Public Use Cryoem at Spring-8

Ph.D. Hideki \ Shigematsu[1], Ph.D. Christoph Gerle[2], Ph.D. Chai Gopalasingam[2]
[1]Japan Synchrotron Radiation Research Institute, RIKEN SPring-8 Center, [2]RIKEN SPring-8 Center
shigematsu@spring8.or.jp

Since October 2021, we have started public use of CryoTEM as an ancillary facility for structural biology beamlines at SPring-8. We have set up a facility with two CryoTEMs, EM01CT for high-resolution data collection and EM02CT for screening, user training, and general purpose. EM01CT produces high-resolution data in a high-throughput manner for single particle analysis by using a CRYO ARM 300 (JEM-Z300FSC, JEOL) which has a cold-field emission gun, an in-column energy filter, a cryo-supporter system and is coupled with a K3 camera (Gatan). EM02CT is a CRYO ARM 200 (JEM-Z200FSC, JEOL) equipped with a K2 summit camera (Gatan). We provide a training course for all new users of the facility to enable them to have an easy start with structural analysis projects by using our CryoTEMs and continue to provide advice throughout their projects to provide the best possible environment for the successful completion of projects.
We have been able to continuously provide productive machine time to the users of SPring- 8 resulting in many high-resolution solution structures in the range of 2 ~ 2.5 Å. The first paper was published by one of our users in June 2022[1]. The authors tried to obtain structures of the gastric proton pump with its known inhibitors to understand its inhibitory mechanism by using X-ray crystallography. In this paper, they succeeded in obtaining the crystal structures with three compounds but could not obtain good crystals with another compound. It was crucial to obtain structures with compounds to compare the interactions between compounds and the protein, therefore they switched to using CryoEM for the complex with the other compound, for which it was difficult to obtain good crystals. This is exactly the situation in which we set up our CryoTEMs as an ancillary facility for structural biology beamlines. In addition to the CryoTEMs, we have been working to establish a facility for compound screening for drug discovery. Since our structural biology beamlines are equipped with an automated data collection system, "ZOO"[2], we plan to utilize its high- throughput data collection capability for compound screening together with the ancillary facility for soaking or co-crystallization equipment. Data collection for the single particle CryoEM is not yet fully automated and the throughput is not high enough, but we would like to offer our CryoTEMs as an option for those who are struggling a lot to obtain good enough crystals. In this presentation, we would like to show our activities in the CryoEM facility at SPring-8 and some of our recent results.

References

{1} Tanaka, S., Morita, M., Yamagishi, T., Madapally, H. V. Hayashida, K., Khandelia, H., Gerle, C., Shigematsu, H., Oshima, A. & Abe, K. (2022). J. Med. Chem. 65(11), 7843.
{2} Hirata, K., Yamashita, K., Ueno, G., Kawano, Y., Hasegawa, K., Kumasaka, T. & Yamamoto, M. (2019). Acta Cryst. D75, 138–150.