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Making Stronger Proteins that Can Tackle Tougher Jobs

Proteins – the building blocks of life – are sensitive beasts. Heat them a few degrees, or otherwise stress them, and they tend to fall apart. Now, Howard Hughes Medical Institute investigator James Bardwell reports a new method for strengthening proteins, with a diverse range of potential applications including improved laundry soap, better nanotechnology, and less expensive cancer drugs.

The advance could also speed basic protein research, says Bardwell, a professor at the University of Michigan. “You’d be amazed how many graduates students beat their heads against the wall trying to make enough proteins for experiments,” he says. “Scientists tend to study the proteins that are easy to make, just for practical purposes. But that excludes a huge number of really interesting proteins.” About half of human proteins are too fragile to be easily made in the lab. For other important organisms, it can be worse -- the number soars to 90 percent with the malaria parasite. “It really does slow progress,” Bardwell says.

In a research article published December 11, 2009, in *Molecular Cell*, Bardwell and co-authors detail the new technique and their success in strengthening proteins. Bardwell credits graduate student Linda Foit for developing the system and his collaborators in England, Sheena Radford and Gareth Morgan, for measuring exactly how strong the proteins became.

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Besides drawing motivation from his own protein-making frustrations, Bardwell’s interest was piqued when his daughter’s daycare teacher came down with Hodgkin’s disease, a type of cancer, and the daycare initiated a

fund-raising drive to pay for Neupogen, an expensive protein-based cancer drug. Neupogen costs about \$1 million a gram “in part because it’s so unstable,” says Bardwell. Finding a way to make a more stable version of Neupogen, and other expensive protein drugs, could drive down manufacturing costs. “If these proteins were easier to make, then a lot more people would have access to them,” Bardwell says.

Laundry detergent enzymes that dissolve stains are also very big-selling proteins. Making more resilient enzymes, which are protein-based catalysts, could improve the cleaning power of detergents. Stronger, more resilient proteins might also boost the nascent nanotechnology industry, which aims to build tiny machines out of proteins. Right now, Bardwell says, nano-motors and other tiny devices made from proteins are often as sturdy as “paper clips made from noodles.”

The method Foit, Bardwell, and their collaborators developed exploits a clever, time-saving trick that enables billions of lab-based bacteria to sort the strong from the weak. Step one is the clever trick: Insert an unstable protein into the middle of another protein, called β lactamase, which confers antibiotic resistance to bacteria. Step two: Randomly mutate that protein with a technique called error-prone PCR. If the inserted protein remains intact, then β lactamase works properly, rendering its host bacteria resistant to the antibiotic penicillin. But if the inserted protein is unstable and it gets digested by cellular proteases, the two halves of β lactamase fall apart and it no longer functions. The bacteria with digested β lactamase—and hence, unstable protein variants—die when exposed to penicillin. “Only the nicely folded proteins will generate bacteria that live, and everything else will die,” Bardwell says.

Finding strong proteins then becomes as simple as spreading all the bacteria – each with a different protein and β lactamase combination – on a plate, exposing them to antibiotics, and heading home for the night. “You come in the next day, and you pick off the bacteria that have lived, which are easy to see because they’ve grown into colonies,” says Bardwell. “Then you look to see what changes happened in that protein that made it stronger.”

Increasing the concentration of antibiotics selects for stronger and stronger proteins. Running a series of experiments with different antibiotic concentrations results in a series of ever-stronger proteins.

There is a caveat: Each protein variation differs slightly from the original, which may hinder its function. For drug development, that means each new variation would have to go through the entire series of FDA-required clinical trials. However, Bardwell says he is working on engineering bacteria to produce the original protein in larger quantities.