Data science skills for referees: I biological X-ray crystallography

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Data science skills for referees: I Biological X-ray crystallography

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Synopsis

There is now a growing wish by referees to judge the underpinning data for a submitted article. It is timely therefore to provide a summary of the data evaluation checks requiring to be done. As these will vary from field to field this article focuses on the needs of biological X-ray crystallography articles, which is the predominantly used method leading to depositions in the PDB.

Abstract

Since there is now a growing wish by referees to judge the underpinning data for a submitted article, it is timely to provide a summary of the data evaluation checks required to be done by a referee. As these checks will vary from field to field, this article focuses on the needs of biological X-ray crystallography articles, which is the predominantly used method leading to depositions in the PDB. These checks necessarily include that a referee scrutinizes the PDB validation report for each crystal structure accompanying the article submission. A referee would also undertake one cycle of model refinement of the authors' biological macromolecule coordinates against the authors' processed diffraction data and assess the model and Fo-Fc electron density maps. If the referee deems necessary, the raw diffraction data images should be reprocessed (e.g. to a different diffraction resolution). The organization of practical referee skills training can be via the crystallography associations.

Keywords: Referee data science skills; Biological X-ray crystallography; Refereeing of data and atomic coordinates; Skills certification

1. Introduction

The level of rigour for refereeing the data underpinning a submitted article in all areas of science is receiving increasing attention. The degrees of such rigour have been classified into four types (see e.g. https://www.springernature.com/gp/authors/research-data-policy/data-policy-types/12327096). The most rigourous is defined as Type 4 for which "a journal would require that all datasets on which the conclusions of a paper rely be available to reviewers and readers. Authors must deposit their datasets in publicly available repositories prior to peer review, or include them as supplementary information files with their manuscript. It is a condition of publication that authors deposit their data in an appropriate repository, and agree to make the data publicly available without restriction, unless reasonable controls on data access are needed to protect human privacy or biosafety." Similarly the International Council for Science points out that "Publishers have a

responsibility to make data available to reviewers during the review process." (http://www.icsu.org/science-international/accord/open-data-in-a-big-data-world-long).

Currently biological crystallography is lagging behind chemical crystallography, which is achieving the Type 4 level of rigour, at least for IUCr journals. It is excellent that the PDB has introduced a Validation report and a Validation service. This is precisely what I pushed for along with IUCr Chester during my time as Acta Cryst Editor in Chief (1996-2005). PDB has responded. This is one half of what is needed in my view. The other half of the required procedure, clearly envisaged within the Type 4 concept, is that referees of biological crystallography articles should routinely also have access to the diffraction data and coordinates as lodged with PDB, as well as the PDB's Validation report.

In judging the data underpinning an article the referee will be assessing the procedure that authors will have followed from raw data to processed structure factors to finalised molecular model coordinates. There are numerous steps and various software programs that can be used. A sensible view will need to be taken of the author's steps, and which may not be the preferred steps that the referee would have taken. There will then be a variance that can be allowed. ***Outside that variance however errors can be determined and which should be clearly described in the referee's report to the editor.*** The descriptions below aim to provide advice to referees of how to evaluate such data involving PDB depositions.

An article will no doubt have particular results to report and the underlying data will be the underpinning evidence. But once in the PDB a wider use of the data might be possible. It is reasonable for a referee to point out deficiencies in either the processed diffraction data or derived molecular model to an editor so as to gain utility beyond the results in an article. Such an approach has worked well in chemical crystallography where that philosophy has been applied.

Let's take an overview now of the PDB Validation report contents and the calculational checks that a referee will make of their own.

Editors of even non-specialist journals now realize that requiring as a minimum the PDB validation reports (Read et al 2010) might prevent the most obvious transgressions of biological macromolecule model plausibility. Thus, for example, Fink (2016) states "The *Journal of Immunology* now requires that the PDB Summary Validation Report …be included with submission of the manuscript so that it is available to editors and reviewers during the review process."

Read et al 2010 have a section especially tailored to advising referees of submitted PDB files and quoting directly, transposing their future tense to present tense syntax:- *"The first page (of the PDB validation report) gives an overall summary, with key percentile scores for global quality on both all-PDB and resolution-relative scales. The first page also gives any "concerns" or "unusual features" present in the structure or data and gives per-chain quality indicators, including mean B-factor, overall RSR-Z, and overall RMS-Z for bonds, angles, and planes. Subsequent pages provide detailed information on residue-based quality indicators, allowing the referee to assess the level of confidence for specific residues discussed in the manuscript."*

Whilst excellent in providing a uniformity of what is assessed, unfortunately in many situations the PDB Validation report is insufficient to pinpoint the validity of an article's claims and models based

on specific electron density interpretations. From a recent informal polling of colleagues a wide variety of journal editors forward to manuscript authors any referee's requests for structure factors and model coordinates to allow full validation of the claims. These requests by referees for access to the underpinning data for a submitted article are now growing in number. Whilst not compulsory this is perceived by many referees as 'good practice' and which I obviously agree with. It is timely then to provide a discussion document of the data evaluation checks required to be done by a referee. This will also guide the crystallography associations in their plans for future continual professional development (CPD) of their members and certifying their data science skills.

2. Methods and Examples

2.1 Refereeing of data and atomic coordinates in practice

There can only be a realistic burden of work on referees in practice. Authors must remain in charge of their papers. But structural biology as a field ***cannot expect a privilege of exemption*** from refereeing of the diffraction data and derived model coordinates underpinning a publication.

Fortunately with the current state of computer laptops and PCs a single cycle of re refinement of a model using e.g. phenix.refine (Afonine et al 2012) yields a readily accessible suite of validation checks of that model and underpinning electron density maps, displayed e.g. in Coot molecular graphics (Emsley et al 2010). So, reasonably easy interrogation in specific detail is therefore possible by a referee.

Where there are concerns from a referee to do with significant difference electron density revealed via their own calculations these would be flagged explicitly by the referee for the attention of the handling Editor for that article, and which could state, as in Acta Cryst C and checkcif:- *'Alert level A: Must be attended to by the authors before acceptance of the article could be considered'*.

2.2 Examples of validation information available from phenix.refine to inform a referee's report on an article's underpinning diffraction data and coordinates

A referee would need to declare that they have read the PDB validation report(s). An example comment to this report might for example be: "This showed several amino acids with RSRZ values flagged as high; these residues need scrutiny by the authors and if no correction proves possible a comment should be included in a Supplementary file to the article."

The rest of a referee's report, as illustrated below, would be based on their own direct calculation checks and would be in addition to their scrutiny of the PDB Validation report described above.

A referee would need to declare that they undertook a round of model refinement using, e.g. phenix.refine [3]. The Fo-Fc difference electron density map peaks displayed in Coot from this can show the referee if it includes some large peaks that need attention by the authors (Figure 1). If so a

comment can be requested by the referee to be made in the authors' Supplementary file to their article, that is, if no reasonably certain chemical assignment is possible. In addition feedback is given in phenix.refine for cases of atom to atom clashes, especially involving non-hydrogen atoms, and of unusually high atomic B factors (eg >100Å^2). The referee would emphasise in their report to the authors the need for these to be scrutinised more carefully in the authors' molecular model. The example shown in Figure 1 is 'anonymised' and used for illustration purposes only. In addition a very helpful refined model diagnostic in phenix.refine is the 'polygon' summary (Urzhumtseva et al (2009)). For a wide variety of examples of the polygon diagnostic, see Figure 4 of Afonine et al. (2012). This can help guide the advice that a referee can give to a journal editor/the authors.

Where there are a quite a number of bound solvent water molecules in the PDB deposited model for which the 2Fo-Fc electron density is below 1.2 rms these should be reviewed as to whether they should really be included in the bound water structure in the PDB file. A further check is the Coot validation of bound waters; this is also informative about questionable waters.

In some cases raw diffraction images may need to be requested via the editor to the authors if the referee deems necessary. This may be necessary for example if the 'Table 1 Model refinement summary' of the article states that truncation of the diffraction data resolution at 'too high' a value of <I/sigI> and / or $CC_{½}$ has been made by the authors. The merits of harnessing the diffraction beyond the 'conventional' resolution limit in the case of biological macromolecule model refinement have been described by Einspahr and Weiss (2011) and by Diederichs and Karplus (2013). An additional reason for reprocessing of just a subset of the raw diffraction mages would be if the difference electron density map shows evidence of undue radiation damage.

2.3 Assessing the precision of displayed distances for non-covalent interactions

The figures of an article that depict non-covalent interactions need to be vetted to ensure a proper precision of any displayed distances. A website tool for achieving this vetting is now available and specific types of examples are provided (Kumar et al 2015).

2.4 A simple viewing by a referee of the derived coordinates and processed diffraction data files

For a referee to simply open the coordinates file and scroll through and read it can reveal unexpected things. One such is the peculiar case of 1LKR where individual amino acid side chain atoms had erroneously variable occupancies, which are obviously physically meaningless. This had not been noticed by anyone in the writing of the paper by the author nor the referees or by the editor or the PDB annotator handling it. For a full description of the case of 1LKR as an example see Helliwell and Tanley (2016).

Another relatively simple data check is to open the processed diffraction data and examine the 'zero layer diffraction zones'. This is straightforward to do using the software suite CCP4 for example when opening an 'mtz' file (Winn et al 2011). This reveals gaps in the data that might exist due for example to physical obscuration of the apparatus of the diffracting solid angle or due to ice rings.

3. Discussion

The Methods and Examples section above documented various areas for data and calculation checks by referees. There are some aspects however where, even amongst highly experienced crystallographers, there is not necessarily a consensus. This can also lead to variations between journal editors as well as authors. These would include:-

* the number of assigned bound waters

* the number of assigned alternate (i.e. split) occupancy amino acid side chains

* the number and type of assigned anions and cations. [The particular category of decision making involving the low atomic number ions is often exacerbated by insufficient experimental data but which is set to improve by new user beamline provision involving utilising long X-ray wavelengths with leadership by Diamond Light Source of the new beamline I23 led by Dr Armin Wagner http://www.diamond.ac.uk/Beamlines/Mx/I23.html .]

* Processed diffraction data poor completeness affects the precision of a refined molecule. Beyond this plain statement there is no community consensus however on what value is reasonable. However it is fair to report that a high resolution shell dropping below 50% in its completeness should at least be commented upon in an article and it should in any case not be allowed to form the basis of a claim for the quoted resolution limit in the title or abstract.

* For electron density map contouring there is no community consensus on the sigma level above which the map is contoured. Typically in publications, indicative therefore of a consensus, Fo-Fc maps are shown contoured from 5 sigma (this being the Coot default value) but from 3 sigma contour level is also common. Anomalous difference Fourier maps are displayed contoured from 3 sigma typically. 2Fo-Fc maps are shown contoured from 1.2 rms. Beyond this there are other practices however. The 2Fo-Fc map can be shown contoured at much lower levels, such as down to 0.8 rms, and where continuity of the map is taken to support an interpretation of a particular molecule or molecular fragment being present. This is questionable practice and a referee would be reasonable to ask that a second crystal be analysed and the same portion of an electron density map be scrutinised. If a basically identical result is obtained then statistically speaking the certainty of the initial interpretation is more confident. Further confidence in a difference map can be obtained by the referee if the same diffraction data is used within two different software packages.

4. Guide to the referee's recommendation options on the data sets underpinning an article

These are really not different from the recommendations that a referee makes to an editor regarding a submitted article namely 'Accept', 'Minor revisions', 'Major revisions' or 'Reject' and will most likely be fairly obvious to the referee in any given case, or decided upon in consultation with the editor.

Overall the referee is not the author and whilst sufficient excursions into the data as described above are made by the referee the actual work of revisions of the data sets, atomic coordinates or structure factors or even raw data remeasurement, is obviously the task of the authors. A list of recommendations to the editor for revisions to be made by the authors would be given by the referee.

5. My experiences in the last 1 1/2 years

When I have been asked by a journal to referee an article submission during the last 1 ½ years, having agreed, I would immediately request that I be provided with the PDB Validation report, the processed diffraction data file and the atomic coordinates with atomic displacement paramters, the ADPs, (the 'pdb' file). I have successfully refereed approximately twelve such article submissions in this time on this basis. These were from seven different journals and four different publishers. In all but one case the PDB Validation report was not automatically provided. In one article submission there were two new protein crystal structures and three existing ones in the PDB. In all five cases, i.e. including the existing three depositions at the PDB, I recommended revisions, and which were made by the authors/depositors. For one publisher there were three refereeing requests to me and two of which did not lead to my receiving the underpinning data files. So I did not submit a referee's report in those two cases but had promptly replied to each reminder from the publisher for my report that I was still awaiting the data files. The publisher signed off with an email saying that they had found another referee but would still like to have my report. However I had not started my refereeing work as I wanted the data to fully understand the authors' article properly. In another refereeing commission the refined structure was an anisotropic ADPs one and the PARVATI web server checks were invaluable (Merritt 1999) in shaping my comments in my report. In another case the handling editor for the publisher eventually sent me, twice, an especially heartfelt thankyou email.

Initially the data files I asked a journal editor for were unspecific. This led to files being sent to me which were not finalised for release by the PDB. The PDB reminded me at the IUCr Congress in Hyderabad in 2017 that as a referee I could ask the author for the to-be-released PDB files since they were provided to the authors. The advantage of this is that all the various PDB checks had been completed by that point; since then those are the data files that I request from the journal editor.

How much time does it take me to do such a referee's report? For the case of five crystal structures that I mentioned above it took me two days. For an article with one new protein crystal structure it takes me approximately a morning or an afternoon. What computer hardware do I have? I have a 15 inch sized screen HP laptop that I keep at home, which I purchased in 2012; it has 8 Gbytes of memory and 1 Tb of disk space. I also have a smaller HP laptop which I travel with to enable calculations I may wish to make during trips. I purchased it in 2014. It has 8 Gbytes of memory and 512 Gbytes of disk space. This hardware has led to the work periods I quote in the previous paragraph. However I have not refereed any ribosome crystal structures for example.

Are there common items that I find out about in my own calculations with the underpinning data? This is an important question because, if so, it may be possible to expand the PDB Validation report to include them. Over the years the chemical crystallographers in sharing their refereeing of data-

with-article experiences have helped expand the checkcif checks (http://checkcif.iucr.org/) of which there are now over 400 in number. Generally I would say that what I state in my report in my data calculations section are in the context of the article's topic and therefore rather specific. Clearly a vital aspect is that my calculations give me access to the whole asymmetric unit electron density map, and other different types of map, rather than selected portions of a restricted type of map.

Has any publisher replied to me on behalf of the authors to say that the data are confidential and so they will not share their data with me at this the refereeing stage? No, they haven't. Also since I now ask for the to-be-released PDB files I imagine that authors would in any case feel protected by their submission date to the PDB.

Should the PDB be the data referee? I get asked this. I think not because it is the publishers that make profits from publishing our science and it is up to them to set up processes which ensure the correctness of what they publish in their journals. Indeed some journals make very large percentage-of-revenue profits.

6. Concluding remarks

The expected referee checks of data underpinning an article have been described with examples. These checks necessarily include that a referee checks the PDB validation report for each structure accompanying the article submission; this check whilst necessary is not sufficient for a complete evaluation. A referee would then be expected to undertake one cycle of model refinement of the authors' biological macromolecule coordinates against the authors' processed diffraction data and look at the various validation checks of the model and Fo-Fc electron density maps in e.g. phenix.refine and in Coot. The referee may deem it necessary (i.e. in the referee's judgement based on the above summarised work) that the diffraction data images should be reprocessed (e.g. to a different diffraction resolution than the authors' submission). This can be requested be done by the authors or if the referee prefers can be undertaken directly by the referee themselves.

A referee wishing to do these data checks may wish to receive a certificate that they have command of these data science skills. The organisation of such voluntary certification training can be via the crystallography associations, and their Continual Professional Development 'CPD' committees.

Overall the aim with data refereeing is to assist with ensuring the FAIR data principles (https://www.force11.org/group/fairgroup) that not only should data be Findable, Accessible and Interoperable but be Re-usable i.e. with as few errors and uncertainties in them as possible. This is the important contribution that a referee can make by also scrutinising the underpinning data of a submitted article.

Acknowledgements

JRH, as Editor in Chief of IUCr Journals 1996 to 2005 saw in detail the exemplary checks undertaken by Acta Crystallographica Section C Coeditors, which included Dr Madeleine Helliwell, using the

checkcif report as well as the chance to scrutinise with their own calculations the underpinning processed structure factors and derived atomic coordinates of each submitted article. Thus JRH is very grateful to all colleagues involved with Acta Crystallographica Section C at the time notably Professor Syd Hall and also Dr Madeleine Helliwell for many discussions.

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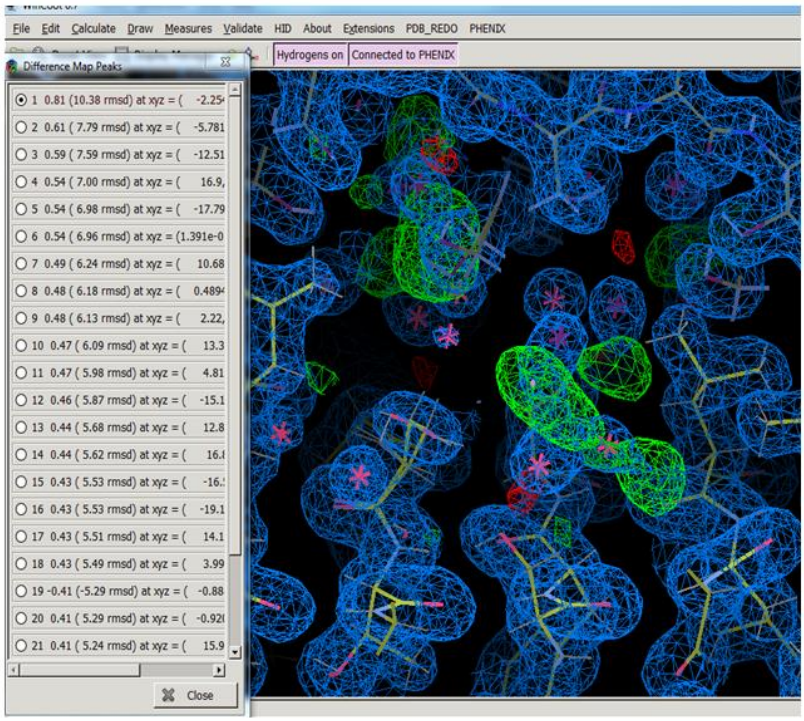
Figure 1. An example of an Fo-Fc peaks list displayed in Coot clearly showing the need for further attention by authors. The Fo-Fc map (without any superimposed model) is contoured from 3σ and the 2Fo-Fc map (with superimposed model) contoured from 1.2 rms. Reproduced with the permission of Dr Paul Emsley.



Notes on contributor

John R Helliwell is Emeritus Professor of Chemistry at the University of Manchester. He was awarded a DSc degree in physics from the University of York in 1996 and a DPhil in molecular biophysics from the University of Oxford in 1978. He was Director of Synchrotron Radiation Science at the Council for the Central Laboratories of the Research Councils (CCLRC). He has served as President of the European Crystallographic Association (ECA).He is a Fellow of the Institute of Physics, the Royal Society of Chemistry, the Royal Society of Biology, and the American Crystallographic Association. In 1997, he was made an Honorary Member of the National Institute of Chemistry, Slovenia. He was elected a corresponding member of the Royal Academy of Sciences and Arts of Barcelona, Spain, in 2015. He was made an Honorary Member of the British Biophysical

Society in 2017. The same year, he became a Faculty 1000 Member, charged with highlighting significant science publications. He was a Lonsdale Lecturer of the British Crystallographic Association in 2011, the Patterson Prize Awardee of the American Crystallographic Association in 2014, and the Max Perutz Prize Awardee of the European Crystallographic Association in 2015. He has published more than 200 research publications and two research monographs.

Subject index

Skills certification

Split occupancies