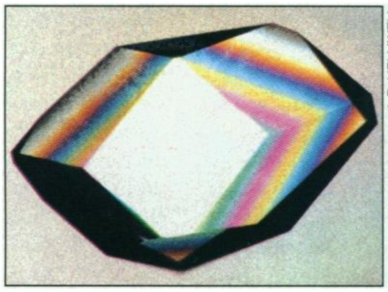
MEETING BRIEFS

Search for Better Crystals Explores Inner, Outer Space

At last month's Sixth International Conference on Crystallization of Biological Macromolecules in Hiroshima, Japan, researchers compared notes on one of the biggest challenges in determining protein structure: growing the high-quality crystals needed for x-ray analysis. Coaxing such crystals to form is still an art, but the meeting saw the beginnings of a more systematic approach. One presentation quantified the advantage of growing crystals in space; another presented techniques for growing better crystals on Earth.

A Heavenly Advantage

Researchers have long suspected that superior protein crystals can be grown in space, but they have never been able to put a number on the improvement. The idea was that the microgravity environment should nearly eliminate the convection and sedimentation that causes flaws, but different groups have reported conflicting results. Now teams at the University of Manchester, England, and the Institute for Crystallography at the University of Karlsruhe, Germany, have shown just how much better high-quality, space-faring crystals can be.


J. R. HELLIWELL

Structured skies. This lysozyme protein crystal was grown last year aboard the space shuttle.

Reporting on data from 1993 and 1994 experiments aboard the Advanced Protein Crystallization Facility operated by the European Space Agency and flown on the U.S. space shuttle, Manchester's John Helliwell said that the resolution of x-ray diffraction for microgravity-grown crystals is dramatically better than for comparable Earth-grown crystals. The latter are actually composites of misaligned blocks of crystal that result in a broadening, or even a splitting, of the x-ray reflections. In space-grown crystals, however, these cells tend to be better aligned throughout the crystal, he said, a feature known as low mosaicity. Reduced mosaicity can improve the signal-to-noise ratio and should result in improved precision in determining crystal structures, Helliwell notes.

"It's really a first: quantifiable findings that show microgravity can make a difference," says Franz Rosenberger, a specialist in crystal growth and director of the Center for Microgravity and Materials Research at the University of Alabama, Huntsville. "These are very important results," adds biochemist Alex McPherson of the University of California, Riverside. The cost of getting into orbit is too high for space-grown crystals to become commonplace, but the results show that crystals can provide Earth-based researchers with a benchmark for measuring the success of their own efforts.

In the first experiment, part of the Spacehab-1 mission, crystals of the protein lysozyme were grown on Earth and in space with identical equipment, conditions, and materials and using what Helliwell calls "standard textbook recipes." An analysis of the diffraction patterns at the Daresbury (U.K.) Synchrotron Radiation Source found that the Earth-grown crystals had a mosaicity three times greater than the space-grown crystals.

The group repeated the experiment last year on the Second International Microgravity Laboratory Space Shuttle and analyzed the data at the new European Synchrotron Radiation Facility in Grenoble, France, which has a beam line with a novel diffractometer of greater precision. The results showed a peak reflection intensity eight times greater for the crystal grown in microgravity, he noted, with the larger size of the space crystal accounting for only part of the improvement. In addition, the intensity curve for the space-grown crystal showed only one peak, suggesting that the crystal was made up of one perfect block, in contrast to the double peaks in some directions for the Earth-grown crystal. The team was able to visualize features of the molecule as small as 1.2 angstroms, roughly the dimensions of individual atoms.

"The mosaicity and diffraction resolution is the best obtained with lysozyme that has been reported so far," says Helliwell, who has a paper in press at *Acta Crystallographica, Section D*. "In essence, we can reproducibly reach the perfect limit."

That sets a standard for Earth-based crystal growers, who hope to minimize the effects of convection and sedimentation sufficiently to approach the quality of space-grown crystals. Helliwell says that such crystals could also serve as references for improving the signal-to-noise ratio of existing diffraction equipment and the apparatus for routine x-ray crystallography.

Greasing the Wheels of Production

Like gems in the window at Tiffany's, space-grown crystals are out of reach for most protein crystallographers. They have to find ways, still mostly trial and error, of doing better on Earth. "The bottleneck in protein structure analysis is growing nice crystals," says Hiroshi Komatsu, a crystal-growth specialist at Tohoku University's Institute for Materials Research and a co-chair of the conference organizing committee. But two groups presented crystal-growing techniques that should boost both efficiency and quality.

Naomi Chayen, a crystal-growth specialist at Imperial College, London, reported on refinements in a technique called microbatch, in which crystals are grown within 1 to 2 microliter drops of a mixture of a protein and a crystallizing agent. In normal batch crystallization, these droplets are left to stand until crystals appear. But typical batch methods can quickly use up a lot of protein material, which is often expensive and scarce, especially when researchers run dozens of samples with varying concentrations to screen for optimal proportions of agent to protein. Using even smaller droplets of solution isn't practical, because the drops dry up before the protein crystallizes.

By covering each droplet with a layer of oil, Chayen and her colleagues found, they could prevent evaporation of the tiny microliter droplets and also protect the sample from contaminants in the air. And that's not all. "When we first developed the microbatch method using paraffin oil, it was an inert way of preventing evaporation," Chayen says. "But the more we use it, the more we realize the oil actually contributes to the experiment."

The group discovered this advantage by accident, as part of an attempt to crystallize an enzyme called β crustacyanin, a protein found in crustacean shells. Another group had failed with two other crystallization techniques, so Chayen's group tried their new microbatch technique. After waiting 2 months for any sign of crystal growth, her team put aside the experiments. But 4 months later, when cleaning out the cabinets, Chayen found the samples had crystallized.

Subsequent attempts to speed up the process proved futile, leading Chayen to conclude that these crystals had grown because of the slow evaporation of solution through the oil layer. Different evaporation rates might favor the growth of different proteins. And the oil-based technique offers a way to tune the evaporation rate by carefully selecting the type and quantity of oil.

Whatever the crystallization procedure, finding the right initial concentration requires crystallizers to set up dozens or even hundreds of samples, each with a slightly differing concentration. Juan García-Ruiz, of the CSIC-University of Granada, Spain, hopes to bypass this tedious process by varying the protein concentration along the length of a capillary tube using what he calls the gel acupuncture technique.

The technique involves inserting a protein-filled capillary tube into a layer of gel placed in the bottom of a vessel. Crystallizing agent is then poured into the vessel. The agent diffuses down through the gel and up into the capillary, mixing with the protein. The gel serves to hold the capillary, and the depth of penetration can be varied to control how quickly the agent diffuses into the protein. The diameter of the capillary minimizes convection. And if sedimentation of the growing crystals is a worry, sealing the vessel and placing it on its side prevents developing crystals from sliding down the tube.

As the crystallizing agent diffuses through the protein, it creates a gradient of concentrations. The hope is that the gradient, at some point, includes the condition of supersaturation that initiates crystal growth. "Unlike other crystallization methods, where you only have one set of conditions in one experiment, [in gel acupuncture] you can scan many, many different conditions of solubility," he says.

García-Ruiz has obtained crystals of several different proteins, some big enough to fill the capillary tube. He believes this approach could be particularly useful for growing larger protein crystals, if he can find a way to control the wave of supersaturation as it travels along the length of the capillary. One possibility is varying the initial conditions so that the speed of the supersaturation wave is slowed to match the rate of crystal growth. In theory, the result would be crystals stretching from the point of initial nucleation to the end of the tube.

But that's a long way off. "We don't know yet how to do it," García-Ruiz confesses. Indeed, Komatsu says that crystallographers are constantly searching for "the guiding principle" of crystal growth. In the meantime, he says, any improvements in trial-and-error methods are "very important."

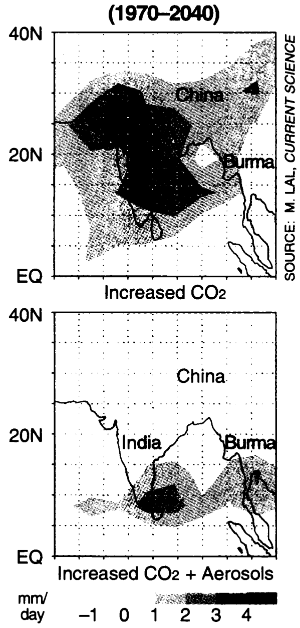
–**Dennis Normile**

CLIMATE CHANGE

Monsoon Shrinks With Aerosol Models

NEW DELHI, INDIA—Sulfur compounds spewing into the atmosphere from burning fossil fuels have long been implicated in acid rain and smog. But it's only recently that climate modelers have also viewed them, along with greenhouse gases such as carbon dioxide, as major determinants of global change. Last month a team of Indian and German scientists showed just how important the aerosols that form from the sulfur compounds may be in affecting climate on a regional scale. Their computer climate model predicts that the cooling effect of aerosols will combine with an increasing greenhouse effect to cause a significant drop in monsoon rainfall over India and parts of China by the middle of the next century.

Changes in Monsoon Rainfall (1970–2040)



Flip-flop. Adding aerosols to climate models dries up monsoon rainfall predictions.

That's a switch from earlier projections based on carbon dioxide alone. "The inclusion of the aerosol effect reverses the rainfall scenario on the Indian subcontinent," says Murari Lal, chief scientific officer at the Indian Institute of Technology (IIT) Center for Atmospheric Sciences and lead author for the study, published in the Indian journal *Current Science* (10 November, p. 752). The research, done jointly with a team at the Max Planck Institute (MPI) for Meteorology in Hamburg, Germany, implies that the combination could reduce rainfall by as much as 10% or more, harming Indian agriculture. The results are similar to those obtained independently by a modeling team at Britain's Hadley Center for Climate Prediction and Research, which are mentioned in last month's report from Working Group I of the Intergovernmental Panel on Climate Change (*Science*, 8 December, p. 1565) but have not yet been published.

The South Asian monsoon is a unique annual atmospheric phenomenon driven by heat differences between the south Asian land mass and the Indian Ocean. During April and May, the land heats up faster than the ocean, creating a land-sea gradient in temperature and pressure that drives the monsoon circulation across the Indian subcontinent. Rainfall during the 4-month monsoon accounts for more than 70% of the annual precipitation over the subcontinent.

A greenhouse warming might be expected to amplify the temperature gradients and the resulting rainfall. But even as greenhouse gases increase, industrialization in China and India is blanketing south Asia in an aerosol haze. The cooling effect of the haze could reduce the land-sea temperature contrast, weakening the monsoon. In addition, a cooler land surface could mean less evaporation and reduced water vapor in the atmosphere.

That's just what the IIT-MPI group found when they ran their model assuming both a 1.3% per year increase in carbon dioxide and a fivefold increase in peak aerosol levels over India by midcentury. The projected decrease in the monsoon ranges from 0.5 millimeters to 1.0 millimeters a day over the fertile north and central Indian plains. That represents a possible drop of 7% to 14% in the average rainfall of 88 centimeters for the 120-day monsoon season. Anything below 10% is considered a threat to agriculture. The monsoon will increase over south India, but by an amount smaller than predicted by models based only on greenhouse gases.

Results from the new model compare well with some observed climate trends. The prediction of a rise of 0.33 degrees Celsius in average near-surface temperatures over India during the past century is close to the 0.4 degrees published recently by the Indian Meteorological Department. The total average predicted rainfall, says Lal, is "marginally lower" than the historical mean.

Beyond its importance to agriculture, the results from the improved model also carry political overtones. "It shows that China can have an important impact on India," says MPI's Ulrich Cubasch, a co-author on the paper. "Specifically, what it suggests is that greater industrialization in China will mean less food for India."

The next step for both groups is joint research on the effect of aerosols—their migration patterns and influence on clouds, for example—and on probable future emission levels. Scientists hope to incorporate that knowledge into improved models of how the rainfall is likely to be distributed during the monsoon, the biggest factor in determining agricultural output.

Despite its uncertainties, the new work is recognized as underscoring the central role of aerosols in climate change. "Two independent groups working with different models and arriving at similar results helps boost confidence in the projections," says Tom Wigley of the U.S. National Center for Atmospheric Research in Boulder, Colorado. "The figures might need to be revised, but the crucial point is that regional models are different when aerosols are included."

–**Ganapati Mudur**

Ganapati Mudur is a science writer in New Delhi.