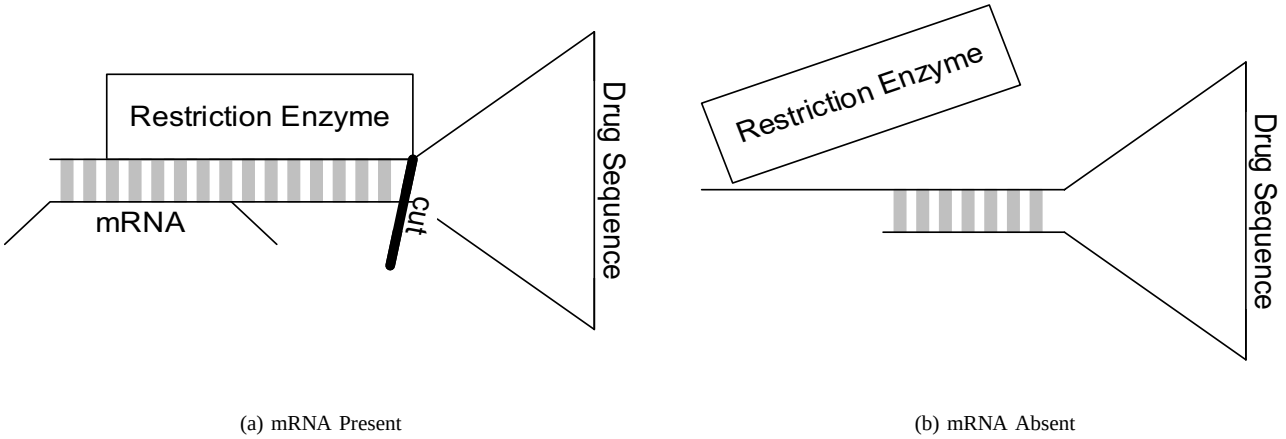
In this paper, we discuss adapting the method Benenson et al. [1] use in vitro, to be used in vivo. While the main underlying idea for local diagnosis and treatment remains the same, almost every mechanism has to be replaced to work within the cell without disturbing its environment. We use the presence of an mRNA in the cell to determine the presence of disease-associated proteins and to expose an otherwise contained drug. We allow for diseases that are detected by the presence of several proteins, as well as diseases that are detected by the presence of one or more out of a set of proteins. The unwrapped drug itself is a DNA strand that codes for a protein that treats the cell (or kills the diseased cell). Thus our approach is applicable to diseases that can be diagnosed by the presence of proteins (which may be viral proteins) in the cell and that do not disrupt translation within the cell.

Additionally, we propose a method for designing a DNA finite state machine. While past methods have required the use of restriction enzymes [2], our approach uses only DNA. It is this finite state machine design that is the main idea behind adapting the diagnosis and treatment system to being used in vivo. We also speculate briefly on drug delivery methods required in vivo.

The rest of this paper is structured as follows: section II discusses work related to ours and how our technique builds on it; section III brings forward the problems in adapting in vitro techniques to work in vivo; sections IV and V discuss the in vivo technique for disease diagnosis and delivery, respectively; section VI discusses future work designing the diagnosis and treatment system; and section VII summarizes our contributions.

II. Related Work

Benenson et al. [1] have developed and experimentally tested an approach for a nanoscale diagnosis and treatment system in vitro. The technique relies on the presence of certain sequences of mRNA that are indicators of a disease. This is a realistic assumption for certain diseases, such as small-cell lung cancer. The technique consists of building a DNA



(a) mRNA Present

(b) mRNA Absent

Fig. 1. Benenson et al. [1] demonstrated a DNA finite state automata that uses restriction enzymes. In the presence of an mRNA for the test-protein (a), the double-stranded region allows the restriction enzyme to connect to the recognition site and free the drug sequence. In the absence of the mRNA (b), the region remains single-stranded and the restriction enzyme cannot attach, leaving the drug sequence unavailable.

complex (see figure 1) that includes a sequence for a drug at the end of a loop of DNA. The DNA complex is a hairpin, made up of a stem and a loop. The only way to access the loop is by cutting off pieces of the double-stranded stem, which can be done using certain restriction enzymes. In order for the restriction enzyme to cut off a piece of the stem, it must land on a double-stranded region known as the recognition site. The initial complex leaves such a recognition site single-stranded but has it pair with an mRNA strand. Thus, only if the mRNA strand is present will the restriction enzyme cut the DNA. For each diagnostic test, the restriction enzymes work only in the presence of certain mRNA strands. Each test only passes if its diagnosis mRNA is present. The drug is released only if all the tests pass. Figure 1 demonstrates how the diagnosis happens. Figure 1(a) shows the case where the mRNA being tested for is present, completing the double helix, and figure 1(b) shows the case where it is absent.

While Benenson et al. have taken the first large step in designing a nanoscale diagnosis and treatment system, they only demonstrate it to work in vitro. Section III discuses some of the difficulties in adapting the approach to work in vivo, and how we plan to attack these problems.

The DNA complex implements a finite state machine. In theory, it is possible to implement not only more complex finite state machines, but ones that compute arbitrary boolean functions for more complex diagnoses [3]. In particular, it is possible to build NOT, AND, and OR boolean gates. Knight and Sussman have proposed and built biological gates that use gene expression to regulate protein concentrations, but their gates introduce a number of proteins and work slowly, over the course of days [4].

Some agents can act to speed up chemical reactions. Such agents are known as catalysts. Turberfield et al. [5] have shown that the placement of a reactive strand of DNA can act as a catalyst. If a strand of DNA that can pair with another strand is on the inside of a loop of a hairpin, it is not easily accessible to pair; however, if the hairpin is opened, the DNA is much more likely to react. It is this observation that allowed Benenson et al. to place the drug sequence at the loop of a hairpin and not concern themselves with it participating in reactions unless the restriction enzymes cut the hairpin stem open and release the drug.

Yurke et al. [6] discussed the idea of strand displacement, which is a dynamic process by which a DNA strand displaces another DNA strand from a double helix. The basic idea lies in the fact that if two arbitrary strands have a complementary subsequences of some length, they will pair; if a third strand has a longer complementary subsequence to one of those two, it will invade off the strand with the shorter complementary subsequence to allow the system to achieve a lower free energy state. The process of strand displacement can be modeled as a random walk driven by free energy considerations, and usually happens very fast. Figure 2 demonstrates how a strand displaces a shorter strand. The displacing strand has a longer region of complementarity and we call that difference in complementarity the *foothold* because the displacing strand attaches there first and uses that region as a starting point to invade off the other strand. We will use the technique of strand displacement to adapt the diagnosis and treatment system to work in vivo.

III. In Vitro Drug

While the technique described by Benenson et al. works in vitro, adapting the technique to work in vivo is not a simple task. Among other problems, there are three major reasons why the technique would not work in the human body: the finite state machine mechanism uses restriction enzymes that may be foreign to the human body and thus may interfere with the human genome and other processes, the in vitro method uses simple diffusion to deliver the drug to the needed locations, and adding certain DNA sequences to the human cell may cause unwanted reactions such as transcription and

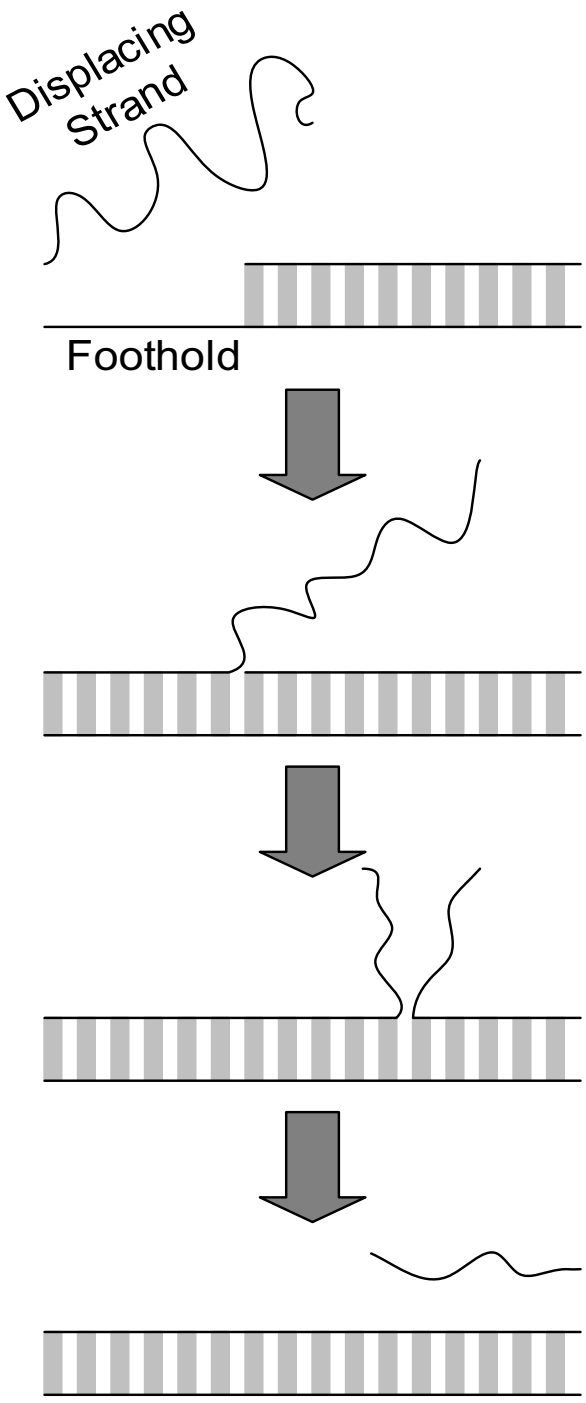


Fig. 2. A DNA strand (displacing strand) with a long complementary subsequence displaces a DNA strand with a shorter complementary subsequence by first attaching at the foothold and then, via a random walk, detaching the shorter strand and achieving a lower free energy state.

the synthesis of unwanted proteins in the cell. The rest of this section will deal with these three problems.

A. Restriction Enzymes

Benenson used restriction enzymes to implement the diagnosis finite state machine, as described in [2]. Restriction enzymes are proteins that can cut double-stranded DNA at specific base sequences. In a living cell, a foreign restriction enzyme may cut the cell's genome and interfere with other cell processes that use double-stranded DNA. Introducing a restriction enzyme foreign to a cell may cause cell death and will likely trigger an immune system response, rejecting the enzyme and thwarting the diagnosis. In addition, other restriction enzymes already present in the cell may interfere with proper functioning of the DNA finite state machine, causing bugs in the execution of logic. Enzymes other than restriction enzymes may also interfere. This may lead to premature drug release or misdiagnosis.

We propose a technique that uses no restriction enzymes for diagnosis. In fact, it uses no enzymes at all. It relies on the process of strand displacement to diagnose the presence or absence of mRNA and release the drug. The technique still uses a DNA complex with the drug sequence in a loop at the end of a hairpin; however, instead of restriction enzymes cutting pieces of the complex off in the presence or absence of mRNA, the mRNA strands themselves invade the complex and gradually open access to the drug portion of the strand. Section IV describes the details of the complex that implements the finite state machine using only DNA and no restriction enzymes.

B. Drug Delivery

Benenson et al. do not concern themselves with the problem of delivering their drug within the body because their technique works in vitro, thus all mRNA and enzymes have easy access to the finite state machine DNA complex. When envisioning such a technique to work in vivo, one must consider a method for delivering the DNA complex and all other required diagnosis and treatment components directly to the cell. The DNA finite state machine in an in vivo setting has to be transported safely to the target cells. Simply consuming it will degrade it by the digestive system, and injection into the blood may trigger an immune system response or never defuse the DNA finite state machine into the proper organs. Drug delivery via viral mechanisms is a possible alternative and a hot and promising area of research [7].

C. DNA Finite State Machine Sequence Design

The in vivo use of a finite state machine, whether made entirely or partially out of DNA, requires the insertion of foreign DNA into the cell. Since the cell has many processes that involve DNA, adding extra strands may cause unwanted transcription and the synthesis of proteins foreign to the cell. Thus, it is important to consider possible side effects of certain DNA sequences in designing the sequences for the finite state machine. While we do not go into details on sequence design in this paper, we acknowledge the existence of this problem.

IV. In Vivo Finite State Machine Design

This section discusses our proposal for adapting the DNA finite state machine mechanism described by Benenson et al. to work in vivo. Our proposal does not require restriction enzymes and works purely on the concepts of strand displacement [6] and DNA catalysis [5].

The DNA complex used by Benenson et al. contains a single-stranded region that allows the mRNA to attach. (Note that RNA-DNA hybridization is energetically favorable to DNA-DNA hybridization.) In the presence of mRNA, the region becomes double-stranded and the appropriate restriction enzyme can attach to its recognition site and cut the DNA complex. In the absence of the appropriate mRNA, the region remains single-stranded and the restriction enzyme cannot attach.

We exploit a similar feature; however, we use strand displacement. Figure 3 illustrates how the finite state machine works. A hairpin at the top of the DNA complex (labeled A) represents the first test of the finite state machine. Because the hairpin is closed, the reaction below it will not begin until this test passes. If a certain mRNA strand is present, it can strand displace the stem, thus opening the hairpin and providing access to the next test. The test passes only if the mRNA strand is present; otherwise the hairpin remains closed. After the first test passes, the second mRNA strand is needed to pass the second test. If the mRNA strand is present, it displaces the lower stem from hairpin A, opening hairpin B and detaching hairpin A completely. Hairpin B then invades off its own tail, which contains the drug sequence. If the second mRNA is not present, hairpin B does not open and the drug is never released.

Figure 3 shows a finite state machine with two tests. If both tests pass, the drug sequence is released, just as in the approach taken by Benenson et al. While figure 3 shows only AND gate logic, figures 4 and 5 shows OR and NOT gates. In figure 4, the OR gate creates a branch in computation. If either of the two (or more) mRNA is present, the computation may continue. Note that the amount of drug released is proportional to the number of computational branches that succeed. In figure 5, the NOT gate is an open hairpin that allows computation to continue, unless a specific mRNA is present to close the hairpin. The computation halts in the presence of such mRNA.

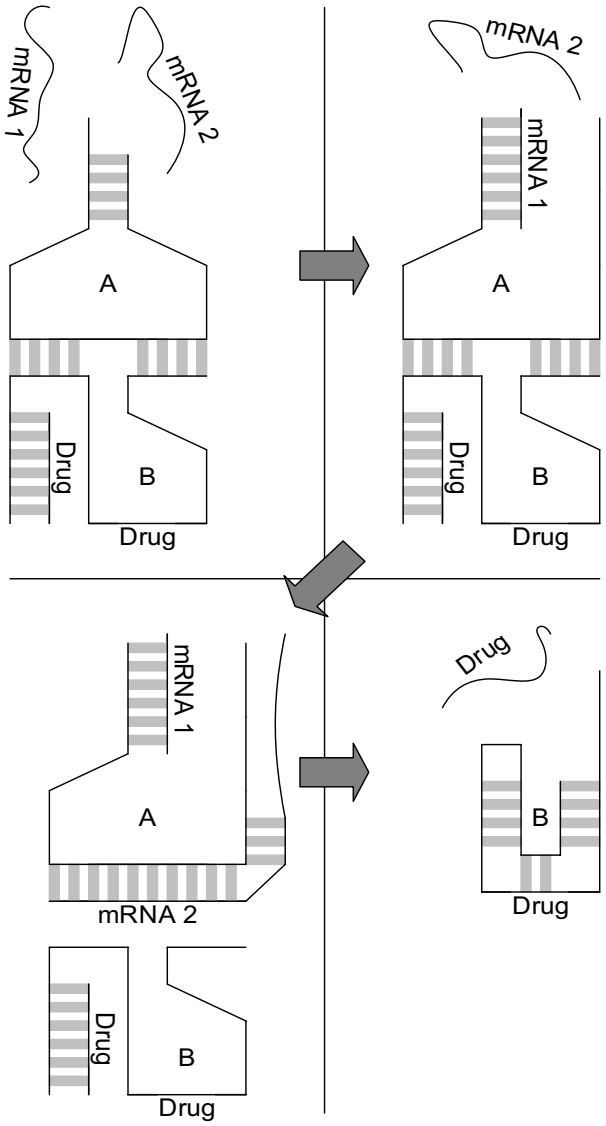


Fig. 3. The DNA finite state machine with two tests (mRNA 1 and mRNA 2). In the presence of mRNA 1, hairpin A opens and allows mRNA 2 access to its foothold. In the presence of mRNA 2, hairpin A detaches and hairpin B opens, allowing the drug's compliment access to its foothold. The drug's compliment closes out its own hairpin, freeing the drug. In the absence of either mRNA 1 or mRNA 2, no drug is released.

V. In Vivo Drug Delivery

We propose a technique for drug delivery that uses a system already common in nature. Many viruses inject cells with DNA that codes for more identical viruses and spread those viruses through the body. The viruses commonly kill the host cell as part of their replication and spreading process. In theory, it may be possible to modify the virus not to replicate but only to inject its DNA into the cell. We propose filling viruses with the diagnosis and treatment systems and injecting the body with an abundance of such viruses to "infect" the target cells.

The delivery of the diagnosis and treatment system will require a vector that is absorbed into the cell efficiently but is also specific for target cell types. Viruses are ideal for the delivery mechanism because they can circulate through the body carrying genetic material much more efficiently than a synthetic DNA carrier, such as a liposome. Due to their small size, protein structure, and biological origins, they can travel through the body without causing a toxic buildup of the carrier or substantial degradation of the DNA [7]. In addition, many of the mechanisms that the viruses have evolved enable them to deliver their genetic material into a cell very efficiently. Typically, foreign DNA would be degraded by the host cell, but viruses have managed to evolve mechanisms that protect their genetic material from degradation, though the DNA may require some modifications.

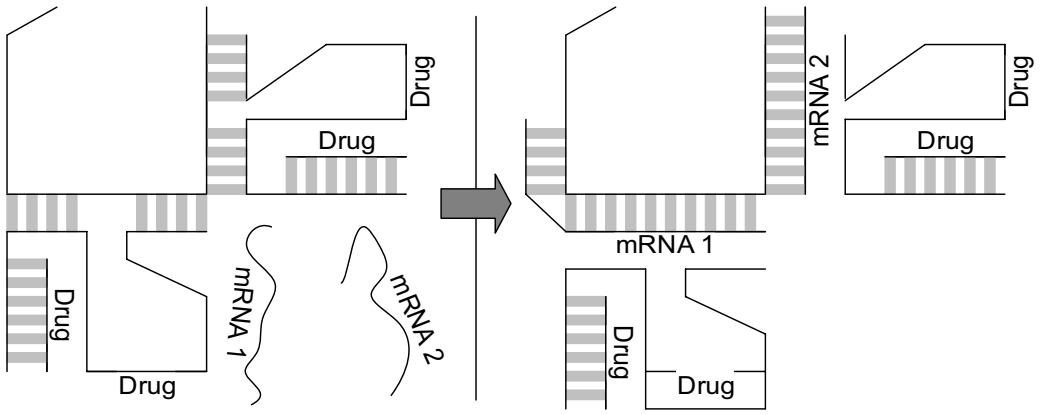


Fig. 4. The OR gate branches the computation into two directions. If either of the two mRNA is present, the computation continues in that direction and the drug is released.

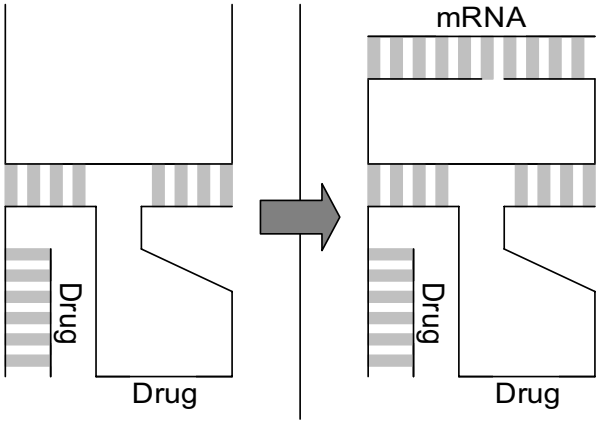


Fig. 5. The NOT gate is an open hairpin which allows the computation to proceed, unless an mRNA strand is present to close the hairpin, thus stopping the computation.

VI. Future Work

In this paper, we discussed a possible approach to adapting the diagnosis and treatment system proposed by Benenson et al. to work in vivo. We removed the need for restriction enzymes to operate the diagnosis finite state machine by using strand displacement and catalysis mechanisms proposed by Yurke et al. [6] and Turberfield et al [5], respectively. We have also speculated regarding a possible mechanism for delivering the drugs to target organs within the human body.

While we have examined what we believe are the some of the serious problems with adapting the approach to work in vivo, there are likely other problems. Further, while Turberfield et al. proposed a mechanism for catalysis that works fairly reliably, it only speeds up a reaction and does not fully control that reaction. In particular, in the absence of an mRNA strand, the reaction will not stop completely, but rather will slow down significantly. Thus it is possible for the drug to be released after a long time even if the finite state machine logic should not allow its release. Note that the same problem is present in Benenson et al.'s original in vitro approach. It may be necessary to include a mechanism to degrade the DNA complex after some time to prevent unwanted drug release.

VII. Contributions

We proposed an adaptation of an in vitro diagnosis and treatment system proposed by Benenson et al. to work in vivo. We discussed some important issues with the technique and proposed solutions to two of them: eliminating the use of restriction enzymes and drug delivery. We see this work as the next step toward building a "doctor in a cell" that is capable of diagnosing and treating diseases on a cell by cell basis.

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