

## Cryogenic Electron Microscope Facility at KEK-SBRC

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The Structural Biology Research Center (SBRC) of the High Energy Accelerator Research Organization (KEK) has been operating MX and bioSAXS beamlines at the Photon Factory for more than 30 years, playing an important role in the structural biology community in Japan. In 2018, in response to the growing importance of cryo-microscopy (cryo-EM) in structural biology, a 200 kV cryo-EM was installed at the KEK-SBRC and applied to a wide range of investigations. In 2022, a new cryo-EM laboratory was built and a 300kV Titan Krios G4 was installed. Our cryo-EM laboratory is a shared facility and provides more than 200 days of machine time per year to external researchers, resulting in about 20 publications in the last three years. The mission of the cryo-EM team at KEK-SBRC is to provide machine time and user support, conduct educational activities, and perform R&D for cryo-EM analysis in order to facilitate the use of cryo-EM in the field of life sciences. In this presentation, we will provide an overview of our activities.

Machine time, user support, and education: Our user support is comprehensive and ranges from grid preparation to single particle analysis (SPA) calculations. We support grid preparation, grid screening, data acquisition, and SPA calculations for structure determination. We also provide consultation on sample preparation. After SPA experiments, we provide users with feedback on the results to address issues and prepare for the next scheduled machine time. In addition, since the SBRC operates beamlines for PX and bioSAXS at the Photon Factory, it is possible to offer structural analysis with cryo-EM as a hybrid analysis. Recently, we have started micro-ED analysis for small molecules, and our micro-ED consultations have been gradually increasing. As an educational activity, the SBRC regularly holds various seminars, including workshops on cryo-EM operation and single-particle analysis. To date, more than 200 researchers have attended these workshops.

Advanced study sessions are also held.

R&D for cryo-EM analysis: R&D for cryo-EM analysis is another important mission of the SBRC. We have developed methods/machines for grid freezing, elimination of preferred orientation, and establishment of an efficient SPA analysis environment. To expand the capabilities of the cryo-EM, we have developed a compact grid-freezing machine that can freeze grids under various conditions, including anaerobic conditions. We have successfully prepared a grid of reduced forms of the ferredoxin reductase BphA4 in an anaerobic chamber. The SBRC has also been involved in the development of a method to avoid the preferred orientation problem in grid preparation. We have designed an amphiphilic peptide that can influence the orientation of protein molecules in the grid. Application of the amphiphilic peptide has improved the preferred orientation in some cases. Another important R&D effort at the SBRC is the construction of an efficient SPA environment using a public cloud. First, we developed an on-the-fly system called GoToFly using Relion. The GoToFly system can sequentially process micrographs to generate the 2D class average of particles during the grid screening. The prepared 2D class average helps to determine the data acquisition parameters and is useful to evaluate the quality of a grid without visual inspection of the acquired micrographs. The GoToFly can also estimate the optimal mask diameter required for SPA analysis of the acquired data. In addition, we build an efficient SPA environment using the public cloud of AWS. Since SPA calculation is a time-consuming process, the high-performance SPA environment would be helpful not only for basic research but also for industrial research, including pharmaceutical science research. We plan to implement automated SPA analysis for SBDD using the public cloud.

In summary, KEK-SBRC is striving to expand the number of cryo-EM users and achieve more results for technology development.