The 1982 Nobel Prize in Chemistry

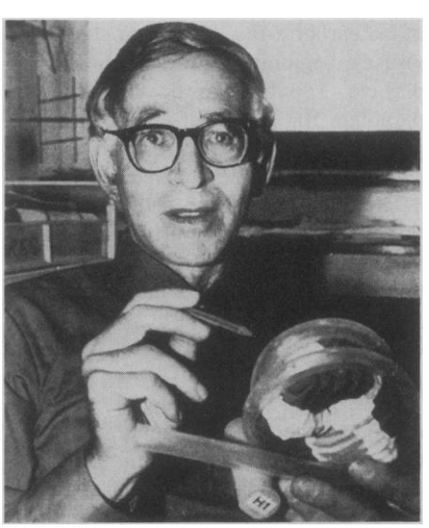
Aaron Klug has been awarded the 1982 Nobel Prize in Chemistry "for his development of crystallographic electron microscopy and his structural elucidation of biologically important nucleic acid–protein complexes." In the 28 years during which he has been actively exploring the architecture of macromolecular assemblies, he has revolutionized the way in which complex biological structures are visualized and their images interpreted. Klug has solved difficult, fundamental problems in the structure of viruses, assemblies of cellular proteins, transfer RNA (tRNA), and chromatin. For the past 20 years, he has carried out his remarkable studies at the MRC Laboratory of Molecular Biology in Cambridge, England.

Klug's career is distinctive in its originality. In the early days of protein crystallography, when solving the structure of a small protein was a formidable task, he embarked on x-ray diffraction analysis of complex virus structures. Images of virus particles from x-ray diffraction, however, were still over a decade away because of the well-known phase problem. Realizing that the electron microscope could provide images directly (or so it was thought), he found ways to visualize virus substructure by electron microscopy when this science had been developed only to the stage where the coarse outlines of the particles could be discerned. Recognizing the underlying physical and mathematical unity in the optics of image formation and diffraction of light, x-rays, and electrons, Klug established the new science of crystallographic electron microscopy or, more generally, Fourier microscopy. Conceptual barriers between different compartments of knowledge do not exist for him.

The success of Klug's work on a dazzling range of structural, methodological, and conceptual problems is a result of his many talents, one of which is developing productive collaborations with people having complementary abilities. The list of his associates is long and distinguished, reflecting the breadth and depth of his many studies. These investigations, ranging through mathematics, physics, chemistry, and biology, all bear the unmistakable imprint of his insight and thoroughness. Those who have worked with Klug can cite, from their own experience, examples of how he attacks and solves problems. Before listing some of his collaborators, we cite examples from our experience.

Interpreting Virus Micrographs

Klug's approach to the interpretation of electron micrographs displays his style of work. He learned what the problems were in using the electron microscope to visualize virus substructure by resolving a controversy about how to count the number of capsomers in papova viral capsids. Some researchers had proposed 42, others 92. The electron micrographs showed fields of particles of uniform dimensions but with a wealth of confusing and variable morphological detail. Klug examined the images, looking for one of the family of patterns of capsomers expected for the icosahedral surface lattices. According to the Caspar-Klug theory of quasi-equivalence, each pattern corresponds to a unique number of capsomers. Klug knew that it was more reliable to determine the pattern and thus the number of capsomers rather than the other way around. Occasionally he could find a particle where the capsomers were discernible, and in all such cases the pattern was unambiguously that of a 72-capsomer structure.



Aaron Klug

Despite the compelling nature of Klug's analysis, the controversy continued.

Klug was well aware that the model had been derived from the small fraction of the images that showed clear detail. He used his model to understand how the images are formed, thereby explaining much of the variability in the appearance of the particles. The confusion of morphological detail arose from a superposition of the capsomeric features on the front and back of the particle. The correct model, if displayed in projection, could account for many, if not all, of the previously uninterpretable images. Was the model unique, or might there be two different models that accounted equally well for the images? Klug knew that uniqueness could be proved by tilts and showed that the correct model predicted the appearance of the virus particles when both were tilted in the same manner. Thus, the model was shown to be unique.

Klug's papers on the papova viruses did more than settle the controversy regarding the number of capsomers: they were a handbook on how to analyze electron micrographs.

Three-Dimensional Reconstruction

While the general principle of tilting to prove the correctness of a model was established, it was not clear exactly how many such tilts were required. Moreover, the task of solving structures by guessing at a three-dimensional model and then producing projections of it was chancy and time-consuming. Was there not some way to directly derive the correct three-dimensional model from the images? There was, and it became known as three-dimensional image reconstruction. Its development arose out of Klug's work on optical diffraction from symmetric structures.

In images of symmetric structures, there will be repeating features. Klug saw the need for a method that would detect all the periodicities in the image. The logical choice was optical diffraction. By taking optical diffraction patterns directly from electron micrographs of symmetric objects, Klug could deduce the arrangement of subunits in the structure; and, for helical structures, he could prove that the images were two-sided, that is, contained morphological detail arising from the front and back surfaces. This clinched the results on the interpretation of the images of papova viruses as two-sided. Based on his analysis of the optical diffraction patterns, he produced filtered images of the front and back

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Aaron Klug (center) early on in his career (1956) at a meeting in Madrid, together with, from the left, Ann Cullis, Francis Crick, Donald Caspar, Klug, Rosalind Franklin, Odile Crick, and John Kendrew. The title of this symposium, "Structures on a scale between the atomic and microscopic dimensions," aptly describes the level of biological organization that Klug has illuminated. His reports at this meeting on the mathematics governing diffraction from symmetric structures and on the structure of tobacco mosaic virus (with Franklin, Finch, and Holmes) anticipate two of the major contributions for which he has been awarded the Nobel Prize.

sides of negatively stained helical structures.

For two-dimensional crystals, the filtered images usually displayed most of the desired information. For other structures, such as the helical T4 phage tail, however, the two-dimensional, filtered image still suffered from the superposition of three-dimensional detail. What was needed for helical structures was a radial filtering scheme in which the radial sections could be separately displayed, thus sorting out the superposition. From his work on the theory of helical diffraction and its application to x-ray diffraction of tobacco mosaic virus (TMV), Klug recognized that for helices such radial filtering could be done on a single particle. For particles with other than helical symmetry, a series of tilted images was required.

The application of the new method to T4 phage tail was published together with a theory of three-dimensional image reconstruction, which could be applied to any structure. The new method has been applied to viruses, muscle proteins and other protein assemblies, and most recently to chromatin. The method has not only fixed the number of views needed to prove a model but, more important, provided a way to generate the correct model directly from the images.

The method was challenged, however, on the grounds that the images seen were not truly projections, as assumed in the theory, but suffered from multiple scattering as well as artifacts due to defocusing. Klug used his knowledge of optics to show that defocusing, rather than being an impediment, actually contributed useful contrast to the image provided the image was subsequently corrected according to his theory. From a through-focus series of electron micrographs of thin crystals of catalase, he showed that a true phase-contrast image of the specimen seen in projection could be obtained. This apparently academic exercise in electron optics was later to form the basis of the dramatic work done by Nigel Unwin and Richard Henderson in producing a 7-angstrom resolution map of the unstained purple membrane from low-dose electron micrographs.

Thus, Aaron Klug has provided for the microscopist an arsenal of powerful weapons with which to attack structural problems. He had discovered them and demonstrated their use as part of his studies of important biological structures.

Tobacco Mosaic Virus Assembly

Klug's work on microscopy grew out of his earlier x-ray diffraction studies on viruses, beginning with the helical TMV particle. Tobacco mosaic virus was the paradigm for the "self-assembly" concept. The structure of the intact particle suggested a simple mechanism for its assembly, namely, the sequential addition of subunits to the growing helix. A variety of polymorphic forms of the protein were found and characterized. A particularly puzzling form was the disk aggregate, which was built of two rings of 17 subunits each.

How could a closed-ring structure be related to the helix assembly, or was it just an oddity? Reconstitution of TMV particles from dissociated protein and RNA was a slow process, requiring hours. Klug hypothesized that, as in crystal growth, the slow step was nucleation. He found that preformed disks could serve as nucleation centers, reducing the time of assembly to a few minutes. Crystals of the disk suitable for x-ray analysis were obtained but, because of the large size of this 34-subunit aggregate, the determination of its structure by crystallographic methods was a formidable technical and analytical problem. After 12 years the structure was solved to atomic resolution. Adjacent to the central hole of the disk there is a gap between the two rings rather like a pair of jaws. The TMV RNA has a specialized region that binds inside a pair of these jaws.

In the nucleation mechanism elucidated by Klug, the disk dislocates into a helical structure after binding a loop of the RNA. Thus, the disk is shown to be an obligatory intermediate in assembly. It initiates assembly of the protective protein coat about the RNA, and it is able to reject foreign RNA by failing to bind RNA's that lack the specialized region. Thus, in terms of the principles of virus construction formulated many years previously, the self-assembly of TMV is a self-checking process dependent on the subassembly of protein disks.

Chromatin Organization

A description of Klug's studies of nucleic acids and proteins must include mention of tRNA and chromatin. The structure of tRNA was determined by classical crystallographic methods, but the analysis of chromatin structure, which is still far from complete, involves novel combinations of chemical, diffraction, and electron microscopic methods.

Klug took up the study of chromatin about 10 years ago, when it had been shown that there were only five main types of histones. The structural problem thus appeared tractable. Initially, x-ray diffraction was used mainly as an assay method to follow the assembly of DNA and histones. These studies showed that the native structure could be reformed readily only if four of the histones were kept together in two pairs. This eventually led to the discovery that chromatin consists of a succession of nucleosomes, each built of two copies of each of the four histones, combined with 200 base pairs of DNA. The crystallization of nucleosomes showed, surprisingly, that DNA in the nucleus is organized at the molecular level in a highly regular manner. The crystals, which diffract to

5-Å resolution, are being studied by x-rays in order to visualize the substructure of the nucleosome.

The initial low-resolution studies have already revealed the outline of the first level of folding of DNA in the nucleosome. Two superhelical turns of the DNA double helix are wound around a shallow ramp made up of the four inner histones. This DNA supercoil is sealed off by the fifth histone, H1, which also stabilizes the next level of coiling of the chromatin filaments, the solenoid.

Klug's work on the solenoidal aggregates found in intact chromatin relates the cellular and molecular levels of organization. These studies exemplify his approach of chemically dissecting out parts of complex structures for detailed analysis by x-ray diffraction, the results of which are correlated with information about macromolecular organization of the intact assembly from electron microscopy.

Colleagues

Klug's interest in the structure of matter was first stimulated while he was a student in South Africa by R. W. James, who had worked with W. L. Bragg in building the foundations of x-ray crystallography. After Klug obtained his Ph.D. at the University of Cambridge, he turned to work on the structure of living matter, joining Rosalind Franklin in J. D. Bernal's department at Birkbeck College in 1954. Franklin had just then switched from studying DNA to studying TMV; Bernal, who had started protein crystallography 20 years earlier in Cambridge, had also begun x-ray diffraction studies of TMV and crystalline viruses. After Franklin's death, in 1958, Klug continued structural studies on viruses with John Finch and Kenneth Holmes and started a collaboration with one of us (D.L.D.C.) on the principles of virus construction. In 1962 he moved to Cambridge, where the scope of his investigations and number of collaborators expanded: in the study of virus chemistry and assembly he worked with Reuben Leberman, Tony Durham, Jo Butler, and David Zimmern; in virus crystallography, with William Longley, Peter Gilbert, John Champness, Gerard Bricogne, and Anne Bloomer; in electron microscopy and image reconstruction, with one of us (D.J.D.), Harold Erickson, Tony Crowther, Linda Amos, Jan Mellema, Nigel Unwin, and, throughout, John Finch; in the structural studies on transfer RNA, with Brian Clark, Jon Robertus, Jane Ladner, and Tony Jack; in chromatin, with Roger Kornberg, Markus Noll, Len Lutter, Daniela Rhodes, Ray Brown, and Tim Richmond. This list is incomplete and it is still growing.

Many of the notable achievements in structural biology have been made at the MRC Laboratory of Molecular Biology in Cambridge. This laboratory has provided an environment enabling long-term structural studies, which may lack the immediate excitement of some other areas of biology, but the knowledge painstakingly gained from these investigations is essential for understanding how living machinery works. Since 1978, Klug has been codirector with Hugh Huxley of the structural division, and together they have kept this laboratory at the forefront of structural biology. In 1962, when Klug joined the MRC Laboratory in Cambridge, Nobel prizes were awarded to Francis Crick, John Kendrew, and Max Perutz, fellow members of the laboratory, for their work on the structure of nucleic acids and proteins. It is fitting on this 20th anniversary of his arrival and of the earlier prizes, that Klug's work on the interaction of protein and nucleic acids has been so appropriately recognized.

Both of us wish to express our appreciation for the knowledge and understanding we have gained from Aaron Klug, and we congratulate him both for his award and for realizing spectacular potentials in the structural biology of macromolecular assemblies.

—D. L. D. CASPAR and D. J. DEROSIER

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The 1982 Nobel Prize in Economics

We get about what we pay for in this world. George J. Stigler has been a walking exception to this popular economic maxim, for the world has long received more from Stigler than it has paid to him. The Nobel Prize Committee's 1982 award helps reduce the imbalance. This is especially gratifying in the case of Stigler because of his insistence on empirical verification of economic maxims. Even a true believer in the importance of evidence can have his belief strengthened by 157,000 tax free data points.

Academic Affiliations and Early Work

Stigler's formal education in economics took place at the University of Chicago during the depths of the 1930 depression. Chicago's economics faculty included some of the leading economists of the time—Frank Knight, Henry Schultz, Henry Simons, and Jacob Viner. Some of the students they lectured also proved to be outstanding—Allen Wallis (President and Chancellor of the University of Rochester and now a senior member of the Department of State), Milton Friedman (1976 Nobel Prize winner) and George Stigler. This rare mixture of talents must have educated and excited students and teachers alike.

With Ph.D. in hand (1939), Stigler began his professional chores at Iowa State College but soon moved on to the University of Minnesota (traditionally possessing an above average economics department). From there he moved to Brown, but his first professional appointment of real duration was at Columbia. There he wrote and taught from 1947 to 1958, concluding the maturing part of his career. This was marked by the publication of his dissertation materials, a successful price theory text that was later to become *The Theory of Price* (*1*), several monographs for the National Bureau of Economic Research, and about 30 articles on a variety of topics. One of these, *Roofs or Ceilings, Foundation for Economic Education* (1946), which was coauthored with Milton Friedman,

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