Proteins at atomic resolution

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Experimental advances in data collection, including bright sources, cryogenic cooling and two-dimensional detectors, have made it tractable to record data to beyond 1.2 Å for several proteins, yielding high-accuracy models and fine details of structure. For small metalloproteins, atomic-resolution data have enabled ab initio solution of the phase problem.

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Introduction

This year marks the 100th anniversary of the discovery of X-rays by Röntgen. Since that discovery the application of X-rays to a broad spectrum of problems, including medical imaging and molecular structure determination, has become standard. The latter field includes a variety of techniques, such as small angle scattering, X-ray spectroscopy, powder diffraction and single crystal X-ray diffraction, with which we are here concerned.

Why are X-rays so well suited to the determination of atomic structures in three dimensions? The answer lies in a combination of their physical attributes. Firstly, X-rays can be generated from appropriate metal anodes with wavelengths of ~1.0 Å, ideal for resolving the positions of individual atoms. Secondly, X-rays penetrate deeply into crystals without being totally absorbed, unlike electrons. Thirdly, they have a reasonable cross-section of interaction with the electrons around the atomic centres (the diffraction efficiency for organic structures is approximately 1 in 10^4) much better than do neutrons. Finally, they are cheap and easy to produce in a conventional laboratory, although intense sources of synchrotron radiation again enter the 'big science' level of funding.

The great advantage of X-ray crystallography is that for normal small molecules data can be recorded to truly atomic resolution, beyond 1.0 Å, allowing the positions of the ordered atoms in the structure to be accurately identified with a typical coordinate error of ~0.002 Å. Moreover, the accuracy relates as much to long as to short interatomic distances within the molecule, unlike many spectroscopic methods which give only short-distance information. Given these attributes, single crystal X-ray techniques have become routine in the small-molecule field. Tens of thousands of structures have been determined, both in academia and in industry. The major limitation to the scope of X-ray studies is the phase problem, as only the amplitudes, and not the phases, of the diffracted rays can be recorded experimentally. This problem is essentially solved for small structures.

Particular problems attend the solution of larger structures, involving not only their sheer size but especially their degree of disorder, factors which make the collection of atomic-resolution data a challenging prospect. The lack of such data causes difficulties at all stages of a structure analysis, from data collection through structure solution to refinement. Several advances during recent years, including two-dimensional (2D) detectors, high-intensity sources and cryogenic cooling, have meant that atomic resolution can be recorded for at least a small subset of well ordered protein crystals.

Although we feel that a review of progress in this field is timely, it is not possible to refer to an extensive set of published results. With the exception of a small number of pioneering studies, such as those involving avian pancreatic polypeptide [1], crambin [2], rubredoxin [3] and insulin [4], data on other structures have been collected within the past three years or so and most are in the process of detailed refinement. We cannot therefore provide a true survey of the work of all groups in the field. Instead, we will give a largely subjective description of our own studies at the European Molecular Biology Laboratory (EMBL) in Hamburg, including the projects of a number of groups that visit the facility. We hope that such an offering will stimulate others to make best use of their crystals and to attain atomic resolution wherever possible.

Advantages of atomic resolution

We heuristically define atomic resolution according to George Sheldrick [5]: the data should extend to at least 1.2 Å, and at least 50% of data in the outer resolution shell should have intensities of >2σ, which roughly corresponds to a merging R factor of ~25%. Data at the resolution edge with errors above this level may prove to be

<table>
<thead>
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<th>Abbreviations</th>
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<td>AkP—Automatic Refinement Procedure; CCD—charge-coupled device; 2D—two-dimensional; EMBL—European Molecular Biology Laboratory; MSD—Macromolecular Structural Database; NCS—non-crystallographic symmetry.</td>
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important in many applications, for example, where data are sparse, or in solving the phase problem using direct methods.

Atomic-resolution data confer two important possibilities. Firstly, for small structures, the phase problem can be solved directly using either Patterson or direct methods. This has also been shown to hold true for small metallo-proteins but not for proteins that lack heavy-atom centres. Secondly, they allow comprehensive least-squares refinement of the structure with anisotropic atomic temperature factors: at 1.0 Å resolution, for example, there are still more than five observations per parameter. The positions of most of the H atoms can be identified in the electron density. The final R factor can be as low as 2% and the residual results largely from the neglect of bonding and lone-pair electrons in the spherical-atom approximation.

Problems of data collection for large structures

Data collection for macromolecules presents two problems, inherent in the nature of the crystals and responsible for all subsequent difficulties in the crystallographic analysis. The first is that the number of X-ray data increases with the cube of the average cell dimension while the average intensity of the reflections decreases (See Table 1). Thus, data collection to atomic resolution is a daunting task even for small proteins. This problem affects all data, both at high and at low resolution. Assuming that the crystals are well ordered, the problem boils down to improving the signal-to-noise ratio, which requires improved counting statistics. This can be achieved by using a higher incident intensity on the crystal combined with simultaneous recording of all diffracted reflections.

<table>
<thead>
<tr>
<th>Table 1. The problem of data collection to atomic resolution (&gt;1 Å) for structures of increasing size*.</th>
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<tbody>
<tr>
<td>Structure</td>
</tr>
<tr>
<td>-----------</td>
</tr>
<tr>
<td>Small organic</td>
</tr>
<tr>
<td>Supramolecule</td>
</tr>
<tr>
<td>Protein</td>
</tr>
<tr>
<td>Virus</td>
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</table>

* Most proteins and all viruses do not in practice diffract to 1 Å resolution. The difficulties arise from the number of reflections increasing as the cube of the average cell dimension and their average intensity decreasing also as the cube.

The higher intensity can be achieved by using modern rotating-anode generators; however, the use of synchrotron radiation is more effective. Rotating anodes may provide up to $10^9$ to $10^{10}$ photons mm$^{-2}$ s$^{-1}$, third-generation synchrotrons approx. $10^{12}$, and fourth-generation sources such as the European Synchrotron Radiation Facility or the Advanced Photon Source should provide at least a further two orders of magnitude. In contrast, efficient recording of the data poses a challenge. Two-dimensional detectors have become standard in most laboratories, and the current detector of choice for the home laboratory is the imaging plate. For synchrotron sources, however, the read-out time is rate-limiting. This problem of detectors with insufficient data acquisition speeds is already substantial at third-generation sources; it can only get worse at fourth-generation ones. At several sites such as CHESS, the imaging plate is being succeeded by charge-coupled devices (CCDs) [6].

The second problem of data collection for macromolecules results from their inherent disorder. The size of the molecules means that they can no longer close pack in the crystal. As a result, the interstices are filled with disordered solvent, although water molecules close to the protein surface do take up an ordered structure. A typical protein crystal is ~50% 'solid' protein phase and ~50% liquid solvent [7]. The lattice forces are weak and often the surface residues of the protein show substantial static or thermal disorder. This exacerbates the weakness of the data at high resolution.

A knock-on effect of the high solvent content is the sensitivity of protein crystals to radiation damage. Although all crystals suffer to a small extent from the direct or primary damage, crystals with high solvent content experience a secondary effect caused by diffusion of the resulting radicals and ions through the solvent channels. This sensitivity of aqueous systems to X-rays is similar to the damage induced in living cells.

In this respect cryogenic freezing represents a fantastic advance. Cryogenic cooling has been applied for several years to small molecules, where the problem of freezing the sample is minimal and typically results in a decrease in average atomic temperature factor and in disorder. For macromolecules these advantages are also to be expected. However even more significant gains can be made in effective elimination of secondary radiation damage. Many protein crystals become in practice immobile in the X-ray beam, within the constraints described by Richard Henderson [8]. Of course, freezing of protein crystals [9] does bring experimental difficulties, involving the formation of ice either within the intermolecular interstices in the crystal or within any residual mother liquor around it. However, the problems are greatly outweighed by the gains. The great majority of crystals can now be frozen successfully after transfer to a cryoprotectant solution. The crystal is rapidly (seconds) scooped up in a fibre loop and exposed to the cold gas stream. For many proteins the process of freezing, while sometimes increasing the mosaic spread, reveals a resolution which could only be presumed to have been lost during the first exposure at room temperature. Experiments suggest that freezing can often increase the resolution by 0.5 Å or more.
Computing hardware, software and algorithms

Ever since the 1950s development in crystallography has relied upon, and indeed has driven, advances in computing. Structure solution and refinement has consistently required the most powerful computers available. The importance of computer power for atomic analysis of proteins lies in three areas of experimentation:

The first of these is data capture and reduction. The fast rates of data acquisition made possible by 2D detectors require substantial computing power and efficient algorithms for data reduction. The former has been effectively addressed through a number of computer programs. The latter has advanced through the development of several software packages, e.g. MOSFLM [10], DENZ0 [11] and XDS [12]. High-resolution data can be rapidly extracted from diffraction images with ease.

The second area is structure solution (the phase problem). This is particularly computer intensive, requiring ever more computer time for advanced algorithms in methods akin to maximum likelihood, pioneered by Gerard Bricogne [13,14], or direct methods such as those advanced by the groups of Woolfson [15], Hauptmann [16] and Sheldrick [17]. For all of these methods the exponential increase in computer power has been vital, although it is still often limiting.

The final area is structure refinement. Algorithms for refinement of small molecules nowadays require minimal computing power; however, the needs increase rapidly as the size of the structure increases. Conventionally, macromolecular structures have been refined at resolutions well below atomic and have required the use of constrained [18] or restrained [19] procedures to either reduce the number of parameters refined or increase the observations using stereochemical restraints. The problems of limited resolution are highlighted in Table 2. With atomic-resolution data, it is possible to return to classic crystallographic approaches.

**Procedures used in our refinements**

For the structures that we are currently refining, a lower-resolution model has generally been available from multiple isomorphous replacement or molecular replacement at lower resolution. We have found the following protocol useful.

**Starting model**

The starting model is first refined against the atomic-resolution data using stereochemically restrained least-squares minimization, with conjugate gradient approximation, which gives a short computing time per cycle. We have used the PROLSQ suite [19] incorporating fast Fourier syntheses [20] from the CCP4 suite [21]. This is a subjective choice and it is clear that other programs such as TNT [22] or X-PLOR [23] should perform this role equally well.

The model is refined with isotropic atomic temperature factors. A serious problem arises when building water structure in proteins. It becomes increasingly time consuming to inspect subjectively the difference map as more and more 'waters' emerge in the improving density at atomic resolution. It is useful to apply at least a semi-objective set of criteria for water selection and we have used the Automatic Refinement Procedure (ARP) of Lamzin and Wilson [24,25], according to which water sites are selected on the basis of distance criteria, electron density and sphericity. This is not the only approach possible, but it is important that some such objective procedure is used. The B values of the waters selected are generally less than 50; all waters are accorded unit occupancy. ARP is used in combination with PROLSQ, but in the subsequent steps with SHELXL-93. Hydrogen atoms riding on their parent atoms are introduced into the model: this generally reduces the R factor by 0.5–1.0%.

The models refined using this procedure typically have R factors in the range 14–18%, which are comparable to those obtained for many proteins at resolutions of ~1.5 Å. One of the questions that we have sought to answer in our studies at atomic resolution was the source of this residual: for small structures, values in the range 2–4% are normal.

**Anisotropic model**

After convergence with PROLSQ, we next use the program SHELXL-93 [26*]. This incorporates many features that make it applicable to macromolecular structures. Particularly important is the existence of

| Table 2. The effects of refining structures at resolution less than atomic. |
|---|---|---|
| Resolution (Å) | Protocol | Features identifiable |
| 1.0 | Full-matrix, anisotropic atoms | Fully resolved atoms |
| 1.5 | The border between isotropic/anisotropic | Hydrogens, disorder; ordered atoms distinguished |
| 2.0 | Isotropic atoms, stereochemical restraints | Some disorder |
| 2.5 | Isotropic model starts to break down | Shape of small groups |
| 3.0 | Rigid groups, some constraints | Shape of fragments, e.g. helices |
| 6.0 | Complete domains as rigid bodies | Globular protein |
flexible options to model atomic anisotropy and of different kinds of restraints on geometry and on atomic temperature factors. Stereochemistry can be restrained against an external library based on small-molecule structures [27–29], or chemically identical units can be restrained to be the same. SHELXL–93 allows double conformations to be refined with complementary occupancies. The stereochemical restraints are only required for the disordered, highly flexible, regions: for well-ordered regions the X-ray terms are sufficient to define the atomic positions to within ~0.03 Å.

We usually run several cycles with isotropic temperature factors upon introducing the model to SHELXL–93. This rapidly converges to an R factor equivalent to that obtained with PROLSQ. Ridding H atoms are included throughout. Subsequently, switching on refinement of anisotropic B values increases considerably the time per cycle. The conjugate gradient algorithm is used: this reduces the time per cycle but more cycles are required to achieve convergence. The R factor generally drops by 5–6% upon introducing anisotropy, leading to final values in the range 8–12%. Selection of waters can be made automatic, incorporating the anti-bumping restraints built into the program. We have continued to use ARP to achieve this.

Finally a few (2–5) cycles of full- or blocked-matrix minimization should be carried out. This is time consuming even for small proteins, and at present necessitates access to a supercomputer for large proteins. The time per cycle for the ribonuclease described below was more than 24 h-cpu on an SGI Challenge.

Validation of the refinement
To confirm the validity of introducing extra parameters for macromolecular structures, the most widely used criterion is the cross validation R_free [30]. A random sample of at least 1000–2000 of the reflections is excluded from the least-squares and the residual for this provides R_free. The introduction of extra parameters should produce a drop in R_free as well as in R: at what point the drop becomes significant in absolute terms is less clear [31**].

At a resolution of, say, 1 Å, introducing anisotropic temperature factors is clearly valid. R_free falls by almost as much as the R factor, about 5–6%. At 1.5 Å, R_free will drop by 2–3%, provided the data are good, i.e. complete and with an Rmerge value of <25% in the outer shell: this is still significant. At 2 Å resolution, however, R_free does not fall at all upon introduction of anisotropy (GM Sheldrick, personal communication) and the model should certainly be isotropic. Having established this protocol it seems unnecessary to assess anisotropy for all subsequent refinements at atomic resolution with R_free.

R_free has proved to be a much less useful validation tool in assessing the fine details of refinement [31**]. Such details include the modelling of alternative protein conformations and alternative models for water, for example, with or without solvent continuum, full or partial occupancies, or with the SHELXL–93 anti-bumping restraints as opposed to ARP. The difference in the real R factor, let alone R_free, is generally minimal between the different models and does not provide an objective overall criterion. It is necessary to return to local criteria, such as the real-space R factor [32] and to detailed inspection of density to validate the models. Overfitting of the data must be avoided.

During the last cycles of refinement all data must be used, including those previously omitted from the computation of R_free.

Non-crystallographic symmetry
The ratio of X-ray observables to parameters is sufficient that it is no longer necessary to impose non-crystallographic symmetry (NCS) restraints. Indeed, doing so might obscure small but significant differences between molecules in different environments in the cell, which will provide general guidelines about how tightly NCS restraints should be imposed at lower resolution.

Results

Structures with atomic-resolution data
At the EMBL in Hamburg data to atomic resolution have been collected on several proteins, although almost all of the results remain to be published. A representative list derived from our own studies and those of visitors to the EMBL is given in Table 3. These studies were initiated after installation of the first imaging-plate scanner. The first structure to be studied as a test for the system was a medium-sized molecule, β-cyclodextrin. It was refined to ~3% R factor at 0.9 Å resolution, i.e. much better resolution than obtained in previous work (previous studies of cyclodextrins had resulted in data to lower resolution, many with R factors in the range 7–15%), setting a benchmark for the approach.

Advantages of atomic-resolution data in refinement
Atomic-resolution refinement can be expected to afford the researcher many advantages:

(a) The final R factor should lie within the range of 8–12% for the anisotropic model including riding H atoms. The R_free is usually 2–4% higher. Moreover, the increase in R factor with resolution is substantially less than for the isotropic model. This has clear implications for the accuracy of the atomic positions [31**,33,34] (DWJ Cruickshank, personal communication).

(b) The difference Fourier synthesis should have a root mean square of 0.05 e Å⁻³, with the largest features not exceeding ±0.4 e Å⁻³. The 'cleaness' of the maps allows much easier visualization of solvent, disordered residues and H atoms.

(c) The positions of C, N and O atoms in the ordered parts of the structure will have estimated errors
Table 3. Representative proteins for which data have been recorded at atomic resolution at the EMBL.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Origin of research group</th>
<th>Asymmetric unit (kDa)</th>
<th>Vm (Å³Da⁻¹)</th>
<th>Resolution (Å)</th>
<th>Current R factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rubredoxin Dv</td>
<td>Seattle</td>
<td>6.0</td>
<td>1.6</td>
<td>1.00 (room)</td>
<td>8.7</td>
</tr>
<tr>
<td>Rubredoxin Dv</td>
<td>Seattle</td>
<td>6.0</td>
<td>1.6</td>
<td>0.92 (room)</td>
<td>7.4</td>
</tr>
<tr>
<td>Rubredoxin Cp Fe</td>
<td>Grenoble</td>
<td>6.0</td>
<td>2.2</td>
<td>1.10 (room)</td>
<td>9.0</td>
</tr>
<tr>
<td>Rubredoxin Cp Zn</td>
<td>Grenoble</td>
<td>6.0</td>
<td>2.2</td>
<td>1.20 (room)</td>
<td>10.7</td>
</tr>
<tr>
<td>BPTI</td>
<td>Hamburg</td>
<td>6.5</td>
<td>1.9</td>
<td>1.08 (room)</td>
<td>10.5</td>
</tr>
<tr>
<td>ROP</td>
<td>Heraklion</td>
<td>7.0</td>
<td>1.7</td>
<td>1.08 (room)</td>
<td>10</td>
</tr>
<tr>
<td>Protein G</td>
<td>Leicester</td>
<td>6.6</td>
<td>2.2</td>
<td>1.10 (room)</td>
<td>9.7</td>
</tr>
<tr>
<td>Cytochrome c₆</td>
<td>Lisbon</td>
<td>9.3</td>
<td>2.3</td>
<td>1.10 (room)</td>
<td>NA</td>
</tr>
<tr>
<td>Insulin</td>
<td>Hamburg</td>
<td>11.0</td>
<td>1.8</td>
<td>1.20 (room)</td>
<td>13</td>
</tr>
<tr>
<td>Insulin</td>
<td>Hamburg</td>
<td>11.0</td>
<td>1.8</td>
<td>0.98 (100K)</td>
<td>13</td>
</tr>
<tr>
<td>RNase P1</td>
<td>Moscow</td>
<td>11.0</td>
<td>2.4</td>
<td>1.08 (room)</td>
<td>11.0</td>
</tr>
<tr>
<td>Lysozyme P1</td>
<td>Seattle</td>
<td>14.3</td>
<td>1.7</td>
<td>0.92 (100K)</td>
<td>17 (iso)</td>
</tr>
<tr>
<td>Cutinase</td>
<td>Marseille</td>
<td>NA</td>
<td>NA</td>
<td>1.05 (room)</td>
<td>9.5</td>
</tr>
<tr>
<td>Trypsin bacterial</td>
<td>NOVO</td>
<td>19.2</td>
<td>1.9</td>
<td>1.10 (room)</td>
<td>7.5</td>
</tr>
<tr>
<td>Trypsin bacterial</td>
<td>NOVO</td>
<td>19.2</td>
<td>1.9</td>
<td>1.02 (180K)</td>
<td>10.8</td>
</tr>
<tr>
<td>Trypsin bacterial</td>
<td>NOVO</td>
<td>19.2</td>
<td>1.9</td>
<td>0.96 (120K)</td>
<td>8.9</td>
</tr>
<tr>
<td>Trypsin bacterial</td>
<td>NOVO</td>
<td>19.2</td>
<td>1.9</td>
<td>0.98 (90K)</td>
<td>10.0</td>
</tr>
<tr>
<td>RNase Sa</td>
<td>Bratislava</td>
<td>21.1</td>
<td>2.3</td>
<td>1.20 (room)</td>
<td>10.6</td>
</tr>
<tr>
<td>RNase Sa recomb</td>
<td>Bratislava</td>
<td>21.1</td>
<td>2.3</td>
<td>1.00 (110K)</td>
<td>NA</td>
</tr>
<tr>
<td>RNase Sa GMP</td>
<td>Bratislava</td>
<td>21.1</td>
<td>2.3</td>
<td>1.15 (room)</td>
<td>10.9</td>
</tr>
<tr>
<td>Xylanase</td>
<td>NOVO</td>
<td>21.8</td>
<td>NA</td>
<td>1.00 (100K)</td>
<td>NA</td>
</tr>
<tr>
<td>Trypsin fungal</td>
<td>NOVO</td>
<td>22.1</td>
<td>2.0</td>
<td>1.07 (room)</td>
<td>11.0</td>
</tr>
<tr>
<td>Trypsin fungal</td>
<td>NOVO</td>
<td>22.1</td>
<td>2.0</td>
<td>0.93 (100K)</td>
<td>11.0</td>
</tr>
<tr>
<td>Savinase</td>
<td>NOVO</td>
<td>26.7</td>
<td>1.8</td>
<td>1.00 (110K)</td>
<td>10.7</td>
</tr>
<tr>
<td>Subtilisin BPN'</td>
<td>P&amp;G</td>
<td>27.5</td>
<td>2.1</td>
<td>1.15 (100K)</td>
<td>NA</td>
</tr>
<tr>
<td>LADH Zn</td>
<td>Stockholm</td>
<td>79.6</td>
<td>2.4</td>
<td>1.10 (110K)</td>
<td>NA</td>
</tr>
<tr>
<td>LADH Cd</td>
<td>Stockholm</td>
<td>79.6</td>
<td>2.4</td>
<td>1.15 (110K)</td>
<td>NA</td>
</tr>
<tr>
<td>LADH Cd/DMSO</td>
<td>Stockholm</td>
<td>79.6</td>
<td>2.4</td>
<td>1.00 (110K)</td>
<td>NA</td>
</tr>
</tbody>
</table>

Full details of the research groups are not provided because none of the structures have yet been published. NOVO represents Novo-Nordisk of Copenhagen, P&G Proctor and Gamble. The temperature at which atomic-resolution data were obtained are indicated (room, room temperature).

of ~0.03 Å. The average for the whole structure may be 0.05 Å, reflecting the substantial errors in the disordered residues.

(d) The significance of the anisotropic atomic model is clear from the R. factors. In addition, analysis of the correlation of thermal vibration with temperature and with distance from the centre of gravity of the molecule (TR Schneider, KS Wilson and F Parak, abstract MO70, Meeting of the American Crystallographic Association, Montreal, 1995) confirms that the B values are physically meaningful.

(e) The positions of many H atoms can be seen in the density maps: for rubredoxin, for example, about two thirds of them could be directly picked up using SHELXL-93.

(f) Dual conformations can be identified for a greater proportion of residues ~10% in our experience. Serines, methionines, lysines, threonines and valines are especially prone to this. Dual conformations have even been observed for tryptophan, and for a tyrosine with two complementary H bond networks. Even at atomic resolution, however, a few residues continue to reveal no clearly identifiable average conformation.

(g) The water structure emerges from the continuum. As the residuals in the difference Fourier decrease, significant features emerge at increasing distances from the protein surface.

The refinement of the bacterial ribonuclease from Strep. aurofaecies illustrates in more detail most of the points made here [35].

Ab initio phasing of atomic-resolution data
This has so far only worked for proteins such as avian pancreatic polypeptide [14], crambin [15], rubredoxin [16*] and cytochrome c₆ [36*•,37•] which contain heavy-atom units such as metals or disulphide bridges. Cytochrome c₆ is the first protein with previously unknown structure that has succumbed to this approach. So far, no all-light atom macromolecular structures have been solved ab initio.
For most of these studies the heavy atoms were first located from the Patterson synthesis, and the initial phase set extended to reveal the bulk of the rest of the structure. For rubredoxin and avian pancreatic polypeptide, classical direct methods were sufficient to produce a meaningful, almost complete model.

George Sheldrick and Durward Cruickshank have both pointed out why the presence of heavy atoms confers such an advantage: the B values of the heavy atoms are so much less than the rest of the structure that they dominate the scattering factors at high resolution, where the lighter solvent and surface residues become insignificant. Thus the structure to be initially defined at atomic resolution becomes rather small and easier to produce via direct methods. It may be a little while before an all-light atom macromolecule is solved ab initio.

Future prospects

When we started this work, we did not have great expectations that any but a tiny number of protein crystals (rubredoxin and lysozyme!) would really provide atomic-resolution data. However, the combination of bright X-ray sources, efficient 2D detectors and cryogenic temperatures has produced a rapidly growing number of structures, and not all of them are small tightly packed systems such as rubredoxin. For example, in Hamburg we have recorded data to 1.1 Å resolution for liver alcohol dehydrogenase, which has two 80 kDa monomers in the asymmetric unit, Elspeth Garman from Oxford has data to 1.0 Å on neuraminidase (also from Hamburg) and Jeremy Tame from York has 1.1 Å data on an oligopeptide-binding protein with a molecular mass of 80 kDa measured at the SRS in Daresbury, UK.

The number of atomic-resolution structures solved will increase and within the next two years several coordinate sets will be deposited in the Macromolecular Structural Database (MSD; currently called the Protein Data Bank [38]) at Brookhaven, providing a wealth of structural information for analysis and comparison of the details of protein structure. Far from simplifying the process of protein structure prediction, such studies are revealing the rules of protein structure to be even more complex than previously envisaged. The proportion of residues for which multiple conformations can be seen is greater at atomic resolution, further complicating the lives of those attempting structure prediction.

The use of fast detectors such as CCDs will without doubt transform the field. For atomic-resolution data on bacterial trypsin, for example, the total exposure time is ~30 min, but data collection takes about 6 h because of the time required for read out of the image plate. The collection of such data in less than 1 h will allow studies to be extended to series of complexes which, together with the use of flash freezing, should allow detailed probing of the enzyme mechanism in tractable times, and at resolutions approaching those of real interest to chemists. The information content of these data sets is phenomenal. It is clear that we cannot at the moment necessarily expect to extract all of the relevant data to produce a definitive model of, say, water structure. Improved algorithms will certainly be developed as more and more models become available. It is crucial that the raw data are not lost but are archived in the MSD. Journal editors must be persuaded that the X-ray data as well as the model should be deposited as a condition of publication.

Direct or Patterson solutions of small metalloproteins is now a reality. We can hope that ab initio solution of normal proteins via maximum likelihood will become a reality during the next years [13]. Restating one of our initial observations, the collection of data to atomic resolution makes all subsequent steps in a crystal structure analysis and refinement easier. It should be pursued whenever possible.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:
• of special interest
•• of outstanding interest


The application of the ‘shake-n-bake’ procedure to the ab initio phasing of a small protein, that lacks a metal atom but possesses only sulphur atoms in disulphide bridges.


26. Sheldrick GM, Schneider T: SHELXL: High-resolution refinement. Methods Enzymol 1996, in press. The paper describes how to use most effectively the SHELXL programme to refine atomic-resolution models of macromolecules. It is essential reading for those embarking on such studies.


This workshop discussed the need for statistical validators of atomic models with regard to the experimental X-ray data. Several procedures were recommended and a number of pitfalls identified.


The first ab initio solution of a previously unknown protein structure obtained from amplitudes alone. Again, the importance of heavier atoms, an iron and three sulphurs, is emphasized.


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