New software dramatically speeds enzyme design

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A Duke University-led team has brought powerful software to the never-ending arms race between antibiotics and germs. Working together, computer scientists and biochemists have developed and laboratory-tested a computer program that can show experimentalists how to change the machinery that bacteria use to make natural antibiotics.

The program -- a set of computer rules known as the algorithm "K*" (pronounced K Star)-- is able to sort through all the possible shapes and changes of a key enzyme that produces a natural antibiotic called gramicidin S, said Bruce Donald, Duke's William and Sue Gross Professor of Computer Science and Biochemistry. The new technique might pave the way toward more automated redesign of old drugs to foil drug-resistance in germs.

"It really excites us that we can redesign enzymes on a computer, make them in the laboratory and have them work as planned," said Donald, who leads the extended research effort and is corresponding author of a new report to be published online the week of Feb 16 in the research journal *Proceedings of the National Academy of Sciences* (*PNAS*).

The work was funded by a grant from the National Institutes of Health.

The search for new antibiotics usually begins by directly modifying existing compounds. But his group instead predicted mutations to enzymes from an antibiotic-making microbe, using K* to search faster and cheaper for the best designs.

"It is essentially a new pathway to make novel antibiotics," Donald said. "There are many possible changes you can make to a protein, but the algorithm can test out orders of magnitude more variations than laboratory experiments alone."

Other protein design algorithms have been proposed, and some of those even attempt to account for the way key parts of real proteins move around in three dimensions. But Donald said the latest version of K* lets protein backbones and side chains wiggle more like they would in real life. Moreover, it simultaneously evaluates what he called "an entire album, or ensemble," of possible shape shifting.

"Thus it not only addresses flexibility. It embraces it," Donald said." "So we might be able to quickly discover things that would take a very long time through purely experimental techniques. It should, in principle, be possible to redesign any enzyme simply by inputting the protein's shape into the algorithm and telling it what you want it to do."

The new report is the latest milestone in a nearly 10-year effort to develop reliable enzyme design algorithms, Donald said. In the process, his group has had a long collaboration with Amy Anderson of the University of Connecticut to biochemically test the algorithm's accuracy using the Gramicidin S Synthetase enzyme system, which produces the natural antibiotic in Bacillus brevis bacteria.

In the new *PNAS* report, Ivelin Georgiev, Donald's computer science graduate student who is one of K*'s designers, used the latest version of the algorithm to redesign the first step in the biochemical assembly line needed to make the antibiotic.

"Redesigning that first step is a big achievement," Donald said. "We are now beginning work on redesigning the half dozen subsequent steps downstream."

The algorithm includes a "dead-end elimination" feature that can run though all possible chemical interactions and flexible molecular architectures to weed out scenarios that cannot work. Calculating just one redesign might take up to a week in the 230-processor computer cluster housed in Donald's lab, he said.

After all the calculations were completed, biochemist Cheng-Yu Chen, another of Donald's graduate students, confirmed the algorithm's predicted designs in Donald's biochemical wet lab, using bacteria to synthesize some "quite big and tricky proteins," Donald said. "It was not at all trivial to do that, and testing the functions of each protein was even trickier."

Georgiev and Chen were both first authors of the *PNAS* report, and the University of Connecticut's Anderson was another co-author. The K* algorithm software is available as open source code for other researchers to evaluate and use.

"While gramicidin S is probably not a really useful antibiotic against emerging infectious diseases, it's a great model system for studying how such enzymes work, because we know a lot about its 3-D shape," Donald said. "It should, in principle, be possible to redesign any enzyme." That might include revamping the machinery that makes other workhorse enzymes in gramicidin's family, such as penicillin and vancomycin.

Vancomycin is considered an antibiotic of last resort for patients infected by methicillin-resistant germs. And Donald's group has now begun to study the proteins involved in its synthesis inside the bacterium Amycolatopsis orientalis. "We believe K* can be used to redesign them too," he said.

Source: Duke University