High-throughput and high-resolution 3D ED structure analysis through AI-based data collection and hybrid approach.

S. Maki-Yonekura1, K. Takaba1, K. Kawakami1, K. Yonekura1,2

RIKEN, SPring-8 center, Sayo, Hyogo 679-5148, Japan, Tohoku University, IMRAM, Sendai 980-8577, Japan
makis@spring8.or.jp

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Electron crystallography, one of the main methods of cryo-EM, is used to determine the detailed structure of samples from their tiny three-dimensional crystals. Since structures with spatial resolution beyond 1 Å can be obtained from various samples in aqueous solution, organic solvents, and powders, this technique called 3D ED or microED is being used not only in the life sciences but also in drug discovery, material sciences, and a wide range of other fields. We have been involved in the development of this technology, and it is now possible to complete the automatic processing and high-throughput structure determination [1] from large numbers of diffraction data collected by AI control of the electron microscope operation [2]. Our recent results include a new double helix structure built by self-assembly of nanographene [3], thin crystals of an organic semiconductor [4], and fibrous crystals of polypeptides related to a neurological disease, amyotrophic lateral sclerosis (ALS) [5]. However, 3D ED has limitations in rotation angles and/or sample thickness, which sometimes hamper structure determination by this technique alone. We have overcome these difficulties by introducing serial XFEL crystallography [6]. XFEL had been used for protein crystals so far, as the correct indexing of diffraction spots needs many diffraction spots per frame. We have shown that the XFEL patterns from small organic compounds are able to be efficiently processed with lattice parameters obtained by 3D ED. This strategy has worked well for various samples including novel compounds for drug discovery and organic semiconductors.

Our cryo-EM system also performs well for high-resolution single-particle cryo-EM, which allowed us to obtain signals of most hydrogen atoms and charges in a test protein [7]. Signals from hydrogen atoms are weak both with X-rays and electrons, but their properties can now be studied by 3D ED, XFEL crystallography, and single-particle cryo-EM. In this microsymposium, I will present and compare our recent results obtained by these methods. Topics also cover advanced applications including AI data collection, dynamical refinement, hydrogen properties, and charge analysis.

Figure 1. AI-based cryo-ED data collection using the yoneoLocr program suite.
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