

Advances in the Structure Analysis from Electron Diffraction Data: Determination of Absolute Structure of Chiral Molecules

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Structure determination by electron diffraction has undergone rapid development in the recent years. It has transformed from a niche technique to a mature method applicable to all types of crystal structures, ranging from intermetallics and metal oxides through minerals, inorganic framework materials and metal-organic frameworks to crystals of organic molecules and even proteins. The experiments have been made faster thanks to the use of automatic procedures and thanks to the advent of new, essentially noise-free direct detection cameras.

One of the problems that limit the applicability of the method is the radiation damage of beam-sensitive materials. Radiation damage inflicted to a crystal during electron diffraction experiment is severely limiting the range of materials whose atomic structure may be solved. In extreme cases only a single diffraction pattern may be collected on one part of the crystal before it is damaged to the extent prohibiting the collection of further diffraction data.

We developed a workflow that allowed us to determine the structures of several organic pharmaceutical materials *ab initio*, including in some cases the determination of positions of hydrogen atoms and determination of the absolute structure of chiral crystals. The method comprises the following steps:

- Collection of diffraction data by means of electron diffraction tomography combined with precession electron diffraction technique. When the crystal is too beam-sensitive to allow collecting a complete data set on one spot, a small beam is sequentially moved on the crystal to collect each frame on a fresh place.
- Determination of approximate unit cell parameters and orientation matrix from the collected data.
- Refinement of the orientation of each frame. This step is necessary especially if the measured crystal is deformed and different diffraction patterns come from different regions of the crystal. An accurate frame orientation algorithm as developed that allows the refinement of the orientation with typical precision better than 0.1° .
- Extraction of integrated intensities by fitting the measured intensities of each reflection on consecutive frames to the expected rocking curve profile calculated from the known precession angle. This approach allows an accurate extraction of intensities even from sparsely and unevenly sampled diffraction space.
- Solution of the structure by established software for the solution of the crystallographic phase problem.
- Extraction of the reflection intensities on individual frames for the purpose of accurate structure refinement by the dynamical refinement method.
- Dynamical refinement of the structure. The dynamical refinement takes into account the multiple scattering effects which are always present in the electron diffraction data and allows the extraction of more accurate structural parameters.
- Localization of hydrogen atom positions by means of a difference Fourier map, if applicable.
- Determination of absolute structure by comparing the fit to the experimental data of both

enantiomorphic structures in case of non-centrosymmetric structures. The determination of absolute structure is only possible if dynamical diffraction effects are taken into account in the refinement. Thanks to the sensitivity of dynamical diffraction effects to the absolute structure, its determination becomes straightforward and robust even for organic materials.

The application of this workflow is demonstrated on the successful solution of the structure of a co-crystal of sofosbuvir and proline, a prodrug of an important antiviral agent. This material is extremely beam sensitive. A dose of $0.07 \text{ e}^- \cdot \text{\AA}^{-2}$ was necessary to obtain a single good-quality diffraction pattern, but most crystals lost crystallinity already after the dose of $<0.1 \text{ e}^- \cdot \text{\AA}^{-2}$ and the most stable crystals withstood only about $0.21 \text{ e}^- \cdot \text{\AA}^{-2}$. The collection of a more complete diffraction data set was facilitated by the fact that the crystals form thin ($\sim 100 \text{ nm}$) and narrow ($\sim 700 \text{ nm}$) ribbons, which are, however, very long ($>10 \mu\text{m}$). Diffraction patterns could thus be collected at different goniometer tilts on a single crystal by sequential moving of the position of the incident beam along the crystal (Fig.1). However, the crystals were bent and twisted ($\sim 0.5^\circ \cdot \mu\text{m}^{-1}$), and the orientation of each frame had to be refined individually for each frame.

The data were measured using the precession electron diffraction technique, with the precession angle 0.65° . Several dozens of crystal were measured. The data were processed with the computer program PETS [1] which allows the refinement of frame orientation and fitting the reflection rocking curves. Datasets from four crystals with the best data quality and the largest coverage were merged (110 frames, completeness 84%, $R_{\text{int}}(\text{obs}/\text{all}) = 20.79/22.76\%$) and the crystal structure was solved ab initio in the space group $P2_12_12_1$ (44 independent atoms, volume of the unit cell $\sim 3100 \text{ \AA}^3$) by the program Sir2011 [2]. Dynamical structure refinement was performed in the program Jana2006 [3] converged to the final $R(\text{obs})$ of 9.86%. The refinement of the inverted structure resulted in R -value $R(\text{obs})$ of 12.43%, *i.e.* 2.6 percent points higher. This difference in R -values was consistently observed for all four refined crystals. Thus, the correct chirality of the molecules constituting the crystal was unambiguously determined [4].

References:

[1] L Palatinus et al., submitted.

[2] M C Burla et al., *J. Appl. Cryst.* **45** (2012), p. 357.

[3] V Petricek, M. Dusek and L. Palatinus, *Z. Krist.* **229** (2014), p. 345.

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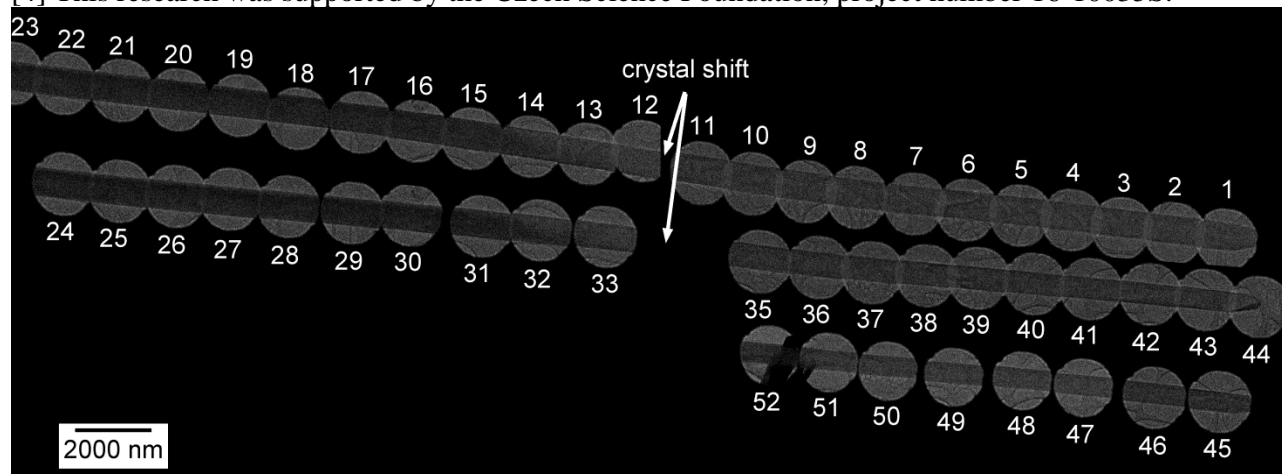


Figure 1. Course of measurement of co-crystal of sofosbuvir and proline showing the shifting of the beam along the long axis of the crystal. The two positions where the crystal was shifted by the stage movement are indicated by arrows.