

Improving evolution

By combining elements of protein engineering and directed evolution, researchers open the door to creating enzymes with diverse catalytic functions in a protein scaffold of their choice.

Evolution is a very powerful process that has yielded the incredible diversity of modern biological species. And yet, some scientists feel there is room for improvement, Hak-Sung Kim from the Korea Advanced Institute of Science and Technology being one of them. His interest originated in protein engineering; he said, "I wanted to use protein engineering technology to understand protein function in more detail." This interest in turn led to the idea that a combination of rational design and directed evolution should be an excellent way of better understanding what evolution has brought forth, or even surpassing it and creating a protein with new catalytic functions.

In a recent article in *Science*, Kim's team describes this approach with the acronym SIAFE: simultaneous incorporation and adjustment of functional elements. As their proof of principle, they chose two members of the metallohydrolase enzyme family that are only distantly related, with similar overall structure but little sequence similarity and no common function: the bacterial metallo β -lactamase, an enzyme that cleaves the β -lactam ring of common antibiotics thus making the bacteria resistant to these antibiotics, and human glyoxalase II (gly II) an enzyme catalyzing the hydrolysis of the thiolester bond of S-D-lactoylglutathione, a critical step in the conversion of cytotoxic 2-oxoaldehyde into 2-hydroxycarboxylic acid. The goal of the researchers was to engineer the catalytic activity of metallo β -lactamase into the scaffold of gly II.

In a first step Kim's team used rational design and removed all sequences from gly II that would sterically hinder the binding of the β -lactamase substrate. As a result, they obtained an inert scaffold with no catalytic activity. They then substituted certain amino acids to create the desired metal binding sites. This left only the cata-

lytic site—the key to the new function—to be engineered. At this step Kim introduced directed evolution into the method. By choosing catalytic site sequences from the metallo β -lactamase family and fusing them to the gly II scaffold by overlapping, error-prone PCR the scientists created various mutated versions of the gly II scaffold with the metallo β -lactamase active site. They then screened these newly created enzymes for their ability to confer antibiotic resistance to *Escherichia coli*. To further improve the catalytic ability of the positive clones, Kim subjected them to multiple rounds of directed evolution, increasing the selective pressure after each step. The end result was an engineered enzyme with the human gly II scaffold and a 160-fold improved metallo β -lactamase activity.

As a proof of concept this is an impressive improvement, if not as a practical application—certainly, nobody will be enthusiastic about bacteria that have a much improved resistance to penicillin and other common antibiotics. What people will be excited about, however, is an approach that allows the introduction of a desired enzyme activity into any protein scaffold of their choice. The possibilities are only limited by the imagination of the researcher, as Kim points out: "This approach can be applied to other protein scaffolds as a means to create a large variety of new catalytic lineages performing diverse reactions."

An even more intriguing possibility than improving the activity of known enzymes is the prospect of creating something entirely new, an activity that has not yet had cause to evolve. Kim speculates, "We might be able to design new hydrolases cleaving C-N or C-S bonds of some chemical compounds that have not been seen in a natural system before." There is a good chance that they will beat evolution to it.

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RESEARCH PAPERS

Park, HS. *et al.* Design and evolution of new catalytic activity with an existing protein scaffold. *Science* **311**, 535–538 (2006).