Protein structure mapped from crystals 2000 km away

Reflections from Crystals

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Prof. Mariusz Jaskólski is head of the Center for Biocrystallographic Research, Institute of Bioorganic Chemistry. With his coworkers he uses crystallography to study the three--dimensional structure of proteins

Dr. Mirosław Gilski applies computational methods in crystallography

Scientists no longer have to have the objects they study within arm's reach. Using the Internet and sophisticated equipment, researchers in Poland have – for the first time ever – remotely investigated the structure of protein crystals mounted at the synchrotron center in Grenoble, all without leaving their own lab in Poznań

Modern protein crystallography was born in 1937, when the young Max Perutz selected crystal structure determination of hemoglobin through the use of X-ray diffraction as his Ph.D. project. Knowledge about the three-dimensional structure of macromolecules, proteins and nucleic acids, was at that time close to nil. It took Perutz 22 years to achieve his ambitious goal. In the meantime, two years earlier in 1957, John Kendrew had solved the crystal structure of the four-times-smaller myoglobin, and in 1953 the structure of the DNA double helix had been discovered, also through experiments with X-ray diffraction. During the 1960s and 1970s protein structure determination was still a formidable task requiring many years of painstaking experiments and calculations, and the accumulation of knowledge about the 3-D structure of proteins was very slow. Through methodological

ESRF

The ESRF synchrotron, set in picturesque surroundings in Grenoble, has a diameter of 0.85 km and the electrons whizzing around its high-vacuum tunnel are accelerated by a potential of six million Volts

developments in protein crystallography and advancements in computing techniques, structural biology has been gaining momentum at an increasing rate. Today, the Protein Data Bank (PDB) stores information on the structure of over 40,000 macromolecules, accumulating over 5,000 new structures annually. All this has become possible thanks to genetic engineering, which supplies protein crystallography with relatively easy-to-obtain recombinant proteins, but also thanks to the widespread use of powerful synchrotron sources of X-ray radiation.

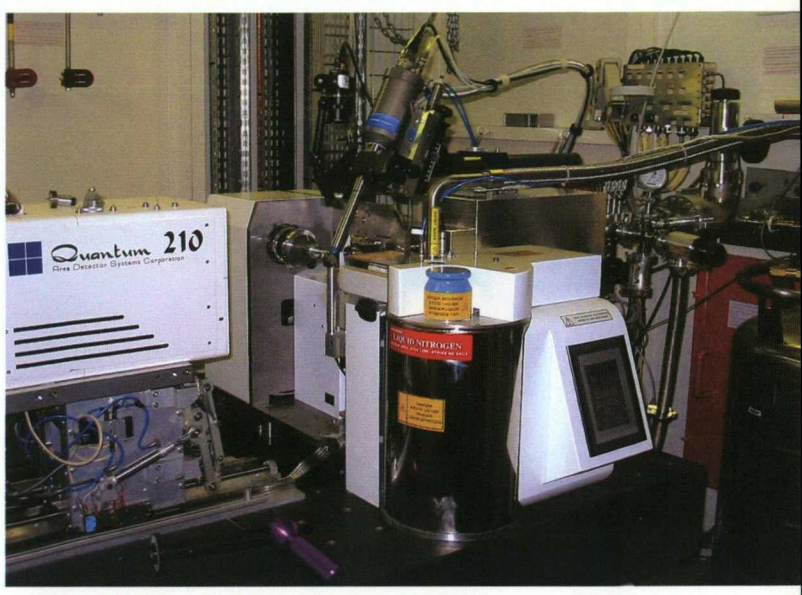
Yet even with this arsenal, protein crystallography hardly meets the demand for protein structure determination, instigated by the explosive growth of genetic data at the beginning of the 21st century. While data about sequences of entire genomes are filling the databases at a nearly astronomical rate, progress in understanding this information is much slower. There is simply no easy way to decode the information encrypted in the ATGC alphabet. The fastest track to understanding how proteins function is through studying their structure. Protein crystallography, as the principal method for protein structure determination, is at its prime again.

Giants at atomic scale

In addition to structural genomics, whose motto is "more structures," modern biocrystallography also works on other fronts. On one of them, crystallographers struggle to crack the structure of huge macromolecular complexes, approaching the size of cellular organelles or even living entities. Indeed, atomic-resolution structures of scores of viruses have been determined, and recently also the structure of the ribosome, the cell's protein synthesis factory. The latter discovery astonished biologists by demonstrating that the ribosome is an RNA machine, unlike most enzymes, which are protein machines.

Protein crystallography also supplies precise structural information to guide rational drug design. Knowing the accurate structure of a macromolecular "target," a protein involved in disease, allows scientists to design efficient "bullets," or drugs that can prevent, revert, or at least stop a pathological process. A spectacular example of this approach is provided by the development of the most successful AIDS drugs, which have been designed as inhibitors of one of the enzymes of the HIV retrovirus.

D. Hall, ESRF



The experimental station ID14 in Grenoble, dedicated to protein crystallography. Suspended in a miniature loop, a small protein crystal is exposed to intense X-rays while being bathed in a cold nitrogen stream (-170°C). The diffraction pattern is captured by a CCD camera

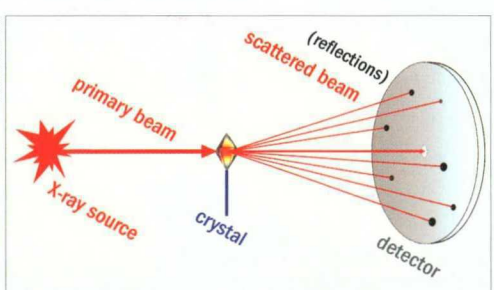
Atomic resolution

Protein crystallography also works on an "in-depth" front where the goal is to determine protein structure with the ultimate accuracy and resolution. Resolution – expressed in angstroms, the units of length in the atomic world (ten angstroms are equal to one-millionth of a millimeter) – defines the level of detail reproduced by a model of a macromolecule. In practice, the higher the resolution, the more experimental data must be recorded. It is not unusual in protein crystallography to collect many millions of individual diffraction data from a crystal as small as 0.1 mm across.

In the beginning there is a crystal...

X-ray diffraction structural analysis requires a well-formed single crystal. Protein crystallization is among the more difficult steps of the structure determination process. Nowadays, protein crystallization experiments

M. Jaskólski, UAM



A small crystal scatters the X-rays into a multitude of sharp reflections, the position and intensity of which are measured by electronic detectors

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M. Strozycki, ICHB PAN

At the historical moment of remote diffraction measurements on 30 November 2006, Mariusz Jaskolski (standing) watches as Mirek Gilski (seated) runs the data collection system from Poznań. Olof Svensson (on screen) watches the experiment at the ESRF site in Grenoble

are carried out by robots which in the volumes of microscopic droplets test thousands of crystallization conditions to find those suitable for a given protein. During X-ray diffraction experiments, protein crystals are "additionally frozen" in liquid nitrogen vapors at -170°C, to protect the sensitive protein material and the layer of water in which it is always shrouded.

The phase problem

The measurement of a diffraction pattern does not automatically reveal the three-dimensional structure of the underlying crystal. The key to solving the latter one lies in the mathematical relation between the diffraction-structure pair, known as the Fourier Transform. To properly solve it, one needs to know the phases of the X-ray waves scattered by the crystal. Those phases cannot be measured experimentally. Crystallographers use, therefore, various tricks to obtain this missing information. One can, for example, use a model of a similar protein to solve a new, unknown structure. In another approach, a very heavy atom can be attached to a protein in the hope that it will mark its presence in the diffraction pattern and facilitate the solution of the phase problem. A variant of this method, MAD (Multiwavelength Anomalous Diffraction), uses an atom that scatters the X-rays in an anomalous way, for example selenium, introduced into proteins as selenomethionine.

Determination of a crystal structure is equivalent to calculating a map showing the distribution of electrons in the crystal. Atoms, represented on such maps as islands of high electron concentration, are then clearly visible, allowing a three-dimensional model of the underlying macromolecule to be constructed. Such models are comprised of thousands of atoms, each of which has pre-

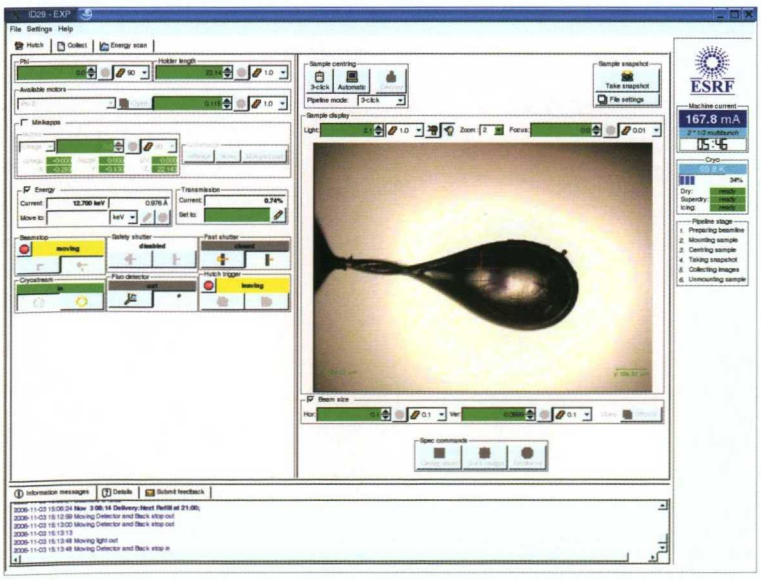
Sz. Krzywda, UAM

An X-ray diffraction pattern recorded for a protein crystal. The intensities of the reflections (spots) contain encrypted information about the spatial disposition of the scattering atoms in the crystal structure

cise 3-D coordinates. Proper understanding of the functioning of such molecular giants requires sound chemical knowledge. Protein crystallographers, therefore, look at their diffraction-physics-derived models through the eyes of a chemist, in order to tackle the most intricate questions of biology.

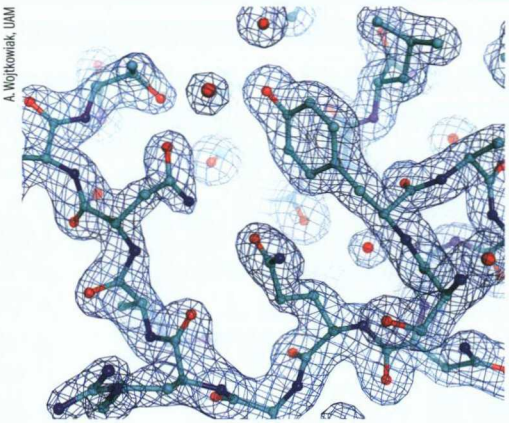
Synchrotron source of... success

In a home laboratory, X-rays are produced in small tubes or somewhat more sophisticated generators. Yet such sources are rather weak, seriously limiting protein crystallographic applications. Currently, diffraction from protein crystals is mostly studied using powerful synchrotron sources of X-ray radiation. A synchrotron is a version of a cyclotron, several hundred meters in diameter, in which a strong magnetic field guides a current of electrons into a circular orbit. The electrons speed along at near-light velocities and emit X-ray photons every time they are forced to change direction along their orbit. The emitted X-rays are of immense intensity, enabling protein crystallographers to collect the diffraction data in a split second. The practical problems are thus of a different nature. Crystallographers have to worry how to transport their precious protein crystals (more valuable than equivalent diamond crystals) to distant synchrotron centers, and how to ensure smooth access to a beamline and trouble-free operation of the highly sophisticated equipment.



BioXHIT

The control panel of the automated data collection program running X-ray diffraction experiments at the ESRF in Grenoble. This program enabled the present authors to remotely control a robot which extracted a crystal from a liquid-nitrogen dewar and inserted it in the X-ray beam. The results of the measurements were displayed less then half a minute later



A. Wojtkowiak, UAM

An electron density map calculated from the diffraction pattern of a protein crystal reveals the atomic architecture of the biomolecule. The high image quality and resolution allow even the water molecules (red spheres), which accompany proteins in crystals, to be visualized

Logistic problems and the high degree of complexity of synchrotron-radiation experiments motivate crystallographers to try to eliminate at least some of these hurdles.

Courier crystallography

One of the solutions proposes to send liquid-nitrogen frozen crystals to a synchrotron center and to supervise the experiments via the Internet. The task of the local staff would be to load the courier-delivered samples into a dewar storage bank of crystals queued for measurements. The BioXHIT project of the EU is consolidating European efforts in this direction. The Center for Biocrystallographic Research (CBB) affiliated with the Institute of Bioorganic Chemistry, Polish Academy of Sciences (Poznań, Poland), has the status of a Training, Implementation and Dissemination (TID) Centre of the BioXHIT initiative. In November 2006, the CBB site conducted an unprecedented test of remote synchrotron beamline operation at the largest European Synchrotron Radiation Facility (ESRF) in Grenoble (France). Through specialized Internet-operated software, a small notebook computer at the CBB could control the operation of a protein crystallography beamline in Grenoble, including guiding a robot which removed the sample from the storage dewar and flawlessly placed it in the X-ray beam! This historical experiment has opened up an exciting possibility for conducting synchrotron-radiation protein crystallography experiments from virtually any place on Earth. ■

Further reading:

Rhodes G. (2006). *Crystallography Made Crystal Clear.* (3rd ed.) Academic Press.