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Acta Cryst. (2011) A67, C104**Validation of small- and macro-molecular X-ray structures: PDB and CCDC collaborations**

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The Protein Data Bank (PDB) and the Cambridge Crystallographic Data Centre (CCDC) have been collecting and curating X-ray structures for almost half a century. Although the databases store different types of structures, macro-molecules and small-molecules respectively, both databases exhibit an exponential growth. This is partly due to experimental techniques becoming more automated and partly due to structure solution software packages becoming more accessible to “non-expert” users.

This raises two issues for both the PDB and the CCDC. First of all we need to design processes and workflows that allow our finite resources to deal with the exponential growth in structures. Secondly, we need mechanisms for flagging honest mistakes made by less experienced (and experienced) users.

In this talk we will discuss issues arising when processing small-molecule and macro-molecule structures. We will also discuss how the PDB and the CCDC have learnt from studying each other’s data processing procedures and how we are sharing information and technologies to improve the quality of data in the databases.

Keywords: validation, PDB, CCDC

MS.43.3

Acta Cryst. (2011) A67, C104**The most powerful crystallographic validation tool: Common sense**

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A recent rush of retractions of protein structures published in high impact journals has led to the call for more rigorous validation of crystallographic protein structure models as well as the underlying structure factor amplitudes [1]. While a posterior validation by an independent repository such as the PDB certainly serves this need (but does not require that the depositor actually corrects the model diligently), a more proactive process is concurrent *a priori* model validation during the building-refinement cycles. This is achieved for example in the model building program Coot [2], where real space geometry and electron density both are used for model improvement.

Interestingly enough, despite the availability of powerful validation tools, the majority of flawed models that passed (or ignored) the tests (and also fooled editors and reviewers) could have been prevented by using the most powerful validation tool at our disposal: the common sense of the person building the model. Basic probabilistic inference models that consider both the crystallographic evidence and the compatibility of the model with all available prior knowledge suffice to intercept almost all major problems - including some rare cases of data fabrication. While such probability analysis provides a solid qualitative measure for model correctness, it still can be fooled by the most insidious problem in biomolecular crystallography: Mental phase (or model) bias often provides an overwhelming desire to find what one seeks. Particularly ligand structures suffer from this inherent temptation, and depend heavily on critical and unbiased plausibility analysis.

Again, such modeler-bias-introduced problems need to be preferably addressed *a priori* in crystallographic education and curriculum [3], with a posteriori validation remaining only the final safeguard against errors. With the great power of modern crystallography comes great responsibility - and that responsibility ultimately rests with the model depositor, irrespective of any automated validation.

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Keywords: Model validation, Bayes’ Theorem, Bias

MS.43.4

Acta Cryst. (2011) A67, C104-C105**Unrestrained reciprocal space refinement can indicate alternative conformations**

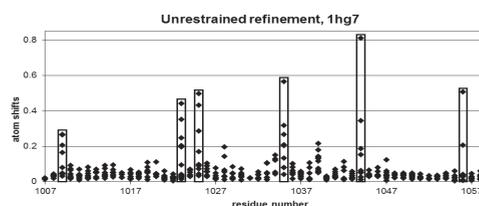
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The reciprocal space refinement with geometrical restraints turned off becomes a common practice when working at sub-atomic resolution. Nevertheless even at ultra-high resolution the stereochemical restraints are usually kept for the residues found to be in alternative conformations. In other case these residues deteriorate significantly. We suggest that this property can be used in an opposite direction as an indicator that can reveal the necessity of alternative conformations for a given residue when applied at early stages of refinement with all residues present in a single conformation. Our tests demonstrated that for the resolution higher than 1.2Å a formal procedure of unrestrained refinement gives a useful hint for which residues might be checked thoroughly with electron density maps as possible candidates for the presence of alternative conformations.

To check this suggestion we designed a pipeline to select structures from PDB with desired resolution and R-factor values (using PDB search engine), download and process experimental data and perform unrestrained refinement with the use of PHENIX [1], and calculate atomic shifts. This analysis allowed to estimate “normal” value for coordinate shifts taking into account resolution and atom properties (main or side chain, protein surface or core residues *etc.*).

The most thorough analysis was performed for structures refined in 1.2-1.1 Å resolution range. It included visual analysis of several electron density maps and comparison with assignments of alternative conformations originally present in PDB files. It was found that usually residues possessing of abnormal atomic shifts after unrestrained refinement either are already present with alternative conformations in PDB file or the electron density map suggests such idea. Some correlation was found as well in magnitudes of atomic shifts and relative occupancies of alternative conformations. The maximal coordinate deviations were obtained for residues that have alternative conformations with equal occupancies.

An attempt of the use of similar procedure at lower resolution had failed, while it worked similarly well at higher resolution [2]. The exclusion from the model of the water molecules resulted in significant growth of shift for the most part of the structure.



Atom shifts for antifreeze protein type III (PDB code 1hg7) are shown. Every column of dots represents shifts of atoms from one residue. Residues marked with rectangles were in alternative conformations with occupancy more than 30% in PDB.

This work was partially supported by RFBR grant 10-04-00254-a.

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Keywords: macromolecule, refinement, conformation

MS.43.5

Acta Cryst. (2011) A67, C105

Validation of B-factor distributions in protein crystal structures

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Many tools for the analysis of protein models from X-ray crystallography are available nowadays. They check the distribution of geometrical and stereo-chemical properties [1], the agreement of the model with the data [2], or both [3]. Despite that, a systematic procedure for the analysis and validation of B-factor distributions is still missing. This is surprising since temperature factors play an important role in model interpretation. Moreover, anomalies in the distribution of B-factors can be symptoms of errors introduced during model building and/or refinement. A tool for the detection of these cases would be useful for the interpretation of protein models available from the Protein Data Bank (PDB) or at the end of the refinement stage.

Here we propose a new approach for the identification of suspicious B-factor distributions. The main assumption is derived from Bayesian statistics and states that isotropic B-factors in a protein crystal structure should follow an Inverse-Gamma Distribution (IGD). A Maximum Likelihood Estimation (MLE) approach is used to estimate the parameters of the IGD that best fits the distribution of B-factors of a given structural model. A Kolmogorov-Smirnov test (K-S test) is then used to evaluate the goodness of fit and compute a p-value.

We developed and tested the new approach on a set of 15998 protein crystal structures selected from the PDB with a resolution of 2Å or higher. We found that for 79% of the PDB structures the p-value was equal or higher than 0.01, indicating a reasonable agreement between the observed distribution and the expected IGD. For some of the structures with a p-value lower than 0.01, their B-factors still satisfied the IGD assumption if polypeptide chains were analysed separately - for single chains from the original set of PDB structures, we found that around 89% of the chains had a p-value equal or higher than 0.01. Furthermore, a re-refinement protocol performed with the experimental version 5.6 of REFMAC [4] was able to rescue some of the outlier structures found with the single chain analysis.

Our work shows that the IGD distribution is a reasonable assumption for the validation of B-factor distributions and the new approach can be used for the detection of suspicious B-factor distributions in protein models.

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Keywords: structure validation, crystal structure analysis, crystal structure properties

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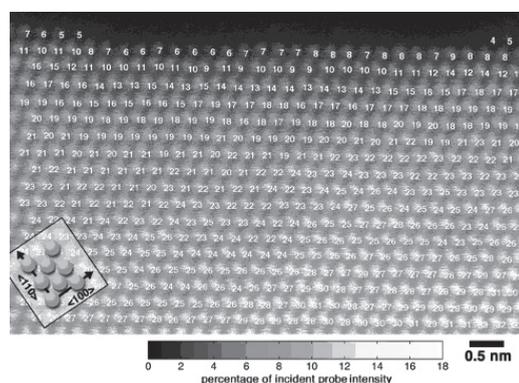
Acta Cryst. (2011) A67, C105

Counting atoms with quantitative scanning transmission electron microscopy

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Atomic scale engineering of materials requires methods that are capable of precisely quantifying the position, type, and number of atoms present. High-angle annular dark-field (HAADF) scanning transmission electron microscopy (STEM) is particularly suited to the task since the images are directly interpretable with intensities depending sensitively on the type and number of the atoms. In this presentation, we will show that when STEM experiments are placed on an absolute intensity scale and combined with accurate thickness determination, direct comparisons between simulation and experiment become possible [1]. Using this approach, we will demonstrate that STEM experiments are in near-perfect agreement with theory, regardless of the material or collection angle [2, 3, 4].

We will show that simulations *alone* can provide the 'calibration standard' necessary to extract the number of atoms contributing to the experimental image intensities. Using this information, we will demonstrate that all the atoms in a wedge-shaped, thin gold foil can be counted [5]. An example is shown in the figure below, where the white numbers indicate the number of atoms in each corresponding atom column. The atom counts are verified by comparing with the specimen thickness determined with position averaged convergent beam electron diffraction patterns (PACBED) [6]. Furthermore, we will show that the finite effective source size can be estimated with this approach. Finally, future prospects of the technique for nanostructured materials will be explored.



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Keywords: STEM, quantitative imaging