CRYSTALLOGRAPHY

New Math Speeds the Search for Protein Structures

An old approach is yielding new recipes for turning x-ray snapshots into 3D maps of proteins in days instead of months

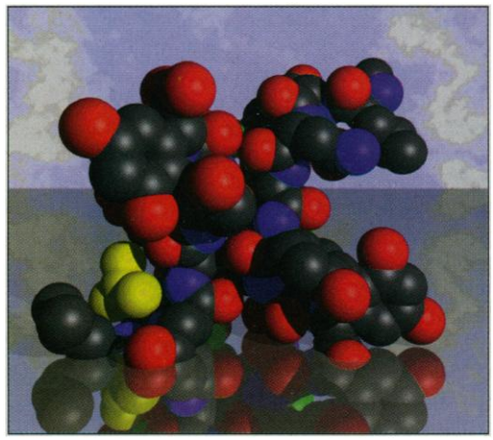
"Fred's Folly" was a contraption that would have made Alexander Calder proud—a box the size of a refrigerator in which a model of a molecule could be neatly strung on piano wire and springs. Designed by Yale University crystallographer Frederic Richards and widely used in the 1970s, the gadget allowed crystallographers who had mapped the atoms in a giant molecule to actually see what it looked like, by tinkering with a molecular mobile until its shadow matched drawings from the data. Visualizing molecules has gotten a lot easier with computer graphics, but the process of figuring out where the individual atoms go is still a laborious enterprise.

The recipe hasn't changed much over the past 2 decades. In one classic method, the molecule—a protein, say—is crystallized and probed with x-rays, then garnished with atoms of heavy metals and probed again. Only then can investigators interpret the x-rays to work out how the thousands of atoms are arranged in space. Now radically simpler recipes are in sight, at least for some molecules.

Two new algorithms, one called Shake-and-Bake and the other—for the moment—Half-Baked, offer a way to look at a single high-resolution x-ray snapshot of a crystal and reconstruct in one gulp how its many atoms are positioned. The basic approach, called direct methods, has been around for decades, but large molecules defeated it in the past. At a recent meeting,* however, researchers reported that the new programs are already helping to unravel full-sized protein structures. And instead of taking years, as other methods do, the new algorithms cracked the structures in a matter of days.

Fittingly, one of the reports comes from Herbert Hauptman, a mathematician who pioneered direct methods and shared the 1986 Nobel Prize in chemistry for the work. Hauptman, president of Hauptman-Woodward Medical Research Institute Inc. in Buffalo, New York, and colleagues have now translated the direct methods idea into a new mathematical language of sorts, and crafted Shake-and-Bake (see www.Hwi.buffalo.edu/SnB). Shake-and-Bake is making short work of test proteins such as lysozyme, which contains about 1000 nonhydrogen atoms. And inspired by Shake-and-Bake, George Sheldrick and colleagues at the University of Göttingen, Germany, have cooked up—although not completed—a similar program called Half-Baked. They have used it to crack a protein with over 2000 atoms and work out several key antibiotic structures.

"It's absolutely incredible," says Suzanne Fortier, a crystallographer at Queens University in Ontario, Canada. "The success of this work is, I believe, a bit of a surprise to everybody." The work has also rekindled interest in a dying discipline. Instead of attracting a few die-hards for a nuanced mathematical debate, the direct-methods session at this summer's meeting was standing room only.



Flash photography. New techniques quickly revealed the structure of the antibiotic vancomycin.

To the uninitiated, trying to figure out a molecule's structure from a diffraction pattern looks about as promising as trying to predict the future by staring at tea leaves. When x-rays pass through a crystal and scatter off the electrons of atoms in the regularly arrayed molecules, they emerge as a constellation of bright dots. Inferring the positions of atoms in the molecule from the intensity and location of those dots is extraordinarily difficult because the dots lack a crucial clue: the "phases," or the relative positions of the crests and troughs, of the x-rays at different dots. Only by knowing the phases can researchers trace the waves back into the crystal to pinpoint the planes of atoms that scattered them.

Introducing heavy atoms can break this impasse because the atoms change the diffraction pattern, giving away their own positions in the crystal. With the x-rays scattered from these atoms as reference points, crystallographers can bootstrap their way into the rest of the molecule, figuring out one phase in the diffraction pattern, then another, and finally revealing the whole structure. But the process can take years and "umpteen" crystals, says Cornell University crystallographer Ashley Deacon.

Direct methods offer a faster route by using computers and equations to puzzle out structure from just a single diffraction pattern. Crudely speaking, the algorithms try different arrangements of atoms, simulating the diffraction patterns they would produce to find an arrangement that makes the observed pattern. Because there is an infinite number of possible configurations, the algorithms have to be clever about how they search. "It's a bit like playing golf, except you don't know where the hole is because someone has removed the flag," Sheldrick explains. "And there are bunkers ... pseudo-solutions you can get trapped in."

As a result, the technique, conceived in the late 1940s, was limited to molecules containing no more than a couple of hundred atoms. "Only once, and with great effort, did we solve a 300-atom structure," says Hauptman. Proteins, which can contain tens of thousands of atoms, were far out of reach until now.

The essence of the new algorithms is that they jump between adjusting or "shaking" the phases, which gives a distribution of atoms, and "baking" in the positions that seem most likely. By shaking and baking repeatedly, they converge on the complete structure. The procedure somehow avoids the bunkers and finishes up with the ball in the hole. "The whole theory is pretty obscure," Sheldrick says. "We don't really understand why it works so well."

Sheldrick, Hauptman, and others caution that the new methods only work for molecules that can be coaxed into producing precise diffraction patterns, with a resolution of about an angstrom—about the size of an atom. Making these precision pics typically requires an unusually high-quality crystal—difficult to get for some molecules, especially large ones containing 10,000 atoms or more. "Currently only about 10% [of large molecules] are suitable," Sheldrick estimates. Making precise diffraction patterns also usually requires the brilliant x-rays available only at the world's few synchrotron accelerators.

Improved techniques and new synchrotrons could bring more molecules into range of the direct algorithms, he says. But in

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* American Crystallographic Association Annual Meeting, 18–23 July, Arlington, Virginia.

the meantime, many crystallographers say, the best hope for figuring out the structures of large proteins lies in combining direct methods with another technique called MAD (for multiwavelength anomalous diffraction) phasing, which has already gone a long way to speeding protein structure determination.

MAD phasing, developed in the early 1990s, works on modified proteins made by inserting the gene for the protein into *Escherichia coli* bacteria, then typically feeding the bacteria a version of the amino acid methionine whose sulfur atom is replaced by a heftier selenium atom. The bacteria incorporate these selenomethionine amino acids into the protein. When synchrotron x-rays are shot through the crystallized protein, they scatter strongly off the heavy selenium atoms. By studying how the diffraction pattern changes as the wavelength of the x-rays is varied, crystallographers can tease out the position of the selenium atoms, which then provides an edifice for determining the phases, and hence, structure of the protein. The procedure resembles the heavy-atom approach, but because it riddles the protein with more precise atomic landmarks, MAD phasing can be done with a single crystal.

Despite MAD's many successes, the technique has bogged down for large molecules, where the positions of many selenium atoms (more than 15 or so) need to be determined. Shake-and-Bake offers a quick way to map large numbers of selenium atoms, Hauptman points out. And for this task, coarse diffraction patterns are often good enough, as the selenium atoms are usually far apart in the molecule. At the meeting this summer, researchers from Cornell University reported using Shake-and-Bake to locate some 65 selenium atoms in a MAD data set, which enabled them to pin down some 25,000 atoms in the structure of a large enzyme that regulates antibiotic uptake in some bacteria. That structure is twice as large as any previously done with MAD, says Cornell's Deacon.

"The beauty of the method is things can happen very quickly," he says. "Once you have the protein and crystal, you can collect data and have the structure in a few days." So far only a few groups have followed suit, however. Says Deacon: "People probably don't realize the magnitude of what can be achieved with this." **–DAVID KESTENBAUM**

ECOLOGY

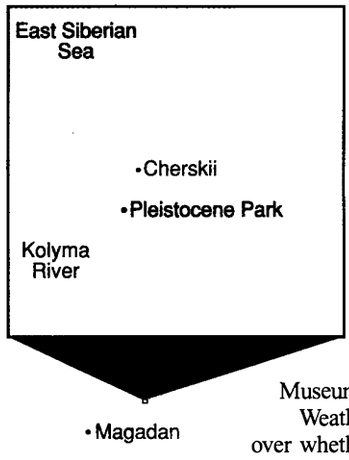
A Bold Plan to Re-Create a Long-Lost Siberian Ecosystem

An international team of scientists will test whether bison, horses, and other large grazers can bring back the mammoth steppe

CHERSKII, RUSSIA—Like a frog hopping from lily pad to lily pad, Sergei Zimov strides from one tussock to the next, wobbling for a moment on each sedge knob rooted in the sodden permafrost. Occasionally he misjudges a tussock's firmness and his leg disappears up to the knee into the marsh water. Within minutes, Zimov has reached higher ground and a carpet of mosses and lichens, birch bushes and scattered larches—hallmark features of a mixed tundra-taiga landscape that dominates much of this region above the Arctic Circle. It is a starkly beautiful, wild land, permeated with the fragrance of alpine sage. Zimov, however, wants to see it torn up and populated.

Zimov is no Soviet-style planner intent on draining the marsh and putting up drab high-rises: He's an ecologist and director of a lonely science outpost in the northeasternmost reaches of Russia. He points to a tangle of birch and willows several dozen meters away, where two of the agents he hopes will carry out his grand scheme are picking their way across a ridge. They are young male Yakutian horses—off-white and pepper-flecked, the color of snow near a Moscow highway. Zimov envisions dozens of these horses, along with moose, reindeer, and a herd of bison imported from Canada, ripping up the moss and shrubs with their hooves and teeth, allowing grasses to move in. Within a few years, he hopes, grazing animals will have supplanted the current ecosystem in a 160-square-kilometer preserve with a grassland resembling one that existed here during the last Ice Age. The idea is to reconstruct a small chunk of the mammoth steppe, a vibrant ecosystem that dominated much of Siberia before vanishing after the Pleistocene epoch ended 11,000 years ago.

In creating what Zimov calls "Pleistocene Park," he, two U.S. ecologists—Terry and Mimi Chapin, a husband-and-wife team at the University of Alaska, Fairbanks—and a group of Canadian and Russian wildlife biologists are embarking on an ambitious experiment that aims to test theories about the forces that shaped, maintained, and ultimately vanquished a long-gone ecosystem. Some experts are calling the experiment a watershed in efforts to study lost ecosystems. "It's a very exciting idea whose time has come," says Paul Martin, who studies Pleistocene extinctions at the University of Arizona, Tucson. The experiment, he predicts, "is going to have a revolutionary effect on how we think about designing nature."



Siberian Serengeti? Site of experiment aiming to restore the Pleistocene-era mammoth-steppe ecosystem.

The project also marks the first attempt to restock Siberia with bison, a species that went extinct in this region at least 2000 years ago. "It makes sense to reintroduce species that have been recently extirpated by human hunting or habitat encroachment," says Paul Koch, a specialist on Pleistocene-era mammals at the University of California, Santa Cruz. "It's just planetary hygiene."

But Koch and others point out that the project's main goal—restoring the mammoth steppe—could be doomed because some Pleistocene elements are impossible to reproduce: the namesake mammoths, of course, and certain climatic features, such as cooler temperatures and less carbon dioxide in the air. "You still don't have analogs for climate," says Russell Graham, curator of vertebrate paleontology at the Denver Museum of Natural History.

Weather is at the crux of the debate over whether Pleistocene Park will succeed. Most experts argue that Siberia in the Pleistocene was much drier than it is today. They point to Pleistocene sediments, which harbor pollen and other remnants of grasses that thrive in dry soil. These grasses, in turn, fed an array of large herbivores, including mammoths, steppe bison, horses, moose, reindeer, and woolly rhinos. Many scientists believe that a sudden and severe climate shift at the end of the Pleistocene—a