18.4.1.2. What is 'atomic resolution'?

Atomicity is the great simplifying feature of crystallography in terms of structure solution and refinement. For a small-molecule structure, accurate X-ray data usually extend to 0.8 Å, and this has three important implications for crystallography.

(1) *Ab initio phasing using direct methods*. Automatic *ab initio* solution of the phase problem depends on the assumption of positivity and atomicity of the electron density. The fact that current *ab initio* methods in the absence of heavy atoms are only effective when meaningful data extend beyond 1.2 Å reinforces the idea that this is a reasonable working criterion for its definition as atomic resolution. In addition, approaches such as solvent flattening and automated map interpretation benefit enormously from such data.

(2) *Resolved atomic peaks in the Fourier maps*. Although some individual peaks can be seen at resolutions beyond ~2.0 Å, they become more fully resolved at around 1.2 Å.

(3) *Refinement of a full anisotropic model*. The number of reflections is sufficient for the minimization of the discrepancy between the experimentally determined amplitudes or intensities of the Bragg reflections and those calculated from the atomic model with up to ten (usually nine) independent parameters per atom. This has been classically achieved by least-squares refinement as described in *International Tables for Crystallography* Volume C, Chapter 8.1 (Prince & Boggs, 2004) or more recently by maximum-likelihood procedures (Bricogne & Irwin, 1996; Pannu & Read, 1996; Murshudov *et al.*, 1997). For small-molecule structures, accurate amplitude data are normally available to around 0.8 Å, giving an observation-to-parameter ratio of about seven for non-centrosymmetric crystals, which allows positional parameters to be determined with an accuracy approaching 0.001 Å. This reflects the high degree of order of such crystals, in which the molecules in the lattice are in a close-packed array. In addition the X-ray data are of high quality, with a high $I/\sigma(I)$ ratio (and hence low merging R value) even in the outer resolution shells.

It is now necessary to define what constitutes 'atomic resolution'. A pragmatic approach has been that data extending to 1.2 Å or better with at least 50% of the intensities in the outer shell being higher than 2σ is the acceptable limit (Sheldrick, 1990; Sheldrick & Schneider, 1997), which means that the statistical problem of refinement is overdetermined. This appears to remain a good working definition for refinement applications and indeed has been put on a more solid theoretical basis (Morris & Bricogne, 2003; Morris *et al.*, 2004). However, for application of direct phasing methods it is advantageous to record even a small fraction of significant reflections beyond this cutoff. These outer shells should be included in the refinement procedure with correct maximum-likelihood weights, but they will not significantly improve the effective resolution.

This is rarely achieved for crystals of macromolecules: as of October 2009 around 1250 out of 52 000 crystal structures in the Protein Data Bank (PDB) had a resolution higher than 1.2 Å compared to 157 out of 13 000 in March 2000. Firstly, the large unit-cell volume leads to an enormous number of reflections for which the average intensity is weak compared to those for small molecules (see Table 9.1.1.1 in Chapter 9.1). Secondly, the intrinsic disorder of the crystals further reduces the intensities at high Bragg angles and usually gives a resolution cutoff which is much less than atomic. Thirdly, the large solvent content leads to substantial decay of crystal quality under exposure to the X-ray beam at room temperature. While the secondary damage (resulting from the migration of ions and radicals produced by the primary absorption event) is largely avoided by vitrification of such crystals, the effect of primary damage has become significant on high intensity beamlines (see Section 9.1.12). The upper resolution limit of the data affects all stages of a crystallographic analysis, but especially restricts the features of the model that can be independently refined (Table 18.4.1.2). Solutions to the problem of refining macromolecular structures with a paucity of experimental data evolved during the 1970s and 1980s with the use of either constraints or restraints on the stereochemistry, based on that of known small molecules. With constraints, the structure is simplified as a set of rigid chemical units (Diamond, 1971; Herzberg & Sussman, 1983), whereas using restraints, the observation-to-parameter ratio is increased by introduction of prior chemical knowledge of bond lengths and angles (Konnert & Hendrickson, 1980).

Table 18.4.1.2
Features which can be seen in the electron density at different resolutions

Disordered regions will not necessarily be visible even at these limiting values. Some features should be included even at lower resolutions, *e.g.* hydrogen atoms at their riding positions can be incorporated at 2.0 Å, but their positions will not be verifiable from the density. The contents of this table should not be taken as dogmatic rules, but as approximate guidelines.

Resolution (Å)	Feature
0.8	Deformation density, *i.e.* deviation from the spherical-atom model
1.0	Hydrogen atoms
1.5	Anisotropic atomic displacement
2.0	Multiple conformations
2.5	Individual isotropic atomic displacement
3.5	Overall temperature factor
4.0	α-Helices and β-sheets
6.0	Domain envelopes

As expected, atoms with different ADPs contribute differently to the diffraction intensities, as discussed by Cruickshank (1999*a*,*b*). The relative contribution of the different atoms to a given reflection depends on the difference between their ADPs $\{\exp[-(B_1 - B_2)s^2]$, where $s = \sin\theta/\lambda\}$. Clearly, if the average ADP of a molecule is small, then the spread will also be narrow, and most atoms will contribute to diffraction over the whole range of resolution. When the mean ADP is large, then the spread of the ADPs will be wide, and fewer atoms will contribute to the high-resolution intensities (Fig. 18.4.1.2).

Three advances in experimental techniques have combined effectively to overcome these problems for an increasing number of well ordered macromolecular crystals, namely the use of high-intensity synchrotron radiation (SR), efficient two-dimensional detectors and cryogenic freezing (discussed in Parts 8, 7 and 10, respectively). These advances mean that there is no longer a sharp division between small-molecule and macromolecular crystallography, but rather a continuum from small through medium-sized structures, such as cyclodextrins and other supramolecules, to proteins. The inherent disorder in the crystal generally increases with the size of the structure, due in part to the increasing solvent content. Thus, it has become tractable to refine a significant number of protein structures at atomic resolution with a full anisotropic model (Dauter, Lamzin & Wilson, 1997; Dauter, 2003). This work of course benefits tremendously from the experience and algorithms of small-molecule crystallography, but does pose special problems of its own. The tech-

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