Comments on Inorganic Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713455155

CURRENT DEVELOPMENTS IN SMALL-MOLECULE X-RAY CRYSTALLOGRAPHY

William Clegg[a]
[a] School of Natural Sciences (Chemistry), University of Newcastle, Newcastle upon Tyne, UK

To cite this Article Clegg, William(2005) 'CURRENT DEVELOPMENTS IN SMALL-MOLECULE X-RAY CRYSTALLOGRAPHY', Comments on Inorganic Chemistry, 26: 3, 165 — 182
To link to this Article: DOI: 10.1080/02603590500201162
URL: http://dx.doi.org/10.1080/02603590500201162

Comments on Inorganic Chemistry, 26: 165–182, 2005

ISSN: 0260-3594 print
DOI: 10.1080/02603590500201162

Taylor & Francis
Taylor & Francis Group

CURRENT DEVELOPMENTS IN SMALL-MOLECULE X-RAY CRYSTALLOGRAPHY

WILLIAM CLEGG

School of Natural Sciences (Chemistry), University of Newcastle, Newcastle upon Tyne, UK

X-ray crystallography is one of the most important and most powerful methods for investigation of the structures of chemical compounds. It has undergone revolutionary changes within the last one or two decades, vastly increasing its speed, extending its scope, and improving its already excellent reliability. This article identifies some of the major advances, discusses current attitudes towards crystallography and uses of it, and considers current challenges and future prospects.

INTRODUCTION

The term "small-molecule crystallography" is really a poor one to describe this subject, since many chemical substances studied by X-ray crystallography are non-molecular (particularly with the burgeoning interest in supramolecular coordination networks, microporous materials, high-temperature superconductors and other ionic compounds) and others are far from small (including multinuclear arrays, oligopeptides and polyprophyrin arrays, for example), but it is well established in chemical vocabulary. It serves as a convenient distinction from macromolecular crystallography, which focuses on the biological realm of proteins, nucleic acids and viruses, and from non-crystalline diffraction techniques used in the investigation of materials such as plastics, muscle, fibers and other polymers. It plays a vital role in modern chemistry,

Address correspondence to William Clegg, School of Natural Sciences, University of Newcastle, Newcastle upon Tyne, NE1 7RU, U.K. E-mail: w.clegg@ncl.ac.uk

organic as well as inorganic, and probably ranks alongside NMR and mass spectrometry as the three most important structural techniques, being less widely applicable than these other two in its requirement for a crystalline solid sample, but far more powerful in the richness of its detailed results when successful. The impact of crystallography in chemistry is immediately obvious in a cursory scan of research journals (especially in inorganic chemistry), as well as in depictions of structures in undergraduate text books, posters and other promotions of chemistry, and the internet.

X-ray crystallography has seen enormous development since the discovery of X-rays in 1895, the demonstration of X-ray diffraction by crystals in 1912, and the application of this in the first crystal structure determination in 1913, almost a century ago. It has been involved in research leading to the award of numerous Nobel Prizes, and its results adorn the front covers of many research journals. A generation or two ago it was commonly regarded as difficult, slow and expensive, and a last resort when more "sporting" techniques had been exhausted, particularly by organic chemists (this attitude has not completely died out!).

Developments in the techniques of crystallography over about the last three decades have brought about dramatic improvements in speed, range of application, and reliability, to the extent that many chemists now see it as the structural method of first choice. This change has probably been most marked in inorganic chemistry (and in the pharmaceutical industry, for different reasons), where the need for detailed geometrical results rather than basic connectivity and qualitative stereochemistry arises from the great flexibility of bonding interactions, variability of coordination geometry, and unpredictability of reaction outcomes.

Improvements in X-ray crystallography have been particularly rapid and marked in the last ten years or so, especially in the technological development of diffraction data collection procedures. Within my own research career of 35 years, the almost complete displacement of photographic and serial four-circle diffractometer techniques by various kinds of area detectors, the transformation of low-temperature data collection from heroic pioneering to routine use, the increasing availability of high-intensity X-ray sources, the move from time-shared mainframe computers and "minicomputers" such as the DEC VAX and Data General Eclipse (ironic name!) to desktop and laptop PCs of far greater power, the progress from punched cards, paper tape and reel-to-reel magnetic tapes to floppy disks, CD, DVD and DAT media and the now ubiquitous

flash-disk or memory stick, the explosion of the internet, and the availability of convenient computer graphics have been revolutionary and breathtaking – except that memories seem to be short, and all these modern advantages are simply taken for granted. "What's a four-circle diffractometer?" ask most young graduate research students on a regular intensive crystallography course I help to teach,[1,2] and X-ray cameras are museum pieces. I wouldn't dream of calling the 1970s "the good old days," of course, and would not wish to inflict visual estimation of photographic intensities on anyone, but unfortunately the positive developments have also brought with them some unwelcome changes in practice and attitude, to be discussed later.

SOME RECENT DEVELOPMENTS

Undoubtedly the most dramatic change in X-ray crystallography over the last ten years has been the widespread introduction of area detectors for data collection. Previously, diffractometers measured diffracted beams serially one at a time, so that the time required for a data collection depended on the size of the structure; a larger structure gives a denser diffraction pattern, the number of independent reflections up to a given maximum Bragg angle being directly proportional to the volume of the "asymmetric unit" of the crystal structure, and hence approximately proportional to the number of symmetry-independent atoms. The sampling of the diffraction pattern only at the expected positions of Bragg reflections by the small detector also meant that it was necessary to determine the unit cell geometry and the orientation of the crystal on the diffractometer before the full data collection could proceed.

An area detector is effectively an electronic version of photographic film: more convenient, cleaner and faster to use, and reusable. Although it was widely used in macromolecular crystallography already, and there were some image-plate and TV-based systems in chemical crystallography, serious uptake began only in 1994, when the first commercial CCD diffractometer became available, consisting of goniometer, detector, control system and software in a complete package. The first shipped system is still in operation in Newcastle, but in the intervening years CCD-based area detectors have become more sensitive and less expensive, and they now dominate the market.

They bring several major advantages. The most obvious is their ability to record many reflections simultaneously, considerably speeding

up the process of data collection; data for a larger structure need take no longer than for a smaller structure, since it simply gives a higher density of reflections. At the same time, many symmetry-equivalent data are usually collected, which would multiply the data collection time with a serial detector, and this has benefits for overall data quality and its assessment, as well as for effective absorption corrections and more reliable space group determination. Since an area detector records the whole diffraction pattern (all of reciprocal space) and not just the Bragg reflections (the reciprocal lattice points), it is not actually necessary to establish the unit cell and crystal orientation before collecting data, and the unit cell determination is usually based on far more observed reflections than with a serial diffractometer, so it is more reliable and less subject to potential difficulties arising from weak subsets of data in cases of pseudo-symmetry, superlattices and other problems. Dealing with multiple diffraction patterns from twins and other imperfect samples is made far more tractable with an area detector, and the speed and sensitivity of the devices have greatly extended the scope of application for weakly diffracting materials and poor quality crystals. Sample screening is rapid (essential at synchrotron sources). Last but not least, teaching and training in crystallography is made easier by the immediate and clear visual display of diffraction patterns on a screen instead of lists of numbers.

A more gradual and less dramatic change, but also important, has been the development of facilities for low-temperature data collection. About 20 years ago, low-temperature devices were unreliable and temperamental beasts, often home-made or modified, and their use was relatively scarce. The most common type of device generates a cold gas stream by evaporation of liquid nitrogen and aims to maintain a constant temperature at the sample by monitoring and feedback control. Formation of ice from atmospheric moisture must be prevented, usually by a concentric dry sheath of nitrogen or air around the coolant gas stream, possibly with the help of other factors such as a dry enclosure and avoidance of draughts. Such devices have become much more reliable and their acquisition and running costs are moderate, so they can now be used routinely and run continuously for months at a time. Temperatures down to about 80 K, just above the boiling point of nitrogen, can be readily achieved and maintained. More recently, helium-driven devices have become available and can reach even lower temperatures; obviously, the costs are considerably higher and the technical requirements are more demanding.

The main advantage of low-temperature data collection is the significantly reduced thermal motion of atoms in the sample, leading to greater intensities, especially at higher Bragg angles, and a more precise structure. Thermally sensitive samples are obviously better handled this way, and flash-cooling of oil-mounted crystals is a much more convenient way of dealing with air-sensitive compounds than sealing them in capillary tubes; even highly sensitive main-group organometallics can be successfully studied without recourse to those cumbersome old methods. The use of low temperatures also helps in the modelling of structural disorder, by giving a clearer resolution of static disorder components and reducing dynamic disorder. It is probably the most cost-effective improvement that can be made in basic diffraction data collection, and ought to be standard practice. The only serious drawback is the occasional experience of an undesirable phase transition on cooling, especially if this involves a major structural change and results in destruction of the single-crystal specimen.

Low-temperature data collection is essential in some specialist areas of crystallography, such as in charge-density analysis and, obviously, in the study of temperature-dependent phenomena such as spin-crossover transitions and superconductivity. It has also made it possible to investigate the solid-state structures of compounds that are liquids or gases at normal room temperature, such as small-molecule derivatives of several main-group elements.[3]

The basic laboratory X-ray tube has changed little in its fundamental design since the early days of crystallography, though modern tubes are more compact, safer and better engineered than the originals. They remain highly inefficient devices, since that is the nature of the physical principle on which they operate, the conversion of electron kinetic energy into X-rays through impact on a metal target with resultant ejection of photoelectrons and secondary emission. Rotating-anode tubes spread the thermal load on the target and hence permit higher electron currents and generate more X-ray intensity, but the gain is usually less than an order of magnitude, as it is with other recent developments in electron-impact X-ray generation. Further intensity gains can be achieved by various focusing optics systems, whereby more of the emitted X-rays are captured and concentrated on the sample instead of going to waste in unwanted directions. The aim of all these attempts is to bring more X-ray photons per second on to the sample crystal, so that the diffraction intensities are greater, either improving the signal or reducing the time for a measurement.

A much greater increase in intensity, of several orders of magnitude in most cases, is achieved by setting up crystallographic diffraction equipment on a synchrotron storage ring. These, however, are major national and international facilities and the venture is a considerable undertaking. A number of technical difficulties have to be addressed, including the polarization properties of synchrotron radiation, which involves building diffractometers on their sides relative to the standard laboratory arrangement, and the variation in intensity from storage rings that are not operated in a constant "top-up" mode, and each synchrotron source operates its own more-or-less bureaucratic system of user access, authorization, training and safety. Nevertheless, the potential advantages of exploiting such sources are enormous and well worth the effort.[4] The dedicated "small-molecule crystallography" diffraction facility of Station 9.8 at the Synchrotron Radiation Source (SRS) at Daresbury Laboratory, UK, has been extremely successful since its construction less than ten years ago, and is oversubscribed by user applications. Since 2001 we have operated a national service there for UK academics eligible for support by EPSRC, who fund the service, so that the experimental work does not require cumbersome formal applications months in advance by individual users for occasional use and so that it is carried out efficiently by trained experts. Up to 12 data sets per day have been collected in this way. The samples, originating mostly from university chemistry departments across the UK, are not amenable to study anywhere else in the country; in many cases, crystals giving barely visible diffraction patterns with long exposures on a rotating-anode system with advanced mirror optics (possibly the most powerful laboratory small-molecule crystallography facility in the world), at Southampton University, produce excellent data within a couple of hours at the SRS.[5]

Small-molecule crystallography facilities at synchrotron sources elsewhere in the world have generally been less successful, mainly because they tend to be shared with other techniques (powder diffraction, spectroscopy, etc.) on the same beam-line, and so are not optimized for this particular use. Plans are currently being developed for an even more powerful facility on the new UK synchrotron source, **diamond**, from about 2008, building on the success of Station 9.8.

While the high intensity is the most obvious advantage of synchrotron radiation for crystallography, there are also potential benefits in the ability to select the X-ray wavelength from a wide range in most cases. Very short wavelengths are useful for reducing effects such as absorption and

extinction (usually at the expense of intensity), and are particularly important for charge-density work, and they can penetrate effectively through sample containers such as high-pressure cells and chambers for controlled atmospheres and *in situ* reaction studies. Alternatively, effects such as anomalous scattering can be maximized and exploited for the determination of absolute configuration of chiral structures.

There are few experimental methods available for the determination of absolute rather than relative configuration. With an appropriate combination of sample composition and quality and X-ray wavelength, it is straightforward and highly reliable with crystallography. Without going into details of the theory, anomalous scattering is the small but significant phase shift that can occur when an atom scatters X-rays with a wavelength close to one of its absorption edges; the effect is negligible for atoms lighter than about Si or P with MoKα radiation, which is most widely used in chemical crystallography, but generally increases for heavier atoms and for higher wavelengths. Anomalous scattering by a particular element can be maximized by wavelength tuning at a synchrotron source. In the presence of significant anomalous scattering, the diffraction pattern of a non-centrosymmetric crystal structure and that of its inverse are not identical, and one of these will fit the observed diffraction pattern better than the other. In the case of enantiomerically pure chiral materials, this corresponds to selecting the correct absolute configuration. Computational and experimental techniques have been developed for optimizing the procedure and assessing the statistical significance of the result.[6,7] It has obvious applications in current research emphases such as asymmetric catalysis and chiral solid-state networks.

Crystallography makes extensive use of computers, from the control of diffractometers and the processing of the measured data, through the solution and refinement of structures, to the graphical, numerical and statistical representation and interpretation of results and their archiving and publication. The rapid development in personal computing power and resources has been very important for the success of new technologies in crystallography, especially with the huge quantity of data generated by area detectors. Powerful, affordable and easily used computer graphics have played an important role in solving and refining complex structures and in understanding them once they are complete; such facilities were an expensive luxury a generation ago. Inexpensive magnetic and optical storage media are also vital if accumulated data and results are not to be lost.

By contrast, the real costs of crystallographic software are not tumbling. There is much time and effort involved in programming new ideas and theories and, under pressures of research assessment, quality assurance measurements and economic difficulties in science subjects, academics are no longer so easily able to carry out such work as a personal interest and make their programs freely available to others. Software, especially in integrated packages, is increasingly being offered commercially rather than in the public domain, and much of the widely used software is showing its age as its originators retire from active research, with few younger researchers able and willing to take over (except in macromolecular crystallography). Commercial software seems to have a greater focus on presentation and automation than on flexibility and user control. These are unwelcome developments.

Crystallographers are fortunate in dealing with data and results that are ideally suited to systematic study, classification and standardization in the form of electronic databases. The maintenance and development of these have been much aided by the adoption of some internationally agreed standard formats for crystallographic data and results, of which the CIF (Crystallographic Information File) has had the biggest impact in terms of information exchange, storage, retrieval and publication.[8] The automatic computer validation of crystallographic results is readily achieved and is a major advantage in submitting them for publication in mainstream chemistry journals as well as specialist crystallographic ones, some of which now operate in an entirely electronic mode from submission, through validation and peer review, to proof checking and publication, achieving very short publication times and reducing both effort and the risk of errors.

Crystallographic databases have become not only the primary exhaustive repository of published crystal structures, but also research areas in their own right, through "data mining," statistical analysis and the discovery of structural patterns and trends. Their use has led to advances in our understanding of such topics as hydrogen bonding, conformational preferences, and coordination geometry.[9–11]

A welcome feature of small-molecule crystallography these days, though one that could be developed further, is a measure of cross-fertilization of ideas with related disciplines. As the subject has expanded its borders into larger and more complex structures through modern technological enabling, it has found more common ground with macromolecular crystallography than previously. Equipment used by the two

communities is often very similar now, and it is interesting to note that designs for macromolecular and small-molecule diffraction on **diamond** have much in common. Problems of low data resolution, extensive disorder of solvent and peripheral groups, and the mounting of very small crystals for synchrotron study in small-molecule crystallography have seen approaches and solutions imported from established macromolecular crystallography techniques. The successful investigation of smaller and smaller crystals has blurred the distinction between single-crystal and powder diffraction and these communities have increasingly shared computational ideas and methods. Information from other solid-state techniques, such as NMR-derived interatomic distances, has been incorporated as restraints in difficult structure refinements. Furthermore, crystallography and theoretical calculations (molecular modelling and quantum mechanics) have made profitable use of each other's results, especially in charge-density studies and through idealized fragment geometries for fitting to electron density maps or as starting points derived from databases for theoretical calculations.

ATTITUDES, USES AND ABUSES

The enormous advances made in crystallography in recent years have inevitably brought with them some problems. Among these, perhaps the most significant are some consequences of the speed and power of the technique, together with a growing "black-box" mentality of automation and a frequent lack of understanding of some fundamental principles. To a large extent these problems are fuelled by the promotion of commercial systems that can supposedly be used (and very often are, without trouble) by non-experts, by funding restrictions, and by the growing use of monitoring and performance indicators, especially in academic research, with their impact on ambitions and working relationships. Also, to a large extent, crystallographers are the victims of their own success.

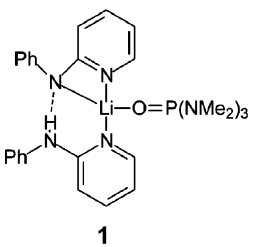
Modern diffractometer systems can generate good quality data sets from suitable crystals within hours, or even in minutes. In many cases, the determination of the unit cell and space group, and the solution and refinement of the structure, proceed smoothly and largely automatically. For a well-defined discrete molecular structure without disorder or other problems, and with no unusual features, the whole process is rapid and simple, and requires no special skill or training. This is push-button crystallography at its most effective. One eminent crystallographer

infamously said a few years ago that crystallography is now so easy that monkeys can be trained to do it, and many chemists might agree. This may be true for straightforward structures, but a lack of real training and a black-box mentality, together with too much haste and a lack of attention to detail, lead all too often to poorly determined crystal structures in which small but significant problems are overlooked or not understood. This may produce structures that are qualitatively correct, but with errors in details of geometry through failure to recognize structural disorder or twinning, the consequences of anomalous scattering effects, poor treatment of encapsulated solvent molecules, incorrect symmetry, or misassigned atom types, among other errors. I have seen many such instances as joint Section Editor of *Acta Crystallographica Section E: Crystal Structures Online*, a purely electronic crystallographic journal that specializes in rapid publication while maintaining high standards, and have come across numerous published results, ranging from poor to outrageous, in highly reputable international chemistry journals, where the work has clearly been done badly and the errors have not been spotted by the authors, referees or editors. Some of these are subsequently addressed and corrected, particularly where space groups have been wrongly assigned, which may have disastrous consequences for molecular geometry when an inversion centre has been overlooked.

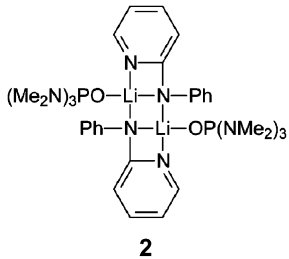
I once had to reject a submitted paper that reported a complex of a highly unusual terminal one-coordinate copper atom, which was almost certainly a bromine atom (the atom bonded to it was also wrongly identified), and other crystallographic referees and editors have reported similar experiences. More recently, an organic structure that would have made the front cover of *Science* or *Nature*, had it been correct, was found, on careful investigation, almost certainly to have fluorine atoms rather than hydroxy groups and a nitro group rather than a carboxylic acid function; the correct structure was, in fact, already known, published and in the Cambridge Structural Database! The purely crystallographic report contained no analytical or spectroscopic characterization and the authors (who will remain anonymous!) had to be persuaded that X-ray crystallography is a very poor technique for chemical elemental analysis. Some cases of mistaken identity have made it into publication and have been subsequently recognized, including nitramine ligands[12] that were actually rather less interesting acetates,[13] and the spectacular case of the amazing compound $[ClF_6]^+[CuF_4]^-$, correctly identified later as the rather mundane $[Cu(OH_2)_4]^{2+}[SiF_6]^{2-}$.[14] Fortunately, such

errors rarely get all the way into publication, because of the inherent self-checking character of much of crystallography and the availability of programs for thorough testing of results.

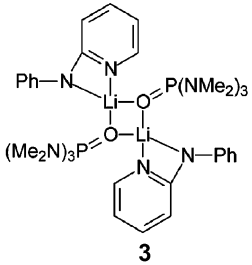
Another, quite different, kind of "mistaken identity" is the case, far from unknown, in which the crystal structure proves to be unexpected. Although this usually indicates that the reaction has proceeded in a different way from that planned or desired, it sometimes turns out to be a minor product (or residual starting material) in the sample. Failure to recognize this may lead to incorrect deductions about the synthesis. This is another reason why many chemical journals refuse to accept a crystal structure as the only evidence for the identity of a product. In order to demonstrate that the selected single crystal is representative of the bulk material, there should also be one or more of chemical analysis, appropriate spectroscopic results, and comparison with a powder diffraction pattern. A good illustration of this, from some 20 years ago, is one of the first results of my collaboration with Ron Snaith, then at Strathclyde University, in lithium chemistry. Crystals were obtained of a product from a 1:1:1 reaction of 2-anilinopyridine (PhNH-2-Py), ^{n}BuLi and HMPA [$OP(NMe_2)_3$] and were mounted in Lindemann capillary tubes because of their air-sensitivity (we were not routinely using low-temperature facilities at that stage). The structure of a selected crystal proved to be that of a 2:1:1 product, (PhNH-2-Py)(PhN-2-Py)Li(HMPA), **1**. Because of good spectroscopic, analytical and cryoscopic evidence that the major product really did have a 1:1:1 stoichiometry, other crystals were examined and a different structure was found. Interestingly, the crystal structure was found to contain two different isomeric dimers, **2** and **3**, one with bridging (and simultaneously chelating) amido ligands and the other with bridging HMPA. The minor product **1**, which was actually present in only small quantities but formed the best crystals, could subsequently be prepared in high yield from a reaction with 2:1:1 stoichiometry of reagents.[15,16]



1



2



3

The advances in crystallography outlined earlier mean that we are able to tackle much more difficult structural problems now than a generation ago; the data collection and computing capabilities are available. However, this is precisely where expertise and time are needed and where automatic approaches fail. We have found with our synchrotron work, tackling samples that come to us as a last resort, that they often demand many hours of painstaking work to resolve problems of pseudosymmetry, extensive disorder or twinning, and the slow emergence of structural details (especially in cases where there are several molecules in the asymmetric unit of the structure), requiring careful use of advanced tricks available in modern software – no room for trained monkeys here! In the final publication of such results, readers will often be quite unaware that the work has required orders of magnitude more time and effort than other structures churned out in a spare couple of hours while thinking mainly about something else. There is often little correlation between the effort involved and the chemical impact and interest of the result!

Sadly, such effort is sometimes quite unnecessary. On the "garbage in, garbage out" principle, a poor quality crystal is usually going to result, even after much hard work, in a structure of limited precision. Although there are certainly many times when this is the only practical approach, the whole difficulty can often be avoided by obtaining better crystals, either through appropriate crystallization techniques, or through the synthesis of a closely related compound. Even a small improvement in crystal quality can make a big difference in the data collection and structure determination. On a number of occasions, I have struggled with a marginal data set, but a subsequent new batch of crystals has led to a simple structure determination from much improved data; a little extra effort and time in sample preparation can save much agony later.

What is meant by "appropriate crystallization techniques"? The usual preparative chemist's approach is with the principal objectives of purity, high yield and speed, rather than with crystal size and quality, and a microcrystalline powder of mediocre quality is often the result. For single-crystal diffraction, we need only one single crystal of suitable size, so yield is an unimportant issue. A suitable size may roughly be defined as somewhere between a typical grain of salt and a grain of sugar, both familiar household substances, though considerably smaller crystals can often be tackled with high-intensity X-ray sources, as mentioned earlier. The key to good crystal quality is usually slow crystallization,

whether this occurs by solvent evaporation, cooling, or the gradual diffusion of two liquids together to reduce solubility (this may occur at a direct liquid interface, or via the vapour phase). Detailed descriptions of methods and practical tips for their use are available in various references.[2,17–20]

In this context it is worth noting that changing the crystallization method or conditions may lead to a different polymorph or solvate (in some instances, better crystals may also be obtained by making a different chemical derivative, such as by changing an unimportant substituent or a counter-ion). Polymorphism, a topic recognized as highly important in the pharmaceutical industry for financial, regulatory, legal and medical reasons, is an area of research in its own right, and has been a great beneficiary of the huge increase in speed of crystallography. Its existence should not be overlooked, however, by chemists in general, who need to recognize that a crystal structure of a compound they have prepared is not necessarily *the* crystal structure; other forms may be possible, in which there are different conformation, different details of geometry, or even grossly different structural features such as overall coordination geometry or degree of association of molecular units in monomers, dimers, up to polymers.

Mention of polymorphism and different solid-state structural forms and isomers is a reminder of the possible consequences of unrecognized problems such as disorder, which can produce unfortunate artefacts if it is not correctly treated. Some, at least, of the examples of "bond-stretch isomerism" discussed vigorously a number of years ago, were unreal, resulting from substitutional disorder of ligands in metal complexes, especially involving partial replacement of different halogen atoms, which have different covalent radii.[21]

Among the important and valuable tools available to the crystallographer in dealing with difficult structure refinements are flexible and powerful constraints and restraints that can be applied to molecular geometry features (such as target bond lengths and angles, ideal ring shapes, and the imposition of planarity on groups of atoms) or to atomic displacements (approximating isotropic behaviour, rigid bonds, and various aspects of similarity for atoms that lie close together in disordered fragments). Like tools in any other trade, they need to be used appropriately, preferably with suitable training and understanding. Inappropriate use can lead to distortions in structural results and, perhaps most dangerously, the imposition of preconceived ideas on the structure. More

than once I have seen authors of manuscripts draw conclusions from the observed degree of planarity around an atom (especially involving NH groups in organic molecules or ligands), after they have actually imposed this planarity through constraints in the refinement! This is usually blatant, but other cases may be more subtle, leading to unjustified conclusions.

Even when data are successfully obtained from a good quality crystal and the structure has been correctly solved and appropriately refined, mistakes can still be made (frequently by "trained monkeys," but also sometimes by experts) in interpreting the results. Some of these mistakes are due to a failure to understand the meaning of precision measures. Virtually every numerical result obtained from crystallography comes with a "standard uncertainty" (also known as "estimated standard deviation" or esd, but the other term is now preferred); these may be rigorously derived by a proper statistical analysis, or they may be estimates made by computer programs or users. One common belief is that crystallographic standard uncertainties are over-optimistic and should always be inflated (multiplication by 3 is frequently recommended) before any conclusions are drawn about the significance of results, such as differences in bond lengths. While there is a basis of truth in this view (many uncertainties are estimated on the assumption of only random and no systematic errors, though this is less true than it used to be in the statistical treatment of diffraction intensity data), the real reason for scaling up crystallographic uncertainties lies in the fact that they are supposed to be estimates of standard deviations of the results (i.e. estimates of what the standard deviation would be for the complete distribution of results obtained if the experiment were to be repeated many times). In making comparisons of results, such as deciding whether two or more bond lengths are significantly different or not, proper significance tests should be made at appropriate confidence levels, based on assumptions of normal distributions. The standard statistical procedures use Student's *t*-test, as described in all basic statistics and analytical chemistry textbooks. Such tests multiply estimated standard deviations by factors depending on sample sizes and on the desired confidence levels, and lead to confidence intervals [conventionally written with $\pm$ symbols, e.g. 1.423 ± 0.009, different from the crystallographic notation with parentheses, e.g. 1.423 (3), where these parentheses enclose the standard uncertainty in the last quoted figure, here 0.003]. The multiplication of crystallographic uncertainties by 2 or 3 is thus a conversion to a rough

confidence interval at the 95 or 99% confidence level, as commonly used for significance testing. In my experience, many chemists do not really understand what "the numbers in brackets" mean at all, or have only a vague idea ("It's the error in the bond length," to which I then ask, "If it's an error, why don't we correct it?"). Consequently, inappropriate statements are made about the supposed significance and reality of small differences in bond lengths and angles, or about the deviation of a group of atoms from a plane, often with complicated arguments to provide reasons for the differences, which actually have no statistical significance at all.

Other mistakes made in interpretation arise from a failure to recognize connections between atoms in the "asymmetric unit" of a structure (the smallest repeat unit, a fraction of the unit cell, from which the complete crystal structure is generated by symmetry operations of the space group) and those in adjacent symmetry-related units. These connections may be covalent, hydrogen bonding, etc., just the same (and just as important chemically and structurally) as those within the asymmetric unit, the choice of which is quite arbitrary when it is not a distinct single connected molecule. Thus dimerization or other oligomer or polymer formation by symmetry-extension of the asymmetric unit may not be recognized. This can have major impact on the assessment of coordination geometry, bridging ligands, polymeric network architecture, hydrogen bonding patterns, and other important structural features. Experienced use of modern computer graphics and other structure analysis programs is vital here.

The final problem resulting from fast modern crystallographic techniques is the fact that many results never get into the public domain. For straightforward structures, formal traditional publication takes far longer than the complete experiment and calculations. Failure to publish results may be caused by the sheer volume of results obtained and time pressures from the effort involved in publication, from the need (real or imagined) to carry out further related studies, from the fact that the results were not as expected, from patent or other commercial considerations, or from a variety of funding, personal and other reasons, good or bad. It has been estimated by some prolific research groups that perhaps 80% or more of fully refined crystal structures remain unpublished, and the stockpile is growing fast. Not only is the work done largely wasted (and, many argue, a misuse of research funding), the community at large is deprived of the benefits (which may be quite unpredictable, through

future database analysis looking for structural trends and patterns), those who have carried out the work receive no recognition (particularly unfortunate for those at the beginning of their research career), and there is a danger that someone else will waste yet more time redetermining (and probably not publishing!) the same structure unnecessarily.

CURRENT CHALLENGES AND FUTURE PROSPECTS

Perhaps predicting the future in this subject really is a case of crystal gazing, but we can be reasonably confident about some likely developments. It will probably not be long before we have the next generation of X-ray detectors. They will be larger than current CCD detectors (for which small size has always been the major disadvantage), probably more sensitive, and (eventually, but not at first) cheaper. X-ray source intensities will be further enhanced, both in the laboratory and at synchrotron facilities, and X-ray free-electron lasers will come along later, though there is still much to be done in this area to make the dream a reality. Computers will continue to increase in power while shrinking in size and cost, of course, but there is unlikely to be anything like the same scale of development in crystallographic software. A major challenge is the attraction of keen and able young scientists into crystallography, and their training and development.

The production of good quality crystals remains a largely unpredictable process, but techniques of multiple screening and automation could be adapted to some extent from macromolecular crystallography. Experiments in robotics for data collection are still rather limited in small-molecule crystallography, and there is scope for much more development here.

Developments in X-ray sources and detectors, speeding up data collection, are beginning to make various kinds of real-time and time-resolved crystallography possible. These include studies of excited states in so-called photocrystallography (where the pulsed nature and time structure of synchrotron radiation can be exploited to make rapid interleaved measurements of diffraction patterns from ground and excited states of molecules in crystalline solids),[22] and real-time monitoring of solid-state reactions.

Low-temperature data collection is now routine down to liquid nitrogen temperatures, and it is becoming easier and more economical to achieve even lower temperatures reliably. Still more adventurous,

but undergoing rapid development, is data collection at high pressures, mainly using diamond-anvil cells, though gas pressure cells are also being explored.

Area detectors have opened up the feasibility of tackling major structural problems such as twinning, in which two or more diffraction patterns occur simultaneously. Even more challenging are incommensurate structures, for which not only careful experimental measurements but also special refinement techniques are needed.

Particular difficulties in structure determination, requiring expertise and advanced software tools, include major structural disorder and structures with several molecules in the asymmetric unit; these often display tricky features such as pseudo-symmetry. In the worst cases of disorder, individual atomic sites can not be modelled, and there is a need for group and whole-molecule orientationally averaged scattering factors, which are not widely available in the most commonly used structure refinement programs.

Success with smaller and smaller crystals, down to micron sizes in favourable cases, has blurred the boundary between single-crystal and powder diffraction as techniques. There is a need for methods of structure refinement that can sensibly combine, with appropriate weighting, data from a range of techniques, not only X-ray single-crystal and powder diffraction, but also neutron scattering, some spectroscopic methods, and computer modelling studies. Crystal structure prediction is still successful in only a limited number of cases, and this is tied up with questions of polymorphism.

The sheer volume of crystallographic results is a challenge for the curators of databases, the structure, contents, maintenance and dissemination of which will need some radical changes if a greater percentage of structures reach the public domain. The publication (taking this word in its widest sense) of crystal structures is currently one of the biggest challenges facing us, and various electronic technologies, such as grid developments and local repositories will have to be developed and embraced (there are pressures from funding agencies for this currently), with proper recognition of the provenance, reliability and significance of the published results.

Small-molecule crystallography has undergone steady development and various revolutions in its century of history. In the future it is certainly not going to stand still.

REFERENCES

1. Blake, A. J., W. Clegg, J. A. K. Howard, P. Main, S. Parsons, D. J. Watkin, and C. Wilson, 2002. *Z. Kristallogr.*, **217**, 427–428.
2. Clegg, W., A. J. Blake, R. Gould, and P. Main, 2001. *Crystal Structure Analysis: Principles and Practice.* Oxford University Press, Oxford.
3. Goeta, A. E. and J. A. K. Howard, 2004. *Chem. Soc. Rev.*, **33**, 490–500.
4. Clegg, W. 2001. *J. Chem. Soc., Dalton Trans*., 3323–3332.
5. http://www.ncl.ac.uk/xraycry
6. Flack, H. D. 1983. *Acta Crystallogr. Sect. A*, **39**, 876–881.
7. Flack, H. D. and G. Bernardinelli, 2001. *J. Appl. Crystallogr.*, **33**, 1143–1148.
8. Brown, I. D. and B. McMahon, 2002. *Acta Crystallogr. Sect. B*, **58**, 317–324.
9. Belsky, A., M. Hellenbrandt, V. L. Karen, and P. Luksch, 2002. *Acta Crystallogr. Sect. B*, **58**, 364–369.
10. Allen, F. H. 2002. *Acta Crystallogr. Sect. B*, **58**, 380–388.
11. Allen, F. H. and R. Taylor, 2004. *Chem. Soc. Rev.*, **33**, 463–475.
12. Öztürk, S., M. Akkurt, N. Demirhan, B. Kahveci, H.-K. Fun, and N. Öcal, 2003. *Acta Crystallogr. Sect. E*, **59**, i107–i109.
13. Ferguson, G. and C. Glidewell, 2003. *Acta Crystallogr. Sect. E*, **59**, m710–m712.
14. von Schnering, H. G. and D. Vu, 1983. *Angew. Chem. Int. Ed. Engl.*, **22**, 408.
15. Barr, D., W. Clegg, R. E. Mulvey, and R. Snaith, 1984. *J. Chem. Soc., Chem. Commun*., 469–470.
16. Barr, D., W. Clegg, R. E. Mulvey, and R. Snaith, 1984. *J. Chem. Soc., Chem. Commun*., 700–701.
17. Jones, P. G. 1981. *Chem. Br.*, 222–225.
18. Clegg, W. 2003. Crystal Growth Methods. In *Comprehensive Coordination Chemistry II*, vol. 1, ed. A. B. P. Lever, Elsevier, pp. 579–583.
19. http://www.xray.ncsu.edu/GrowXtal.html
20. http://www.cryst.chem.uu.nl/growing.html
21. Parkin, G. 1993. *Chem. Rev.*, **93**, 887–911.
22. Cole, J. M. 2004. *Chem. Soc. Rev.*, **33**, 501–513.