

## PREFACE



In the course of the last several decades, biology went through a dramatic development. A new branch of natural sciences – *molecular* biology – emerged from a blend of biology, biochemistry and biophysics, bearing the ultimate promise of a scientific understanding of life processes. The scope of molecular biology is to find a chemical rationalization of all material transformations in living organisms, i.e. to understand such processes in terms of molecular reactions and molecular interactions. Macromolecules play a central role in living systems and the elucidation of structure and function of proteins – the major constituents of living systems – as well as nucleic acids – the molecules controlling genetic processes – were among the highlights of biochemical and biophysical research. The most powerful technique for the determination of the structure of macromolecules is still X-ray crystal structure analysis, which – under favourable circumstances – permits an elucidation of the structure of crystals of macromolecules down to atomic resolution. However, despite the efforts in many excellent laboratories, it has not been possible to obtain crystals suitable for X-ray analysis from all important macromolecular biomolecules by any means. However, most of them can be prepared in the form of pure solutions, in which case analysis by small-angle scattering with X-rays or neutrons can be used. This technique works without any destructive or modifying effect on the investigated substance, and – compared to crystal structure analysis – is better suited to investigate intermediate products of biochemical reactions. It is also much less time consuming.

Judging from the number of studies reported in the literature and from the variety of results, small angle scattering with X-rays dominates neutron scattering. The reasons are probably not scientific. Many applications would benefit immensely from the use of neutrons, but the fact that only few suitable neutron sources exist around the world prevents wider use.

Another very powerful technique applicable in this area should, of course, be mentioned in this context: electron microscopy. In its classical form, it has the great advantage of yielding directly interpretable pictures, while, for any X-ray scattering technique, the structure has to be computed from the angular dependence of scattered intensity. This can be quite a formidable task, which, however, has been dramatically facilitated by modern computer technology. Considerations of computer time are, therefore, today no great obstacle to a successful application of small-angle scattering. The advantage of direct interpretability of electron micrographs has to be paid for by the price of a possibly destructive or modifying sample preparation, which involves such steps as precipitation, metal-plating, etc.

There are still other methods which, similar to small-angle scattering, are applicable to solutions and which work non-destructively. We mention such important techniques as ultracentrifugation, Staudinger viscosity, coherent light scattering, etc. Each of these techniques yield one or two parameters characterizing the investigated molecules.

Does the small-angle method have any significant advantages over these techniques? How does it work? – An extremely thin bundle of X-rays passes through the sample containing a solution of macromolecules, whose dimensions (100-10000 Å) are immensely large compared to the X-ray wavelength (~ 1 Å). According to the law of the reciprocity of optics, the diffuse scattering of the large particles is concentrated in a small angular range around the primary beam; the larger the particles are, the smaller the scattering angle is.

Today, highly specialized instruments, such as small-angle cameras, proportional counters, position sensitive detectors and, if very short recording times are required, high-power X-ray sources are available for the routine detection of this scattering.

The small-angle technique is particularly powerful for the investigation of homodisperse macromolecular solutions. In its total effect, it is comparable to microscopy, but with improved resolution by three orders of magnitude. Thus, in addition to obtaining the average size of macromolecules, one also obtains more precise information. We shall discuss that in four steps:

(1) The technique allows direct determination of about a dozen parameters. Among them are the radius of gyration of the electron density, which corresponds to the radius of gyration in mechanics and which characterizes the particle size. For elongated particles, one obtains the radius of gyration of the cross section (perpendicular to the long axis of the particle), for plate-like particles, one obtains the radius of gyration of the thickness, and, therefore, also the thickness itself. Moreover, one can determine volume and surface area of the particle, and-for elongated particles-the area of the cross section. Finally, by Fourier inversion of the scattered intensity, the distance distribution function, i. e. the distribution of distances between all pairs of electrons in the particle, can be obtained. In addition to these geometrical parameters, it is possible (from a determination of the ratio between scattered intensity and primary intensity) to deduce the particle molecular weight and, for elongated and plate-like particles, the masses per unit length and per unit area, respectively.

(2) Even more detailed information about the particle geometry is accessible by a trial-and-error procedure. Based on the above parameters and, possibly, on additional information from other techniques (electron microscopy, chemistry, etc.), one postulates a particle shape. Its theoretical scattering curve can easily be computed, and from the deviation between theoretical and experimental curve one obtains indications about modifications of the model. Repetitions of this procedure may, eventually, converge to a model which is equivalent in scattering to the investigated molecule. Frequently, hundreds of models have to be tested, which, however, is no serious problem in view of the performance of modern computers.

(3) So far, we talked about homogeneous particles; however, particles may contain inhomogeneities whose dimensions are not negligible compared to the particle size. Direct characterization of such inhomogeneities is possible for particles with certain symmetries: for nearly spherical particles and for particles with approximate cylindrical symmetry, one can compute the radial electron density distribution. For plate-like particles with a mirror plane parallel to the particle plane, the average electron density distribution perpendicular to the mirror plane is obtainable.

(4) Additional information is accessible if markers-consisting e.g. of heavy atoms or groups of heavy atoms-can be specifically attached to the molecule. Comparison of the scattering curves of marked and unmarked particles will then yield the distances between the markers. This technique is particularly powerful in neutron small-angle scattering, where particles with deuterium instead of hydrogen, differ significantly in their scattering behaviour. Thus, if large particles consisting of many subunits are under investigation (e.g. ribosomes containing many protein molecules), it may be possible to replace two subunits by their deuterated isomers. The two deuterated subunits will then behave as markers, and their distance can be computed. Repetition with other pairs of subunits will eventually yield the spatial arrangement of all subunits. This process, which is the three-dimensional analogon of the work of land surveyors, has been termed "triangulation" in both areas.

We tried to demonstrate the role of small-angle scattering in the concert of modern biophysical methods for the elucidation of macromolecular structure. However, the technique is, of course, not restricted to soluble biomolecules. Any colloidal system can be investigated by small-angle scattering, yielding valuable results in such diverse areas as dissolved and solid natural and synthetic high polymers, colloidal powders, glasses and alloys. In fact, small-angle scattering has established itself as an invaluable tool in colloidal research.



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