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.

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Keywords: macromolecule, refinement, conformation

MS.43.5

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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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Counting atoms with quantitative scanning transmission electron microscopy

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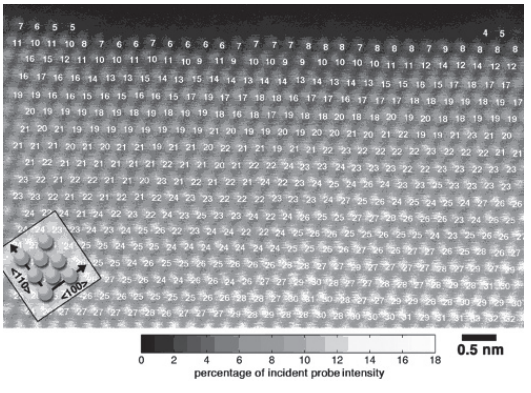
Atomic scale engineering of materials requires methods that are capable of precisely quantifying the position, type, and number of atoms present. High-angle annular dark-field (HAADF) scanning transmission electron microscopy (STEM) is particularly suited to the task since the images are directly interpretable 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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