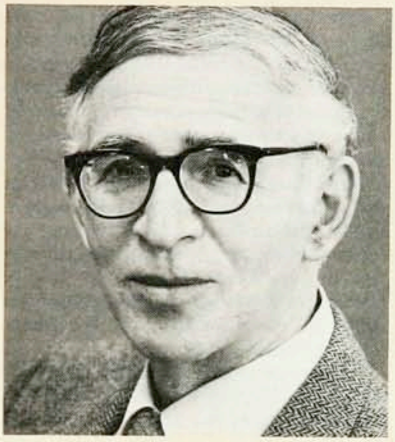
Aaron Klug wins Nobel prize in chemistry

The Royal Swedish Academy of Sciences has awarded the 1982 Nobel Prize in Chemistry to Aaron Klug, "for his development of crystallographic electron microscopy and his structural elucidation of biologically important nucleic acid–protein complexes." Klug's academic degrees are in physics. After taking a master's degree at the University of Capetown in x-ray crystallography, he received his PhD in solid-state physics at Cambridge in 1952. Since 1962 he has been at the (British) Medical Research Council's Laboratory of Molecular Biology in Cambridge.

Through "an ingenious combination of electron microscopy with principles from [x-ray crystallographic] diffraction methods," the Swedish Academy tells us, Klug has devised a set of techniques for determining the three-dimensional structure of biologically functional macromolecular aggregates about which straightforward x-ray diffraction or raw electron micrographs can tell us little. At the MRC Laboratory, he brought to electron microscopy insights gained from his x-ray crystallographic work on viruses during his years at Birkbeck College (London), 1954–1962. Until her untimely death in 1958, Rosalind Franklin was his principal collaborator at Birkbeck. She is best known for having discovered the B-helical form of DNA by x-ray diffraction. Klug told us that Franklin was the most important formative influence of his early scientific career.



KLUG

Although x-ray diffraction has succeeded in revealing the structure of many biological macromolecules since the beginnings of protein crystallography at Cambridge in the 1930s, complicated molecular aggregates such as viruses, membranes, muscle fibers and chromosomes cannot in general be obtained as highly ordered, three-dimensional crystals suitable for x-ray diffraction analysis. Electron microscopy, on the other hand, has its own severe limitations. A transmission electron micrograph is in effect a two-dimensional projection of the three-dimensional electron distribution of an extended object. The random orientation of samples under the microscope and the strongly orientation-dependent appearance of superposition patterns (similar to Moiré patterns) of front and back surface structures projected on top of one another brings considerable confusion to the electron microscope images of viruses, for example. Furthermore, the predominance of light elements in biological systems has usually required the application of heavy-metal-salt stains to give electron micrographs reasonable contrast. These stains, together with the electron beam itself and the instability of proteins in the anhydrous vacuum environment of the microscope, tend to generate distortions, granularity and damage in the biological system under study.

"Klug has turned the interpretation of electron micrographic images of macromolecular aggregates into something quantitative and precise," we were told by David DeRosier (Brandeis), who worked with Klug at the MRC Laboratory. Previously, he told us, such interpretation was highly subjective, primarily because of the confusion of superposed surfaces. Identical objects appeared bewilderingly diverse under the microscope. The determination of molecular arrangements was largely guesswork. "He found ways to visualize virus substructure by electron microscopy at a time when this science had been developed only to the stage where coarse outlines could be discerned."

The mathematical reconstruction of three- (or two-) dimensional distributions from their two- (or one-) dimensional projections is a problem that transmission electron microscopy shares with a number of other disciplines—radio astronomy and x-ray tomography among others. The analytical (if not always the *practical*) solution is basically the same for all such problems. Surprisingly often, however, workers in one field must rediscover mathematical techniques already known in other fields. Allan Cormack, for example, was unaware of the 1917 solution of Johann Radon when he solved this problem for CAT scanners in 1963. (He won the 1979 Nobel prize in medicine for this work. See PHYSICS TODAY, December 1979, page 19.) Klug and DeRosier solved the problem for transmission electron microscopy by applying the mathematical apparatus of x-ray crystallography.

Under the transmission electron microscope, electrons are scattered out of the field of view by the electrons of the sample under scrutiny. Because of the instrument's great depth of field, the resulting image is essentially an attenuation projection of the sample's electron density distribution; the incident brightness at each point in the image plane is reduced by the density distribution integrated along the beam direction. The key to the reconstruction of a three-dimensional distribution from such two-dimensional projections is a "projection theorem" well known to x-ray crystallographers. The theorem tells us that the Fourier transform of a two-dimensional projection corresponds to a two-dimensional slice through the full three-dimensional Fourier transform (through the origin in transform space) of the original distribution. Thus one can reconstruct the full transform, and hence the three-dimensional distribution in configuration space, by making sufficiently many projected images in different directions—by rotating the sample to different orientation under the microscope.

0031-9228 / 83 / 0100 17-06 / $01.00

Beginnings. This work on image reconstruction had its beginnings in 1964, with an attempt to discern the discrete symmetries of macromolecular aggregates from electron micrographs, despite the confusion engendered by superpositions. Klug hit upon the novel idea of generating and examining the optical diffraction patterns of the micrographs themselves. At the MRC laboratory, he and Jack Berger shone light from high-pressure mercury lamps (later it was done with laser light) through negatives of the electron micrographs and photographed the resulting diffraction patterns. The diffraction pattern is essentially the Fourier transform of the original image, averaged over many unit cells. Translational periodicities (for example helical structures or membrane lattices) will manifest themselves as sets of spots or lines, just as they do in x-ray crystallography.

The following year Klug and DeRosier developed a technique for emphasizing these symmetries on the micrograph images by filtering aperiodic noise and other obscuring features out of the diffraction pattern before refocusing the original micrograph. They accomplished this optical filtering by making a mask of the diffraction pattern; they cut holes in an opaque screen corresponding to strong diffraction spots; aperiodic background noise was ignored and missing reciprocal-lattice spots that could be deduced from the diffraction symmetry pattern were added. Replacing the film in the diffraction plane by this mask, they once again illuminate the original electron micrograph with laser light and focus the light that passes through the holes in the mask on an image plane. The resulting image is effectively a filtered version of the original micrograph, with symmetry patterns stressed and aperiodic noise removed. They had effectively performed two successive Fourier transforms on the original image, leaving only the strongly periodic features of the first transform to highlight the symmetry properties of the macromolecular array.

The filtering procedure was also used to disentangle symmetry features that become obscured by superposition. Regularities of front and back virus surfaces, for example, might be well separated in the diffraction pattern while their spatial images were obscured by projection. Thus Klug and his colleagues were able to examine one surface (or one of several overlapping membrance layers) at a time by masking out the diffraction spots generated by the regularities on the other surface.

These procedures point up an important advantage of electron microscopy over straightforward x-ray diffraction analysis. Because x-ray diffraction is a non-imaging technique, one has only the intensity pattern in the diffraction plane; the phase information of the diffraction pattern is lost. Reconstruction of the original array is therefore laborious and subject to troublesome phase ambiguities (PHYSICS TODAY, November, page 17). In Klug's procedure, on the other hand, the light passing through the masks retains all the diffraction phase information; it can be refocused to produce an image that makes the symmetries of the original array readily apparent and permits one to examine the different patterns of overlapping surfaces one at a time.

Three-dimensional images. The essence of the optical filtering procedure can be computerized and exploited to produce a three-dimensional reconstruction of a quite general macromolecular aggregate, even in the absence of strong periodic symmetries. For spherical or icosahedral virus shell, for example, one learns little from examining the diffraction pattern or using it to make a filtering mask. But the diffraction pattern is nonetheless the Fourier transform from which the projection theorem lets one determine the three-dimensional structure.

That is in fact how Klug, DeRosier and their MRC colleague John Finch began to generate three-dimensional reconstructions in 1966. They took several electron micrographs of a highly symmetric sample from different angles and digitized each image with a microdensitometer. Instead of using laser light to generate the diffraction pattern (effectively an analog computation of the Fourier transform), they computed the Fourier transform of each projected image (amplitude and phase) from each digitized projection. By the projection theorem, each such transform is a slice through the full three-dimensional Fourier transform of the original aggregate. With sufficiently many projections one has an adequate sampling of the full transform, which one can then invert to produce a three-dimensional model of the original object. The minimum number of projections that will suffice to reconstruct the sample without loss of detail can be deduced by a geometric criterion for adequate sampling. Klug and Tony Crowther have shown that this geometric criterion remains valid despite the noise and inconsistencies that are always present in real projection data.

Some applications. An important application of this union of x-ray diffraction techniques with electron microscopy is the elucidation by Klug and Roger Kornberg (now at Stanford) of the structure of the nucleosome, a button-shaped aggregate of histone proteins, each associated with a stretch of chromosomal DNA, that Kornberg discovered at Cambridge in 1974. Kornberg described it to us as "an elementary particle of the chromosome." By x-ray diffraction of a crystalline array of these nucleosomes and complementary electron-microscope studies of their isolated histone cores, Klug was able to determine that the constituent histones are arranged in a helical ramp, with a two-turn loop of the chromosome's DNA molecule wound around each nucleosome. The large-scale helix formed by this looping of the DNA around successive nucleosomes is not to be confused with the finer double helix intrinsic to the DNA itself. Such investigations, the Swedish Academy says, "have yielded a detailed picture of the functional arrangment of nucleic acid–protein complexes that has already provided clues to the problem of cell differentiation."

Viruses are also nucleic acid–protein complexes. Klug began studying the tobacco-mosaic virus with Franklin at Birkbeck. They determined that the virus's RNA was imbedded in a coat of several thousand identical protein macromolecules helically arrayed with 17-fold rotational symmetry. At Cambridge, Klug expanded the analysis to include electron microscopy. Klug and Jonathan Butler were able to determine in astonishing detail the mechanism by which the TMV assembles itself out of RNA and protein macromolecules. A special hairpin-shaped "initiation region" of the TMVs long RNA molecule generates dislocation on a self-assembled disk of protein macromolecules, giving the disk the appearance of a lock washer. This dislocation then serves as a nucleation site from which the disk grows into the full TMV helix. This self-assembly mechanism of a primitive biological entity, Klug points out, bears a surprising resemblance to the nucleation and growth of crystal lattices.

For TMV and other helical viruses, the diffraction patterns generated from electron micrographs yield considerable information about the symmetry structure of the molecular aggregate. For spherical viruses, on the other hand, the diffraction patterns themselves yield relatively little direct information. The shell design of spherical viruses was not understood, Klug told us, until he and Donald Casper (now at Brandeis) produced a theory in 1962 predicting that the protein molecules should be arranged in icosahedral arrays—rather like geodesic domes. Every spherical virus species studied in subsequent years by Klug and Finch has in fact been found to have one or another of the icosahedral designs predicted by the Caspar–Klug theory.

In 1970, Klug and Harold Erickson did a theoretical analysis of electron microscopy, addressing in particular concerns, expressed in some quarters, that multiple scattering and defocusing might introduce serious distortions in Klug's reconstruction technique. Klug and Erickson were able to show that multiple scattering is not a serious problem, and that the purposeful defocusing employed in low-resolution (20 Å) electron microscopy to enhance contrast does indeed provide useful imaging information.

At the time Klug regarded this analysis as a largely academic exercise. But in 1975 Nigel Unwin and Richard Henderson at the MRC Laboratory made dramatic use of Klug and Erickson's analysis of defocusing. Defocusing is employed to produce electron-micrograph contrast when imaging transparent objects, because one cannot do phase-contrast electron microscopy. Klug and Erickson pointed out that computer processing of the images thus distorted can retrieve the "true" image.

Unwin and Henderson have developed new sample-preparation techniques that permit them to apply these defocusing ideas to high-resolution electron microscopy—dispensing with heavy-metal-salt stains and achieving striking contrast and resolution with very low electron beam intensities. Averaging over many unit cells, they have succeeded in reconstructing the three-dimensional configuration of proteins in a photosynthetic membrane with a resolution of only 9 Å. This is the first time that anyone has seen a membrane protein *in situ* with anything like this resolution, DeRosier told us. He regards this work as "a dramatic culmination of Klug's many years of work in electron microscopy."

"The great breadth of Klug's structural work on a number of very important molecular aggregates has contributed greatly to our understanding of how biological complexes are constructed," DeRosier said. "He had revolutionized the way such structures are visualized and their images interpreted." Casper points to "the dazzling range of his accomplishments, which bears the unmistakable imprint of his talent and insight in mathematics, physics, chemistry and biology. Conceptual barriers between different disciplines do not exist for him." —BMS

A look at the future of particle physics

US particle physicists have recently been debating where they will find the most exciting physics and what future facilities will best enable them to pursue it. The dialog intensified last summer at a study sponsored by the American Physical Society Division of Particles and Fields and continued through the Division's annual meeting at the University of Maryland at the end of October. About 150 high-energy physicists participated in the DPF Summer Study on Particle Physics and Future Facilities, which was held in Snowmass, Colorado (near Aspen), from 28 June to 16 July. Its stated purpose was to assess the physics topics that might be interesting in the future, to explore the limits of technological capabilities and to consider the nature of future facilities for particle physics in the US. Charles Baltay (Columbia University), chairman of the organizing committee, stressed to us that the role of the summer session was to study the physics but not to arrive at any specific conclusions.

Although the DPF Summer Study drew heavily on previous studies dealing with future physics and accelerators, it differed from them somewhat in content and structure: First, the study did not focus on any one particular facility but was more generally concerned with all future US facilities and experimental programs. Second, its organizational structure promoted interchanges among particle physicists from different areas of specialization. The entire atmosphere stimulated what Maury Tigner (Cornell) termed "free-wheeling" discussions.

Working sessions at Snowmass were determined by a matrix of topics. Each participant attended morning sessions in one row group and afternoon sessions in one column group. The four rows were labeled by topics in physics: testing the standard model, beyond the standard model, accelerator technology, and novel accelerator ideas and novel detector ideas. The columns denoted five types of facilities: lepton–lepton, lepton–hadron and hadron–hadron, colliders, fixed-target accelerators and non-accelerator experiments.

Existing and planned accelerators set the stage for discussions of physics still to be explored and facilities yet to be built. The US has a number of major facilities in operation, under construction or in the planning stages. Fermilab is in the midst of installing its Doubler/Saver, a ring of superconducting magnets below its existing main ring, and hopes to have all magnets in place and cooled by this spring. The project is the first of its size to use superconducting magnets. Fermilab will extract a 1000-GeV proton beam from this new ring and operate the machine as a fixed target project called Tevatron II. By 1985, Fermilab plans to have completed an antiproton source plus auxiliary cooling and storage rings so that it may operate the facility as a proton–antiproton collider. This project is called Tevatron I. TEV I will have a center-of-mass energy of 2000 GeV (or 2 TeV) and expected luminosities on the order of 10^{30} cm^{-2}sec^{-1}.

Brookhaven currently operates a 30-GeV Alternating Gradient Synchrotron but has had to slow construction drastically on Isabelle, its planned 400 on 400 GeV proton–proton collider (PHYSICS TODAY, April 1982, page 20). However, Isabelle, or a less expensive version of its original design, may resurface under the more generic name, the Colliding Beam Accelerator. Brookhaven has been asked to study several alternative designs and to narrow the choice to one by March. The leading candidate for this accelerator is a p–p colliding-beam facility with beam energies of 400 GeV and luminosities on the order of 10^{33} cm^{-2}sec^{-1}, according to Brookhaven's director, Nicholas Samios. Other contenders are a 20 on 400 GeV electron–proton or a heavy-ion collider. Paul Reardon (formerly associate director of the Princeton Plasma Physics Lab) recently became the project head of the Colliding Beam Accelerator and associate director for high-energy facilities at Brookhaven.

SLAC operates three lepton accelerators at present: the 33-GeV linac and two electron–positron colliders, SPEAR (4 on 4 GeV) and PEP (18 on 18 GeV). SLAC is conducting an R & D project on a linear electron–positron colliding-beam machine with 50 GeV in each beam that could be built by 1986 or 1987. This SLAC Linear Collider will help determine the feasibility of using linear e^+e^- accelerators to attain still higher-energy lepton collisions.

Cornell University has an 8 on 8 GeV lepton collider called CESR. Cornell has proposed building a 50 on 50 GeV successor, but in October Cornell announced that this project is not being pursued any longer at an Ithaca site.

In Europe, two major new facilities are making their debut at CERN. That center recently began running its proton–antiproton collider, which has a center-of-mass energy equal to 540 GeV and a design luminosity of 10^{29} cm^{-2}sec^{-1}. CERN is also constructing an e^+e^- colliding-beam machine (LEP) that will initially have beam energies of 50 GeV and that will eventually be upgraded to produce beams of 130 GeV each. At DESY two e^+e^- colliding-beam devices are in operation. DORIS has 5 GeV on 5 GeV. PETRA is just starting to run at 20 on 20 GeV and is expected to run at 22.5 on 22.5 GeV next spring. DESY is seeking approval for its plans to build HERA, a 30 on 800 GeV e–p collider.

Future facilities. Of the accelerators now funded, approved or under construction, the major ones to be operat-