Oral presentation

Approaches to quantum chemistry using microcrystals: electron and XFEL crystallography

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Since the 2010s, three-dimensional electron diffraction (*3D ED*) has been developed for the structure determination of micro- to nano-meter sized crystals. During the same period, synchrotron X-rays have also used to determine the structure of undersized crystals. Serial femtosecond X-ray crystallography, *SX* or *SFX*, with pulses generated by free electron lasers (FELs), is a particularly effective method for macromolecular crystals. While the resolution of *SX* has not previously high enough to reveal sub-atomic structures, some researchers have recently applied *SX* techniques to small molecular crystals [1-3]. Therefore, it is expected that the resolution will inherently exceed atomic resolution (≤ 1.2 Å).

In order to proceed with crystallographic assessment toward quantum chemistry, the higher resolution of the structure is essential. Sufficiently large (≥ 100 μm) crystals need to be prepared for conventional X-ray diffraction measurement. When neutron diffraction is used to determine nucleus positions, even larger crystals are required. However, growing crystals is not always possible, and X-ray irradiation can sometimes cause structure degradation, especially for organic crystals. These factors are the challenges for quantum crystallography. Therefore, it is anticipated that *3D ED*, *SX* and/or a combination of these diffraction methods will expand the opportunity to access sub-atomic information using microcrystals.

We have improved *3D ED* and *SX* applications to various types of targets, using a JEOL electron microscope and at SACLA, the XFEL facility in Japan [2, 4, 5]. Through these developments, we have been able to evaluate the advantages and disadvantages of these methods depending on characteristics of the crystals. In the case of a model sample, rhodamine-6g, we were able to collect both *3D ED* and *SX* data at resolutions of 0.90 and 0.83 Å, respectively. According to the respective scattering origins of electrons and X-rays, we could differentiate how these beams visualize hydrogen atoms [Fig. 1]. Based on the revealed densities we were also able to evaluate atomic charges and valence electrons, respectively. In this presentation, we will introduce the latest updates on our experimental and analytical processes, and discuss the current limitations and prospects for quantum crystallography with microcrystals.

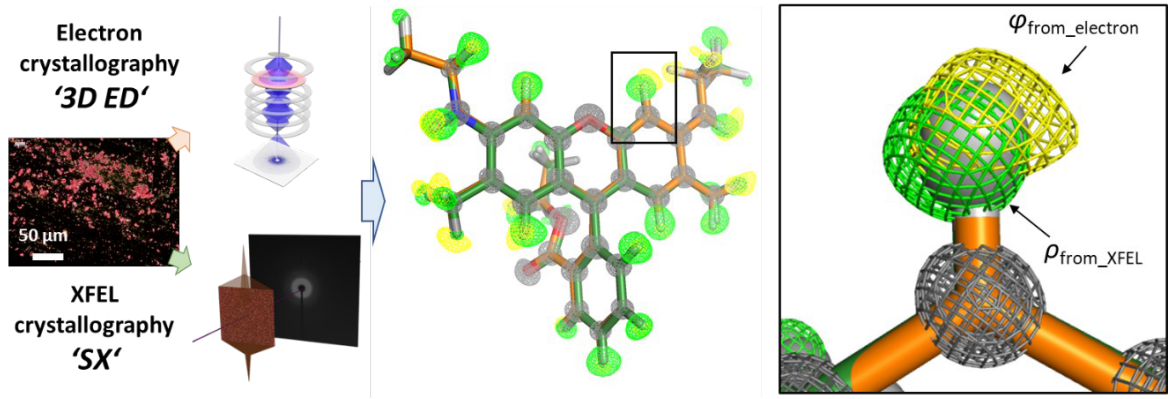

Figure 1. Scheme and visualization of *3D ED* and *SX*. The structures and maps of rhodamine-6g are shown. The Coulomb potential (φ, yellow) and electron density (ρ, green) maps for hydrogen atoms were obtained from *3D ED* and *SX*, respectively [2].

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[3] Støckler, L. J. *et al.* (2023). *IUCrJ,* **10**, 103–117.

[4] Takaba, K., Maki-Yonekura, S. & Yonekura, K. (2020). *J. Struct. Biol.*, **211**, 107549.

[5] Takaba, K. & Maki-Yonekura, S. *et al.* (2024). *J. Am. Chem. Soc.*, **146**, 5872–5882.

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