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'Computational evolution' offers riboswitch solution

A computational strategy for the fast and efficient design of sensitive, sequence-activated ribozymes could dramatically reduce the time and benchwork investment for scientists interested in engineering specialized RNA-based tools.

The 'RNA world' hypothesis, which postulates that the earliest 'biological' functions and reactions were strictly RNA-mediated, has been around for decades. Subsequent support of this hypothesis would come with the discovery of actual catalytic RNA molecules, or ribozymes, fueling interest in research efforts to engineer specialized RNA molecules with a variety of catalytic and regulatory properties. In many cases, these *in vitro* advances would quickly be overshadowed by the discovery that nature had beaten scientists to the punch, and that many organisms already use a wide array of such RNA-powered systems.

"I think the days of demonstrating that RNA can work as a molecular switch are past," says Yale University researcher Ron Breaker, a pioneer in the engineering of catalytic RNAs. "We know that RNA can work as a switch—biology has proven it, and molecular engineers have proven it. Now let's make usable systems." Some of Breaker's early work involved the development of riboswitches, RNAs with functional properties that can be activated or inhibited by a target molecule, studies that typically used multiple rounds of *in vitro* evolution to generate specialized molecules that are optimized to perform a function of interest. Now, Breaker and postdoc Robert Penchovsky have moved this screening process from the dish to the disk, developing a fast and efficient computer-based system capable of rapidly screening millions of RNA sequence and structure variants to identify allosterically regulated ribozymes capable of responding sensitively to the presence of specific DNA sequences.

The framework for their computational system relies largely on the adaptation of existing algorithms for calculating the energy profile of RNA molecules. Says Breaker, "It turns out that there's enough information known about how RNAs fold and the stabilities of certain RNA sequences to nicely predict sequences that will adopt two different states—one in the presence of the target, and one in the absence of the target." Starting with the well-characterized, self-cleaving hammerhead ribozyme, they identified sites into which oligonucleotide sequence recognition sites could be engineered; they then used their algorithm to identify ribozyme variants in which target binding would change the ribozyme's energy profile in a manner that favors a secondary structure shift that turns cleavage 'on' or 'off'.

Using this approach, they engineered several allosteric ribozymes, based on the principles of digital logic gates, which they then tested experimentally. These included YES-1 (binding induces cleavage), NOT-1 (binding inhibits cleavage), and AND-1 (two separate oligos must both bind to induce cleavage). All variants performed as expected, with surprising sensitivity—in a final test, Breaker and Penchovsky set up a basic 'pathway', in which activation of YES-1 releases an oligonucleotide which in turn triggers the activation of a related ribozyme, YES-2.

Their computational approach trims a process that can take weeks of labwork down to several hours of computing time and, according to Breaker, it should offer a generalizable tool for virtually any RNA design project. To this end, Breaker is looking to make their system publicly available online as soon as possible. "I would love to see researchers now come up with useful genetic switches that can control genes inside cells or that act as molecular sensors inside cells," says Breaker, "and they're not derived from natural components… we're making them brand-new from scratch."

Michael Eisenstein

RESEARCH PAPERS
Penchovsky, R. & Breaker, R.R. Computational design and experimental validation of oligonucleotide-sensing allosteric ribozymes. *Nat. Biotechnol.* **23**, 1424–1433 (2005).