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Commentary and Perspective

Technical development and sharing of high-resolution cryo-electron microscopes

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The BINDS program, Basis for Supporting Innovative Drug Discovery and Life Science Research (https://www.binds.jp/en/), has been running since 2017 for five years to promote drug discovery in academia at pre-clinical phase and basic life science research by the AMED, Japan Agency for Medical Research and Development. Total 59 research groups were selected in diverse fields, such as Pharmaceutics, Medicine, Chemistry, Genomics, Structural Biology, Informatics, and Computer Science, from all over Japan with a total basic budget of about 3 billion yen every year. The BINDS program has a characteristic feature to support researchers even outside of the program for drug discovery and general life science. So far, about 3,000 research projects have been supported.

In particular, the BINDS program strongly promotes technical development and sharing cryo-electron microscopes (cryo-EMs) to solve high-resolution structures of proteins and their complexes. Since 2017, high-end cryo-EMs have been installed with equipment grants by the BINDS project. In addition, eight more cryo-EMs are being installed in 2021 at several laboratories in Japan. To promote cryo-EM usage among many researchers, the BINDS program created the cryo-EM network (https://www.cryoemnet.org/), so that most of the cryo-EM machines in Japan are shared by users with the well-organized schedule as much as possible. These new shared cryo-EM facilities enable higher-resolution and higher-throughput structural analysis, together with the recent technological progress, including the development of new grids and methods for online remote cryo-EM operation. At the 59th Annual Meeting of the Biophysical Society of Japan in November 2021, six speakers are invited to share the recent results of single particle analysis, tomography, and micro-ED by cryo-EMs, and to discuss the issues to be overcome by technical development. This symposium is cosponsored by the AMED-BINDS.

Dr. Masahide Kikkawa from the University of Tokyo reviews the studies by cryo-EMs at the University of Tokyo for structural analysis to researchers and companies throughout Japan. At present, nearly 50 projects are being supported by them, and six companies are also using the facility. In their facility, they use three cryo-EM methods to perform cross-scale observation ranging from atoms to cellular structures. Single particle analysis is used to observe biological molecules, microED is for atomic resolution structures of tiny crystals [1, 2], and cryo-electron tomography is used for cellular structures. In the symposium, he shows the recent results using the three methods and discusses what is necessary to utilize the cryo-EM shared facility further.

Dr. Keiichi Namba group from Osaka Univ. and RIKEN developed a cryo-TEM named “CRYO ARM” with JEOL over the last decade. The groups determined the structure of apoferritin at 1.53Å from about 900 images collected in one day in 2019 [3]. Since then, they developed a new TEM control software, multi-hole imaging with a GATAN K3 camera, which now allows collecting 22,000 images in one day and determining the apoferritin structure at 1.29 Å from images collected over 15 hours. In addition, they developed an epoxidized graphene grid to solve denaturation and preferred orientation problems at the air-water interface. As a result, the GroEL structure was solved at 1.99Å from just 500 images

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collected in 1 hour.

Dr. Toshiya Senda from KEK reviews the cryo-EM network with the support of AMED-BINDS. The cryo-EM network is led by Dr. Senda to accelerate the shared use of cryo-EMs in Japan. In addition, he has organized an academia-industry collaboration in the non-competitive area of pharmaceutical research [4]. In the fiscal year of 2021, cryo-EM machines that pharmaceutical companies can use are being installed at the TARA center of Tsukuba University, and he presents the current status and future direction of the collaborations.

Dr. Katsumi Maenaka from Hokkaido Univ. introduces the activities of CRED (Center for Research and Education on Drug Discovery) of Hokkaido University, which has been established for an integrated drug discovery system. Concerning structural analyses, they started a collaboration to use cryo-EMs with Univ. of Oxford in 2013. From 2017 to 2019, they introduced the 200 kV cryo-EMs, Glacios, and FIB/SEM Aquilos. After 2020, they have introduced two 300 kV cryo-EMs, Krios G4, at BSL2 and BSL3 facilities with the support of AMED-BINDS. They are conducting single particle analysis, cryo-electron tomography for viruses, cells, and tissues, and MicroED for organic compounds to develop therapeutics and vaccines against COVID-19 [5-7].

Dr. Seizo Koshiba from Tohoku Univ. talks about a new 300 kV cryo-EM system (CRYO ARM 300 II, JEOL), which was recently installed at Advanced Research Center for Innovations in Next-Generation Medicine (INGEM) in Tohoku University. Now, many high-quality data have already been obtained. Their cryo-EM system cooperates with the supercomputer system of Tohoku Medical Megabank Organization, where cryo-EM data are directly transferred to the storage system of the supercomputer, and the users can use the powerful computer resources including GPUs for the image analysis. They open their cryo-EM system to the public in the autumn of 2021 so that many institutes and companies widely use their system.

Dr. Kouta Mayanagi from Kyushu Univ. talks about the two high-end cryo-EMs (200 kV and 300 kV) to be set up in the fiscal year of 2021 at the Pharmaceutical Research Institute, Kyushu University, which is the Library Screening Section organized by Dr. Shigehiro Ohdo. Using these two new cryo-EMs together with the cryo-EM Polara at Medical Institute of Bioregulation, Kyushu Univ. will promote research support for researchers in academia and pharmaceutical companies in the areas of Kyushu and West Japan. Taking advantage of the characteristics of the above Library Screening Section, they aim to focus on research support for drug discovery research, such as structural analysis using libraries of chemical compounds. In addition, they also plan to utilize the supercomputer ITO (https://www.cc.kyushu-u.ac.jp/scp/system/ITO/) to build a standard single particle analysis environment, easy and convenient for beginners.

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