Accelerating CAK Complex Inhibitor Design through Cryo-EM: A Workflow for High-Throughput Screening and High-Resolution Data Collection Using 200 kV and 300kV cryo-TEMs

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Abstract

Cryo-electron microscopy (cryo-EM) has revolutionized structure-based drug discovery by allowing accurate and rapid visualization of drug-target interactions at high resolutions[1]. In this study, we demonstrate a workflow using a 200 kV Glacios cryo-transmission electron microscope (cryo-TEM) equipped with a Selectris X imaging filter and Falcon 4i detector for high-throughput screening of drug binding on CDK-Activating Kinase (CAK) complex. With just one hour of data collection, we obtained structures at 4 Å, sufficient to identify lead compound binding pocket and density. With four hours of data collection, the structures approached 3 Å, enabling visualization of lead compound conformation. Best grids imaged using the 300 kV Krios cryo-TEM resolved the structure to 2 Å, allowing the modelling of ordered waters to inform lead compound optimization. Our study demonstrates that 200 kV cryo-TEMs can deliver the high productivity needed for structure-based drug discovery and design, while targeted imaging on 300 kV cryo-TEMs can provide even higher resolutions.

Apoferritin benchmark

To test the limits of the 200 kV Glacios Cryo-TEM with Selectris-X and Falcon 4i, we defaulted to the apoferritin benchmark. Here, we achieved 1.6 Å (Fig. 1) with ~7 hours of data collection at ~650 images/hr, which encouraged us to look at the more challenging CDK-Activating Kinase complex.

Figure 1. Cryo-EM reconstruction of apoferritin at 1.6 Å. Left panel shows the cryo-EM density map; right – a zoomed in density with the apoferritin atomic model coordinates fitted into the map.
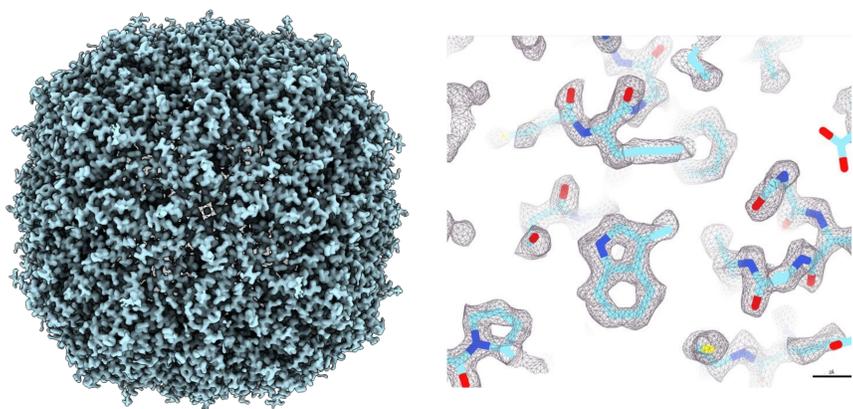
CDK-activating kinase

The human CDK-activating kinase (CAK) complex is an interesting target for cancer drugs due to its involvement in transcription initiation control and the cell cycle[2]. To enable the discovery and rational design of next-generation therapeutics with increased potency and reduced off-target effects, structural data permitting the application of structure-based drug design approaches are instrumental. We therefore set out to structurally characterise complexes of CAK bound to a range of both commercially available molecules and the series of compounds developed and characterized alongside ICEC0942[3], aiming to uncover the structural basis of CDK7 inhibitor selectivity to pave the way towards next-generation therapeutics.

Experimental workflow

Glacios; 1 hr
3.5 - 4.5 Å

Glacios; 4 hr **or** Glacios; 22 hr
2.9 - 3.2 Å **2.3 Å**

Krios; 10 hours
1.8 - 2.1 Å
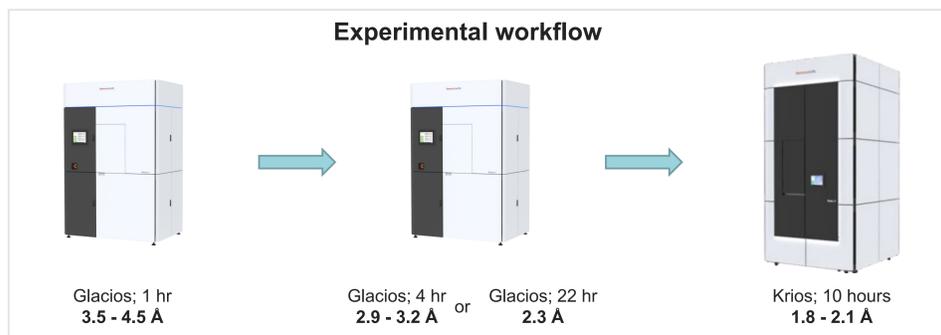
CAK compound screening

In a high throughput screening workflow, multiple CAK complexes with different small molecule ligands were imaged for 1 hour initially (Fig 2), then on promising samples were imaged for 4 hours. Within the first hour of imaging, we averaged 3.5 - 4.5 Å resolution, sufficient to identify grids with preferential orientation issues and/or presence of compound density.

Figure 2. Live processing of 1-hour Glacios datasets. Top panels show a view of the 3D reconstruction (CDK7 grey, cyclin brown, MAT1 orange) and a close-up view of the density for bound inhibitors (purple). Middle panels show the resolution (FSC = 0.143). Bottom panels show orientation distribution plots from cryoSPARC.
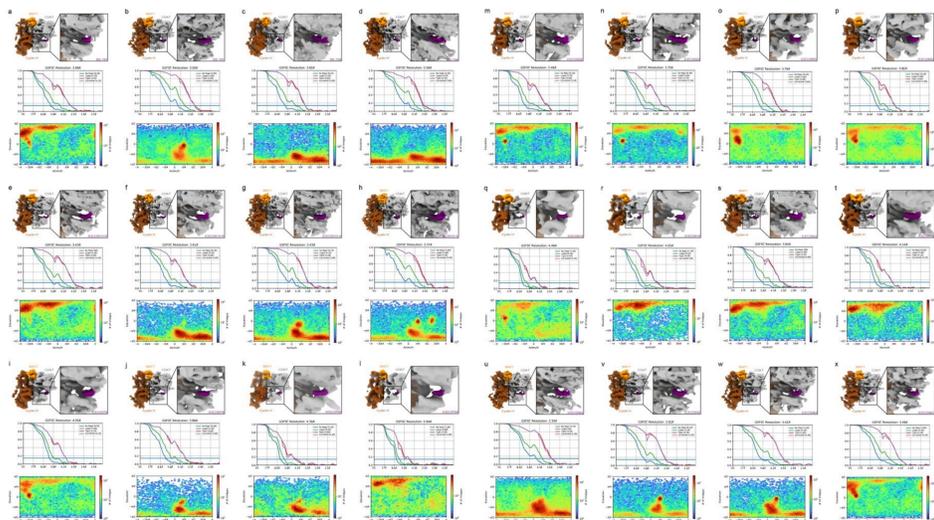
High-resolution structures of the CAK:Ligand complexes

Aiming to resolve CAK-bound inhibitors at high resolution to provide highly accurate molecular models and identify water molecules that may contribute to inhibitor binding and specificity, we used the 300 kV Titan Krios G4 for high-resolution data collection. Data collections lasted for approximately 10 hours and yielded roughly 5,000 micrographs for each sample.

Our high-throughput screening and collection workflow enabled us to visualise 12 CAK inhibitor complexes at 1.8-2.2 Å resolution and gain insight into inhibitor selectivity (Fig 3)[4].

Figure 3. Cryo-EM structural characterization of the CAK:Ligand complexes. Top left – diagram of the human CAK complex and 2.3 Å reconstruction from Glacios 2 data. Top right - high-quality zoomed-in cryo-EM density at 1.9 Å resolution from Krios data. Bottom (a-l) - structures of inhibitors bound to CDK7.

85 kDa CAK complex
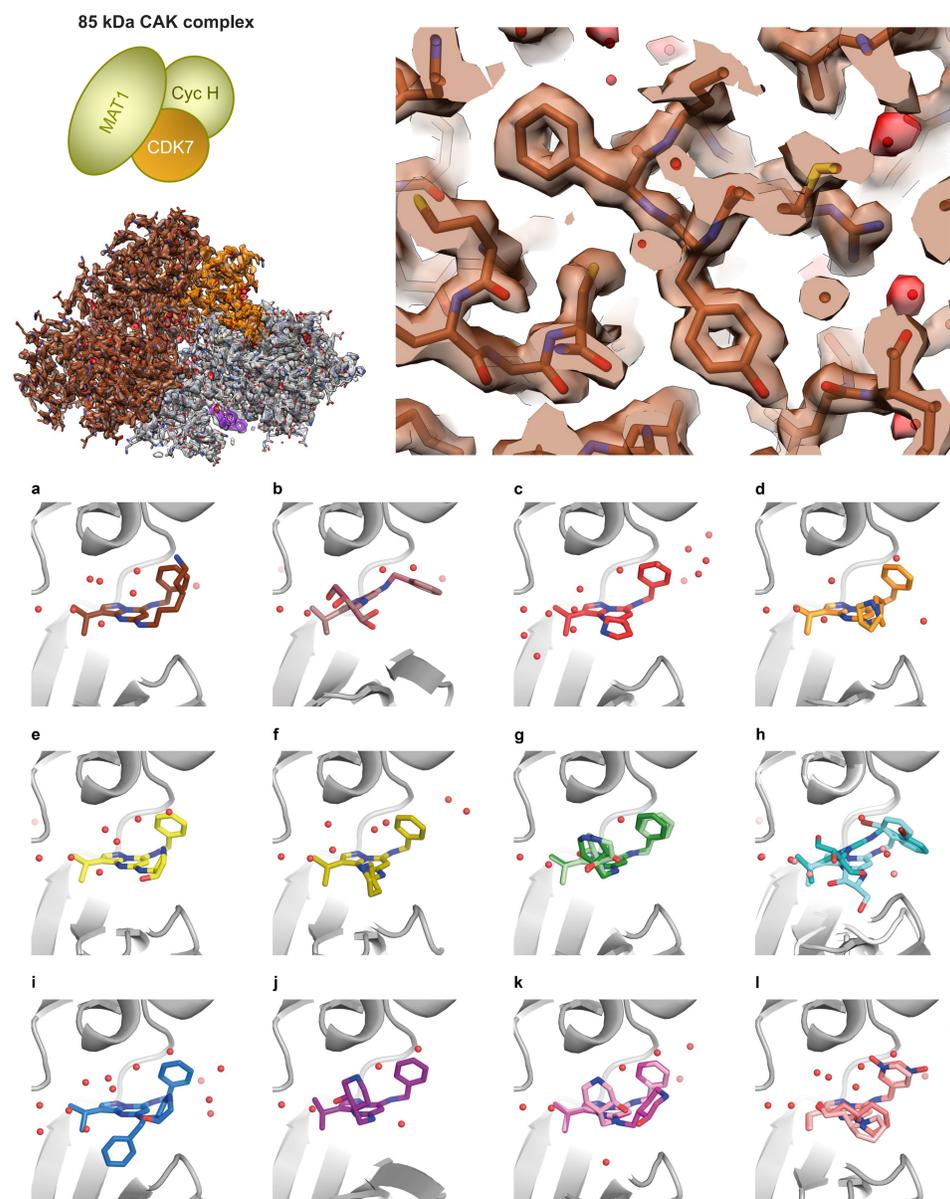
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Conclusions

This study reports the determination of cryo-EM structures of multiple CAK complexes with different small molecule ligands, which represents a significant step forward in the design of CDK7 inhibitors.

Our results demonstrate the potential of cryo-EM in structure-based drug discovery, and highlight the productivity of both 200 kV and 300 kV cryo-TEMs.

The 200 kV Glacios 2, equipped with a Selectris energy filter, is a versatile tool that can provide high-quality cryo-EM data for high-resolution studies or function as a high-throughput screening instrument to prepare grids for use with a 300 kV Krios, enabling even higher resolution imaging.

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