

Automata make antisense

Anne Condon

Information-carrying DNA strands can be used to perform simple computations, but have so far been little more than toys. Can molecular computers be more broadly useful — in medicine, for instance?

People have long been fascinated with automata, fashioning them from any available materials to create mechanical creatures or musical devices, or to carry out simple computations. The materials used have even included biological molecules: in 2001, for instance, Yaakov Benenson and colleagues¹ built a tiny automaton from DNA strands and enzymes. On page 423 of this issue², Benenson *et al.* suggest how such molecular automata might be used to augment so-called antisense technologies, carrying out a diagnosis *in vivo* (that is, in a living cell) that automatically controls drug delivery.

Antisense drugs are oligonucleotides — short, single-stranded DNA molecules — that offer the promise of treating diseases caused by the expression of a harmful gene, for example a cancer-causing gene³. These drugs are designed to prevent the expression of such a gene: they bind specifically to the messenger RNA (mRNA) strand that is transcribed from the gene, thereby inhibiting the translation of the mRNA into a protein.

Benenson *et al.*² were motivated by the idea of achieving the ‘conditional’ release of such an antisense drug: *if* certain diagnostic conditions are true, such as low expression levels of certain mRNAs and high expression levels of others, *then* the drug is released. The *if-then* mechanism is the new element of computation introduced in their scheme.

Benenson and colleagues’ diagnosis proceeds in specific steps (transitions), one for each of the diagnostic conditions. After each transition, the state of the diagnosis is either positive (‘yes’), indicating that all conditions tested to date are true, or negative (‘no’), indicating that at least one of the conditions tested so far is false. The diagnosis can thus be viewed as a sequence of transitions of a molecular automaton that can exist in two types of state (Fig. 1). Each transition tests for high or low levels of a particular indicator molecule.

In Benenson and colleagues’ experiment, which was done *in vitro* (that is, in an artificial environment rather than in a cell), the drug is enclosed in the loop of a hairpin-shaped oligonucleotide structure called the diagnostic molecule. Initially, the stem of the diagnostic molecule is composed of four guards, one for each of four diagnostic conditions that the authors test (Fig. 2a). A positive diagnosis for each condition results

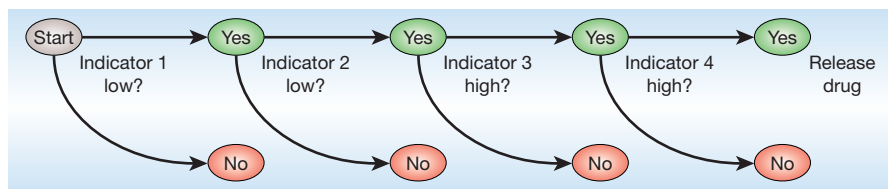


Figure 1 Automaton in abstract. Benenson *et al.*² devised a molecular automaton that theoretically tests for certain diagnostic conditions (high or low concentrations of particular indicator molecules) in cells. After each transition, the state of the diagnosis is either positive (‘yes’), indicating that all conditions tested to date are true, or negative (‘no’), signifying that at least one of the conditions tested so far is false. Should all four conditions be satisfied, indicating a positive diagnosis overall, a drug would be released.

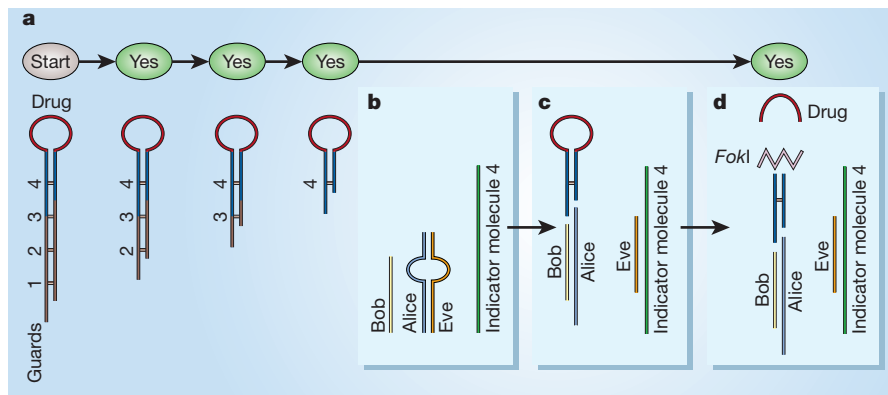


Figure 2 Benenson and colleagues’ molecular diagnostic automaton. a, The start state of the automaton is a hairpin structure. The drug is a nucleotide sequence within this structure that is protected by four ‘guard’ sequences. Upon each ‘yes → yes’ transition, indicating low or high levels of an indicator molecule as appropriate, a guard is cleaved from the stem of the hairpin, yielding a new diagnostic state. b, Initial configurations of the members of the cast of oligonucleotides needed for the final ‘yes → yes’ transition. c, To effect the transition, allegiances shift among the oligonucleotides: Eve binds to indicator molecule 4, leaving Alice to bind to Bob. The Alice–Bob combination then binds to guard 4. d, The *FokI* enzyme (jagged line) recognizes the sequence formed when the Alice–Bob pair binds to guard 4, and then cleaves guard 4, producing the final ‘yes’ state in which the drug is released.

in removal of the guards, one at a time. The changing diagnostic molecule, which gets shorter with each transition as guards are sequentially removed, is the physical realization of the state of the automaton as the diagnosis progresses.

Physically, each transition is a carefully orchestrated game of shifting allegiances among several oligonucleotide ‘players’. Consider the final ‘yes → yes’ transition, which should diagnose a high level of indicator molecule 4. In addition to the diagnostic and indicator molecules, the cast of players includes a pair that we will call Alice and Eve, and a lone ranger, Bob (Fig. 2b). Eve prefers to pair with indicator 4 rather than with Alice. So if all of the players are added to a

solution in which indicator 4 is present, then Eve deserts Alice and pairs with indicator 4. As a result, Alice settles for Bob (Fig. 2c). Furthermore, the Alice–Bob pair can dislodge the final guard, thereby releasing the drug (Fig. 2d). In the absence of indicator 4 in the solution, Alice and Eve would remain paired and the drug would remain captured.

How can the oligonucleotides be designed so that they are likely to shift allegiances according to this plot? Pairing, or formation of a duplex, between two oligonucleotides occurs when a sequence of nucleotides in one oligonucleotide binds to a complementary sequence in the other. Very roughly, the greater the number of nucleotide bonds between the two oligonucleotides,



100 YEARS AGO

Messrs. D. Schulte and Co. have submitted a sample of their self-lighting Bunsen burner, in which the well known property of finely divided platinum igniting under the influence of a stream of hydrogen is employed. The burner proper is of the usual type, but is furnished with a bypass tube at the side, controlled by a cross stopcock. At the top of the bypass, close to the open end of the burner, there is fitted a small bracket holding the bundle of several fine platinum filaments, so constructed that the thin stream of gas from the bypass tube impinges on the stretched wires... The arrangement works very readily, and if the old difficulties with regard to the durability of the delicate portions can be surmounted, the apparatus should be of considerable convenience to laboratory workers.

ALSO

At a sale recently held by Mr. Stevens in King Street, Covent Garden, a great auk's egg in fine condition was sold for two hundred guineas... This is a considerable falling-off from the three hundred guineas obtained for the last specimen sold by Mr. Stevens, the reason being attributed to the fact that several other fine examples are in the market. From *Nature* 26 May 1904.

50 YEARS AGO

It has been shown that as a rule the deoxyribonucleic acid content of the interphasic nuclei of tissues of a given species corresponds to a constant equilibrium value, which is double that of the deoxyribonucleic acid content of the spermatic nuclei of the same species. Consequently, at each mitotic division, synthesis of deoxyribonucleic acid must take place in order that the quantity of this substance should be restored in the nuclei of the daughter cells at a 'normal' level... According to some authors, this occurs immediately before mitosis, so that the content reaches double that of the normal value: after division each nucleus of the daughter cells receives also a normal content. According to other authors, synthesis occurs soon after mitotic division when nuclei of the daughter cells which receive only half of the normal content restore the latter. We have undertaken the study of this question in the thyroid cell of the white rat... These measurements show clearly that in our material deoxyribonucleic acid is synthesized immediately before the onset of mitosis. From *Nature* 29 May 1954.

the more stable the duplex. Eve is designed to be complementary to a relatively long unpaired region of indicator 4, whereas Alice is complementary only to a sub-sequence of Eve. So, Eve binds more stably with indicator 4 than with Alice. Also, the Alice and Bob oligonucleotides have (somewhat shorter) complementary sub-sequences, which are likely to form a duplex once Eve has bonded to indicator 4. The game of shifting allegiances plays out somewhat differently when diagnosing low, rather than high, concentrations of an indicator molecule, although the principles are similar.

Also needed is a mechanism for cleaving the guard in the presence of the Alice–Bob pair. This pair can bind to the guard (again, via bonding between complementary single-stranded regions of Alice and the guard, called sticky ends; Fig. 2c). One additional molecule, an enzyme called *FokI*, recognizes a sequence pattern formed when the Alice–Bob pair is bound to the guard. *FokI* then cleaves the guard from the hairpin loop, releasing the drug.

Unfortunately, the specific mechanisms proposed by Benenson *et al.*² would not work in a living cell: unwanted side effects of the

cast of supporting molecules (particularly the *FokI* enzyme) would be one major problem⁴. Nevertheless, getting an experiment of this scale to work *in vitro* is a real achievement. Perhaps more importantly, the work takes a conceptual step forward, by linking the development of molecular automata to antisense therapies. It is plausible that different molecular mechanisms can be found to create diagnostic automata in the cell, building on progress made so far in the use of antisense therapies or cellular computation^{5,6}. Developing such mechanisms would certainly be a good direction for further research. ■

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Granular materials

The brazil nut effect — in reverse

Troy Shinbrot

In a box of mixed nuts, the brazils rise to the top. In granular mixtures in general, depending on their size and density, the 'brazil nuts' may sink instead. This reverse effect has now been explored further.

Every farmer can attest to the curious fact that the largest crop each spring is the boulders that appear, untended, on open fields. Common wisdom holds that this crop is loosened from the soil by frost heave, and rises because small pebbles can slip beneath large boulders, but not vice versa¹. This is the 'brazil nut effect' — named for the fact that, in a container of mixed nuts, the brazil nuts always seem to rise to the top (Fig. 1). Because similar processes and effects occur in pharmaceutical, chemical and food processing, the problem of granular segregation has earned serious attention² — and now, in *Physical Review Letters*, Huerta and Ruiz-Suárez³ add the latest piece of the puzzle.

The first complication to the simple picture of pebbles slipping beneath boulders (termed 'percolation') was the demonstration that a tapped bed of grains 'conveys' in a regular pattern: a wide swath of grains rises in the centre of a container, and thin margins correspondingly sink⁴. According to the convection picture, large 'intruder' particles rise with the surrounding bed, and then find themselves simply unable to fit into narrow

downwelling margins. This mechanism was confirmed by a clever experiment in which the convection rolls were reversed and, as predicted, large particles migrated to the bottom of vibrated beds⁴. Later confirmations came from magnetic-resonance-imaging experiments that conclusively demonstrated the presence of segregating convection rolls⁵, and from meticulous computational comparisons that revealed that convection dominates over percolation in producing segregation in deep beds⁶.

Over the past decade, however, our understanding of the segregation of large particles in vibrated beds has been challenged by experiments revealing that although large heavy 'intruder' particles can indeed rise in vibrated beds of finer grains, equally large light intruders can sink, contrary to expectation and common experience. Now termed the 'reverse brazil nut effect', this observation⁷, made by myself and Fernando Muzzio, is explained by neither the convection nor the percolation description. It is so counterintuitive that a reviewer of the original manuscript reporting the effect insisted that it could not be correct; and the