Crystal Clear A master practitioner initiates young scientists into the arcane craft of crystallography.

A CRYSTAL IS A THING OF BEAUTY, BUT TO A PROTEIN scientist it is that and much more. The crystal form of a material embodies its essence—the unique framework of atoms and chemical bonds that determines the molecule's function and how it interacts with other molecules.

But growing the crystal from a protein involves an uncommon blend of science, art, and tenacity—qualities that HHMI investigator and protein biochemist David Eisenberg strives to convey in his course on x-ray crystallography at the University of California, Los Angeles.

"At any of several steps, you can get stuck for months at a time," says Howard Chang, a student in the course. "All you can do is continue to screen the hundreds or thousands of different conditions that influence crystallization in hopes that one will work. Even under optimum conditions, a crystal can take years to grow."

But the payoff is huge, Chang adds, both in scientific terms and as a kind of pure wonder. "Seeing the molecules in the greatest detail possible, it's beautiful how these intricate atomic-level 'machines' evolved by nature operate."

Atoms are too small to see with visible-light microscopes. Instead, scientists infer the positions and orientations of atoms indirectly by using x-ray diffraction. They fire x-ray beams into the crystal, which are scattered in different directions by the atoms and captured on a detector surface. An experienced researcher can then devise a structural model of the protein from the resulting pattern. Knowing the structure gives insights, for example, into how amyloid proteins cause neurological disease (see "Chasing Amyloid," page 10). And structure-based drugs, such as the protease inhibitors created to treat HIV/AIDS, are becoming more common.

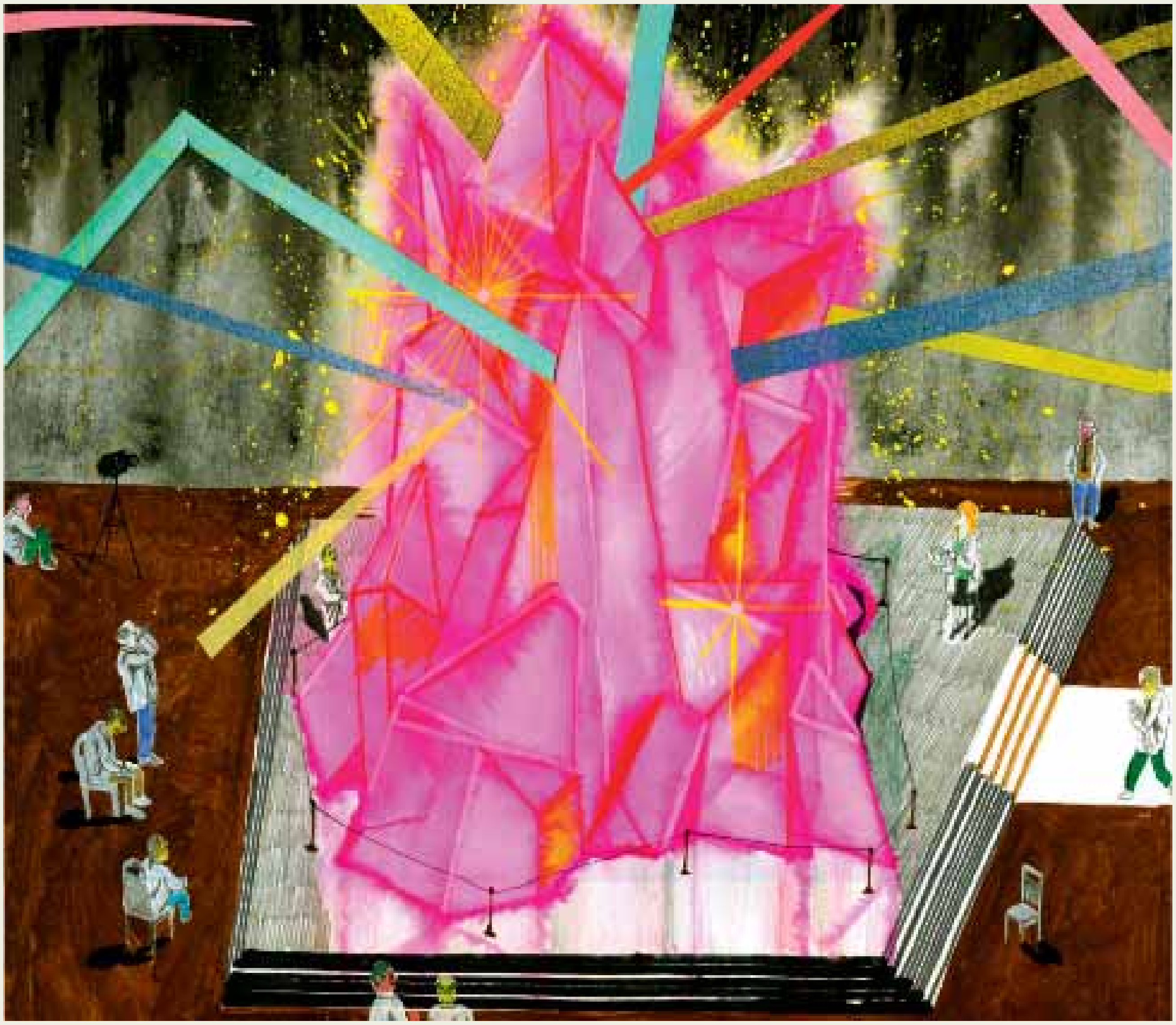
Eisenberg, whose career has witnessed—and contributed to—many milestones of protein crystallography over the last three decades, puts a great deal of thought, personal experience, and enthusiasm into his course, which is taken by about 20 researchers—mostly graduate students—each year.

"We feel that x-ray crystallography is an extremely important tool for young scientists to acquire—it is no longer the province of specialists," says Eisenberg, who is director of UCLA's Institute for Genomics & Proteomics. The multi-instructor course includes the history of x-ray diffraction techniques, their theoretical underpinnings, hands-on practice in crystallizing a protein, "shooting" it with x-rays, and interpreting the data.

First-year graduate students Chang, 25, and Anni Zhao, 23, who recently joined the Eisenberg lab, started with little knowledge of the field. The learning curve was steep, but both were impressed by the instructors' creativity and skill in conveying the needed information. For example, to appreciate the various kinds of symmetry crystals can adopt, students begin by identifying symmetrical elements in the mathematically inspired artwork of M.C. Escher.

In the sequence of instruction, the first part explains x-ray crystallography as well as nuclear magnetic resonance (NMR) and electron microscopy—techniques that can be applied to atomic structure. "We teach them how to read a paper, how to know if a structure is reliable, and what assumptions have been made," Eisenberg says.

Next come two five-week segments delving deeper into the actual mechanics of x-ray and NMR crystallography—collecting and reducing data and then determining the structure from the data. Along with the lectures, students practice what they've been learning with a set of laboratory problems. "Because it's such a complex concept to teach, it really helps that Dr. Eisenberg is able to bring in six instructors who specialize in different areas," says Zhao.



Drew Beckmeyer

Finally, it's time to put it all together: the students are given a protein from which to grow crystals, freeze them, mount them in the x-ray apparatus, gather the data, and interpret the structure. "To get a good crystal that gives you a nice diffraction pattern is a big deal," says Zhao. "The initial formation is difficult: there are many combinations of factors—concentration, pH, temperature, salts, buffers—and the possibilities go up exponentially."

Zhao praises Eisenberg for his supportiveness in helping students weather the ups and downs. "He does not push you for results, but when you need direction or help, he is always available and extremely helpful," she says. "People can go into his office very frustrated and walk out the door more motivated than ever."

In practice, much of the tedious trial-and-error pipetting is now done with automated equipment, Eisenberg says, but going through the process manually is part of the learning experience. After completing the course, students are well prepared to join a lab group that relies on x-ray crystallography for structure analysis.

"Some of our students go on to become real experts in the field—they far surpass me in mastering the software and the hardware," he says. "Others just use it for their research as biochemists. What I like about our training system is that it provides a basis for both of those careers." ■ –RICHARD SALTUS