corroborated in melanoma patients that peptide-specific T-cell responses are increased after intranodal injection as compared with intradermal or intravenous routes[10].

In their new paper, de Vries *et al*. studied direct injection of antigen-loaded dendritic cells into the lymph node of humans. They took advantage of the high phagocytic capacity of immature dendritic cells, which, they show, take up FDA-approved magnetic nanoparticles with no adverse effects on their interactions with T cells *in vitro* and *in vivo*.

Eight evaluable stage-III melanoma patients received an intranodal injection, under ultrasound guidance, of a mixture of dendritic cells labeled with ^{111}In or superparamagnetic iron oxide (SPIO) and pulsed with tumor peptides at a ratio of 1:1, two days before radical dissection of regional lymph nodes. The cells were successfully tracked *in vivo* using a noninvasive 3T MRI system. In 4 out of 8 patients, MRI demonstrated that the dendritic cells were actually delivered into the perinodular fat (and not into the lymph node), whereas scintigraphy detected a single spot at the injection site. MRI could detect up to 1.5×10^5 migrated cells and image up to 5 individual lymph node sites (whereas scintigraphy detected two sites in most cases). By co-injecting equal numbers of dendritic cells labeled with ^{111}In or with SPIO particles, they demonstrated that MRI is as sensitive as scintigraphic imaging for the detection of dendritic cells *in vivo*.

The major advantage of MRI over scintigraphic imaging is the presence of high-resolution anatomical background contrast, allowing for a precise anatomical localization of SPIO-labeled cells, first at the injection site and then at distant sites. Thus, MRI proved valuable for monitoring the migratory capacity of dendritic cells. Remote lymph nodes containing SPIO-labeled cells could be visualized individually in patients *in vivo*, and this result was confirmed by histological analysis of lymph node biopsies. The high spatial resolution of MRI and lack of saturation of images represent significant advantages over scintigraphy. However, according to de Vries *et al*., scintigraphic imaging is superior to MRI for quantifying the number of cells that have migrated to lymph nodes. Therefore, the authors argue that both techniques should be combined to achieve accurate quantitative and qualitative information on the fate of adoptively transferred dendritic cells.

The current work is of biological and medical significance. Proper inoculation of dendritic cell vaccines should improve our knowledge of dendritic cell biology and, more importantly, should boost the clinical response to antigen-loaded dendritic cells, obviating the need for repeated vaccinations. The study by de Vries *et al*. was not designed to monitor the correlation between correct dendritic cell inoculation and immune response, and future studies must address this question. However, it may be expected that monitoring of dendritic cell application to patients, as outlined here, will contribute to the optimization of vaccination schedules and the quality control of the vaccines (**Fig. 1**). It is also easy to envisage that MRI tracking of transplanted therapeutic cells will be adapted to other cell types, such as tumor-specific lymphoid cells or stem cells. Whatever the cell type, the ability to monitor the cells' fate *in vivo* will be essential to the development of effective cell therapies.

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Boolean calculations made easy (for ribozymes)

Adam A Margolin & Milan N Stojanovic

A new algorithm facilitates the design of ribozymes that encode logical operations.

The basic electronic devices that perform Boolean calculations are called logic gates. Elementary logic gates, such as AND, NOT and OR, are organized into more complex electronic circuits that, together with other devices, are used to build computers. Molecules can function as logic gates in that their behavior (outputs) is influenced by the presence of other molecules (inputs). The construction of a reliable set of molecular-scale logic gates that can communicate with each other would allow the engineering of molecular circuits capable of performing complex logical operations in solution. From this perspective, the contribution by Penchovsky and Breaker[2] in this issue is groundbreaking; they describe a precise protocol to construct and test a complete set of ribozyme-based logic gates whose self-cleavage activity is modulated by the presence of oligonucleotide inputs. This work also provides us with a tantalizing glance into how these elementary units can be organized into more complex circuits.

To construct molecules that perform basic Boolean operations in solution, Penchovsky and Breaker generate allosteric ribozymes using the hammerhead ribozyme secondary structure motif (**Fig. 1a**). For each logic gate, a computer program searches millions of potential sequences, and a partition function algorithm identifies those sequences that are most likely to form a dominant active or inactive secondary structure and robustly switch to the alternate state in the presence of one or more oligonucleotides.

For a sensor (YES) gate, the ribozyme must assume an inactive conformation except in the presence of a particular oligonucleotide, which triggers activity. For a NOT gate, the effect of the oligonucleotide is reversed; that is, it deactivates the ribozyme.

For the two-input logical operations AND and OR, the partition function must consider four possible states corresponding to the presence or absence of two inputs.

All gates designed by Penchovsky and Breaker consist of a catalytic module that undergoes self-cleavage if the gate is in the active form, and a recognition module that binds one or more input oligonucleotides (**Fig. 1a**). Recognition modules can be altered to exhibit different input specificities with a high likelihood that they will maintain robust switching

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properties. Inputs that favor alternative secondary structures or have unfavorable kinetics can, in most cases, be eliminated through the sophisticated algorithm described in the paper, which takes advantage of existing RNA folding programs. Moreover, by engineering logic gates such that the self-cleavage output of one gate functions as the input triggering activity of a downstream gate, the authors demonstrate the possibility of designing molecular circuits capable of implementing arbitrarily complex logical operations.

Although oligonucleotide-sensing deoxyribozyme-based logic gates[3], their cascades[4,5] and automata[6] (machines whose responses to all permissible inputs are specified by a set of states and a set of rules for passing from one state to another) have been previously reported, the computational search method for rational design described in the current work improves on previous approaches in several ways. First, the extensive search procedure allows the rapid identification of many potentially effective sequences and the generation of a large library of potential gates as off-the-shelf reagents for building circuits; all eleven predicted sequences that were tested experimentally demonstrated the expected robust switching behavior. Second, activation ratios for all reported gates were consistently high, and all gates had similar catalytic rates in their active forms. Finally, the use of an extensive computational search enabled the first successful implementation of an OR logic gate, which had previously been difficult to engineer by manual design in a single nucleic acid enzyme molecule.

Penchovsky and Breaker's results will influence a number of related fields, including several emerging directions in biological chemistry. Ensembles of molecular scale logic gates are one example of the very few artificial molecular systems capable of analyzing environmental signals and performing computations autonomously (that is, without human input, or being interfaced with macroscopic electronic devices). Although, for example, more advanced forms of existing automata[6] may find applications in massive analyses of complex oligonucleotide mixtures, in the long run it is perhaps more important that the outputs of computation can be connected to other molecular devices[5]. This characteristic is opening up prospects to use such automata to establish control over various DNA-based conformational machines and walkers[7]. This is the same type of control that computers exert over macroscopic mechanical devices, and it should eventually lead to complex, stimuli-sensitive behaviors of assemblies of molecules.

Of course, such systems will require further technological development, in particular, reliable gates capable of reversing cleavage activity (to reset the devices)[4] and gates powered by small-molecule fuels, such as ATP. The idea of coupling molecular motors with molecular computation is the key to the success of the fledgling field of nucleic acid–based 'molecular robotics'[7]. The procedure described by Penchovsky and Breaker empowers us to generate large libraries of dependable components that could be used in such projects.

Another equally futuristic prospect is to use such logic gates in therapeutic and diagnostic molecular automata. Such automata would consist of networks of molecules programmed to release a drug or diagnostic reporter *in vivo* in response to multiple disease markers. To date, two systems based primarily on nucleic acids have been proposed for this purpose. In a powerful proof-of-concept experiment, automata consisting of cascades implementing input-directed restriction enzyme activity (a modification of Shapiro-Rothemund's automata) were shown to release antisense molecules (or anti-antisense molecules as protective fac-

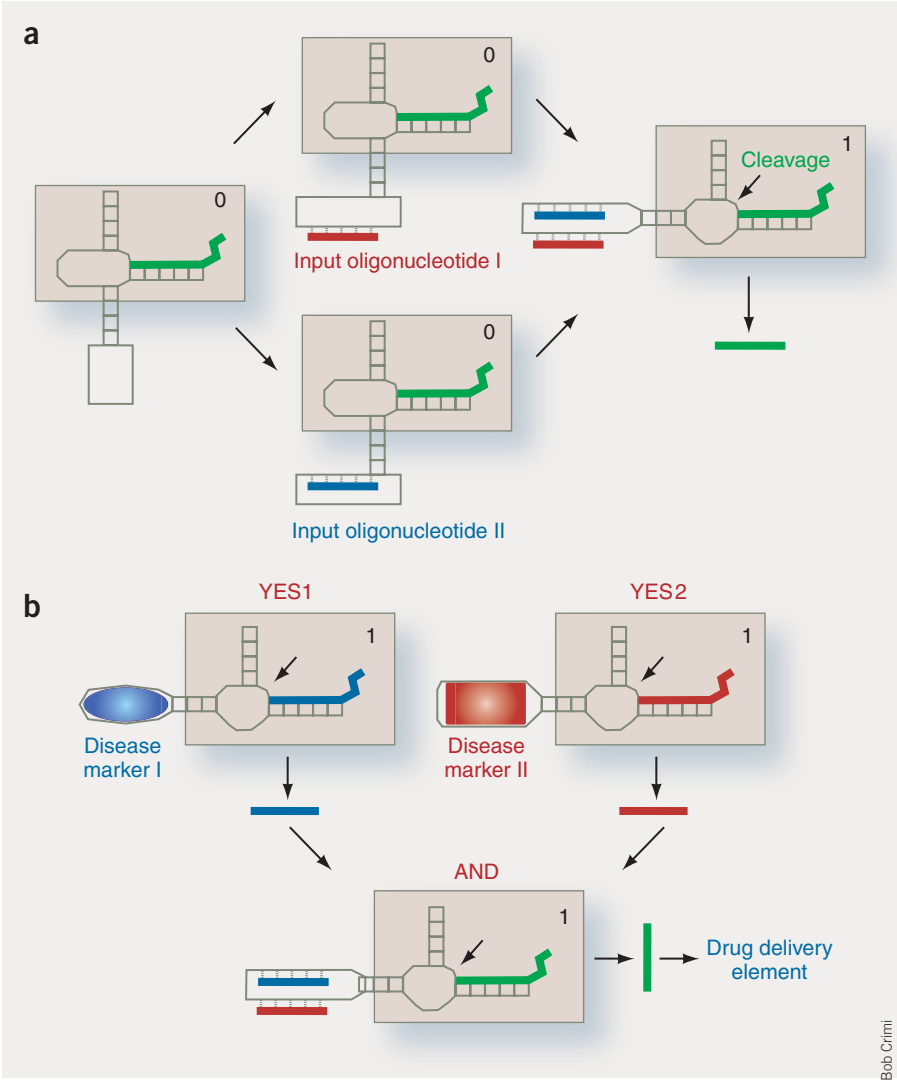

Figure 1 **a**, Schematic representation of an AND gate with the hammerhead ribozyme structural motif. The AND gate recognizes two oligonucleotides, I (blue) and II (red) and has four possible states. Three states exhibit no self-cleavage activity (inactive or output 0) owing to misfolded catalytic modules (boxed). Only the state with both oligonucleotides bound to the recognition module contains the properly folded hammerhead catalytic module that exhibits self-cleavage activity (active or output 1). **b**, Operation of a rudimentary therapeutic automaton implementing AND logic and analyzing two disease markers. Two upstream sensor (or YES) gates recognize two protein disease markers (ellipse and rectangle), become active (1) and release oligonucleotides (blue and red) through autocatalytic action. The two released oligonucleotides activate a downstream AND gate, and release an oligonucleotide (green) that activates a downstream drug delivery element[5].

tors) in response to the presence or absence of several input mRNAs[8].

The second system, developed in our laboratory[3,6], uses circuits of nucleic acid–based logic gates like those described by Penchovsky and Breaker. In this approach, a series of (deoxy)riboswitches would recognize multiple disease markers and release oligonucleotides that would serve as inputs for downstream circuits of logic gates, which would in turn trigger the release of drugs[5] (**Fig. 1b**). Again, a protocol for high-throughput, automated design of reliable molecular switches brings this application one step closer to reality.

Penchovsky and Breaker's work will also make a significant contribution to the field of synthetic biology and engineered intracellular circuits. For example, the recent demonstration in a mouse model of ribozymes as oligonucleotide-responsive gene expression control devices[9] foretells next-generation experiments in which ribozyme logic gates organized in molecular cascades can reprogram cellular behavior in response to combinatorial molecular inputs.

Finally, this work supports the contention that it might be easier to design complex intracellular circuits made of RNAs than of proteins; currently, computational predictions based on extensive search of potential sequences is possible only for nucleic acid–based systems, for which the principles of Watson-Crick base pairing are well understood, the thermodynamic parameters are well studied and catalytic activity can be reliably predicted through knowledge of the secondary structure. However, cells are adept at assembling protein-based molecular circuits from individual protein gates. Recent progress in understanding the underlying principles of cellular signaling network organization, in particular the ability to construct new protein gates using principles of modular allostery and to rewire networks using engineered scaffold proteins[10], argues that proteins may be equally suited to the construction of artificial circuits. Thus, although nucleic acids seem to have the initial advantage in this field, the eventual outcome of this rerun of the old evolutionary battle between two biopolymers is not easy to predict.

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Life, diversity and the pursuit of haplotypes

Jeanette McCarthy

The complete haplotype map of the human genome provides an unprecedented view of human genetic variation.

The completion of the Human Genome Project revealed, among other things, that sequence variation in the human genome is abundant[1,2]—so abundant, in fact, that identifying disease-causing variation from among the vast amount of inconsequential variation poses a formidable challenge. Understanding how genetic variation is organized in the genome would greatly improve the efficiency with which we can identify alleles underlying common diseases. In a recent paper in *Nature*, the International HapMap Consortium reports on its genome-wide survey of common genetic variation in the human genome and reveals the underlying architecture of genetic variation in human populations[3].

It is estimated that there are 10 million polymorphic sites in the human genome, the result of mutations in the DNA of our ancestors that have been transmitted through the human population over time. It has also long been recognized that genetic variation at different nearby sites is correlated, or in linkage disequilibrium, but the degree of correlation varies between any two sites. The patterns of linkage disequilibrium in the genome reflect, among other things, the process of chromosomal recombination, whereby segments of DNA are swapped between maternally and paternally derived chromosomes. The segments of chromosomes that remain intact, without disruption from recombination, are inherited in blocks (**Fig. 1**). The delineation of these blocks, and the genetic diversity represented within them, is revealed in the human genome HapMap.

The International HapMap Consortium identified a set of over one million common (>5% frequency of less common allele) single nucleotide polymorphisms (SNPs) evenly spaced throughout the genome at an average distance of one per 5 kb, which they genotyped in 269 individuals from four diverse populations. Their analysis of haplotype structure and diversity confirmed that recombination rates vary extensively throughout the genome, with some regions (centromeres for example) exhibiting little or no recombination and others, hotspots, demonstrating recombination rates dramatically higher than background rates. For example, within one 5-Mb region, 80% of all recombinations occurred in 15% of the sequence.

The result is that most of the genome omprises long blocks of DNA that are disrupted by recombination sites. These blocks harbor many sequence variants, but because these variants are in strong linkage disequilibrium, the diversity of the block is actually quite limited. On average, for a given block, there are only four to six haplotypes or unique combinations of alleles at adjacent sites. Furthermore, the investigators report that common and rare haplotypes are often shared across ethnic populations, although the frequency of a particular haplotype may vary between populations. As it turns out, the average common SNP is in strong linkage disequilibrium with three to ten other SNPs, indicating that the redundant SNPs add no new information. Therefore, a set of less than 500,000 SNPs is sufficient to capture information on all common variation in the human genome.

How will this information be used? One of the goals of the HapMap project was to improve the ability to carry out genome-wide association studies for the purpose of identifying gene variants underlying quantitative traits, common diseases and response to therapeutics (pharmacogenomics). By surveying the entire genome, the genome-

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