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Recent developments in the field of X-ray crystallography, e.g. 3rd generation synchrotron radiation of increased intensity and improved detectors, facilitate macromolecular structure determination of biological samples at high resolution. Several protein and DNA structures are known with a resolution better then 1.0 Å. High-resolution diffraction data reveal electron density features more clearly and enable the use of non-spherical scattering factors. Such data also allow to resolve static disorder that remains undetected at lower resolution or when using data of low quality. In order illustrate the benefits of combining high-resolution crystallography and non-spherical scattering factors we studied the 16-residue thiopeptide Thiostrepton and a DNA structure by invariom refinement [1]. For this purpose complete and redundant Bragg data from the thiopeptide Thiostrepton were measured at the Swiss Light Source synchrotron at a temperature 100K to a resolution of 0.65 Å and compared to laboratory data to 0.81 Å. Furthermore Dauter et al. kindly provided a 0.55 Å resolution dataset from a Z-DNA structure [2]. These datasets were initially evaluated with the independent atom model (IAM) and afterwards re-refined using non-spherical scattering factors of the invariom database [1],[3] which is based on the Hansen-Coppens multipole model [4]. High resolution single-crystal diffraction data evaluated with invarioms provide not only detailed and accurate molecular geometries, but also information on the electron-density distribution and on properties derived from it. With a view to biological, structural and medical functionality of Thiostrepton as well as DNA, an analysis of the electrostatic potential and the molecular dipole moment is especially relevant, and both properties will be reported.

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MAIN 2011: Refining against all diffraction data – free of R-free

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Macromolecular molecular models are subjected to multiple cycles of model building and refinement before the structure is considered determined. In real space the model see and feel the electron density maps which contain structure factors corresponding to all measured as well as missing data, whereas in the refinement stage a share from 5 to 10% is sacrificed to enable cross-validation. The model thus toggles between steps where it feels all the data and those where it does not. Within the last few years real space refinement and model building tools of MAIN have reached the point where model building sessions are decreasing the gap between the TEST and WORKING set of diffraction data thereby diminishing the usefulness of the TEST set for the maximum likelihood target function which relies on the TEST set independence. Several approaches can be used to address the problem: Ignoring it.

Trying to make model independent from the TEST set by randomization

Use all the data in refinement throughout the whole structure determination process.

Following the route 1 one assumes that the fitting of the model to the electron density maps by the modeling programs was not efficient enough to affect the TEST set independence and R-free. The assumption is without proper validation based on hopes only.

Following the route 2 the model can be refined using multiple randimization cycles between rounds of refinement. In MAIN kicking is used, molecular dynamics based annealing is equally efficient.

Following the route 3 one should include all data in refinement. In order to avoid overfitting one should use target functions which do not rely on independence of the TEST portion of diffraction data as the maximum likelihood function yet provide similar outcome. For these the uses of averaged Fobs-Fmodel kick maps as target functions have been explored in refinement. The kick map approach has been used to calculate model less biassed electron density maps. Averaged kick maps are the sum of a series kick maps, where each kick map is calculated from atomic coordinates modified by random shifts. As such they are a numerical analogue of maximum likelihood maps. Analysis has shown that they are comparable and correspond better to the final model than σA and simulated annealing maps[1]. In the presented analysis we have explored kick map uses in refinement and structure validation and compared the outcome of the approaches 2 and 3. (For MAIN reference "http://www-bmb.ijs.si/").

[1]J. Pražnikar, P. Afonine, G. Gunčar, P. Adams, D. Turk *Acta Cryst.* **2009,** *D65,* 921-931.

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Automatic identification of alpha-helices in Patterson maps

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Protein crystal structure solution is often challenging due to limitations of current phasing methods, occurring at low data resolution and/or high structure complexity. Ab initio and SAD/MAD phasing methods are also hampered by the lacking of heavy atoms in the crystal, while molecular replacement is ineffective when low homology models are available. Recently brute force methods have been developed, which use minimal apriori structural information to drive the phasing process towards solution [1]. They find all possible positions of alpha-helices in the crystal cell by molecular replacement and explore systematically all of them. Knowing in advance the orientations of the alpha-helices would be a great advantage for this kind of approach. This is exactly the aim of the method we developed, which consists in a fully automatic procedure to find the orientations of alpha-helices within the Patterson map. The method is based on pattern recognition techniques, specifically addressed to the identification of helical shapes in low resolution Patterson maps. This approach has been first outlined in [2]. In our implementation, Fourier filtering techniques operating on Patterson maps described in polar coordinates supply a list of candidate orientations, which are then refined by using proper figure of merits based on the local comparison between the experimental Patterson map and that calculated from a template poly-alanine helix, calculated along each candidate direction. The first step has been optimized to work at 3Å resolution, while the second operates at 5Å resolution. The algorithm is complementary to the molecular replacement approach