Charge density study on cyclosporine A

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Cyclosporine A is a cyclic undecapeptide with seven N-methylated amino acids. It is an antifungal antibiotic from *Tolypocladium inflatum* [1]. The compound is widely used as an immunosuppressant drug in transplantation surgery. It blocks intracellular signal-transduction in T-lymphocytes [2].

The electron density study using X-ray diffraction experiments on cyclosporine A ($C_{62}H_{111}N_{11}O_{12}$) has been done in order to explore the currently available experimental possibilities and analysis methods for complex organic structures. A thorough evaluation of this method revealed the need for the development of an oblique incidence correction for intensity data from high resolution crystal structure analysis intensity datasets collected using area detectors [3].

For the electron density study on cyclosporine A, X-ray diffraction experiments with single crystals have been carried out at low temperatures (T = 5 K and T = 90 K) at the Swiss Light Source (SLS) (d_{min} = 0.55 Å and d_{min} = 0.6 Å) [4]. Very accurate datasets have been attained ($R_{mrgd}(F)$ = 0.008 resp. $R_{mrgd}(F)$ = 0.0011). The oblique incidence correction was applied to these datasets. This correction is mandatory for high resolution X-ray diffraction experiments using area detectors.

In order to take into account the aspherical density deformation due to chemical bonding or

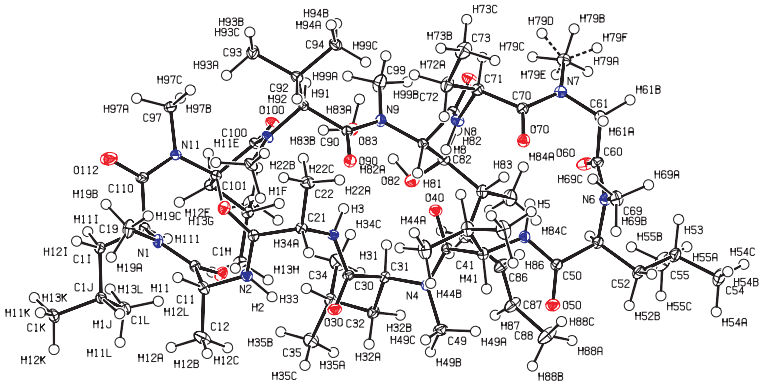

Figure 1: `ORTEP`-presentation (50 % probability, after multipole refinement of cyclosporine A, measured at T = 5 K.

charge transfer, the structure of cyclosporine A has been refined using the multipole model by Hansen and Coppens [5] (R(F) = 0.0246 resp. R(F) = 0.0193) (see Fig. 1). The atomic displacement parameters of those atoms with an inharmonic potential have been modelled using the Gram-Charlier-expansion up to the third order. Detailed electron density studies and resulting physical and chemical characteristics, such as the topology of the electron density (see Fig. 2), the electrostatic potential (see Fig. 3), and the atomic volumes and charges have been quantified.

The results are in very good agreement with the expectations of chemistry and studies from the literature. Both datasets, which were measured at different temperatures, have been evaluated and differ only in the achieved resolution and in atomic displacement parameters.

It is well known that a spherical atom model for structure refinement is not sufficient to model the real electron density distribution in presence of e.g. bonding effects. However, due to the large number of parameters and limited number of reflections, a multipole refinement is not always feasible for larger structures. Databases with calculated multipole parameters enable the refinement for more complex molecules at lower resolution ($d_{min} \sim 1$ Å). The quality of the approximation using tabulated multipole populations from two databases (Invariom [6] and UBDB [7]) have been compared with a full multipole refinement for cyclosporine A. The entries of both databases are

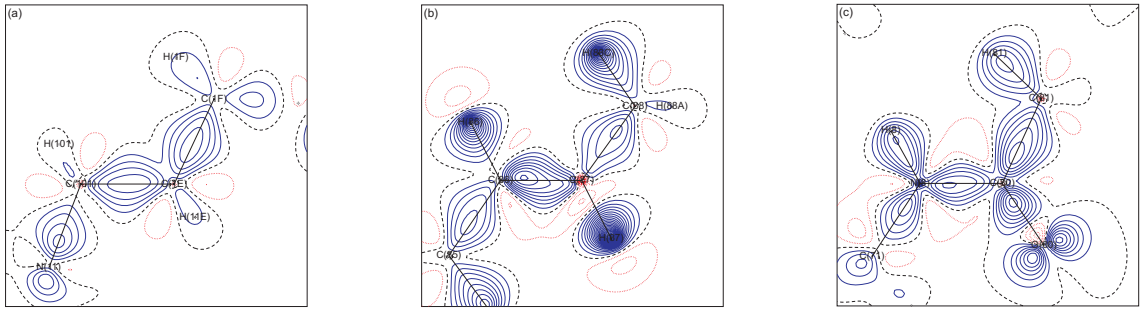

Figure 2: Deformation electron density maps, for different types of chemical bonding (a) C–C–C, (b) C=C–C, (c) N–C–C of cyclosporine A (measured at T = 5 K). The dashed lines represent the zero line, the solid line positive contours and the grey line negative contours (plot contours 0.1 $e\mathring{A}^{-3}$).

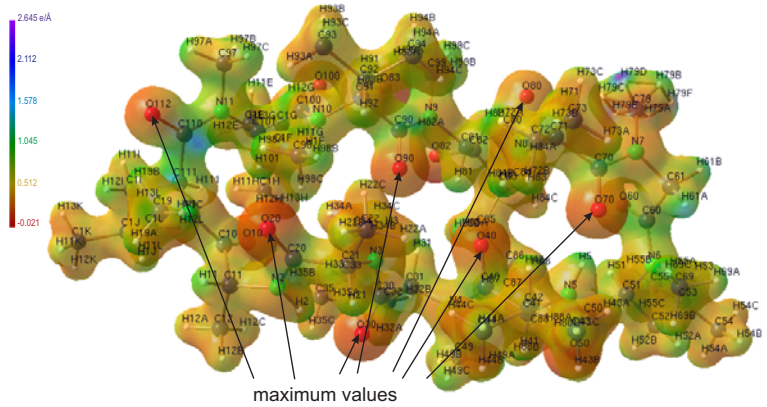

Figure 3: Electrostatic potential of cyclosporine A, mapped on a 0.5 $e\mathring{A}^{-3}$ isosurface of the electron density using `MolIso` [8]. Legend is shown in units of [$e\mathring{A}^{-1}$].

comparable in quality. Disordered atoms, which are found more frequently in more complex structures, can be modeled by the tabulated multipole parameters in these databases, but the accuracy of their topological analysis is only limited.
The electron density study on cyclosporine A shows that even a molecule with almost 200 atoms in the asymmetric unit can be analyzed using high quality and high resolution datasets. Considering the actual technical conditions, the feasibility of such analyses of larger molecules is confirmed. Regarding the growing interest in high resolution protein structures, this work provides a basis for future electron density studies on larger structures.

References

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