A new way to synthesize 'natural' proteins

Nature seems to do it so effortlessly — forge proteins, that is.

These complicated bundles of amino acids, elegantly aligned to fold into three-dimensional sculptures of exact proportions, perform essential biological tasks. Yet synthesizing proteins has proved extremely difficult.

Chemists have likened the process to building a minuscule house in the dark with molecule-size bricks.

Now, Stephen B.H. Kent, a biochemist at the Scripps Research Institute in La Jolla, Calif., and his colleagues report a new technique for fashioning proteins in the laboratory. Their report of "native chemical ligation" appears in the Nov. 4 SCIENCE.

"Making large peptides or proteins is very hard to do if you try to build them one amino acid at a time," says Philip E. Dawson, a chemist at Scripps and a coauthor. "We're good at making peptides with 50 to 70 amino acids. But if you want to make a protein with 100 to 200, even 300, amino acids, you need to be able to join larger units together."

Recent attempts at building bigger proteins have involved several types of chemical ligation, whereby chemists link up peptides, or protein fragments, in series, says Dawson.

Trouble can arise in the links, though. Synthetic methods typically use "unnatural" bonds, rather than the types normally found in proteins, to glue chains of amino acids together.

With this new method of protein synthesis, the bonds holding together the protein's key components resemble nature's own — hence the phrase "native chemical ligation."

To exemplify the new procedure, Kent and his crew cobbled together two large peptides — one with 33 amino acids and the other with 39 — to create an exact replica of human interleukin-8, an immune system protein with 72 amino acids.

This technique should work for any series of amino acids or any protein, says Dawson. "So the next step is to generalize this work and apply it to many kinds of biological problems to learn more about how enzymes work.

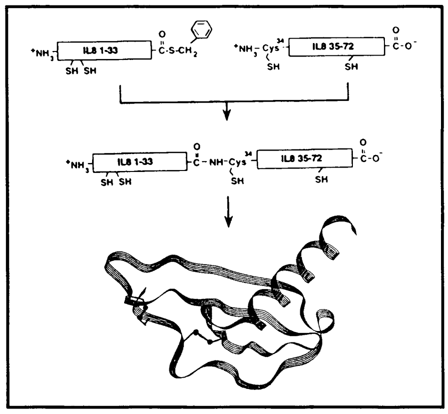
"In this paper, we showed how to connect two peptides," Dawson adds. "But in theory, this technique can join four or five peptides in series, which would allow someone to make larger proteins."

In time, this ligation technique will help scientists probe enzyme function. "To really understand how a protein or enzyme works, you have to make small changes in its structure and observe the consequences," Dawson says. "The finer the changes, the more carefully you can tune your experiments."

Tom W. Muir, a biochemist at Scripps, points out that this new way of joining peptides will augment another, DNA-based tool for making proteins. "There are things that this method can do that DNA recombination technology can't do and vice versa. So the two techniques complement, rather than compete with, each other."

"Many proteins in nature are made of well-defined [pieces]," says Muir. By using this method to link together those pieces, researchers will be able to synthesize types of proteins "that weren't possible before." — *R. Lipkin*

Using native chemical ligation, chemists link two peptides to yield interleukin-8.



Dawson *et al.* /SCIENCE

Small amounts go down for the count

For the past century, researchers have noted that people can quickly and accurately report the number of a small set of items — up to about four — without explicitly counting them. But it remains unclear whether this process, known as subitizing, relies simply on speedy counting or on brain processes that operate outside the realm of mathematics.

A new study supports the latter view. It finds that one type of brain damage disrupts the ability to count four or more objects but spares accurate numerical estimates of up to three items. The key to subitizing may lie in several networks of brain cells that work simultaneously to isolate and keep track of objects in a visual scene, argue Stanislas Dehaene of the National Center for Scientific Research and Laurent Cohen of Salpêtrière Hospital, both in Paris.

"These patients suffer from a fundamental inability to use spatial tags to keep track of previously explored locations," the two psychologists conclude. "Counting [is] virtually impossible for them, suggesting that their preserved subitizing abilities are not based on serial counting."

No consensus currently exists on how, or whether, subitizing relates to more complex mathematical feats. Experiments suggest that infants (SN: 8/29/92, p.132) and some nonhuman animals (SN: 5/23/87, p.334) calculate small quantities by employing either basic counting skills or nonmathematical subitizing mechanisms.

Dehaene and Cohen studied five brain-damaged adults suffering from a condition known as simultanagnosia. These individuals recognize single objects in a scene, but they lose track of an object after scanning it and fail to perceive the scene as a whole. They also find it difficult to count items presented visually.

Five healthy adults also took part in experiments.

In a series of trials, each volunteer viewed white rectangles flashed on a computer screen for one-fifth of a second. These rectangles contained from one to six smaller black rectangles, half the time arrayed in random patterns and half the time in patterns such as those found on dice.

Participants also completed "visual search" tests of their ability to scan from one to six rectangles for changes in color and orientation.

All five patients performed well, and in several cases nearly flawlessly, when quantifying sets of one, two, or sometimes three rectangles, Dehaene and Cohen report in the October JOURNAL OF EXPERIMENTAL PSYCHOLOGY: HUMAN PERCEPTION AND PERFORMANCE. But the same individuals nearly always erred when shown four or more rectangles, they contend, indicating a retention of subitizing skills and loss of more complex counting abilities.

Responses to visual search tasks indicated that patients suffered mainly from an inability to keep track of already viewed objects.

Patients subitized equally well in response to random and systematic patterns of rectangles. Characteristic arrangements of one, two, or three objects apparently do not foster accurate subitizing, the scientists argue.

Numerical distinctions between sets of one or a few items may still play a role in the type of quantification displayed by these brain-damaged patients, holds Karen Wynn, a psychologist at the University of Arizona in Tucson.

"Dehaene and Cohen's evidence looks convincing for a parallel visual process that keeps tabs on small numbers of items," Wynn remarks. "But subitizing also requires the activation of a correct numerical concept for those items." — *B. Bower*