



Validation and interpretation of results

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1 Introduction

Firstly, we will consider the important step of validation of results, the tools available, how to use the feedback from such tools and how to check aspects of the results of a structure determination that validation tools cannot assess.

Secondly, we must consider how to *interpret* the results and decide which information is meaningful, if our original questions and reasons for determining the structure can be answered and if new information might be gleaned. In the lecture on the derivation of results, we saw how to move from the primary data of atomic coordinates and atomic displacement parameters to the secondary data of geometrical parameters of interest. We also considered errors, accuracy and precision. Now we will discuss how we might detect patterns, common features, similarities and significant differences and variations within and between structures, and make deductions on the basis of the observed geometry. Some deductions can extend beyond the structural properties to the enlightenment about the chemical properties of the material being studied. Questions to be asked might include: How do we compare sets of results? How similar are two molecules? How short should an interatomic distance be before we call it a bond or a significant intermolecular interaction? Such questions cannot always be answered definitively and are sometimes open to interpretation.

2 Validation

2.1 What is validation about?

Validation means something is correct, appropriate and defensible. Various degrees of validation and evaluation should be done at all stages of a structure determination from initial assessment of the diffraction quality of a crystal onwards. This might include, for example, considering figures of merit output by data collection software or an improvement in the refinement results after the model has been changed. Here we will focus on the validation carried out at the end of the structure refinement. *It is important to fully validate your results at the moment you complete the refinement* while you are still focussed on the structure and not leave it until you are trying to publish the result. It can be difficult to rectify problems later on when interest has waned, the methods and reasons used to get to the existing result have been forgotten about, or the sample is no longer available. Occasionally, seeking fresh crystals might be the best option – hence the need to think about validation issues while the sample is available!

The refinement of your structure is finished, it looks great and you are confident that the work is correct, it agrees with the chemistry, the result is the best possible and everything is in order. Are you sure? *Are you really sure?* How do you know? Validation is your best friend for ensuring you have not accidentally overlooked something trivial or less trivial – easy to do, even for a really experienced crystallographer. *Is your data and/or model adequate for the purpose of your investigation?* Is there unexpected twinning, untreated disorder, or an accidentally misassigned element? Are too few/too many H-atoms present? There are a myriad of things one needs to keep an eye on during a structure

determination and we cannot remember to check absolutely everything all of the time. The online IUCr checkCIF tool (<http://checkcif.iucr.org>) or the local variant, the PLATON validation button (Spek, 2003, 2009), are tools to help simplify the checking of our results. Crystallography is such a numerical science that it is quite easy to develop software to help us detect unexpected "features" or "outliers" in our structures, alert us to them and give us an opportunity to correct or at least think about the issues. These tools help us ensure we have not overlooked or forgotten anything, encourage us to keep up best practice and ultimately help avoid the risk of publishing something embarrassing. However, there are caveats: checkCIF cannot necessarily detect every error or issue with a structure, so you must remain vigilant at all times and get into the habit of doing some additional checks.

2.2 checkCIF

Remember: checkCIF is a tool designed to help you, not one to make life hard or frustrating and not one to prevent publication. Try not to be frustrated by the feedback it delivers. Think carefully about each alert, even if apparently trivial, and react coolly and scientifically. Three myths surrounding checkCIF:

- There are no significant alerts, so my structure must be correct.
- There are A alerts, so the structure cannot be published.
- I must manipulate my data and/or model until all (serious) alerts go away.

None of the above is true. Firstly, there can be errors in a structure that checkCIF cannot detect, *e.g.* charge balance issues, or a misassigned element in isolation, such as putting H₂O where NH₄⁺ exists. Don't forget the checks suggested in §2.3. Secondly, while major alerts frequently suggest some remedial action is required (even new crystals), there are cases where they indicate an outlier or non-standard procedure that is quite acceptable for the case in hand. Of course, such "features" must be documented properly in the CIF, as they are important experimental details. The CIF sections `_refine_special_details` and `_exptl_special_details` are ideal for this and can be alluded to if you need to insert a Validation Response Form (VRF) into the CIF. Thirdly, one should never manipulate the contents of the reflection file or massage the model, such as omitting poorly agreeing reflections or applying restraints, simply to remove alerts about calculated/observed F^2 mismatches or poor ellipsoids. There should be a good scientific reason for taking such action. Again, document such actions so everyone is aware of it (*i.e.* nothing is hidden).

The use of checkCIF will have been demonstrated during the single-crystal hands-on sessions and the slides for this lecture show the categories of tests, the significance of the alert levels and alert types and how to address and respond to them. While checkCIF can be used with powder CIFs, it is not optimised for all situations (some tests skipped) and some powder programs do not create an optimal CIF without significant hand-editing.

2.3 The limitations of checkCIF; other checks that can be done

checkCIF is only as good as the people developing the algorithms and some tests might not have been thought of yet or are too difficult to implement in the software. Some mistakes cannot be detected from the data in the CIF (*e.g.* the crystal colour is in the shape item). Sometimes seemingly correct entries in the CIF are nonsensical when considered together with other entries (*e.g.* "synchrotron" and "fine-focus sealed tube" do not go together).

Other checks can assess things that checkCIF might not detect or only flags related or even seemingly unrelated items with low significance. Looking at a displacement ellipsoid plot while rotating it can be very revealing to the eye if there are problems with the model. Check charge balance, sensible chemical stoichiometry, that the obtained structure is and

looks chemically reasonable, especially if the molecule is unexpected, that the H-atoms are present in the correct numbers and have sensible geometry, that the empirical formula of the model matches that of the expected true structure, including solvent molecules when present, and that the bond lengths and angles are appropriate for the elements concerned, the bond type and the environment, *e.g.* the C–C bonds in a *t*-butyl group should be similar and not highly varied, otherwise disorder treatment or restraints might be needed.

3 Interpretation of results

3.1 Inorganic compounds

For the structure assessment and classification of inorganic solid-state compounds like salts, oxides and intermetallic phases, a wide range of approaches is available. The interpretation of bond lengths (*e.g.* determination of the bond order) can be very challenging and complex. In simple cases Pauling's formula might help: $d(n) = -c \log n + d(1)$, where $d(n)$ is the bond distance for a bond of the order n , $d(1)$ is the single bond distance and c is a constant, empirically determined to be approximately 0.6976. In many inorganic compounds the bond orders are not integers. Bond distances in compounds with a high ionic character can be related to the bond valence (Brown & Altermatt, 1985; O'Keefe & Brese, 1991). Band-structure calculations can be indispensable for an assessment of the bonding pattern in these systems (Hoffmann, 1988).

An analysis of the coordination polyhedra present in a structure is very useful for detecting similarities between structures. For ionic compounds, Pauling (1960) and Baur (1970) defined a very useful set of rules for rationalising the observed coordination numbers, distances and interconnections. The electrostatic valence rule of Pauling can help distinguish between species with different ionic charge, such as H₂O, OH⁻ and O²⁻, which cannot easily be distinguished on the basis of the crystal structure analysis only.

The concept of determining the Atomic Environment Types (Daams *et al.*, 1992 and subsequent articles) is very useful for the description of intermetallic compounds. Here the coordination polyhedra around a central atom are determined and classified using the number and type of polyhedron faces. The breakdown of a complex structure into smaller polyhedra, probably known from other structures, can help in describing and visualizing complex structures. An example of such a decomposition into smaller, known fragments, in an extremely complicated intermetallic phase Al_{55.4}Cu_{5.4}Ta_{39.1} is given by Conrad *et al.* (2009). Surfaces of various styles (periodic minimal surfaces, equi-potential surfaces, periodic nodal surfaces, periodic hyperbolic surfaces) can be used for a partition of space and for a description of the topology of complex structures, which are otherwise hard to comprehend. Symmetry and group-subgroup relationships can also be used for classifying crystal structures (Bärnighausen *et al.*, 1980). Here the relationships between crystal structures are expressed as family trees, starting from a highly symmetric aristotype. The program Structure-Tidy (Gelato & Parthe, 1987), included in PLATON, can be used to standardize crystal structure data, thus enabling a direct comparison of structures in order to identify related structures.

3.2 The interpretation of bonds and interatomic distances

In most organic molecules, bond lengths between most types of atoms fall within fairly narrow ranges. Multiple bonds are shorter than single bonds and bond lengths deviating considerably from 'standard' values are rare, except in very strained molecules like small rings, polycyclic molecules and those with extreme steric crowding.

In organometallic and inorganic structures, bond lengths between atoms of the same pair of elements are much more variable, and formal partial bond order is a common feature.

The variation of geometrical parameters within a structure, or between different structures, and deviations from 'expected' values can be assessed statistically, but how do we assess these deviations *chemically*? At what distance do two atoms have a significant interaction and what distance indicates a formal bond?

These questions might be answered by comparing distances with sums of some kind of atomic bonding radii. Thus a 'normal' bond length should be about equal to the sum of two covalent radii, and a significant non-bonded interaction is indicated by a distance shorter than the sum of relevant van der Waals radii; see Pauling (1960) and Bondi (1964). Compilations of the bond lengths observed in various chemical environments in organic and organometallic compounds are available in Volume C of International Tables for Crystallography or by an analysis of the data in the Cambridge Structural Database.

There are, however, many difficulties associated with deciding on whether or not two atoms are bonded or interacting. Firstly, it is not always clear which are appropriate radii to use. Depending on the material under investigation, the radii may be covalent, ionic, or metallic, and these can differ enormously for some elements. Secondly, the radii are only approximate, and they are dependent on the environment. For example, ionic radii tend to increase with coordination number, so longer bonds are to be expected around an octahedral centre than around a tetrahedral one. Radii also vary with oxidation state [*e.g.* Fe(III) has a smaller radius than Fe(II)] and with electron configuration [*e.g.* high-spin Ni(II) has a considerably larger radius than low-spin Ni(II)]. It has been demonstrated that van der Waals radii can be markedly anisotropic (Nyberg & Faerman, 1985), so that a distance that represents a significant interaction in one direction may be nothing at all in another direction in the same crystal structure. Thirdly, some radii are by no means certain, and considerable variation is found in published tabulated values; consider the van der Waals radius for Hg, for which a wide variety of values has been proposed. It is unwise to mix values of van der Waals radii from different compilations, as these may not be consistent with each other. It is particularly among the heavier elements that considerable variation of interatomic distances is found, with a wide range of strength of secondary interactions, as well as direct primary bonding. Attempts to bring some quantitative order to this difficult area of interpretation include formal definitions of coordination number, and various measures of bond valence and other bond strength criteria. Bond length – bond order correlations are, however, notoriously unpredictable and wayward.

Interactions involving H-atoms require additional considerations. The positions of H-atoms are not very precisely determined by X-ray diffraction, because of their low electron density, so the low precision of geometric parameters involving H-atoms should not be overlooked in interpreting the significance of distances. In addition, H-atoms are frequently constrained (riding) or restrained to positions considered optimal for modeling against X-ray diffraction data, but not representative of the true nuclear positions. Weak hydrogen bonding is sometimes postulated when the experimental precision simply does not support it and hydrogen-bonding H...A distances should be always be interpreted cautiously.

For organic and organometallic compounds, nearly every structure in which OH or NH groups are present exhibits some form of hydrogen bonding, either as intramolecular or intermolecular interactions. Acceptor atoms are electronegative atoms like N, O, S, F, Cl and Br. These are generally referred to as classical hydrogen bonds and often play a role in determining how the molecules are packed into the crystal lattice. Weaker interactions like C-H...O, C-H... π , π - π and halogen...halogen interactions are often of interest these days. Keep in mind that the border between the presence and the absence of a non-bonded

interaction is very fuzzy indeed. The sum of the van der Waals radii of the involved atoms is often used as a limiting criterion, but interactions do not switch on or off as this border is crossed. Interactions become weaker and less significant as the distances grow longer, until eventually the interactions become negligible. Keep this in mind in any analysis of intermolecular interactions and tread cautiously when working in the region of very weak interactions. In addition to the distances between the atoms involved, the D-H...A angles are important and depend on the type of interaction involved – linear is not always best (Desiraju & Steiner, 1999). Possible hydrogen bonding which does not fit in with widely recognized patterns should be examined very carefully, before too much significance is attached to it

3.3 Graph set descriptors for hydrogen bonds

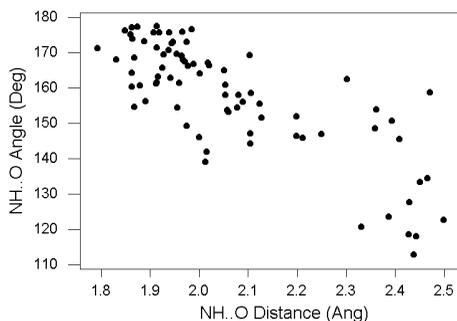
A useful system has been developed to categorize hydrogen bonding patterns in terms of simple motifs known as graph sets (Etter *et al.*, 1990; Bernstein *et al.*, 1995). These can be used to distinguish interactions that lead to different types of chains, loops and intramolecular patterns. It is often found that quite different structures have complex hydrogen bonding networks built up from the same basic motifs. The paper by Bernstein *et al.* gives full details of the definition and use of graph set motifs, particularly for complex patterns. A brief introduction follows.

At the beginning, one looks at just one of the hydrogen bonds a molecule is involved in as a donor and follows the continuation of just that same donor interaction in any subsequent molecule that acts as an acceptor of that interaction. The graph set motif is derived from the type of pattern thereby formed and the number of atoms one has to count through until the pattern repeats all over again. The general form of the motif descriptor is $G_d^a(n)$ where G represents the type of motif formed upon perpetuation of the single interaction [chain (C), ring (R), intramolecular (S), or the interaction stops at the acceptor molecule (D)], a and d are the number of acceptor and donor atoms that appear in one repeat of the motif and are omitted if both are unity, and n is the number of atoms in one repeat of the motif. Some examples are in the lecture slides.

Combinations of motifs are possible when one considers more than one symmetry-unique hydrogen bond in a structure together, see Bernstein *et al.* (1995). Two examples. If both H-atoms on a water molecule form hydrogen bonds with the same acceptor atom in an adjacent molecule, the ring motif R1,2(4) can be envisaged. If a normal chain motif is interrupted by the insertion of a water molecule (as an acceptor, then donor) between two main molecules, then a *binary* graph set motif is used, because now two different H-bonds are being tallied together. If it takes 9 atoms to get from the original donor atom to itself further down the chain, then the binary graph set motif is C2,2(9).

3.4 Structure correlation

It is often necessary to place a particular crystal structure determination in the context of a series of other related structures that have been determined. Where two parameters are of interest, a simple plot of one parameter against another may reveal a pattern that is called *structure correlation*. For example, the variation of NH...O distance with NH...O angle in hydrogen-bonded amide-alcohol systems shown at right. It is quite clear that, although there is some scatter,



hydrogen bonds become more linear as the distance decreases.

There are several well-documented examples of structure correlation studies in the literature. Perhaps the best known is the Bürgi-Dunitz examination of nucleophilic addition to a carbonyl centre (Bürgi *et al.*, 1973); other examples have been given by Gilli (1992). Another illustration is a study (Crabtree & Lavin, 1986) of the carbonyl exchange pathway between iron atoms. Some crystal structures of iron carbonyl derivatives contain terminal carbonyls, some have the carbonyl ligands symmetrically bridging two Fe atoms, while others contain asymmetric semi-bridging carbonyls. The bridged compounds are snap-shots of the carbonyl exchange process. The process can be parameterised in terms of the Fe-Fe-C and Fe-C-O angles and the two Fe-C distances, which can then be shown to be correlated, indicating the exchange pathway.

3.5 Limitations of comparisons between structures

The overlay and least-squares fit of molecules from the same or different structures was discussed in the derivation of results lecture. Normal probability plots can also be used (Abrahams & Keve, 1971); a gradient of one and a zero intercept indicate that the two sets of parameters are essentially equivalent and their s.u.s are correctly estimated. A gradient greater than unity indicates that one or both sets of s.u.s are underestimated. Systematic errors will probably produce non-linearity and possibly a non-zero intercept.

In comparisons, it is important to know what you are really comparing and if the chosen data are representative of the parameters of interest. For example, it is unlikely to be useful to extract the mean of *all* bond lengths between C and N atoms in structures from the CSD and compare it with a C-N bond in your structure, because no distinction is made between single, double, triple and aromatic C-N bonds, which are separate distributions.

Two detailed analyses of structural comparisons, with special reference to the Cambridge Structural Database, indicate the need for caution when comparing structures. Firstly, since structural parameters are usually reported to the number of decimal places dictated by the magnitudes of the s.u.s, differences between pairs of these parameters will necessarily be rounded off to the same number of decimal places. If the differences are small, their distribution will be more discrete than continuous. This artifact of rounding may produce uncertainties in the application of statistical tests (Taylor & Kennard, 1985).

Secondly, analysis of 100 pairs of independent determinations of the same structure (Taylor & Kennard, 1986) supports the widely held view that crystallographic s.u.s tend to be over-optimistically low: a factor of about 1.4 underestimation is indicated on average for the positional parameters, and consequently for the derived geometrical parameters, in these particular structures. The effect is greater for heavy atoms. An even greater factor is found for unit cell parameters, particularly those obtained from area detector (CCD) diffractometers. With such diffractometers, the refinement of unit cell parameters from several thousand reflections frequently leads to s.u.s for the unit cell edges on the order of 0.0001 Å. In reality, though, a repeated data collection from the same or different crystals of the compound may reveal reproducibility barely better than 0.01 Å.

These examples show that care must be exercised in making decisions about the significance of differences in geometrical values, and it is probably better to err on the side of caution by not claiming as genuine a difference whose significance in a statistical sense is marginal. In the literature, one sometimes sees attempts to make a big issue of deriving chemical or electronic significance from differences that are hardly different at all.

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