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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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Keywords: macromolecules, biocrystallography, charge density

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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/").

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Keywords: refinement, R-free, kick map targets, maximum likelihood maps

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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

and its outcomes may be also used to constraint the generation of more reliable structural models. The method has been applied to a large number of protein test structures, showing a good discriminant power with respect to the complexity of the structure, the space group symmetry and the presence of additional beta domains. The accuracy in the determination of the direction of the alpha helix depends on its length, and only helices greater than ten residues may be found with a reliable precision. The automatic procedure has been tested in Matlab and will be included in the software package ILMILIONE, devoted to protein crystal structure solution [3].

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Keywords: alpha-helix, patterson map, pattern recognition

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Structural and computational analysis of 3,6-dioctyloxiphenyl-2,5-dimethyl-1,4-diketopyrrolo[3,4-c]pyrrole-1,4-dione

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Diketodiphenylpyrrolopyrroles are industrially important red pigments[1]. The success of these compounds as pigments relies, in part, on their high light fastness and very low solubility in most common solvents.

The title compound was synthesized, structural and spectroscopic properties were investigated. Molecular and crystal structure of 3,6-dioctyloxiphenyl-2,5-dimethyl-1,4-diketopyrrolo[3,4-c]pyrrole-1,4-dione, $C_{36}H_{48}N_2O_4$, have been determined by single crystal X-ray diffraction study. The title compound is triclinic, with *a*=6.2419(6) Å, *b*= 9.5847 (9) Å, *c*=13.4568 (8) Å, α=106.573(7) °, β=94.244(6)°, γ=93.154(8)°; Z=1, D_x=1.24 g/cm^3, μ(CuK_α)=0.08 mm^{-1}, and space group is P-1. The structure was solved by direct methods and refined to a final R=0.055 for 3214 reflections with I>2σ(I). The structure is devoid of classical hydrogen bonds. However, there are two intra-molecular weak interactions between C9-H9···O2 and its inversion counterpart C9^i-H9^i···O2^i (Symmetry code i: 4-x, 1-y, 1-z), with the geometrical parameters: D-A=3.06 Å, H···A= 2.18 Å, D-H···A=158 °.

Optimized geometry and NMR spectra of the title compound were investigated and analyzed using DFT at B3LYP functional by 6-31g(d) basis set. Experimental and computational NMR spectra were determined and compared.

Computational and crystallographic results, NMR spectra and molecular geometry, are in good agreement. RMSD (Root Mean Square Distance) value between crystallographic result and optimized geometry is 9,507·10^{-3}Å. The geometry of diketopyrrolopyyrole ring is in agreement with previous study [2, 3].

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Keywords: diketopyrrolopyrroles, crystal structure, dye-pigment.

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Automatic structure determination for small molecule crystallography

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When the automatic structure determination algorithm was proposed over two decades ago [1], it had some technical problems. For example, it took more than a half day to solve a structure because of the limited capability of computers at that time. Recently, the situation has dramatically been changed. We can solve the structure of a small molecule several tens of seconds or sometimes in a few seconds. We have developed a software, AutoSolve, for small molecule crystallography to determine structures automatically.

AutoSolve was designed for organic and metal-organic compounds. This software uses SIR [2] as a direct method and SHELXL[3] as a refinement tool. In addition, we have incorporated a feature to switch among direct methods, SIR, SHELXS and Superflip[4] when a method does not give an initial structure.

In the initial step, AutoSolve assumes all atoms as carbons except heavy atoms and executes refinement by using SHELXL. After that, AutoSolve assigns atoms by taking temperature factors (Biso/Beq), bond distances, and chemical valencies into account. AutoSolve decides when to convert temperature factors to anisotropic and when to add hydrogen atoms by checking the R1 value in every refinement cycle. Finally, the hydrogen atoms are generated by using the SHELXL's HFIX command. Our algorithm follows the conventional structure determination procedure but it gives good results.

In order to evaluate the performance, we picked up 50 samples from Acta Cryst. C and ran AutoSolve. We did not get good results for inorganic compounds. However, AutoSolve gave structures close to final structures for organic compounds with success rate of about 100% and 60% for organic and metal-organic compounds, respectively.

AutoSolve can reach nearly a complete structure in a few seconds or several tens of seconds, except for inorganic compounds.

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Partial observations, partial models and partial residuals in least squares refinement

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Certain features of the standard use of least squares methods should cause concern as they are the result of a number of misconceptions. In particular one has to seriously question the use of a global scale, *K*, to assess errors in variables by creating a variance-covariance matrix **M** as *K* times the inverse of the matrix used to describe the least squares equations. There is also the oversight of not calculating variances for components of the model of the observations, the failure to take sufficient notice of how information is distributed in the observations and how easy it is to make mistakes in the description of twins, powders and pseudo symmetric structures that will not self correct during refinement. Ideas have been developed using partial observations, partial models and partial residuals that suggest how to better identify