

## Laboratory for Biomolecular Computers

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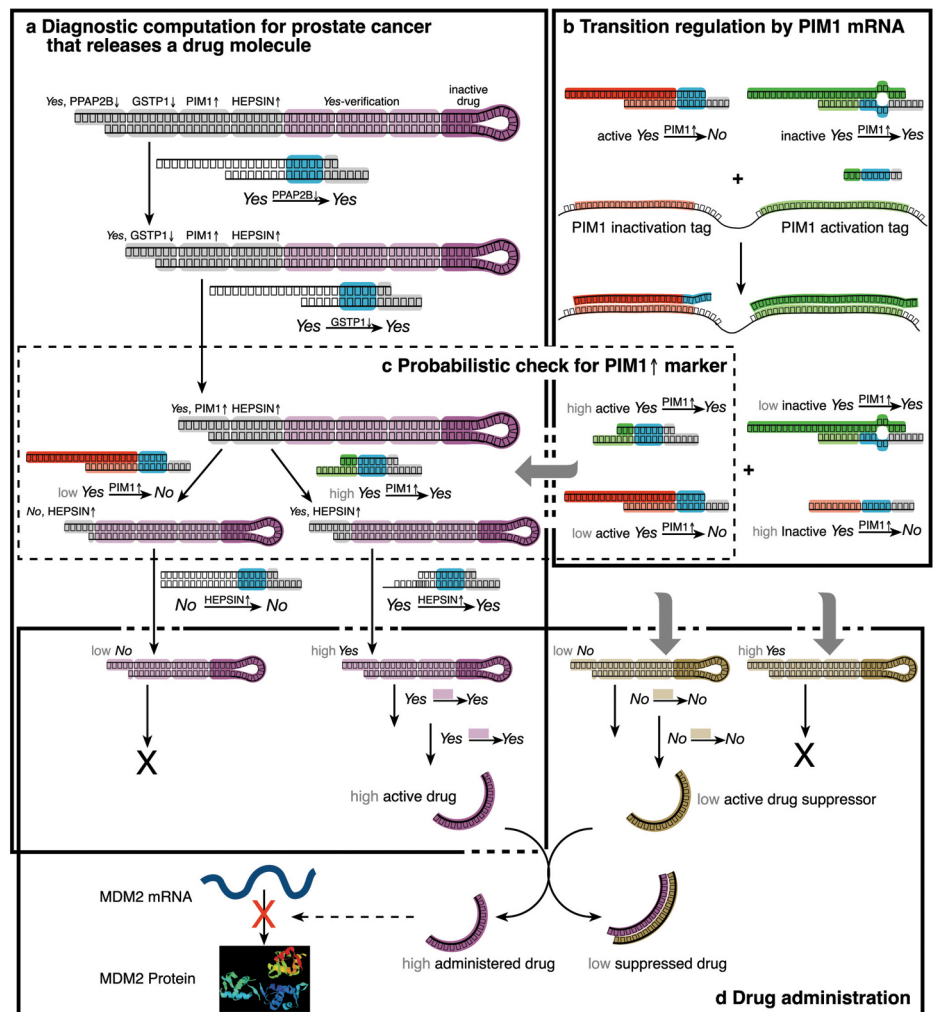
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Electronic computers and living organisms are similar in their ability to carry out complex physical processes under the control of digital information - electronic gate switching controlled by computer programs and organism biochemistry controlled by the genome. Yet they are worlds apart in their basic building blocks - wires and logic gates on the one hand, and biological molecules on the other hand. While electronic computers, first realized in the 1940's are the only "computer species" we are accustomed to, the abstract notion of a universal programmable computer, conceived by Alan Turing in 1936, has nothing to do with wires and logic gates. In fact, Turing's design of the so-called Turing machine has striking similarities to information-processing biomolecular machines such as the ribosome and polymerases. This similarity holds the promise that biological molecules can be used to create a new "computer species". Such computers can have direct access to a patient's biochemistry, a major advantage over electronic computers used for medical applications. Eventually, progress in the development of the molecular computers may lead to a "Doctor in a Cell": A biomolecular computer that operates inside the living body, programmed with medical knowledge to diagnose diseases and produce



**Fig. 1 a.** The overall process: The gray section is made up of four symbols, each of which checks for a different disease marker. The middle module (purple or beige) verifies the final diagnosis, and facilitates the release of either a drug or drug suppressor (darker colored loop at the end). **b.** A demonstration of the detection of a present marker (mRNA) that changes transitions concentrations. **c.** Demonstration of the stochasticity. **d.** Output operation; the final active drug concentration depends on the ratio between drug and drug-suppressor release.

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the requisite drugs. Our laboratory embarked on the attempt to design and build these molecular computing devices and lay the foundation for their future use for biomedical applications.

"DNA computing" experiments were pioneered by L. Adleman from USC and originally aimed at competing heads-on with electronic computers by solving compute-intensive problems using human-assisted, laboratory-scale manipulation of DNA. With a "Doctor in a Cell" vision in mind, we pursue a different paradigm: autonomous, programmable simple computers called finite automata. It turns out that the features of autonomous operation and programmability are much more important than sheer computational power when it comes to potential applications. At the [first stage](#) we designed, implemented and extensively studied the molecular realizations of two-state finite automata. In these automata the input is encoded as a double-stranded (ds) DNA molecule, software, called transition rules, is encoded by another set of dsDNA molecules, and the hardware consists of DNA manipulating enzymes. A computation commences when all molecular components are present in solution, and proceeds by stepwise, transition-rule directed, enzymatic cleavage of the input molecule, resulting in a DNA molecule that encodes the output of the computation. An automaton can be stochastic, namely have two or more competing transitions for each state-symbol combination, each with a prescribed probability, the sum of which is 1. A stochastic automaton is useful for processing information that is uncertain or probabilistic in nature, like most biological and biomedical information. Recently, we have [demonstrated](#) a stochastic version of our molecular automaton (Fig. 1).

The "Doctor in a Cell" concept calls for an autonomous molecular-scale computer that can be programmed to check for disease symptoms; to diagnose these symptoms according to medical knowledge; and to administer, upon diagnosis, the requisite drug at the required dosage and timing (Fig. 2). Recently, we have developed a molecular computer that can perform these operations *in vitro* on simplified molecular models of diseases (Fig. 1). The disease models consist of a combination of several molecular disease markers, including over-expressed, under-expressed and mutated genes that indicate cancer and hereditary diseases. The computer operation is governed by a diagnostic rule that encodes medical knowledge in simplified form. For example, the diagnostic rule for prostate cancer states that if the genes PPAP2B and GSTP1 are under-expressed and the genes PIM1 and HEP SIN are over-expressed then administer the ssDNA molecule GTTGGTATTGGACATG, a purported antisense drug.

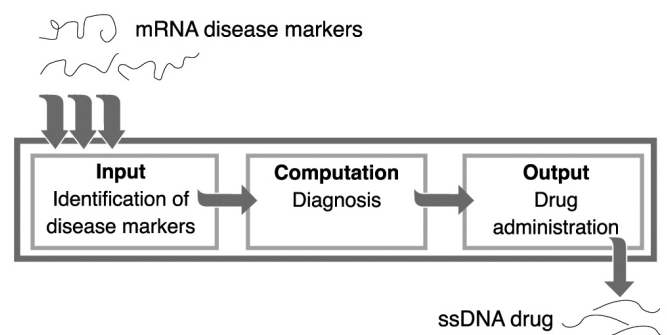
The diagnostic component of the computer is a stochastic molecular automaton with positive (Yes) and negative (No) states. The computation starts in the positive state and if it ends in that state the result is positive diagnosis, otherwise negative diagnosis. The set of markers is represented as a string of symbols, one for each molecular marker. For each marker symbol the automaton has three types of transitions: Yes→Yes (positive); Yes→No (negative); and No→No (neutral). A novel molecular mechanism regulates the probability of each positive transition by the corresponding marker. The presence of the marker increases the probability of a positive transition and decreases the probability of its competing negative transition and vice versa if the marker is absent. High ratio of Yes to No final output means that all positive transitions have high probability and hence all sampled disease markers exist in the biochemical environment.

Instead of releasing a drug on positive diagnosis and doing nothing on negative diagnosis, we opted to release a drug suppressor on negative diagnosis. This allows fine control over

the diagnosis confidence threshold beyond which an active drug is administered. Rather than using a single automaton for both tasks, we implement this by using two types of automata, one that releases a drug molecule upon positive diagnosis; and another that releases a drug-suppressor molecule upon negative diagnosis. The ratio between the drug and drug-suppressor molecules released by a population of automata of these two types determines the final active drug concentration.

As for current research, recent data showed altered expression of specific micro-RNA (miRNA) genes that contributes to the initiation and progression of cancer. miRNAs may function as perfect disease markers, of the bio-molecular computer, due to the fact that they biologically function as regulators of a large set of protein-coding genes. Furthermore, their simple secondary structure, high level of expression and their biological activity as single stranded molecule that hybridizes with a complementary sequence suit the bio-molecular computer demands. We are developing an autonomous bio-molecular computer that can logically analyze the levels of two miRNA species, and in response produce a molecule capable of effecting levels of gene expression designed to operate *ex vivo*. Apart from biomedical applications, such system, operating *ex vivo*, might enable the detection of miRNAs levels in single cells and could thus be used as a significant tool to measure miRNA levels and their combinations for research purposes. Another aspect of our research is, thus, to examine the possibility of performing the computation inside a living entity, i.e. human cell line (*ex vivo*).

Computers made of biological molecules are a computer species in search for a "killer application". Our results may encourage the speculation that "smart drugs" - molecular computers that roam the human body and diagnose a diseased cell, tissue or organ and administer the appropriate therapy *in situ* might be this application. However, radical changes will probably be required for our computer to operate *in vivo*. Adapting the medical computer to the *in vivo* environment is our major challenge that lies ahead.



**Fig. 2** The computer consists of three, semi-independent modules. To a certain extent, changes may be made in one of the modules without affecting the other two.

### Selected publications

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