KN5 Femtosecond Protein Nanocrystallography with an X-ray Free-Electron Laser.

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The ultrafast pulses from X-ray free-electron lasers (FELs) are of high enough intensity and of sufficiently short duration that individual single-shot diffraction patterns can be obtained from a sample before significant damage is apparent at the atomic scale. We have applied this "diffraction before destruction" method to determine the molecular structures of proteins in the form of micron to sub-micron crystals, with pulses up to 10^{18} W/cm^2 [1,2] Using the Linac Coherent Light Source we recorded millions of diffraction patterns from a flowing stream of protein crystals. Our measurements show that sample destruction at 1.9 Å resolution can be avoided with pulses of 30 fs or shorter. This style of macromolecular crystallography provides many advantages: data are collected from samples at room temperature, avoiding possible biases caused by cryogenic freezing of samples; sub-micron to micron sized crystals are more easily formed than macroscopic crystals, overcoming a major bottleneck in sample production; natural (in vivo) protein crystals can be studied; radiation-sensitive samples such as proteins with metal centers can be examined; higher quality diffraction is obtained when the crystal size is comparable to the long-range order length-scale of the crystal; and anomalous diffraction signals, which can be used for phasing, are enhanced by the extreme X-ray intensities. The method also opens up the possibility of time-resolved measurements of reversible or irreversible reactions, either by stopped-flow techniques (actually continuous flow, but the short pulse freezes all motion) or by photo excitation.

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[2] Boutet, S. et al. (2012). Science May 31.

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KN6 Absolute Structure Determination.

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The ability to distinguish one absolute structure from its inverted analogue depends on the resonant (or anomalous) scattering effects having sufficient magnitude to lead to measurably different intensities for reflections with indices *hkl* and $\overline{hkl}$ (and their symmetry-equivalents). These *Bijvoet differences* depend on the elements present in the crystal and the wavelength of the X-rays used to collect data.

In an absolute structure determination one absolute structure is refined competitively against the inverted alternative. The result is expressed by the Flack parameter $x(u)$, which for can be interpreted as the mole fraction of the alternative enantiomer in the crystal.[1] The physical range of x is 0 to 1; Flack and Bernardinelli have shown that the standard uncertainty (u) should be less than 0.1 before any firm conclusions can be drawn.[2]

The most important practical application of absolute structure determination is in the identification of absolute configuration of enantiopure chiral compounds, particularly in organic and pharmaceutical chemistry. However, Bijvoet differences for light-atom organic compounds are very small even for data collected with Cu-K radiation, and meeting Flack and Bernardinelli's precision criterion is difficult. Strategies will be described which address this problem.

In addition to a number of post-refinement statistical procedures [3,4], two methods which can be applied during refinement will be described. In one method is based on the quantity

$$D(\mathbf{h}) = \frac{I(\mathbf{h}) - I(\text{-}\mathbf{h})}{I(\mathbf{h}) + I(\text{-}\mathbf{h})} = (1 - 2x)\frac{F^2(\mathbf{h}) - F^2(\text{-}\mathbf{h})}{F^2(\mathbf{h}) + F^2(\text{-}\mathbf{h})}$$

which is used to define restraints based on observed and calculated values of $D(\mathbf{h})$. Systematic errors in the intensities, such as absorption, tend to cancel out (in an average way) so that measured values of $D(\mathbf{h})$ should be more accurate that the values of the measured intensities.

The second method is based on leverage analysis, which yields quantities which measure the influence that observations have on the precision of a specific parameter.[5] A weighting scheme has been developed where data which strongly influence the Flack parameter are systematically up-weighted.

Both methods yield significantly more precise values of the Flack parameter than conventional refinement. In the case of alanine, conventional refinement yielded x = 0.12(21), whereas the restrained refinement yielded a value of 0.00(8). A refinement based on the leveraged weighting scheme yielded x = -0.02(6).

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[5] Parsons, S., Wagner, T., Presley, O., Wood, P.A. & Parsons, S. (2012). *J. Appl. Cryst.* **45**, 417-429

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