MEETING REPORT

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Report of a Workshop on the Use of Statistical Validators in Protein X-ray Crystallography*

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1. Aim of the meeting

Investigators from many of the major groups involved in developing structure and phase refinement packages were present. There were also representatives from the Brookhaven Protein Data Bank (PDB) and the International Union of Crystallography (IUCr), as well as other interested crystallographers. The program is given in the *Appendix*. The objectives were to review methods of refinement, with particular reference to new techniques and to evaluate methods for assessing the correctness and accuracy of refined atomic parameters. In addition efforts to assess density-modification routines and their power of phase improvement were discussed: articles by the three major contributors in this area are being published in this volume and are not addressed in detail here.

2. Questions addressed

2.1. *Why is refinement important?*

Reliable structural information and a proper awareness of the significance of the atomic parameters is needed to extract physical, chemical and biological information (energy, bond lengths, folding pathways, binding and enzymatic mechanisms and interactions) sensibly from the structure. All of these are distorted by errors in coordinates. An X-ray experiment is complicated and time consuming and it is unlikely that the results will be repeated independently by other investigators. This places an unavoidable responsibility on the crystallographer carrying out the research to ensure that the final model is a realistic representation of the available data.

2.2. *Why is protein refinement difficult?*

Small-molecule crystallographers manage analysis and refinement of their structures with very few problems. Why are proteins different? Macromolecular crystals present several particular problems in refinement.

For macromolecular crystals, the unit cell is big, and there is a very large number of X-ray data to collect, all of which are very weak. The signal-to-noise ratio is low. It is, therefore, not usually possible to collect data to atomic resolution as is normal for small molecules. The data available often suffer from both systematic and random errors. These arise because of the crystal size, problems of mounting, absorption, crystal decay and sometimes non-isomorphism between different crystals.

Protein crystals have an additional problem. There is usually a high solvent content, and the crystal forces are weak. Some parts of the chain may not be crystalline at all, and others may have high thermal motion. This means that not all the unit cell can be properly parameterized. This is true for almost all proteins, not just those which diffract to lower resolution. This problem particularly reduces the intensity of the high-resolution data. In addition it leads to severe effects of radiation damage. The distribution of amplitudes is not Wilsonian, and, therefore, scaling is more difficult.

These two problems mean that experimental data extend to limited resolution, typically to a maximum limit in the range 3–2 Å. Some structures generate data to 1.5 Å or better and allow more detailed analysis.

This means that the ratio of observations to parameters to be fitted is too low for conventional least-squares minimization to converge.

3. Improvement in refinement possibilities for proteins

3.1. *Data quality*

There has been a general improvement in the quality of diffraction data obtained over the past five years. Several factors have contributed.

Two-dimensional detectors, especially image plates, are now routinely used, they have a greater dynamic range than film, and are less prone to some of the systematic errors.

Cryogenic freezing techniques mean that crystal lifetime is now no longer a problem, and the quality of the diffraction does not degenerate significantly during the experiment. They often extend the limiting resolution obtainable.

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Synchrotron radiation sources mean that much smaller crystals can be used and higher resolution data obtained. The use of short-wavelength radiation greatly reduces absorption effects.

Techniques for data processing are advancing. There is also a growing awareness that the estimation of the standard deviations of observations is important. It is essential to carry our extensive refinement of some high-resolution structures to be able to assess the agreement between observed and calculated amplitudes. Only this can give a feel for the reliability of their empirically assigned standard deviations.

3.2. *Computing power*

The computing power available to crystallographers is immensely greater now than it was ten years ago and this is coupled with excellent graphics facilities. New refinement methods have developed side by side with this, incorporating different algorithms for convergence, simulated annealing, anisotropic temperature-factor refinement and now maximum-likelihood refinement. The 'prior knowledge' (*e.g.* bond lengths and angles) utilized by the refinement programs has been extracted from the Cambridge Structural Database for organic molecules and is very reliable. The new model building programs exploit databases derived from high-resolution structures and routinely provide better starting models which expedites refinement.

3.3. *Better protocols*

The value of using complete data during refinement is becoming more appreciated. This is not surprising since one can think of refinement as differential synthesis, and maps from complete data sets are obviously better than those from incomplete ones, especially when the missing data are systematically distributed. Examples of this were clearly demonstrated during the meeting. Cowtan used the classic diffraction from a duck (Cowtan, 1995, World Wide Web URL, http://www.yorvic.york.ac.uk/~cowtan/fourier/fourier.html) and showed how systematic elimination of data from the reciprocal-space pattern severely affected the image of the duck. Tronrud expanded on this with a two-dimensional crystal analogue. The low-resolution terms are especially important for placing the missing parts of the structure, and there is always some part missing, even if it is only the solvent. Sheldrick has shown that his high-resolution refinement benefitted from including reflections from high-resolution bins where the merging R was up to 40%.

4. Better validation indicators

4.1. *Global indicators*

The crystallographic R factor (R_{cryst}) is the most widely accepted indicator of the general quality of a crystal structure, but it is well recognised that significant errors can still be associated with acceptable values of this criterion (Kleywegt & Jones, 1995*a*). It can be manipulated by excluding some data or adding parameters inappropriately, and it must be considered as a function of resolution and completeness of the experimental data.

An independent indicator of the quality of the fit between the observed and the calculated amplitudes is provided by the cross-validation factor 'R_{free}'. This is based on the general statistical principle of cross validation where the model is required not only to reproduce those experimental data included in its estimation but also a set of excluded data. R_{free} was introduced to crystallography by Brünger (Brünger, 1992, 1993). This uses a randomly chosen sample of observations not employed in the refinement for cross validating the atomic parameters, scale *etc*. There was discussion on how many reflections were needed to give a reasonably reliable indicator, and Brünger showed that the deviation in R_{free} was roughly proportional to its value divided by the square root of the number of excluded reflections. A test set of about 1000 reflections should be acceptable. More complete cross validation requires repeating calculations with each subset excluded in turn, and then there is an obvious advantage in taking 10% of the data at a time. This type of cross validation was shown to be valuable for density-modification methods. (For ultimate cross validation one should omit each reflection in turn: this is not tractable.)

R_{free} is certainly useful for judging protocols and progress in refinement. It is particularly valuable as a tool for detecting overfitting, *i.e.* trying to use too many parameters in the minimization. At two extremes its meaning is clear. If the introduction of new parameters gives the same drop in R_{cryst} and in R_{free}, then they have given a significantly improved model. In contrast if R_{cryst} falls, but R_{free} remains the same or increases, then the new parameters overfit the data and should not be introduced. A problem arises in the intermediate case where R_{free} falls, but by less than R_{cryst}. At what level is the fall significant? An example is the introduction of anisotropic B values. At 1.0, 1.5 or 2.0 Å resolution introduction of anisotropic B values reduces R_{cryst} by about 6%. At 1.0 Å R_{free} also falls by 6%. At 2.0 Å R_{free} does not fall. At 1.5 Å it may or may not fall depending on data quality. An enigma: when is the fall significant? We need a quantitative validator to assess this. The expected value of R_{free} is a function of resolution and data quality. Cruickshank mentioned the expected value of the free R factor (EFRF) could be estimated as,

$$\mathrm{EFRF} = [N_{obs}/(N_{obs} - N_{par})]^{1/2} R_{cryst},$$

where N_{obs} is the number of observations, N_{par} is the number of parameters. In protein crystal structures at low resolution N_{par} may exceed N_{obs} if only X-ray observations are included.

Non-crystallographic symmetry (NCS) will reduce the discrepancy between R_{cryst} and R_{free} due to relationships between reflections through the G function. Different types of NCS will have different effects. Any pseudo lattice where the NCS does not increase the reciprocal-space sampling cannot easily be utilized for refinement. In contrast Rossmann (Chapman & Rossmann, 1996) has shown that for some virus structures where it is only tractable to collect a very limited subset of data, but where there is a high degree of NCS, the R factors are very similar for all such subsets irrespective of which was used for minimization.

Both R_{cryst} and R_{free} are global measures which cannot detect local errors. If atoms are placed in correct positions the R factor will decrease even if they are chemically inappropriate.

4.2. *Estimates of coordinate precision*

We assume that,

$$B = 8\pi^2\langle\mu^2\rangle,$$

where $\langle\mu^2\rangle$ is the mean-square atomic displacement, *i.e.* the mean-square amplitude of vibration in an ordered structure or the combination of vibration and disorder in a slightly disordered structure such as a protein. A B of 20 Å^2 corresponds to an r.m.s. amplitude of vibration of 0.5 Å. For a coordinate precision of <0.25 Å it is required that B should be <5 Å^2. Since in most proteins a substantial fraction of the atoms have much higher B values, coordinate precision cannot be better than this. In spite of the enormous range of B factors in proteins it is helpful to give some overall indicator. Cruickshank pointed out that the widely used Luzzati plot (Luzzati, 1952) is not appropriate. For one thing it can give similar limits for refinements carried out at very different resolutions. He suggested using a formula dependent on R factor, number of refined parameters, resolution, and completeness of the data, which he showed gave a better estimate of the agreement between independently refined structures for those parts with low or moderate B value,

$$\sigma_d(x) = 0.7(N/p)^{1/2}C^{-1/3}d_{min}R,$$

where N is the number of non-H atoms in the asymmetric unit, p is N_{obs}–N_{par}, d_{min} is the limit of the resolution in Å, C is the fractional completeness of the data from infinity to the limiting resolution and R is R_{cryst}. $\sigma_d(x)$ is the expected positional error for an atom with average B factor. Hence $\sigma_d(x)$ may be of use as a diffraction precision indicator. The errors of indivual atoms depend strongly on B value and atomic number, see *e.g.* Daopin, Davies, Schlenegger & Grütter (1994).

Another frequently reported estimate of coordinate error is derived from the σ_A plot introduced by Read (1986). This too reflects the agreement between observed and calculated amplitudes, and is, therefore, also susceptible to overfitting. With a different normalization algorithm it may be possible to use either of these formulae to estimate precision from the R_{free} set. Brünger showed that the cross-validated Luzzati and σ_A plots correlate better with the actual coordinate errors (Brünger, 1996).

As more high-resolution structures are refined it is becoming possible to use the classic least-squares matrix inversion at the completion of refinement to give e.s.d.'s for the parameters describing the well ordered parts of the cell. This will allow a formula such as Cruickshank's to be properly tested.

4.3. *Maximum likelihood*

Bricogne, Murshudov and Read emphasized how the use of maximum likelihood is a self-validation technique. They also pointed out the importance of searching for satisfactory self-validation techniques in principle. Least-squares refinement with the same weights for all X-ray observations assumes all errors are independent and all measurements are equally accurate. In addition least-squares refinement assumes that errors in $|F|$ or $|F^2|$ are distributed normally. Of course this is not true. We need, first, to minimize observational errors (better data) and, second, to get better estimates of σ. In theory, maximum likelihood with correct weighting of prior and experimental information should be a better technique for estimating atomic parameters. It should allow separation of errors arising from a poor and incomplete model from experimental errors. This should lead to better convergence, and early tests are encouraging.

4.4. *The Hamilton R test*

Another self-validation technique was described by Lamzin & Bacchi (Bacchi, Lamzin & Wilson, 1996), namely an attempt to extend the Hamilton R-factor ratio test (Hamilton, 1965) to protein structures, including the effects of restraints applied during the refinement. Sheldrick pointed out the limitations of the original Hamilton test. Nevertheless, it is clear that some self-validation tools should be developed.

4.5. *Peptide and protein geometry.*

There is very satisfactory software for assessing main-chain and side-chain stereochemistry within new structures. However, this does not give direct information about the precision of a refinement, by which we mean the consistency of the model with the experimental data. Properties such as r.m.s. deviations of bond lengths may well be determined more by the refinement protocol than the precision of the experimental fit (Kleywegt & Jones, 1995*a*,*b*). The software is invaluable, nevertheless, for detecting gross errors in interpretation. It is important *not* to include some sensitive error detectors as prior knowledge during refinement. Sheldrick pointed out that

luckily it is relatively difficult to minimize against the Ramachandran plot or side-chain rotamer databases and thus these are ideal quantities to use for later validation of the structure. In other words, when a quality indicator is monitored and used in refinement and rebuilding, it can no longer be used as an *a posteriori* check on the quality of the model.

4.6. *Electron-density maps*

The final map allows us to evaluate visually and to quantify to some extent how well the coordinates lead to correct amplitudes and phases, and measures based on it such as map correlation and real-space (free) R factors give some estimate of the precision of different parts of the structure. Unfortunately, assessment of map quality is still hard to quantify absolutely on a computer. Memory effects (ghost peaks) in the Fourier synthesis remain a serious problem.

4.7. *'Cross validation' using common sense*

A large amount of validation requires common sense. Tronrud's article (Tronrud, 1996) emphasises this point. For example, a good structure will not have most of its main-chain torsion angles in strange parts of the Ramachandran plot and this criterion alone would clearly have allowed the rejection of most of the bad structures discussed at the meeting (Kleywegt & Jones, 1995*b*, 1996*a*). Other obvious, but very useful, checks include the following.

Are there unacceptable symmetry contacts between adjacent molecules?

Are the B factors sensible, *e.g.* higher at the surface than in the core, not wildly divergent between adjacent atoms and similar for the same atoms in NCS-related molecules?

Does the chemistry make sense?

Do the hydrogen-bondable groups actually make hydrogen bonds?

Are there charged groups buried in hydrophobic environments?

Do the maps show the expected features, *e.g.* do omit maps reveal the missing atoms, do difference maps show substrate atoms?

Is there a suspicious divergence from NCS? (Kleywegt & Jones, 1995*a*.)

Does the reported detail of the model reflect the resolution of the data? For example, at 3 Å resolution one must be suspicious of models with individual B factors, alternate conformations, and/or unrestrained NCS. (Kleywegt & Jones, 1995*a*,*b*, 1996*a*,*b*.)

Most programs flag many of the above. Most users ignore these flags. At the end of the refinement it is essential that laboratories have routine and easy-to-use local checks for such problems. Education is important. Laboratories have a duty to ensure that people do not run automatic packages mindlessly.

5. Detecting gross and overall errors

Serious mistracing is rare but can happen. Kleywegt demonstrated some classic examples of serious errors from the recent literature (Kleywegt & Jones, 1996*a*) and some synthetic examples, for instance with the chain artificially traced backwards (Kleywegt & Jones, 1995*a*). The existing tools [$R_{\rm free}$, some stereochemical checks as applied in *PROCHECK* (Laskowski, MacArthur, Moss & Thornton, 1993), and *WHAT-IF* (Vriend & Sander, 1993)] easily detect such errors if applied sensibly. Actually the Ramachandran plot alone is a powerful tool for cross validation to identify such gross errors since the dihedral angles φ and ψ are not usually used as restraints in refinement programs.

Local errors such as loops out of register can easily be overlooked or attributed to disorder and ignored. They can be identified by the real-space R factor and B values as a function of residue number, inspection of the maps and comparison with related structures (Kleywegt & Jones, 1995*a*).

5.1. *The limitations of model building*

We know that proteins contain structural features such as disordered groups and bound solvent, regardless of how well they diffract X-rays. These features affect the scattering amplitudes of all the data, but our model-building techniques are not powerful enough to recognise them without high-resolution data. This limitation results in the commonly observed situation where a model refined against high-resolution data will agree better with the low-resolution data, than one refined against the low-resolution data alone. Although we can predict the existence of these structural features in all models their validation will always be a problem.

5.2. *The limitations of refinement*

All algorithms currently used, except full-matrix least squares, require in theory that many more cycles be run than there are parameters in the model. The large amount of computer time required means that this cannot be carried out. The major components ignored in the minimization procedure are the correlations between parameters. These produce the greatest problems when there are multiple conformations, with interpenetrating models. Such models require many more cycles of refinement to reach the same quality of convergence as ones with no overlapping atoms.

6. Bad practice: many examples

Many people now entering the field of structural molecular biology do not have a strong training in and background knowledge of crystallography. Meanwhile, the programs for processing data and refining structures give

the appearance of becoming more and more automated. This results in their being used blindly as black boxes. Many program packages have examples and defaults which are misused by inexperienced (and often by experienced) users. This is not a good situation. Obvious pitfalls to avoid include the following.

6.1. *Not using all the available data*

Do not use a low-resolution cut-off, *e.g.* many structures are still reported as being refined with data in the resolution range 5–2 Å and with a σ cut-off. The data within the infinity to 5 Å shell contain a wealth of important information about your structure. (You may need to use an appropriate bulk solvent correction before the solvent network is assigned.)

Do not use a 2σ cut-off on your observations during processing or refinement. Sigma cutoffs inevitably reduce the completeness more seriously at higher resolutions and are usually unhelpful. However you need to make a sensible decision about the upper resolution of your data. This is less important if you are using more sophisticated weighting schemes.

Make sure you have not lost all the strong (often low resolution) terms through detector saturation, especially important with image plates at synchrotrons. Make a second data-collection pass, or even a third, to avoid this. The big terms dominate all steps in your structure analysis.

If you can possibly avoid it do not leave a large wedge of data uncollected. Offset your crystal by up to 15° to avoid a blind region. Make sure you cover the appropriate rotation range, and start at an appropriate orientation.

Use all data in the calculation of electron-density maps.

Illustrations supporting all the above were provided by Kevin's duck.

6.2. *Attempting refinements when the observation-to-parameter ratio is too low*

At about 2.8 Å for a protein crystal with about 50% solvent, the number of observations is equal to the number of positonal (*xyz*) atomic parameters. Even at this resolution the least-squares minimum is no longer well defined. Unless there is NCS (or extremely high solvent content) it is, therefore, foolish to 'refine' automatically against data sets at resolutions below 2.8 Å. If there is NCS this limit can be relaxed with care, always ensuring the number of parameters is less than the observations: this absolutely requires the NCS to be imposed. Caveat: if your NCS is close to pseudo crystallographic symmetry (*e.g.* $P2_12_12$ but pseudo $I222$, or $P6_5$ but pseudo $P6_522$), then it is less powerful and you will have special problems. In particular in these cases it is the weak data which shows the deviation from exact symmetry. Including phase information or carrying out torsion-angle refinement may improve the observation to parameter ratio.

Do not try to refine individual isotropic atomic B values until you have enough observations, about 2.5 Å or better. How to estimate reasonable B values remains a problem.

The significance of introducing extra parameters should be cross validated using R_{free}.

Use all prior information; for instance if you have already refined a native structure at high resolution use it to restrain the refinement of a low-resolution mutant. (Kleywegt & Jones, 1996*b*)

Do not use the same protocols for refining at different resolutions. The parameters used sensibly at one resolution, such as relative X-ray to stereochemical weighting, are very likely to be totally inappropriate at a substantially different resolution. This problem is compounded by differing data quality. A so-called 2.5 Å data set where half the intensities are less than one σ will require different treatment from one where essentially all the data are greater than 10σ.

7. The future

7.1. *Improve and use your data*

This is sometimes not so difficult and is the best way to improve refinement. Always collect and use complete data to the highest possible resolution. This will make all subsequent steps in your analysis easier. The low-resolution data, to as low a resolution as technically possible, should be recorded. Freezing should be used as a default. It is often worth waiting for synchrotron time rather than wasting time and effort.

7.2. *Match parameters and data*

Reduce the number of parameters as far as possible when refining (moving the atoms around by unstable least-squares) at low resolution. Use domains *etc.* as rigid bodies initially or throughout, *i.e.* use constraints instead of restraints to reduce the number of parameters. The most powerful reduction in parameters is the imposition of NCS. Kleywegt claims that 50% of low-resolution structures have NCS. Use grouped or overall atomic temperature factors. Torsion-angle refinement can be used to reduce the number of parameters. Monitor the progress of the refinement with R_{free}, until a better self-validation tool is developed.

7.3. *New algorithms*

We still need new software to be developed in several areas.

Maximum-likelihood algorithms which should allow correct weighting of prior and experimental information are being developed. This approach will make it easier to

include information from various sources, for example, experimental phase information or data from multiple crystal forms.

Better approaches for scaling experimental and calculated amplitudes or intensities are required.

Most macromolecular least-squares algorithms use the same weighting for all X-ray observations, effectively assuming all errors are independent and all measurements equally accurate. We need firstly to minimize observational errors (better data) and secondly to get better estimates of σ. Refinement algorithms should utilize these estimated experimental errors in their weighting schemes.

Torsion-angle refinement has been developed which will reduce the number of parameters needed to describe a structure. This may be sufficient to allow refinement with data sets which only extend to 3 Å or below (Rice & Brünger, 1994).

7.4. *Improved dictionaries*

These should be expressed in a more rational form. In the future the mmCIF format will produce something not unlike the *TNT* format. We need tools to generate entries automatically from the small-molecule database coordinates.

7.5. *Map interpretation*

This is becoming rapidly a problem of the past with rapid advances in graphics programs and in automated building procedures.

8. Questions still to answer

How can we deal with anisotropic B factors sensibly? It is essential to have the facility to mix anisotropic B factors for well defined atoms, and not to use them for atoms with high B factors. Cruickshank showed the relative contribution to the scattering power from atoms with different B factors at different resolutions. It may be best to use reciprocal-space calculation for the few atoms with anisotropic B factors and atoms with significant anomalous-dispersion factors.

Better estimates of scales are needed for both maximum likelihood and least-squares algorithms.

How to estimate errors and σ's? Are they really Gaussian?

How do we estimate the number of observations and the number of parameters? This is especially difficult when carrying out restrained refinement.

Can we derive a satisfactory precision parameter like Cruickshank's to represent the effective resolution or even better the information content of data extending to some nominal resolution, with a given completeness and I to $\sigma(I)$ ratio ? What we finally want to achieve is a criterion for the information content of a model.

9. Deposition requirements

The Macromolecular Structure Database (MSD) must have sufficient information to validate the model deposited against the experimental observations. This means that it is essential that the experimental observations are deposited as well as the coordinates.

If users of the MSD are to be able truly to judge the quality of a model, then sufficient information must be present in the data bank. This requires not only experimental X-ray data and model, but a complete description of the structure determination. Those data used in the cross validation during refinement must be indicated in a manner standardized by the community. This will allow refinement with a different (hopefully improved) algorithm to be compared directly with the original procedure. In order to reproduce the final maps it is also desirable to include calculated F's and phases since different programs handle such things as scaling and bulk solvent differently.

There is so far no requirement or proper mechanism for reporting experimental data quality in the data bank. mmCIF will address this need. It is again essential that the MSD supported by the community enforce the deposition of such information. mmCIF will thus require details of the experiment including $R_{\rm merge}$, completeness, multiplicity, $I/\sigma(I)$ in resolution bins.

Pressure needs to be applied at the highest levels, *i.e.* through the IUCr and related bodies, to the editors of journals to enforce these deposition requirements.

APPENDIX

Program and speakers

Friday 7 April

09:30 K. Wilson, Opening

09:40 G. Kleywegt, The big picture

Session I. Cross validation. Chair: G. Bricogne

09:55 A. Brünger, Cross-validation and free R value

10:45 D. Cruickshank, Re-examination of conventional measures of accuracy and precision.

11:30 G. Sheldrick, Use of $R_{\rm free}$ in high-resolution refinement

12:15 V. Lamzin, Are $R_{\rm free}$ and mean phase error always correlated?

14:00 P. Metcalf, Validation of real structures

14:30 G. Kleywegt, Applications and limitations of $R_{\rm free}$

Session II. Residuals. Chair: E. Dodson

16:00 R. Read, Maximum likelihood *versus* least squares

16:45 G. Bricogne, Maximum-likelihood structure refinement with *BUSTER* and *TNT*

17:30 G. Murshudov, Comparison of refinements with different residuals

Saturday 8 April

Session III. Convergence. Chair: D. Cruikshank

09:00 D. Tronrud, Least-squares minimization

09:30 A. Brünger, Simulated annealing: why does it work?

10:15 G. Sheldrick, Convergence and false minima

Session IV. Density modification. Chair: P. Main

11:00 A. Roberts, Phase improvement by cross-validated density modification

11:45 K. Cowtan, Phase combination and cross-validation in iterated density-modification calculations

14:00 J. Grimes, Symmetry averaging.

14:45 J.-P. Abrahams, Phase validation in the absence of an atomic model: applications in density modification.

Session V. Resolution and data quality. Chair: Z. Dauter

16:00 E. Dodson, Comparison of resulting structures after refinement against different data sets.

16:30 Z. Otwinowski, Statistics of experimental data.

17:00 V. Lamzin, Effect of data completeness.

17:30 G. Kleywegt, Low-resolution refinement.

Sunday 9 April

Session VI. Miscellaneous aspects of refinement and rebuilding. Chair: G. Dodson

09:00 D. Tronrud, Modelling disordered solvent

09:30 Z. Dauter, High-resolution structures

10:00 C. Smith, Refinement of DNA structures

11:00 P. Fitzgerald, Refinement, validation and mmCIF

11:30 N. Manning, Refinement, validation and the PDB

Session VII. Free discussion. Chair: K. Wilson

All attendants were encouraged to raise issues for discussion during this session with topics from the pragmatic to the philosophical. Examples of topics: relative weights of restraints and crystallographic terms in refinement; validation at the MSD; effect of data scaling on refinement; estimating coordinate error; use of databases during rebuilding; should we reject papers that describe unvalidated structures? Model validation; are all water molecules really water molecules? How to model temperature factors at different resolutions; stereochemical dictionaries (proteins and hetero-compounds); how to deal with NCS.

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