

AUGUST 16, 2007

Researchers Evolve Artificial Enzymes in the Laboratory

Living cells are not the only place where enzymes can help speed along chemical reactions. Industrial applications also employ enzymes to accelerate reactions of many kinds, from making antibiotics to removing grease from clothing. For the first time, scientists have created a completely new enzyme entirely in vitro, suggesting that industrial applications may one day no longer be limited to enzymes that can be derived from natural biological sources.

HHMI investigator Jack W. Szostak and Burckhard Seelig, a postdoctoral associate in his Massachusetts General Hospital and Harvard Medical School laboratory, show in a paper published in the August 16, 2007, issue of the journal *Nature* the steps they took to create the artificial enzyme, an RNA ligase that catalyzes a reaction joining two types of RNA chains.

“The real achievement of our work,” said Seelig, “is that we have, with very simple methods, made a completely new enzyme - one that you cannot find in nature.”

"The real achievement of our work is that we have, with very simple methods, made a completely new enzyme – one that you cannot find in nature."

- **Burckhard Seelig**

Enzymes are already widely used for research and commercial purposes, with many other potential applications under study. Chemical and pharmaceutical synthesis often relies on enzymes, as do “green” technologies such as those used to clean up chemical and oil spills and other environmental hazards. The ideal enzyme for such applications cannot always be found in nature, however, and in these cases, scientists would like to turn to artificial enzymes. For example, therapeutic applications might include enzymes that degrade commonly abused drugs or that replace missing enzymes in the case

of metabolic diseases.

“When you look at the complex and beautiful structures of natural enzymes, it can be hard to imagine how the first enzymes evolved,” said Szostak. “However, our experiment suggests that small, simple enzymes can evolve rather easily.”

So far, most scientists experimenting with artificial enzymes have started with naturally occurring enzymes, and used genetic mutation and screening to evolve variants that work under different conditions or catalyze a slightly different reaction. In a few cases, computationally intensive molecular modeling has been used to design new enzymes, placing amino acids in carefully selected positions on a scaffold protein. This process creates active enzymes that can be subsequently optimized by mutagenesis and screening.

However, these model-based approaches require a detailed understanding of an enzyme's structure and catalytic mechanism that researchers do not always have. Even when they do, computational design remains a difficult art.

Szostak's approach relies instead on evolution. The technique enabled the researchers to generate a new RNA ligase without any pre-existing model of how it would work. According to Szostak, “There is no known biological enzyme that carries out this reaction.”

To create one, the researchers assembled a library of 4 trillion small protein molecules - each with slight variations on an initial protein sequence -- then subjected those molecules to evolutionary selection in the laboratory. “Here,” Szostak says, “the hard work is in designing a good starting library, and an effective selection process. Since we do not impose a bias on how the enzyme does its job, whatever mechanism is easiest to evolve is what will emerge.”

The enzyme that emerged from the group's experiments is what Szostak characterizes as “small and not very stable, and not very active compared to most biological enzymes.” Nevertheless, Szostak's group is optimistic about their ability to select for versions of the enzyme that are more stable and more active.

Szostak's and Seelig's successful creation of an enzyme in the laboratory was the culmination of a long series of developments, beginning 10 years ago with the development of messenger RNA-display, which binds proteins to the messenger RNA molecules that encode them. Szostak could then select proteins with certain characteristics, such as binding to a specific target molecule, and produce more of that protein. Six years ago Szostak's team described the first isolation of a completely artificially evolved non-biological protein, an ATP-binding protein. Three years ago his group published the first of several papers on the optimization of that new protein. “We have been working on the enzyme evolution project for the past five

years,” says Szostak, “and it has been a long hard struggle.”

Szostak says the next step is to optimize the new RNA ligase enzyme. The goal, he says, is to “see how much we can improve it, and also to try to get a nice stable version that we can use to solve the three-dimensional structure of the enzyme. We think we can get even better activity - another thousand-fold improvement might be possible.”

If this works out, he says that the next challenge will be to pick an enzyme activity with an interesting application, and try to create it. He says, “I am sure that future improvements in the technology will make this process faster and easier. I hope that eventually many more people will get involved in the evolution of new and useful enzymes.”