Crystal Clarity

Making Sense of the Genome Requires Analyzing Pure Protein Crystals

Just about everyone has heard by now of the Human Genome Project, the massive scientific effort of the 1990s that catalogued the *entire* genetic blueprint of *Homo sapiens*—a sort of how-to guide for the construction of the species. But now the really hard work begins. Scientists are painstakingly translating that genetic plan into an understanding of the day-to-day workings of cells, including cancer cells. At the forefront of Einstein's effort is the Structural Biology Center. Key personnel at the Center include its Director, Dr. Mark Chance of the Department of Biochemistry, as well as his departmental colleagues Drs. Steve Almo, Anne Bresnick and Mark Girvin.

The genome is like a book of about three billion letters—a run-on sentence to put Faulkner to shame—each letter representing one of the four basic building blocks of DNA. Deep in that alphabet soup are something like 30,000 genes, which are the blueprints for the proteins that do the cell's chores. You might say that, with the completion of the Human Genome Project, scientists have bought the genome book, but they've barely opened it. Now that researchers have found the genes, they have to figure out what the proteins coded for by these genes look like (the three-dimensional structure), what their jobs are, how they do those jobs, and what role those proteins might play in disease.

The most versatile technique for determining protein structure is called x-ray crystallography, and scientists like Dr. Chance are pushing the envelope on how quickly it can be done. In the simplest terms, researchers shine x-rays on a crystal of protein, which perturbs the light, or diffracts it. To the untrained eye, what you get in return looks like nothing more than a bunch of spots. The trained eye, however, also sees a bunch of spots, but knows what to do with them. Powerful computers translate the unique patterns of spots into a picture, in three-dimensions, of the protein that must have created that particular spot pattern—the protein's overall shape, its internal geometry, the juxtaposition of its parts. And all of this sheds light on how the protein works.

Protein crystallographers have a hard lot. Proteins can be fiendishly tough to obtain in large quantities, to purify and to get into crystal form. The data are hard to gather—the more detail you want, the longer that process takes, and over time the crystal loses its structural integrity. In addition, the calculations are numbingly complex. The months or years it can take to nail down the structure of a single refractory protein have led many a crystallographer to the brink of despair.

Good news for crystallographers—and bad news for their therapists—is the revolution going on in crystallography that is being led by scientists such as Dr. Chance and his colleagues. The instrument of their salvation is a behemoth called a synchrotron light source. Chance uses Einstein-owned parts of the synchrotron at the Brookhaven National Laboratory on Long Island. There, electrons race near the speed of light in a doughnut-shaped tunnel ringed by powerful magnets. As the magnets guide them in their circular path, the electrons emit intense light—a nuisance to particle physicists, a bonanza for crystallographers. The light is sent down lines called beams, to be focused on dozens of simultaneous experiments in a room that literally hums and throbs with electronic equipment. The College of Medicine owns four of the beam lines, and shares synchrotron data and research projects with the National Cancer Institute, Rockefeller University and the Memorial Sloan-Kettering Cancer Center.

EINSTEIN'S BEAM LINE FACILITY AT BROOKHAVEN NATIONAL LABORATORY.

MISSION CONTROL FOR THE BEAM LINES AT BROOKHAVEN.

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The appeal of the synchrotron to the protein crystallographer has three major elements. First, it's fast. "The speed at which we can collect the data is up by a factor of at least 100 to 1000, because of the brightness of the synchrotron beam," explains Dr. Chance. "The analogy is that of a light bulb to a laser. While a light bulb radiates light in all directions, with the laser beam you can get all of its radiation focused onto a small spot. So we can get data in hours as opposed to days."

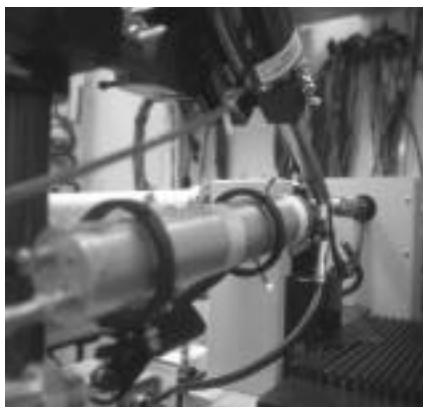
Moreover, the fact that the synchrotron beam can be tuned to different wavelengths of light—colors, as it were—lets crystallographers play some mathematical tricks that remove the old necessity of making multiple types of crystals for each protein, thus saving headache, heartache and time. Though the technique is called MAD (for multiwavelength anomalous dispersion), it makes crystallographers smile. Finally, the pictures the synchrotron gives are high-resolution, often revealing details down to near a ten-millionth of a millimeter (which translates to about three-billionths of an inch across).

Dr. Chance and his colleagues have had some spectacular successes with the synchrotron, and numerous members of the Einstein community benefit. A group studying tumor cell motility, led by Dr. John Condeelis, pushed one protein structure from data collection to the cover of a scientific journal in a mere six weeks. (Such work literally took years only a few years ago.) "They finished the structure before they left the beamline that day," Dr. Chance notes. "Another group was working on it and we beat them because of the technology available here at Einstein." The synchrotron's resolution also is critical, for example, for Einstein immunologist Dr. Stanley Nathenson's projects in immuno-oncology (the immune system and cancer) and for biochemistry chairman Dr. Vern Schramm's research into cancer drug design. (See article on page 1.)

So far there is no Rosetta stone of protein structure—researchers cannot simply look at the DNA sequence in the gene and know the structure of the protein encoded for by that gene. They still have to tackle each new gene and its protein the hard way. But as more and more structures are solved, and computers recognize patterns, the job is likely to get easier. Genes from other organisms will be helpful as well—sometimes structure determination is easier with a non-human protein, letting researchers make inferences about the human version.

A case is point is *xeroderma pigmentosum* group C protein, studied by Einstein's radiation oncology group and found in radiation sensitive cells at levels 13 times as high as in radiation resistant cells. When researchers tried to produce the protein in bacteria, to obtain large quantities for crystals, the bacteria all died. But it turns out the analogous protein in the flatworm *C. elegans* is easy to work with. "Do we have any idea why?" Dr. Chance asks. "No. Do we care? No. All we care about is that we have a handle on a protein that's very close to the human protein, so we're going to push ahead with that." Other Einstein researchers are using a similar approach to study DNA repair genes that are defective in colon cancer.

To speed the conversion of genome information to protein structure, Einstein has teamed up with the Brookhaven National Laboratory, Rockefeller University, Mount Sinai School of Medicine and Weill Medical College of Cornell University to form the New York Structural Genomics Research Consortium, which the National Institutes of Health is funding to the tune of $25 to $30 million over the next five years. Dr. Chance compares the endeavor to efforts like the Manhattan Project and the space program, in which multiple investigators and institutions are brought together for a common purpose. And he expects Einstein's Structural Biology Center to lead the way. Æ



DETAIL OF THE EINSTEIN CRYSTALLOGRAPHY APPARATUS AT BROOKHAVEN.