Small-angle X-ray scattering was eyeing micron-level structures before they were “nano”.

With the current focus on all things nanoscale, chromatographers and spectroscopists have been busily adapting their small-molecule techniques to examine increasingly larger molecules. Imaging technology is also evolving to capture features in the size range between electron and optical microscopy, but one nanoscale analysis method has been around since the 1950s—small-angle X-ray scattering (SAXS). Unlike diffraction techniques, which are inherently limited to length scales up to a few tens of angstroms, small-angle scattering methods are ideally suited to examining structural features a few tens to a few thousands of angstroms across—the region of interest for nanoscience. SAXS cameras, which use the same type of X-ray sources as diffractometers (and often share a source on the same instrument) are a common sight in polymer research labs, but several national laboratories have installed small-angle instruments at their neutron and synchrotron X-ray sources as well.

Rings, Chains, and Blobs

SAXS is familiar to the polymer science community as a method for looking at particle sizes and distributions and for determining molecular weights and nanoscale structural features (see box, “How It Works”). SAXS scans and images show periodic ordering arising from crystalline or partially crystalline regions in the sample, in much the same fashion as a diffraction pattern, but on a longer scale. However, SAXS also shows noncrystalline features, such as strain and molecular alignment, that are characteristic features of a polymer’s processing history (Figure 1). SAXS shows particle sizes, shapes, and distributions in block copolymers and blends, and it characterizes inclinations such as elastomer particles in polymer matrices. This information is useful in studying crystallization kinetics, miscibility, mechanisms and effectiveness of compatibilizers, phase separation, dendrimer growth, and chain conformations.

One of the most common uses of SAXS is determining the radius of gyration ($R_g$) of a nanoscale structural feature such as an inclusion in a polymer matrix, a self-assembled molecular ring or layer structure, a micelle, or a protein that can fold and unfold. $R_g$ is an indicator of how the mass of a particle is distributed around its center of mass, a useful parameter for comparing irregularly shaped particles. It is defined as the root mean square of the distances between points in the particle (taking mass into consideration) and the center of gravity. $R_g$ is found using a Guinier plot; that is, a plot of scattered X-ray intensity versus the square of the scattering vector (which is a function of the incident wavelength and the scattering angle) ($\theta$).

Researchers from Nara, Japan, recently used SAXS measurements of $R_g$ to confirm that a complex that they had prepared was actually a ring composed of six porphyrin dimers (3). Ryoichi Takahashi and Yoshiaki Kobuke constructed this complex to mimic the light-harvesting antenna compounds used by photosynthetic organisms. Their SAXS measurements, taken at a synchrotron X-ray source, allowed them to calculate $R_g$ values for the complex that corresponded to a hexameric ring model. Their synthetic assembly, a closed ring formed by six imidazolylporphyrinatozinc (II) dimers connected by 1,3-phenylene spacers, closely resembles the light-harvesting ring assemblies found in photosynthetic purple bacteria.

"Green" Solvent for Methyl Orange

In an earlier study, Joseph Desimone and colleagues at the University of North Carolina at Chapel Hill equipped a SAXS instrument with a high-pressure cell to characterize dendritic surfactants for extracting hydrophilic compounds from water into liquid CO$_2$ (4). In recent years, CO$_2$ has been touted as an environmentally friendly replacement for various organic solvents, but only a few types of polymers are soluble in either liquid or supercritical CO$_2$. Desimone’s group assembled micelles based on hydrophilic dendrimer polymers functionalized with a fluorinated outer shell that would allow the micelle to dissolve in CO$_2$. Methyl orange, a watersoluble ionic dye, can be encapsulated in these micelles and transferred from an aqueous phase to liquid CO$_2$ at 24 °C and 340 atm pressure. High-pressure SAXS measurements showed that $R_g$ for the fluorinated dendrimer micelles (30 ± 1 Å) stayed constant regardless of their concentration in the CO$_2$ phase, an indication that the micelles were not aggregating. The SAXS data were also used to calculate the relative molecular mass of an individual micelle (33,500 amu), a value consistent with values obtained using proton NMR and elemental microanalysis. Pressure cells equipped with optically trans-
**How It Works**

SAXS works in much the same way as its more familiar cousin, X-ray diffraction (XRD); in fact, some instruments are set up to perform both techniques, using the same X-ray source and two different cameras. Both techniques use a narrowly collimated beam of monochromatic X-rays as a light source, which illuminates the flat surface of a sample. The sample can be rotated through a range of angles with respect to the beam to produce a linear scan, or a two-dimensional detector can capture the entire scattering pattern at once.

Diffracted X-rays are scattered elastically by the electron clouds surrounding the atomic nuclei in the sample (i.e., the X-rays lose no energy when they “bounce off” the electron clouds, much like two billiard balls colliding). If the atoms are arranged in a crystal lattice, the scattered X-rays form an interference pattern as they exit the crystal. The pattern depends on the incident wavelength, the scattering angle, the crystal structure, and the chemical elements that make up the sample. The larger the distance between two crystal planes, the smaller the angle at which a diffraction maximum, or peak, occurs in the diffraction scan. Typical diffraction scans can measure scattering at angles down to a few degrees, a measurement that corresponds to a few tens of angstroms when a conventional (non-synchrotron) X-ray source is used.

SAXS starts where XRD leaves off, measuring the scattering in the angular range from a few degrees down to a few hundredths of a degree between the X-ray beam and the sample surface. This gets past the length scale between crystal planes and into the much-investigated “nano” region, from a few tens of angstroms to several hundreds of angstroms—or thousands of angstroms, using the right optics. (Some instruments can get down to less than 0.001 degree to measure nm-sized features.) Thus, SAXS patterns give information on structural ordering arising from crystalline and noncrystalline features. SAXS patterns arise from inhomogeneities in the distribution of electrons in the material under study. These differences can be between inclusions and the surrounding matrix, between types of atoms in a chemical compound, or between individual molecules or molecular assemblies.

One primary difference between SAXS and XRD instruments is the pinhole mask or slit collimator needed to shield the SAXS detector from the incident beam. In a typical XRD pattern, low-angle scattering is obscured by a “halo” coming from the incident X-ray beam. A narrow pinhole or slit collimator (or a series of collimators) between the X-ray source and sample creates a very small, highly parallel incident beam, which allows scattering to be detected at very small angles without being washed out by the intensity of the direct beam. Of course, this also reduces the intensity of the signal coming to the detector, and improved detector technology has been critical to the development of the technique.

Newer instruments also rely on more intense X-ray sources (including rotating anode and synchrotron sources), highly effective monochromators, and evacuated beam paths (to reduce stray scattering by air molecules). Cross-coupled Göbel mirrors use multilayered optical coatings to produce an intense, highly parallel beam (6). High-speed area detectors capture an entire two-dimensional scattering pattern simultaneously, making it possible to do time-resolved studies and to examine short-lived structural features.

**Beta Sheet Shows Its Face**

Synthetic polymers are networks of long carbon chains with various functional groups, built up from small-molecule building blocks. So are proteins. Thus, it was only a matter of time until the protein research community adopted the SAXS techniques that originated in polymer research. A team of researchers from the University of Rochester (NY) Medical Center and Yale University studied the size and shape of a soluble form of the immunogenic lipoprotein “outer surface protein K” (OspA) for the Lyme disease spirochete bacterium *Borrelia burgdorferi* (5). The protein was complexed with the Fab fragment of a monoclonal antibody (the part of the antibody that contains the binding site for antigens). The OspA structure has a central single-layer β-sheet connecting globular N- and C-terminal domains. It is unique in that the β-sheet is exposed rather than having its hydrophobic face buried in an interior hydrophobic core, as is the case for similar protein structures. The researchers wondered if the β-sheet in OspA was held in an exposed position by the bound antibody or the crystal lattice packing and whether this conformation would change when the protein molecules were in solution. Using previous crystal structure studies and NMR studies of the local structure of the OspA for comparison, they looked at the global conformation of OspA in solution using SAXS. They found that all three techniques yielded a similar structure, showing that the OspA structure observed in the crystal form was also stable in solution. SAXS measurements were used to calculate Rg, the length distribution function P(r) (a direct measure of global conformation), and the maximum dimension Dmax (confirming that the molecule was as rigid in solution as it was in crystal form).

SAXS has been sorting out shapes and sizes in the nano zone for many years, providing polymer chemists with a tool to look at crystallization and processing effects. It was only natural that researchers working on biomimetic polymers would take up the technique, and from there, it was a short hop to biological molecules themselves.

**References**


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