Revolutionizing Small Molecule Drug Discovery Pipelines with Cryo-EM: A Workflow for High-Throughput Screening and High-Resolution Data Collection

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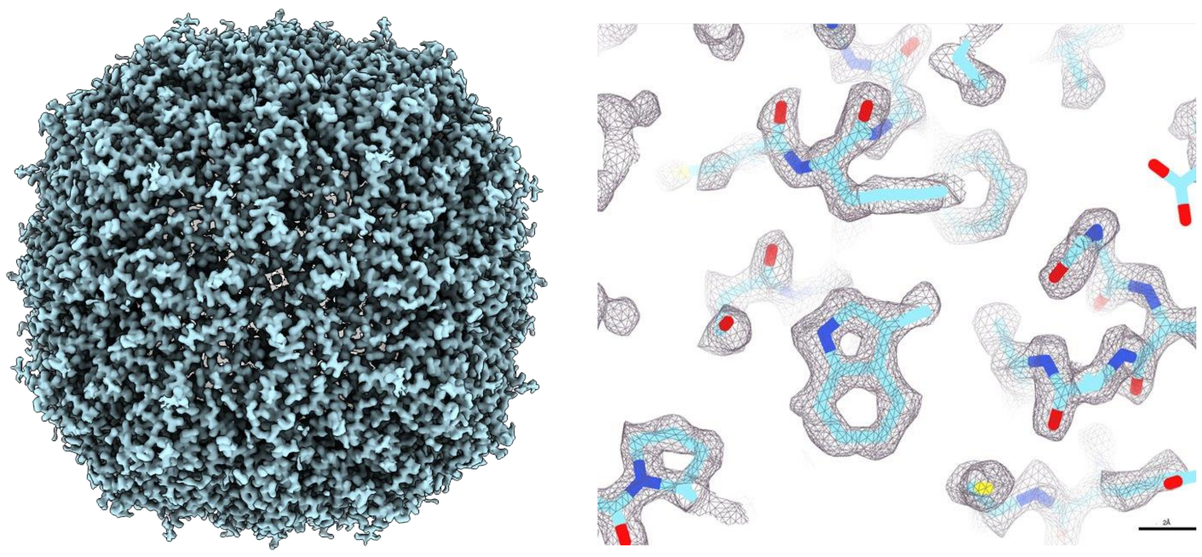
Abstract

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 Thermo Scientific™ Glacios cryo-transmission electron microscope (cryo-TEM) equipped with a Thermo Scientific™ Selectris X imaging filter and Thermo Scientific™ 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 Thermo Scientific™ 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

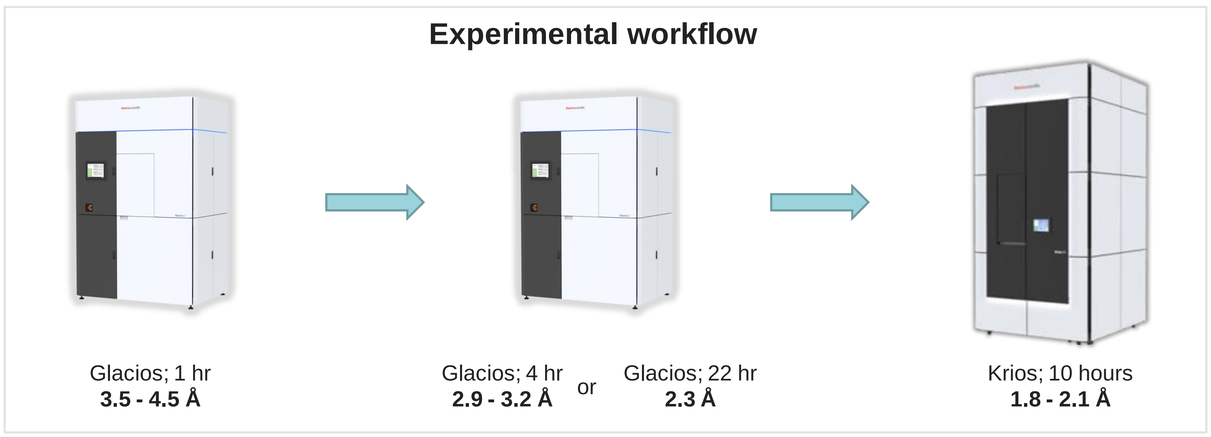
To test the limits of the 200 kV Glacios Cryo-TEM with Thermo Scientific™ Selectris X and Falcon 4i, we defaulted to the apoferritin benchmark. Here, we achieved 1.6 Å (Fig. 1) with ~7 hours of data collection at ~650 images/hr, which encouraged us to look at the more challenging CDK-Activating Kinase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

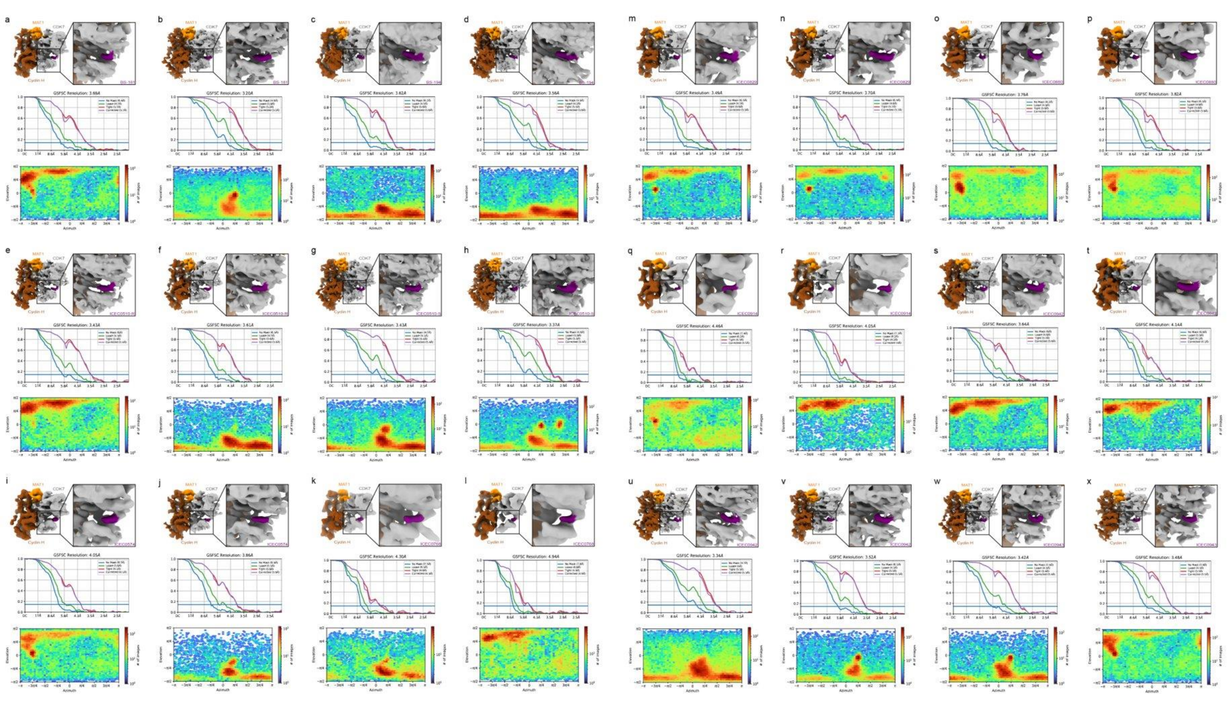
The human CDK-activating kinase (CAK) complex is an interesting target for cancer drugs due to its involvement in transcription initiation control and the cell cycle[2]. To enable the discovery and rational design of next-generation therapeutics with increased potency and reduced off-target effects, structural data permitting the application of structure-based drug design approaches are instrumental. We, therefore, set out to structurally characterize 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.



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), and then promising samples were imaged for 4 hours. Within the first hour of imaging, a resolution of 3.5 - 4.5 Å is achieved, sufficient to identify grids with preferential orientation issues and/or presence of compound density.

Figure 2. Live processing of 1-hour Glacios datasets. The top panels display a view of the 3D reconstruction with CDK7 in grey, cyclin in brown, and MAT1 in orange. Additionally, a close-up view of the density for bound inhibitors is shown in purple. The middle panels illustrate the resolution (FSC = 0.143). Lastly, the bottom panels exhibit orientation distribution plots obtained from cryoSPARC.

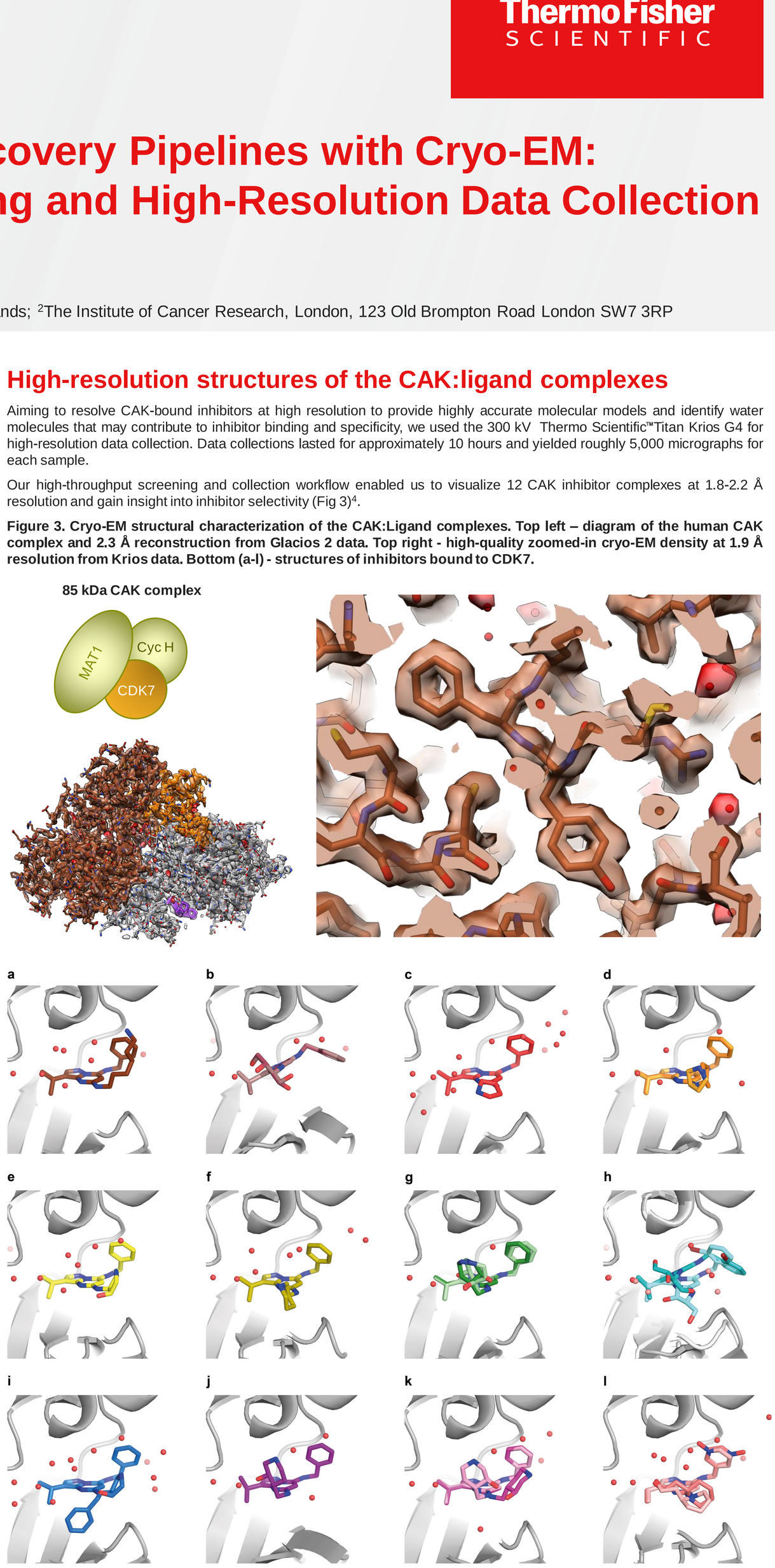


High-resolution structures of the CAK:ligand complexes

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 Thermo Scientific™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 visualize 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.



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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 advancement 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 X energy filter, is a versatile tool that can provide high-quality cryo-EM data for high-resolution studies. It can also function as a high-throughput screening instrument to prepare grids for subsequent usage with a 300 kV Krios, enabling even higher resolution imaging.

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