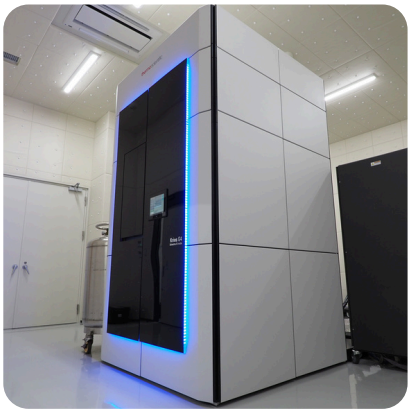
KEK×CRYO-EM
ACTIVITY REPORT

VOL.7 April 2024 - March 2025

KEK IMSS SBRC
Cryo-Electron Microscope

KEK Titan Krios



Instrument Name: Titan Krios G4
Acceleration Voltage: 300 kV
Electron Source: XFEG
Stage: AutoGrid-compatible side-entry stage
Phase Plate: Volta Phase Plate
Energy Filter: Selectris-X
Detector 1: Falcon 4i
(*130k = 0.96 Å/px, 165k = 0.75 Å/px, 215k = 0.58 Å/px, 270k = 0.46 Å/px)
Detector 2: Ceta-D
Software: EPU, EPU-D

KEK Talos Arctica

Instrument Name: Talos Arctica G2
Acceleration Voltage: 200 kV
Electron Source: XFEG
Stage: AutoGrid-compatible side-entry stage
Phase Plate: Volta Phase Plate
Former Detector: Falcon 3EC
(*92k = 1.13 Å/px, 120k = 0.88 Å/px, 150k = 0.69 Å/px)
Detector 1: Falcon 4
(*92k = 1.08 Å/px, 120k = 0.84 Å/px, 150k = 0.66 Å/px)
Detector 2: Ceta 16M
Software: EPU, EPU-D

Grid Freezing Device
Model: Vitrobot Mark IV

Glow Discharge Unit
Model: PIB-10
Current: Soft mode ≈ 7 mA, Hard mode ≈ 11 mA

Operational Focus of the KEK Cryo-EM Facility

The KEK Cryo-Electron Microscopy Facility operates a Thermo Fisher Scientific 300 kV cryo-EM Titan Krios G4 and a 200 kV cryo-EM Talos Arctica G2 (Falcon 4) to fulfill the following three missions:

1. Provide over 200 days of machine time annually to academic and corporate users.
2. Support grid freezing and data acquisition (including single-particle analysis when needed).
3. Facilitate the adoption of cryo-EM experimental techniques.

Weekly Schedule

Monday: Maintenance
Tuesday: 1-day slot (screening & data collection 800-3,000 images)
Wednesday: 1-day slot (screening & data collection 800-3,000 images)
Thursday: 1-day slot (screening & data collection 800-3,000 images)
Friday, Saturday, Sunday:
3-day slot (screening & data collection 3,000-10,000 images)

Daily Schedule

9:30 - Meeting about Grid preparation
9:45 - Start of experiment
Morning - Grid freezing (up to 6 grids)
Afternoon - Screening measurement (approximately 1 hour per grid)
Around 18:00 - Start of overnight/over weekend measurement
End of experiment

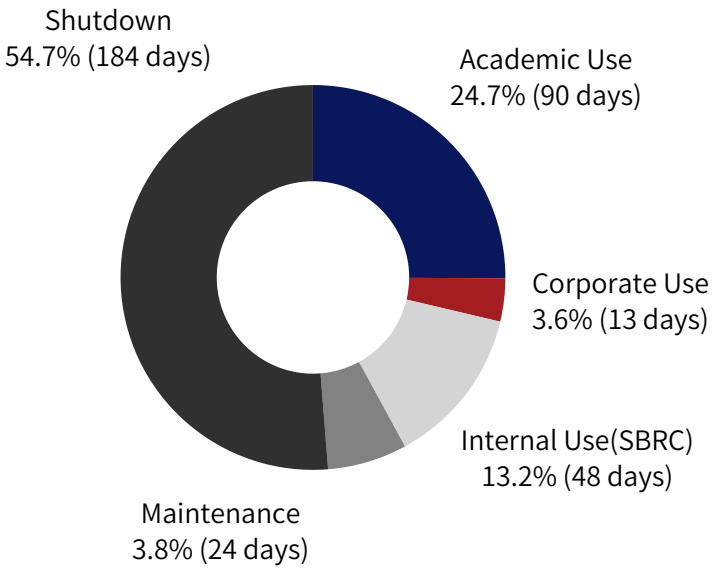
On the next day, KEK staff will collect the data and send it to the client.

What to bring on the day

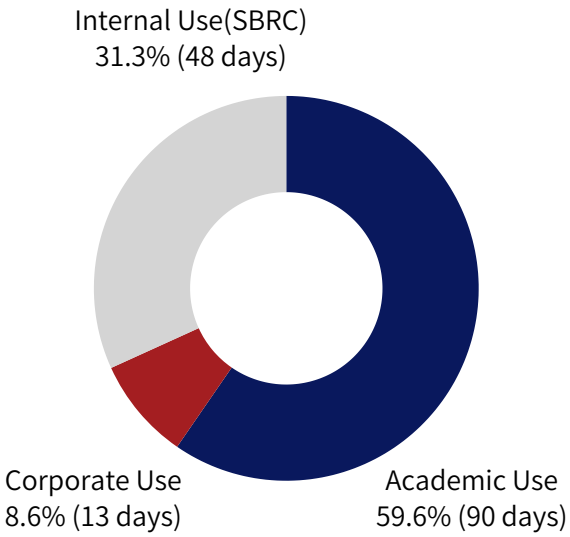
Samples
Dilution buffer
4-8 TB HDD

KEK Cryo-EM Usage Statistics

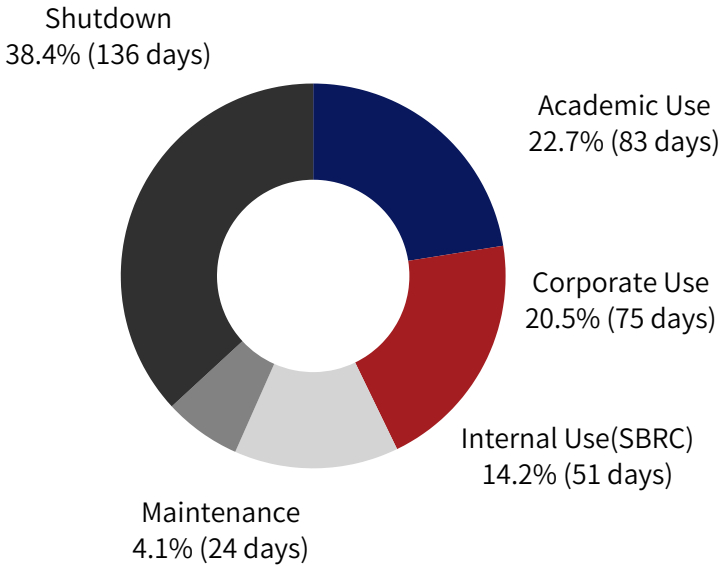
Talos Equipment Usage Status 2024 (365 days)



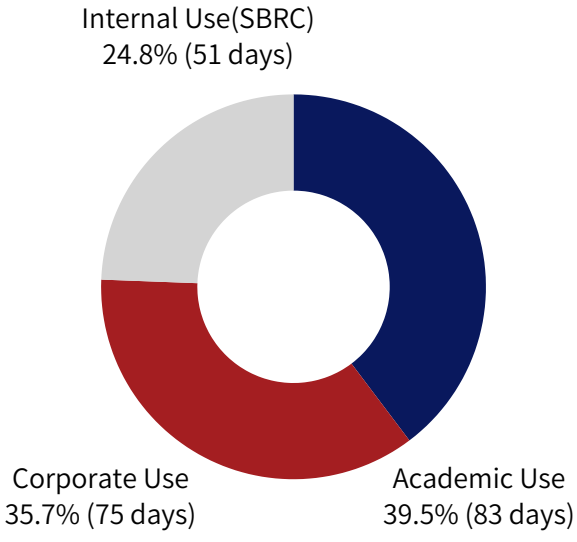
Talos Equipment Usage Status (only operating days) 2024 (151 days)



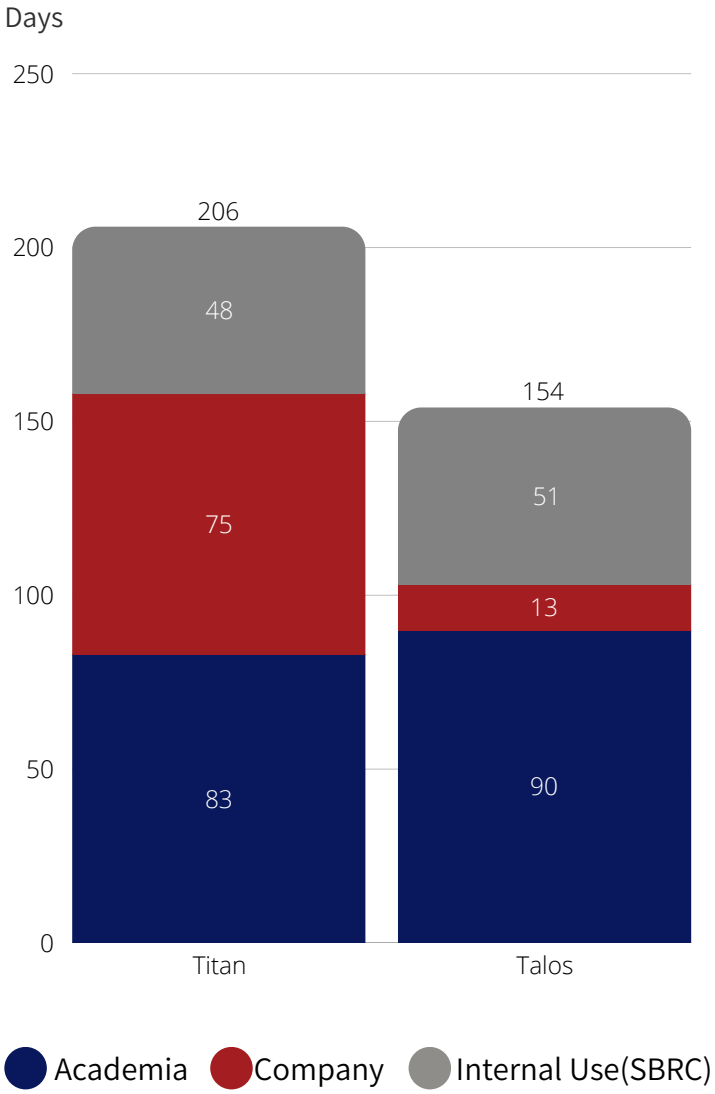
Titan Equipment Usage Status 2024 (365 days)



Titan Equipment Usage Status (only operating days) 2024 (209 days)



Titan and Talos Machine Time Allocation (FY2024)



Note: Figures include data from Vol. 1–6 for annual calculation.
Note: Titan Krios is not included in the FY2022 data as it was under test operation.

Accelerating novel drug design based on Cryo-EM protein structures by cloud computing

Cryogenic electron microscopy (Cryo-EM) single-particle analysis (SPA) is widely adopted as one of the methods for visualizing protein 3D structures at atomic-level resolution. Cryo-EM is a specialized type of transmission electron microscope that allows imaging of rapidly-frozen biological specimens and other soft materials. The samples are kept at liquid-nitrogen temperatures to prevent damage from the vacuum environment and electron radiation. SPA reconstructs 3D structures of proteins by using numerous 2D projection images of protein particles with identical 3D structures, captured from various orientations. This computational method involves detecting the positions and estimating the 3D projection angles of each particle image appearing at a random position and orientation in the measured 2D data for the 3D reconstruction.

Cryo-EM SPA offers unique advantages over other structural determination methods: (1) It can be applied to proteins that are difficult to crystallize, such as large membrane proteins or supramolecular complexes, which are often drug targets but unsuitable for other techniques like X-ray crystallography; (2) It allows the separation of different structural states captured simultaneously during imaging through computational image processing; and (3) It enables high-resolution visualization specifically of substructures directly related to a research interest, such as a drug-binding site. The dynamics information is fundamental data for revealing mechanisms of conformational changes that regulate protein function, and has the potential to become valuable intellectual property, comparable to genomic data, with wide-ranging applications from next-generation life sciences to industrial use.

The Structural Biology Research Center (SBRC) of IMSS, KEK, actively supports Cryo-EM SPA to promote structural biology research. The Cryo-EM at KEK serves as a shared-facility, widely accessible not only to academic researchers but also to users from industry. In addition to data acquisition, the center also provides user support for data analysis.

However, to determine a large number of protein 3D structures at atomic-level resolution within a practical timeframe, the use of large-scale computational environments is essential. Furthermore, with recent advancements in Cryo-EM, vast amounts of data can now be acquired in a short period of time. As a result, not only the data analysis itself but also securing an adequate amount of computational resources has become a bottleneck. To address this issue, we have taken notice of the secure and scalable computational resources offered by cloud computing (Cloud) and have built a Cloud-based platform called “GoToCloud” on Amazon Web Services (AWS) to support advanced data analysis and management associated with Cryo-EM SPA (Fig. 1) [1].

GoToCloud enables users to easily execute each step of Cryo-EM SPA using multiple computers through the power of AWS. As a result, both the facility and users no longer need to maintain their own hardware and can dynamically adjust computing resources as needed. GoToCloud leverages AWS managed services (e.g., Elastic Compute Cloud (EC2), ParallelCluster), enabling effective account management for security and billing, cluster management, and flexible data storage utilization. Additionally, we developed custom shell scripts to automate the complex steps for the construction and setup of the platform, reducing it to just three simple steps.

We selected RELION (Fig. 2) as the main Cryo-EM SPA software package, and built multiple executables for a given version of RELION, each of which is optimal for a different type of hardware, considering whether or not it is equipped with GPUs, to ensure efficient performances of major RELION jobs. We also present benchmark results obtained using GoToCloud, providing guidelines for selecting the most suitable computing resources based on processing time and cost. With the support of collaborators from AWS, we have set up and manage a dedicated AWS account, where we maintain the software packages required for Cryo-EM SPA data processing and make these tools readily available to each user within their own AWS account (Fig. 1). In this way, users can focus solely on data analysis, relieving headaches caused by software maintenance or hardware optimization.

Our study also demonstrates significant reductions in processing time and cost. By tailoring a Cloud environment to meet a specific research objective, the GoToCloud platform can serve as a promising foundation for accelerating structure-based drug design (SBDD) using Cryo-EM SPA. SBDD is a method that utilizes the 3D structural information of protein molecules to rationally design novel drug candidates by allowing researchers to predict molecular interactions and develop more effective and specific drugs. Thus, GoToCloud as a research infrastructure is expected to make a substantial contribution to the medical field as well as the life sciences.

Currently, to enhance the effectiveness of the GoToCloud platform, we are introducing high-speed data transfer technology (Zettar zx Unified Data Mover) at our Cryo-EM facility, enabling rapid data transfer over the internet between our facility and AWS. We also support users in deploying GoToCloud with their own AWS accounts. Since July 2021, we have held multiple hands-on GoToCloud workshops for small groups (1-4 participants per group), targeting external users from both academia and industry. Some of the participants have already been utilizing the GoToCloud platform for over a year, and trial usage as a part of user support has also begun internally at KEK.

Given the ongoing increase in data size and computational demand across various experimental techniques and medical diagnostics, the design philosophy of GoToCloud is likely to be beneficial not only for Cryo-EM, but also for other fields, where researchers and medical professionals are longing for easy access to Cloud-like computational resources and immediate use of related data processing software, without the need for acquiring specialized knowledge in managing such resources.

Toshio Moriya
Project Associate Professor

Structural Biology Research Center,
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High Energy Accelerator Research Organization (KEK)

References

[[1] T. Moriya, Y. Yamada, et al., Commun. Biol., 7(1), 1320 (2024).

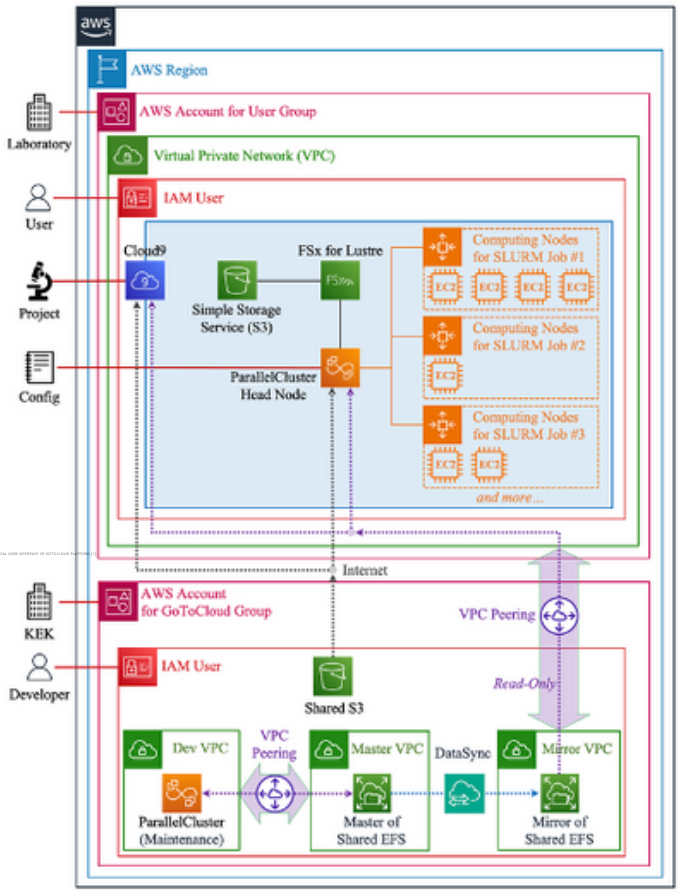


Fig. 1. Design of GoToCloud Platform [1].

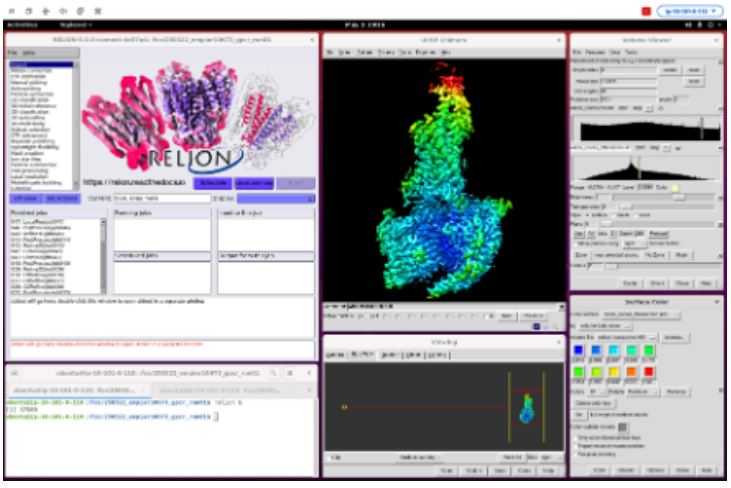


Fig. 2. Running RELION and UCSF Chimera with graphical user interface of GoToCloud platform [1].

Cryo-EM Usage Fees

Titan Krios

Academic

Equipment Use: ¥3,000/hour* (Usage unit: 9 or 24 hours/day)
Measurement and Analysis Assistance/Guidance: Free of charge
（装置利用：3,000円/時間＊（利用単位は9時間/日または24時間/日）
測定解析補助：無料）

Corporate

Equipment Use: ¥15,000/hour (Usage unit: 24 hours/day)
Measurement and Analysis Assistance/Guidance: ¥30,000/hour (Usage unit: 8 hours/day)
（装置利用：15,000円/時間（利用単位は24時間/日）
測定解析補助：30,000円/時間（利用単位は8時間/日））

Talos Arctica

Academic

Equipment Use: ¥2,000/hour* (Usage unit: 9 or 24 hours/day)
Measurement and Analysis Assistance/Guidance: Free
（装置利用：2,000円/時間＊（利用単位は9時間/日または24時間/日）
測定解析補助：無料）

Corporate

Equipment Use: ¥10,000/hour (Usage unit: 24 hours/day)
Measurement and Analysis Assistance/Guidance: ¥30,000/hour (Usage unit: 8 hours/day)
（装置利用：10,000円/時間（利用単位は24時間/日）
測定解析補助：30,000円/時間（利用単位は8時間/日））

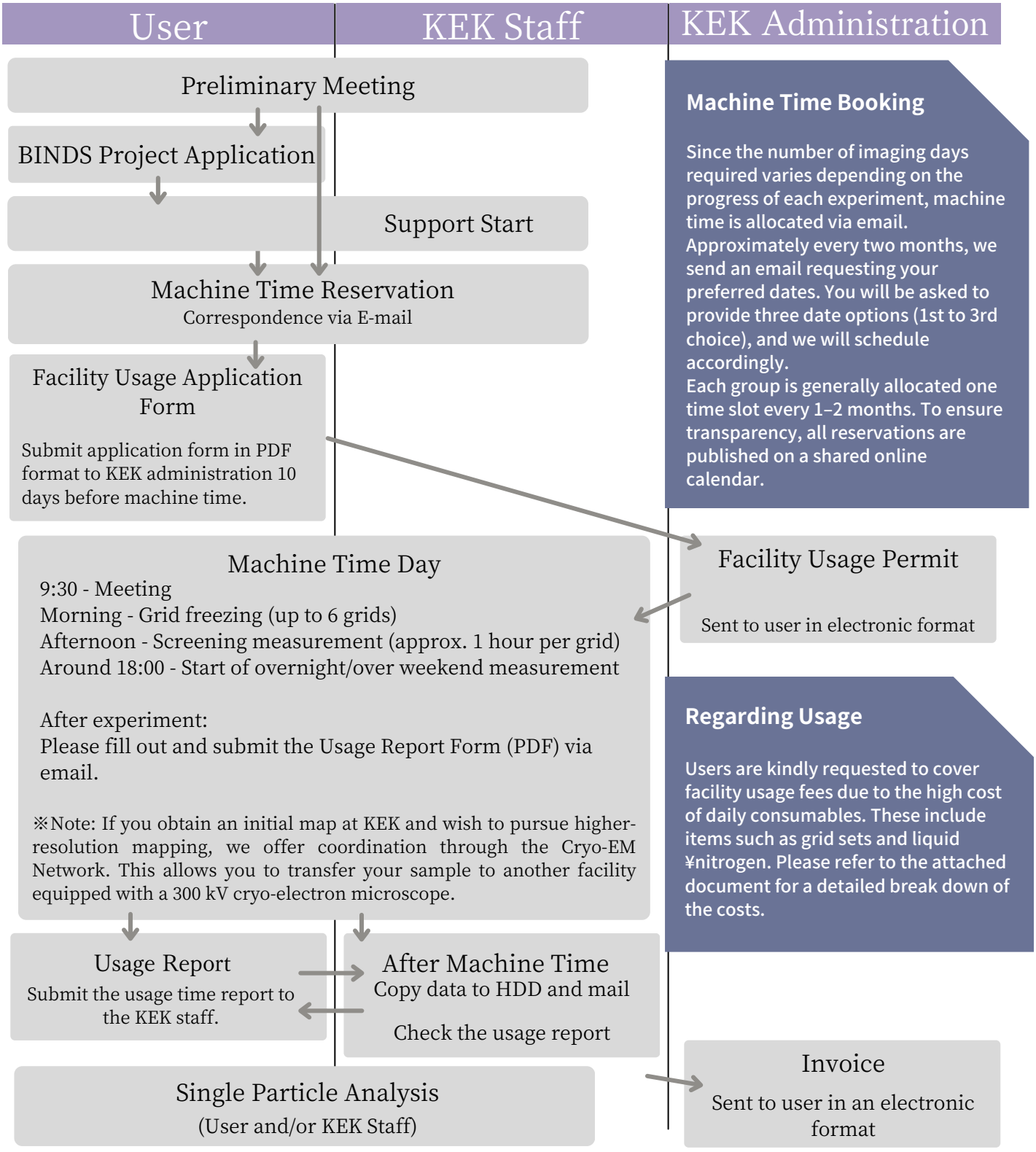
Optional

Initial Analysis (optional)：¥125,000 x number of days
初期解析 ：125,000円 x 日数
Detailed Analysis (optional)：¥250,000 x number of days
詳細解析 ：250,000円 x 日数

*The amount is equivalent to the actual cost of consumables (grids, C-clip, C-clip rings, filter paper, liquid nitrogen, HDD, etc.). We sincerely appreciate the cooperation of our users.
*消耗品実費相当の金額となっています（グリッド・C-clip・C-clipリング・ろ紙・液体窒素・HDDなど）。ユーザーの皆様のご協力に心より感謝申し上げます。

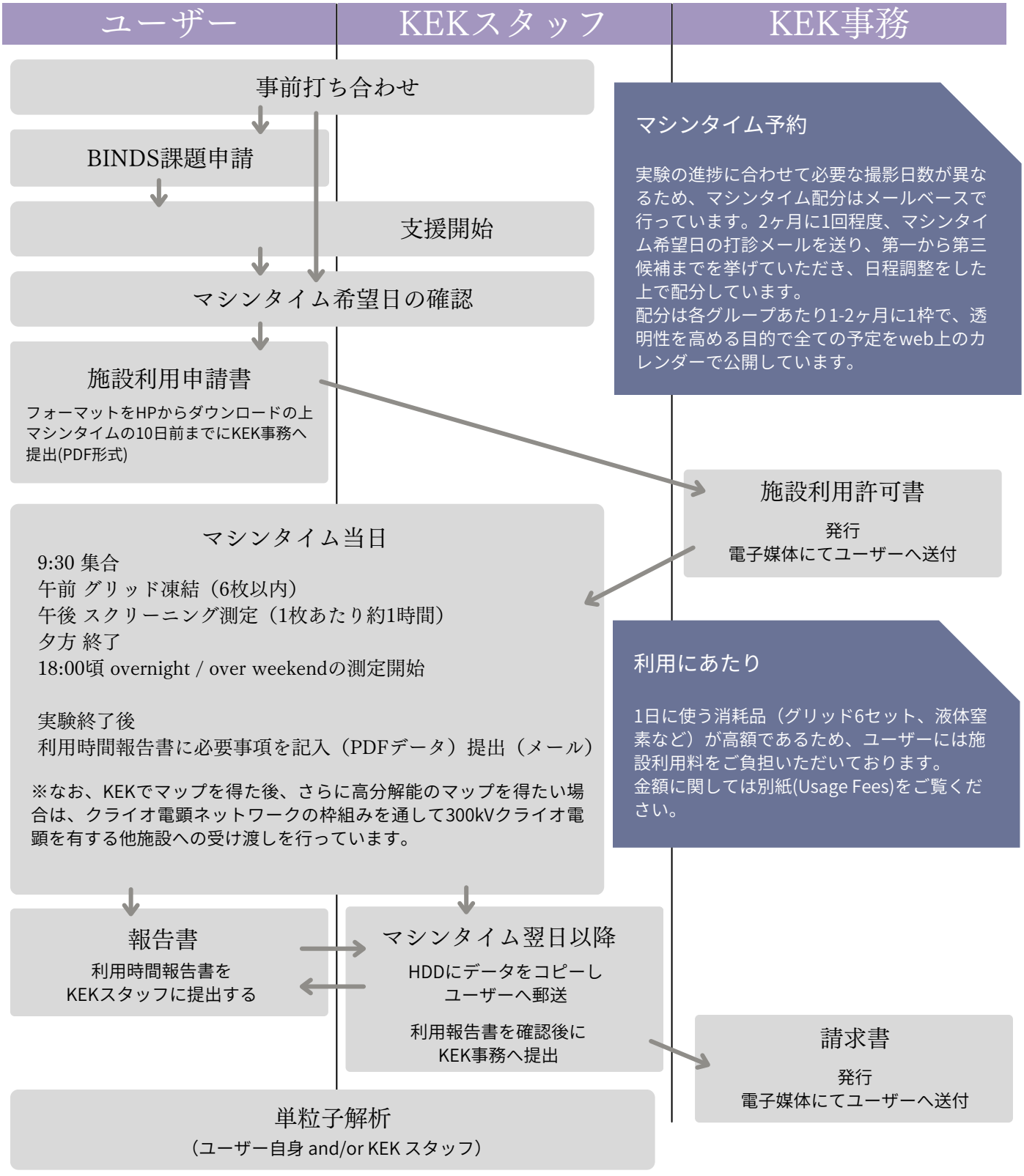
Flow of Using KEK Cryo-EM

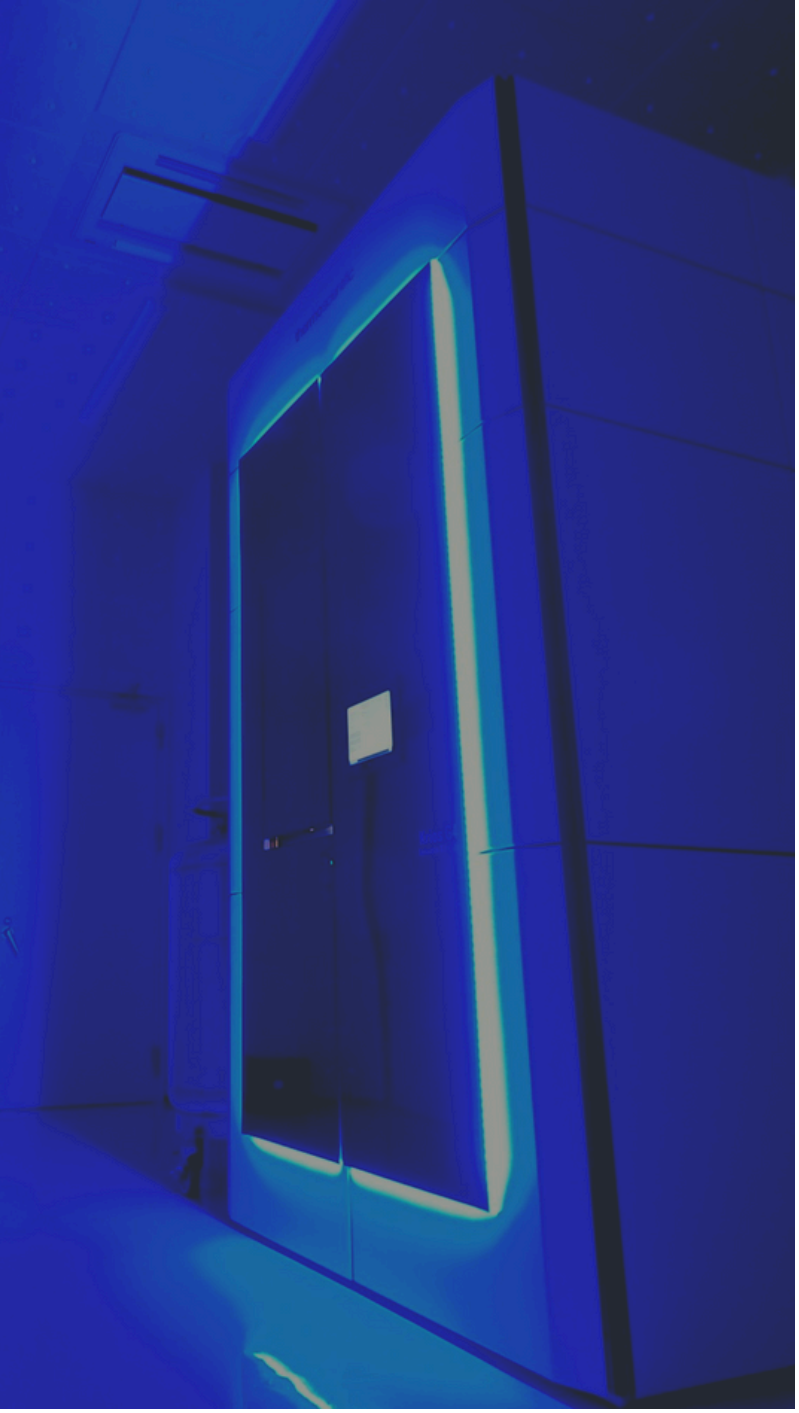
KEK Cryo-EM is available to academic users via BINDS, and to corporate users through joint research or consulting agreements.
For academic users, the typical flow is:
Preliminary meeting → BINDS application → Support starts → Machine time allocation → Experiment day
As required imaging days vary by project, machine time is coordinated via email. About every two months, users are asked to submit three preferred dates, and the schedule is adjusted accordingly.
Each group is usually assigned one slot every 1–2 months. All reservations are published on the KEK Cryo-EM website for transparency.



KEKクライオ電顕利用の流れ

KEKクライオ電顕の利用は、アカデミアユーザーはBINDS経由で、企業ユーザーは共同研究契約・学術指導契約などの枠組みで可能です。
アカデミアユーザーの初回利用までの流れは「事前打ち合わせ→BINDS課題申請→支援開始→マシンタイム配分→マシンタイム当日」が一般的です。実験の進捗に合わせて必要となる撮影日数が異なるため、マシンタイム配分はメールベースで行っています。
2ヶ月に1回程度、KEKからユーザー宛にマシンタイム希望日の打診メールを送り、第一から第三候補までを挙げていただき、日程調整をした上でマシンタイムを配分しています。配分は各グループあたり1-2ヶ月に1枠で、透明性を高める目的で全ての予定をKEKクライオ電顕のweb siteで公開しています。





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Editor's Note

Thank you for picking up this issue of our Activity Report.

Starting from this issue, we have switched to an English edition. We know there may still be areas for improvement, and we would appreciate your understanding and support as we continue to refine it. We hope you enjoy reading it.

Masuda

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KEK：高エネルギー加速器研究機構
IMSS：物質構造科学研究所
Structural Biology Research Center：構造生物学研究センター

SBRC