

is buried. Researchers release the electrons by heating the sample, then measure the intensity of the brief glow produced to estimate the number of freed electrons.

The team found the artifacts in sediments they dated at between 116,000 and 176,000 years old. And, says co-author Head, a chunk of boulder bearing the circles lay in a stratum dated to between 58,000 and 75,000 years old. That would make the circles perhaps twice as old as the oldest known human art, the cave paintings at Chauvet in France.

All this has major implications for such issues as the definition of "modern" humans, because the ancient people must have boated to Australia from Indonesia—a feat that required technology and social organization, says Head. It also bears on how and where humans arose. Many researchers follow the "Out of Africa" theory, that modern humans arose in Africa, then spread across the globe sometime after 100,000 years ago, reaching Australia 40,000 to 60,000 years ago. Others, however, suggest that *Homo sapiens* evolved from precursors spread around the world, and the Jinnium dates add evidence for this "multiregional" hypothesis, says University of Michigan paleontologist Milford Wolpoff. "This kind of finding makes ultimate sense to me," he says.

But such conclusions depend on the dates, and TL dating is problematic because researchers can never be sure when the sample's "clock" started ticking. For example, a grain broken off from a long-buried chunk of sandstone might be mixed with the artifacts at the time they were buried. Because the sandstone's clock would already be running, it could make the artifacts seem much older than they are. Co-author Price defends the validity of his team's TL dates, noting that radiocarbon dates of charcoal fragments in the upper layers of the Jinnium site match the TL dates. But at the deepest levels, there are no controls such as bones or teeth that can be dated by other methods. "I can't imagine anyone calling this Australian Stonehenge or Australian anything at this stage," says archaeologist Henry Schwarcz of McMaster University in Ontario, Canada.

Still, earlier dates have been turning up for other Australian sites, notes anthropologist Alan Thorne of Australia National University. More data may come in soon, as Jones and Richard Roberts of La Trobe University in Melbourne are dating the Jinnium samples with a slightly different dating method, called optically stimulated luminescence (OSL). This method is similar to TL, but may be more reliable because the clock used is more likely to be reset to zero by even brief exposure to light, says Schwarcz. If the OSL dates confirm the TL ages, then the scientific debates will begin in earnest.

—Constance Holden

PROTEIN CHEMISTRY

Between the Sheets: Why Do Protein Strands Line Up?

The three-dimensional structures of proteins are as different as the sentences in a language. But just as different sentences share the same set of words, protein structure has a standard vocabulary: of helices, U-turns, and so-called β sheets. To understand how protein chains fold up into their complex structures and mimic them to create new drugs and materials, researchers have to master that basic vocabulary. And that means learning how the properties of individual amino acids—the letters that spell out a protein—determine which structures form as a protein folds up into its final shape.

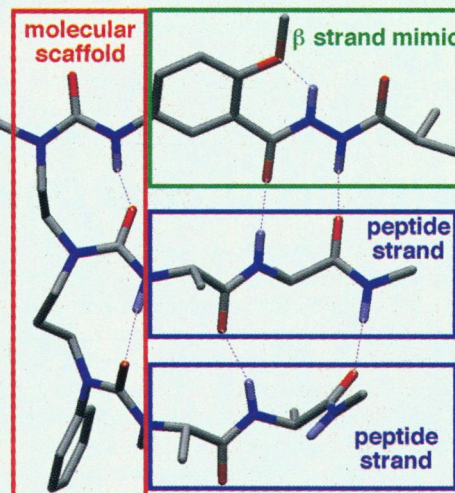
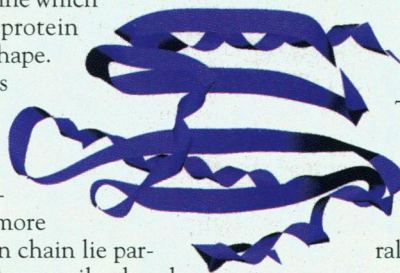
By now researchers know most of the rules governing the formation of helices and U-turns. But β sheets—regions where two or more segments of the protein chain lie parallel to each other, like pencils placed side by side—have been more reluctant to give up their secrets. And so, in a growing subfield of protein chemistry, researchers are learning by doing: making their own small β sheets or disrupting natural ones in hopes of learning the key factors that hold them together.

One of the latest milestones in this effort came in late August at the American Chemical Society (ACS) meeting when researchers at the University of California, Irvine (UCI), reported designing a molecular scaffold that holds a trio of protein strands in a sheetlike arrangement—the largest artificial β sheet to date. By changing the chemical makeup of the strands to see whether the artificial sheet unravels, the UCI researchers hope to learn more of the principles of β sheet formation. Other researchers are taking a different approach toward reading the same rulebook: isolating the parts of natural proteins that fold into β sheets and systematically varying the amino acids at particular positions to see how the changes affect the fragment's sheet-forming ability. With researchers now beginning to reap the benefits of these efforts, "this field is really at the mountaintop right now," says Jeffrey Kelly, a chemist and β sheet expert at Texas A&M University in College Station.

To look at a β sheet's simple arrangement of parallel chains, you might not think it would hold many mysteries. But although the neighboring strands in a sheet some-

times lie adjacent to each other along the linear protein chain, simply connected by a sharp hairpin turn, more often they are from distant regions of the protein. They only become neighbors when the protein folds into its final structure and weak hydrogen bonds form between the strands, locking them together. As a result, few such sheets can simply be clipped out of a protein and studied separately. "That's what makes these things so hard to study," says James Nowick, who led the Irvine team.

But in a trio of studies over the last 2 years, Peter Kim and his colleagues at the Massachusetts Institute of Technology's Whitehead Institute analyzed one fragment of an immunoglobulin-binding protein known as G(GB1) that does naturally wrap itself into a β sheet.



Designer sheets. Parallel strands make up β sheets in the thioredoxin protein (top) and a model compound (above).

They genetically engineered bacteria to produce variants of the fragment in which one or more of the sheet-forming amino acids had been altered. They then looked at the structures of the resulting proteins to see whether the β sheets formed normally or not—and what amino acid properties were key to the difference. So far, they have learned that context is crucial: Amino acids such as valine and isoleucine, for example, seem to play a strong role in holding β sheets together when they are located in the middle

of the sheet. But when they lie near the edge of the sheet, changing them has little effect on sheet stability.

Instead of relying on natural sheets to learn the rules of β sheet formation, the scaffold-builders position peptide strands side by side in an artificial scaffold, then see whether the strands hydrogen-bond into a sheet. And although Kelly admits that "peptide model systems are not absolute mimics of proteins," he and other model-builders maintain that the models make up for that drawback, because peptide strands can be chemically altered in ways that natural proteins cannot.

In 1994, for example, Kelly and his Texas A&M colleagues built scaffolds containing hydrophobic chemical groups—groups that flee from water's presence in the surrounding solution. The A&M researchers then used the scaffolds to anchor a pair of peptide strands, each of which also contained a hydrophobic group near the scaffold. The result, the researchers found, was that the hydrophobic groups clustered together, drawing the peptide strands close and encouraging them to form β sheets. Because this result echoes similar findings that hydrophobic interactions promote β sheet folding in natural proteins, "it suggests that hydrophobic interactions appear to be key for making β sheets," says Kelly.

Nowick and his colleagues have built larger scaffolds that can hold more peptide fragments side by side. At the ACS meeting, Nowick's graduate student Eric Smith told researchers that they had built a scaffold able to hold two peptide strands next to a so-called β strand mimic—a rigid, rodlike chemical group that forms hydrogen bonds with its flexible peptide neighbor to hold it in an elongated sheet formation. And because the second peptide strand formed similar hydrogen bonds with the first, all three strands ended up woven into a β sheet.

The group is now varying the amino acids in the peptide strands to tease out more secrets of β sheet formation. And to speed such comparative studies, the Nowick group has developed a technique for mass-producing variants of these β sheet mimics. Using standard "combinatorial chemistry" techniques, they attach their molecular scaffold and β strand mimic to each of thousands of polymer beads. They then split the beads into batches and vary the peptide strands that they add to each batch (*Science*, 31 May, p. 1266). "This allows us to juxtapose different amino acids next to one another to see how it affects the structure and stability" of the sheets, says Nowick.

Even before they have a complete book of rules for making β sheets, these chemists are thinking ahead, to how they might tailor their molecules to make them useful as everything from novel materials to drugs. In an article

earlier this year in the journal *Macromolecules*, for example, Kelly and his colleagues reported that by changing pH, they could coax their two-strand β sheets to line up and link side to side. The result was long fibrils with a molecular architecture resembling that of portions of natural rubber or silk. These first fibrils had little of the strength or flexibility of the natural materials, but Kelly says that he and his colleagues are now trying to intersperse segments of their β sheet fibrils with regions of standard polymers in hopes of improving their properties.

Kelly and others are also thinking about how they might exploit the rules of β sheet

formation in a possible treatment for Alzheimer's disease. Researchers suspect that runaway β sheet assembly could be what causes "beta amyloid" proteins to agglomerate into dense plaques in the brains of patients with Alzheimer's disease. By engineering β sheet-forming compounds that could bind selectively to the amyloid proteins and block other amyloids from binding, Kelly, Nowick, and others hope to come up with a compound that might arrest plaque formation. If such efforts succeed, artificial β sheet-makers will have passed their language lesson with top marks.

—Robert F. Service

CLIMATE

Ice-Age Rain Forest Found Moist, Cooler

What happens to a hot, wet tropical forest when an ice age grips the world? In the case of the Amazon rain forest, the answer is crucial to two long-running scientific debates, one in climatology and one in evolution. Climate researchers have argued for years about whether the tropics cooled a little or a lot during the height of the last ice age 18,000 years ago (*Science*, 17 February 1995, p. 961). Their interest is more than academic curiosity, for the answer will offer clues to the sensitivity of Earth's climate system to the strengthening greenhouse effect.

On an entirely different front, evolutionary ecologists have squabbled for decades over how the Amazon flora and fauna became so stunningly diverse. One long-standing theory has suggested that glacial drying shrank the Amazonian rain forest to a few isolated patches surrounded by dry grasslands. New species arose in these refugia, then spread outward when the climate changed again and the rain forest expanded. But other biologists counter that there is precious little direct evidence of refugia—and plenty of alternative ways to generate high diversity.

Now, a single study of a couple of meters of lake mud from deep within the Amazon rain forest sheds new light on both of these controversies by pointing toward a cool but still wet ice-age Amazon. On page 85 of this issue, pollen specialist Paul Colinvaux of the Smithsonian Tropical Research Institute in

Panama and his colleagues present fossil pollen evidence that tropical South America cooled a lot—5° to 6°C—during the last ice age, but remained relatively wet and as densely forested as ever. This undercuts the role of ice-age refugia in Amazonian speciation and implies a relatively sensitive climate system. Researchers debating glacial climates welcome this first long pollen record from deep within Amazonia, but urge caution in interpreting data from a single site. "It's provocative," says limnologist Kerry Kelts of the University of Minnesota—but a lone pollen core isn't likely to end either of these protracted disputes.

Even getting this much data was a struggle. Colinvaux has been working for more than 10 years to get ice-age temperature and ecological data from a single spot deep within the Amazon lowlands, which comprise an area nearly the size of the continental United States. The problem was finding a place where sediment was deposited continuously, such as a lake, that had been around since the height of the ice age. "It's a classic needle-in-the-haystack problem," says Colinvaux. "There's lots and lots of water, but which is an ancient lake?"

Colinvaux's eventual answer was Lake Pata, one of several lakes nestled on a low rise just north of the equator in northwestern Brazil. The site is remote—he and his colleagues carried coring equipment and rubber



Window on the past. The pollen record buried in the mud of this lowland Amazonian lake reveals a cooler, moist ice-age climate in the tropics.