HAL
open science

Ultrahigh-resolution crystallography and related electron density and electrostatic properties in proteins

Claude Lecomte, Christian Jelsch, Benoît Guillot, Bertrand Fournier, Angélique Lagoutte

To cite this version:

Claude Lecomte, Christian Jelsch, Benoît Guillot, Bertrand Fournier, Angélique Lagoutte. Ultrahigh-resolution crystallography and related electron density and electrostatic properties in proteins. Journal of Synchrotron Radiation, 2008, 15 (3), pp.202-203. 10.1107/S0909049508000447 . hal-01713023

HAL Id: hal-01713023

https://hal.science/hal-01713023v1

Submitted on 20 Feb 2018

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

electronic reprint

Journal of
Synchrotron Radiation
ISSN 0909-0495
Editors: **Å. Kvick, D. M. Mills and T. Ohta**

Ultrahigh-resolution crystallography and related electron density and electrostatic properties in proteins

Claude Lecomte, Christian Jelsch, Benoît Guillot, Bertrand Fournier and Angélique Lagoutte

J. Synchrotron Rad. (2008). **15**, 202–203

Copyright © International Union of Crystallography

Author(s) of this paper may load this reprint on their own web site or institutional repository provided that this cover page is retained. Republication of this article or its storage in electronic databases other than as specified above is not permitted without prior permission in writing from the IUCr.

For further information see http://journals.iucr.org/services/authorrights.html

Synchrotron radiation research is rapidly expanding with many new sources of radiation being created globally. Synchrotron radiation plays a leading role in pure science and in emerging technologies. The *Journal of Synchrotron Radiation* provides comprehensive coverage of the entire field of synchrotron radiation research including instrumentation, theory, computing and scientific applications in areas such as biology, nanoscience and materials science. Rapid publication ensures an up-to-date information resource for scientists and engineers in the field.

Crystallography Journals Online is available from **journals.iucr.org**

Journal of
Synchrotron Radiation
ISSN 0909-0495

Received 20 July 2007
Accepted 7 January 2008

Ultrahigh-resolution crystallography and related electron density and electrostatic properties in proteins

Claude Lecomte,* Christian Jelsch, Benoît Guillot, Bertrand Fournier and Angélique Lagoutte

Laboratoire de Cristallographie et de Modélisation des Matériaux Minéraux et Biologiques (LCM^{3}B), CNRS ESA 7036, UHP Faculté des Sciences, BP 239, 54506 Vandoeuvre-les-Nancy CEDEX, France. E-mail: claude.lecomte@lcm3b.uhp-nancy.fr

With an increasing number of biological macromolecular crystal structures measured at ultrahigh resolution (1 Å or better), it is necessary to extend to large systems the experimental valence electron density modelling that is applied to small molecules. A database of average multipole populations has been built, describing the electron density of chemical groups in all 20 amino acids found in proteins. It allows calculation of atomic aspherical scattering factors, which are the starting point for refinement of the protein electron density, using the *MoPro* software. It is shown that the use of non-spherical scattering factors has a major impact on crystallographic statistics and results in a more accurate crystal structure, notably in terms of thermal displacement parameters and bond distances involving H atoms. It is also possible to obtain a realistic valence electron density model, which is used in the calculation of the electrostatic potential and energetic properties of proteins.

© 2008 International Union of Crystallography
Printed in Singapore – all rights reserved

Keywords: electron density; protein refinement; high-resolution crystallography.

The details observable in protein crystal structures depend on the experimental diffraction resolution. In X-ray diffraction (XRD), the experimental resolution (Å) is defined by $d = \lambda/(2\sin\theta)$, where θ is the Bragg angle and λ is the wavelength. At the usual resolution for macromolecular crystallography, $1.6 < d < 3$ Å, the number of Fourier components $F(h, k, l)$ of the electron density (ED) as obtained by

$$\rho_{\rm exp}(x, y, z) = V^{-1} \sum_h \sum_k \sum_l |F(h, k, l)| \exp i\varphi(h, k, l) \times \exp[2\pi i(hx + ky + lz)] \quad (1)$$

is not high enough to estimate all the atomic parameters. In the ultrahigh-resolution case, we will show that bonding interactions and electrostatic properties may be quantified in relation to biological activity.

In contrast to the independent atom model (IAM), where all atoms are assumed to be neutral, spherical and independent, the crystal static valence electron density is modelled by a sum of multipolar pseudo-atom densities, $\rho^{\rm at}_{\rm mult}(\mathbf{r})$, located at atomic centres (Hansen & Coppens, 1978), while the atomic core density, $\rho_{\rm core}(\mathbf{r})$, remains unchanged,

$$\rho^{\rm at}_{\rm mult}(\mathbf{r}) = \rho_{\rm core}(r) + \kappa^3 P_{\rm val}\, \rho_{\rm val}(\kappa r) + \sum_{l=0}^{l_{\rm max}} \kappa' R_{nl}(\kappa' r) \sum_{m\mp} P_{lm\mp} y_{lm\pm}(\theta, \varphi), \quad (2)$$

where the term $\rho_{\rm val}$ represents the spherically averaged free atom Hartree Fock valence density. The third term of the summation describes the non-spherical part, in which the radial functions used are of Slater type: $R_{nl}(r) = Nr^{nl}\exp(-\xi r)$. The functions y_{lm} are spherical harmonics in real form; the (θ,φ) coordinates are expressed in an atom-centred local axis system, which facilitates the application of chemical similarity. The refinable parameters are the κ and κ' coefficients, which describe the expansion–contraction of the perturbed valence ED (Coppens *et al.*, 1979), and the population parameters $P_{\rm val}$ and P_{lm}.

To apply this formalism to ultrahigh-resolution protein diffraction data, we have proposed a multipolar parameters library (Pichon-Pesme *et al.*, 1995; Zarychta *et al.*, 2007). High-resolution XRD data have been collected at the Laboratoire de Cristallographie et de Modélization des Matériaux Minéraux et Biologiques (LCM^{3}B, Nancy, France) on a large group of mono- or polypeptides to determine precisely the ED distribution of all natural amino acids (neutral or charged). Multipole refinement of the related structure factors allowed the building of an experimental database of multipolar parameters for each protein atom type in a given chemical environment (Jelsch *et al.*, 1998), from which a specific atom-type nomenclature has been developed. The static electron deformation density,

$$\Delta\rho_{\rm static}(\mathbf{r}) = \sum_{j=1}^{N_{\rm at}} \left[\rho^{\rm at}_{j\,\rm multi}(\mathbf{r} - \mathbf{r}_j) - \rho^{\rm at}_{j\,\rm IAM}(\mathbf{r} - \mathbf{r}_j)\right], \quad (3)$$

can be calculated from the experimental database in all amino acids and natural peptides. The database parameters were shown to be transferable to the protein amino acids and permit the calculation of aspherical atomic scattering factors to be used for protein refinement.

electronic reprint

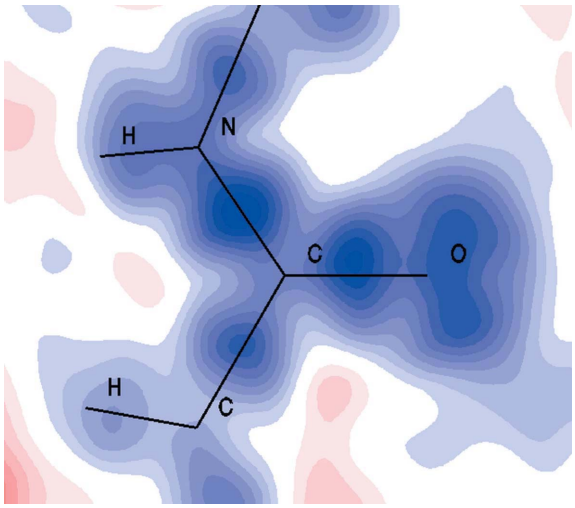

Figure 1
Dynamic valence electron density map computed in the Val5–Val6 peptide bond of the eel pout type III antifreeze protein RD1. Positive and negative electron density is, respectively, plotted in blue and red contours of ± 0.08 e Å^{-3}. Values between -0.08 and $+0.08$ e Å^{-3} are not represented (white colour).

The validity of these aspherical scattering factors was checked more than ten years ago (Jelsch *et al.*, 1998) and has been confirmed since then by several studies. These factors have been successfully used to refine ultrahigh-resolution protein structures: a scorpion toxin (Housset *et al.*, 2000), crambin (Jelsch *et al.*, 2000) and hAR (Muzet *et al.*, 2003). As shown in these papers, the use of aspherical scattering factors improves all least-squares statistical indices and, consequently, leads to more physically meaningful bond distances and thermal anisotropic displacement parameters. Aspherical features, such as, for instance, non-bonded density on a C═O O atom, are also taken into account.

This finding led us to the development of the *MoPro* package of crystallographic programs (Guillot *et al.*, 2001; Jelsch *et al.*, 2005). In *MoPro*, for any chemical type of atom belonging to a protein-type molecule, automatic assignment of the corresponding aspherical scattering factor is performed, and these scattering factors can then be further used in the refinement process. Hence, a stereochemical and anisotropic thermal motion parameters refinement following the assignment of aspherical scattering factors can be performed on protein atoms, leading to the deconvolution of thermal motion and valence electron density deformation effects. This allows the computation of dynamical deformation valence electron density, smeared out by thermal vibrations, as routinely performed in small-molecule crystallography. Fig. 1 shows an example of a dynamical deformation electron density map computed in the plane of a well ordered peptide plane of the 0.62 Å structure of an eel pout type III antifreeze protein RD1 (Ko *et al.*, 2003; Protein Data Bank code 1ucs). The bonding electron density features are clearly visible on each covalent bond, as well as electron density peak accumulation corresponding to the carbonyl O atom electron lone pairs.

Such a generalized refinement, which is not necessarily a *charge density* refinement as the aspherical scattering factors can be fixed parameters (as in the above example), should also be generalized to all small molecules and protein refinements in the future. Furthermore, as shown below, this atom transferability allows estimation of electrostatic properties at least as accurately as when usual force fields are used (Muzet *et al.*, 2003).

Another approach, based on high-level theoretical calculations, was later proposed by Koritsanszky *et al.* (2002; SUNY Buffalo database). The aim of their theoretical database is different from ours because the also transferable multipolar parameters will be used to estimate properties such as protein–protein or protein–ligand interaction energies based on theoretical ED reconstruction, while our approach is based on experimental ultrahigh-resolution XRD data. A comparison between our experimental database and the SUNY Buffalo theoretical database is in progress (Pichon-Pesme *et al.*, 2004) and will be published soon. A similar project has been proposed by Dittrich *et al.* (2004).

To summarize, we have at our disposal an experimental database that contains a set of transferable multipolar pseudo-atoms that can be used for protein refinement and electrostatic properties estimation. These transferable pseudo-atoms have their own topology and may therefore be considered as experimental topological atoms as defined by Bader (1990).

Drs A. Podjarny and Virginie Pichon-Pesme are gratefully acknowledged. AL and BF thank the French Ministry of Research for a doctoral fellowship.

References

Bader, R. F. W. (1990). *Atoms in Molecules: A Quantum Theory*. Oxford University Press.
Coppens, P., Guru Row, T. N., Leung, P., Stevens, E. D., Becker, P. J. & Yang, Y. W. (1979). *Acta Cryst.* A**35**, 63–72.
Dittrich, B., Koritsanszky, T. & Lüger, P. (2004). *Angew. Chem. Int. Ed.* **43**, 2718–2721.
Guillot, B., Viry, L., Guillot, R., Lecomte, C. & Jelsch, C. (2001). *J. Appl. Cryst.* **34**, 214–223.
Hansen, N. K. & Coppens, P. (1978). *Acta Cryst.* A**34**, 909–921.
Housset, D., Benabicha, F., Pichon-Pesme, V., Jelsch, C., Maierhofer, A., David, S., Fontecilla-Camps, J. C. & Lecomte, C. (2000). *Acta Cryst.* D**56**, 151–160.
Jelsch, C., Guillot, B., Lagoutte, A. & Lecomte, C. (2005). *J. Appl. Cryst.* **38**, 38–54.
Jelsch, C., Pichon-Pesme, V., Lecomte, C. & Aubry, A. (1998). *Acta Cryst.* D**54**, 1306–1318.
Jelsch, C., Teeter, M. M., Lamzin, V., Pichon-Pesme, V., Blessing, R. H. & Lecomte, C. (2000). *Proc. Natl Acad. Sci. USA*, **97**, 3171–3176.
Ko, T. P., Robinson, H., Gao, Y. G., Cheng, C. H., DeVries, A. L. & Wang, A. H. (2003). *Biophys. J.* **84**, 1228–1237.
Koritsanszky, T., Volkov, A. & Coppens, P. (2002). *Acta Cryst.* A**58**, 464–472.
Muzet, N., Guillot, B., Jelsch, C., Howard, E. & Lecomte, C. (2003). *Proc. Natl Acad. Sci. USA*, **100**, 8742–8747.
Pichon-Pesme, V., Jelsch, C., Guillot, B. & Lecomte, C. (2004). *Acta Cryst.* A**60**, 204–208.
Pichon-Pesme, V., Lecomte, C. & Lachekar, H. (1995). *J. Phys. Chem.* **99**, 6242–6250.
Zarychta, B., Pichon-Pesme, V., Guillot, B., Lecomte, C. & Jelsch, C. (2007). *Acta Cryst.* A**63**, 108–125.

electronic reprint