Towards diffract-and-destroy electron crystallography: ab-initio structure determination of the pharmaceutical co-crystal Sofosbuvir-L-proline from single nanocrystals

Brazda, P.[1], Palatinus, L.[1] and Babor, M.[2,3]

[1] Institute of Physics of the Czech Academy of Sciences, Na Slovance 2, 18200 Prague 8, Czech Republic, Czech Republic, [2] University of Chemistry & Technology, Technicka 3, 16628 Prague 6, Czech Republic, Czech Republic, [3] Zentiva, U Kabelovny 130, 10237 Prague 10, Czech Republic, Czech Republic

Radiation damage inflicted to a crystal during electron diffraction experiment is severely limiting the range of materials whose atomic structure may be solved. In analogy with serial x-ray crystallography[1], a method of serial electron crystallography appears to be a possible solution for the radiation damage problem. In this method a series of diffraction patterns from different crystals is collected and merged into a single data set. Merging of frames from serial electron crystallography experiment is complicated by the presence of dynamical effects, which alter the kinematical intensities. A method circumventing the problem of frame orientation dependent intensities was proposed by Smeets and Wan and it is based on ranking the reflections using their intensities. The potential of the method was shown on simulated data. Using few hundreds of randomly oriented frames, the ranking-merged data of zeolite structures were suitable for structure solution.[2]

We performed an electron diffraction experiment on a co-crystal of sofosbuvir and proline, a prodrug of important antiviral agent, and faced a problem of extreme beam sensitivity. A dose of 0.07 $e^-.Å^{-2}$ was necessary to obtain a single good-quality diffraction pattern, but most crystals lost crystallinity already after the dose of <0.1 $e^-.Å^{-2}$ and the most stable crystals withstood only about 0.21 $e^-.Å^{-2}$. Therefore, an approach of serial electron crystallography appeared necessary to solve its structure.

In the case of sofosbuvir proline the approach was facilitated by the fact that the crystals form thin (<100 nm) and narrow (~1 μm) ribbons, which are, however, very long (>10 μm) (Fig. 1). Individual snapshots could be thus taken from only a handful of crystals at different goniometer tilts. However, the crystals were bent and twisted (~0.5°.μm^{-1}), and the orientation of the frames had to be refined individually for each frame.

The data were measured using the precession electron diffraction technique, with the precession angle 0.65°. The key ingredient to the successful structure determination was the merging of intensities. Our method relies on a precise determination of frame orientation, with estimated standard deviation about 0.1° (Fig. 2). The orientation and reflection intensities are used for reconstruction of an average rocking curve as a function of diffraction vector **g** (Fig. 3), which is then used for frame scaling and for intensity extraction by rocking curve fitting. Several tens of ribbon-shaped crystals were measured. Finally, datasets from four crystals with the best data quality and the largest coverage were merged (110 frames, completeness 84%, Rint(obs/all) = 20.79/22.76%) and the crystal structure was solved *ab-initio* in the space group $P2_12_12_1$ (44 independent atoms, unit cell volume ~3100 $Å^3$) by the program Sir 2011. Dynamical structure refinement converged to R(obs/all)=10.45/16.78% and identified correctly the absolute structure.

References

1 H.N. Chapman et al., Nature 470 (2011) 73 - 77.

2 S. Smeets and W. Wan, J. Appl. Cryst. 50 (2017) 885 - 892.

Acknowledgement

The authors acknowledge the financial support of the Czech Science Foundation, project no. 16-10035S.

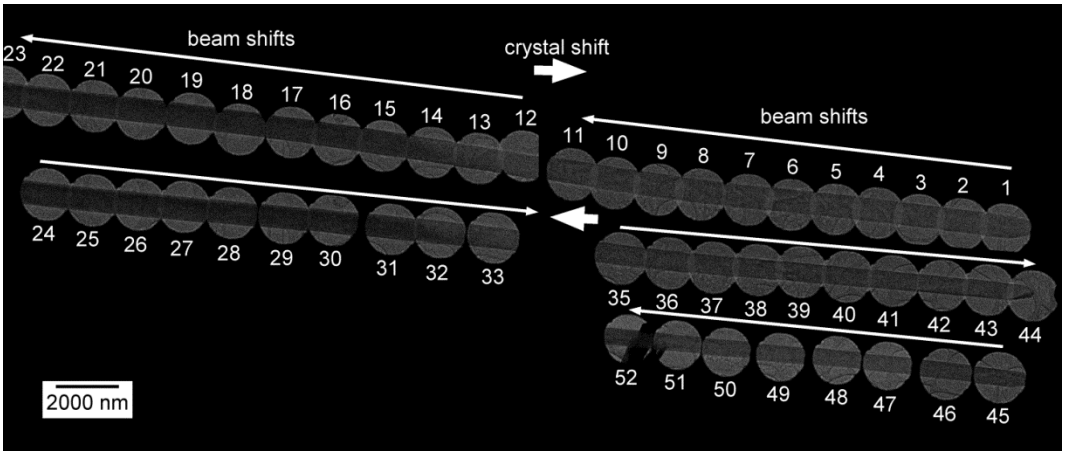

Figure 1 Course of measurement of co-crystal of sofosbuvir and proline (total length of 33 μm). Measured spots are numbered consecutively. The position of the beam was changed by beam shifting. After reaching the edge of field of view the crystal was shifted (between frames 11 and 12 and then between 34 and 35). There are two turning points - frames 23 and 44. The goniometer was rotated by 1° after each measurement.

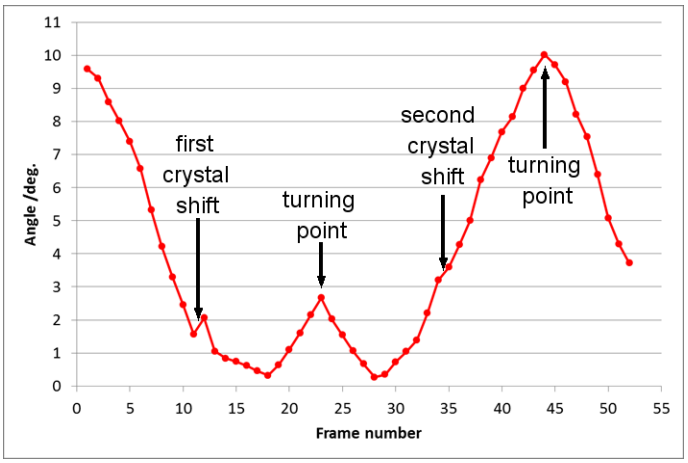

Figure 2 Absolute rotation angle between reference crystal coordinate system and actual crystal coordinate system at measured spot (frame numbers correspond to Figure 1). There are two turning points corresponding to frames 23 and 44. The crystal was scanned by beam shifts and two crystal shifts (between frames 11 and 12 and between frames 34 and 35). The first crystal shift induced a lash of the stage.

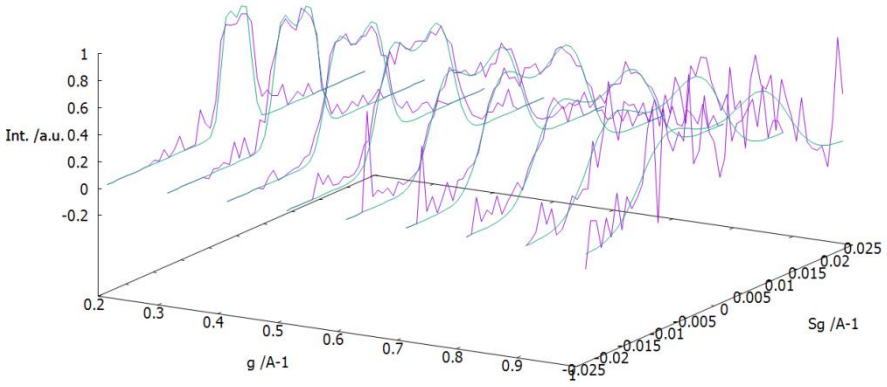

Figure 3 Average rocking curve (purple) and fitted rocking curve (green) of sofosbuvir proline co-crystal.