

Routine high-throughput screening and high-resolution data collection workflow for structure-based drug discovery using 200 kV and 300kV TEMs

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Abstract

Structure based drug discovery and design has relied on X-ray crystallography for many years, due to its well-established workflow and high-throughput when the targets for study would crystallize reproducibly in the presence of leads/fragments. Today, cryo electron microscopy (cryo-EM) can accurately and rapidly visualize to high resolutions the interactions between drug and receptor of a multitude of samples, even ones that resist crystallization to enable informed and accelerated drug discovery and design. Advances in TEM technology, automation and GPU-accelerated high-performance computing have boosted productivity of cryo-EM from every angle. This is all while simultaneously decreasing the required level of expertise for routinely obtaining high resolution results. While state-of-the-art 300 kV cryo-transmission electron microscope (Cryo-TEM) continues to deliver the highest-resolution single particle analysis (SPA) structures to date, we believe using 200 kV Cryo-TEMs in an integrated workflow can deliver high-throughput and high-resolution screening followed by targeted imaging on 300kV Cryo-TEMs can deliver extremely high productivity needed for structure-based drug discovery and design. Here, we show how the 200 kV Glacios Cryo-TEM equipped with the Selectris-X and Falcon 4 was used to rapidly screen for particle distribution and drug binding on a small 85kDa CDK Activating Kinase (CAK) complex. With just a single hour of data collection, it is possible to obtain structures at $\sim 4\text{\AA}$, sufficient for identifying lead compound binding pocket and lead compound density. With 4 hours of data collection, the structures approach 3\AA and lead compound conformation can be visualized. Best grids imaged in the 300kV Krios resolves the structure to $\sim 2\text{\AA}$, where ordered waters can be modelled to inform lead compound optimization.

Introduction

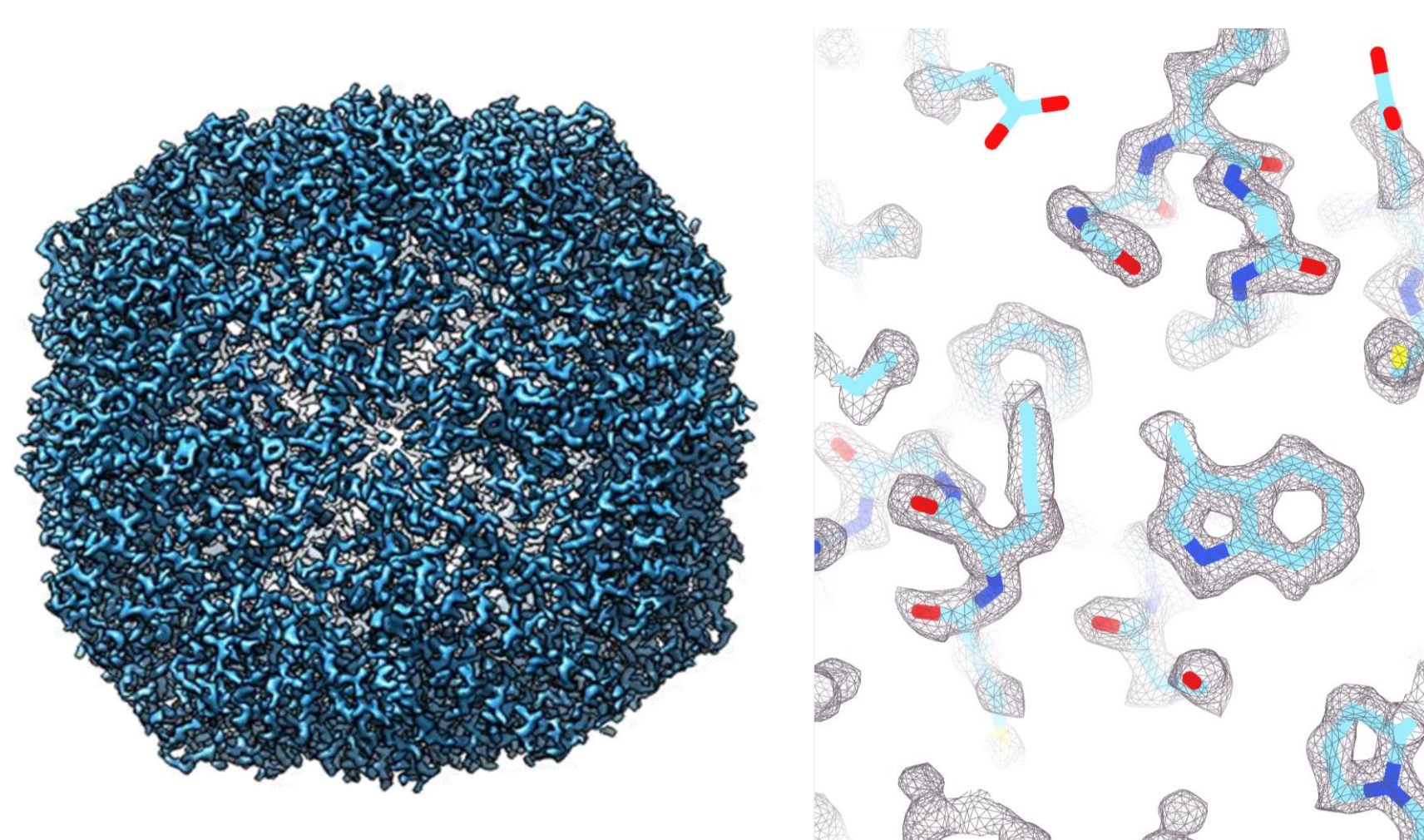
CryoEM SPA has, over the last decade, benefited from a multitude of technological advances leading to significantly improved ease-of-use, data collection throughput and resulting map quality. With its ability to resolve structures previously intractable via X-ray crystallography, it presents a valuable opportunity for studying a wider range of drug targets.

Here, we present a workflow using both 200 kV Glacios and 300kV Krios Cryo-TEMs both equipped with Selectris-X and Falcon 4i for high-throughput screening of difficult samples to high-resolution data acquisition for reconstructions of a range proteins frequently targeted for structure based drug discovery and design.

Yet another ApoF benchmark

To test the limits of the 200 kV Glacios Cryo-TEM with Selectris-X and Falcon 4i, we defaulted to the trusty Apoferritin benchmark. Here, we achieved 1.6\AA with ~ 7 hours of data collection at ~ 650 images/hr, which encouraged us greatly to look at the more challenging samples.

Apoferitin @ 1.6\AA with Glacios 2



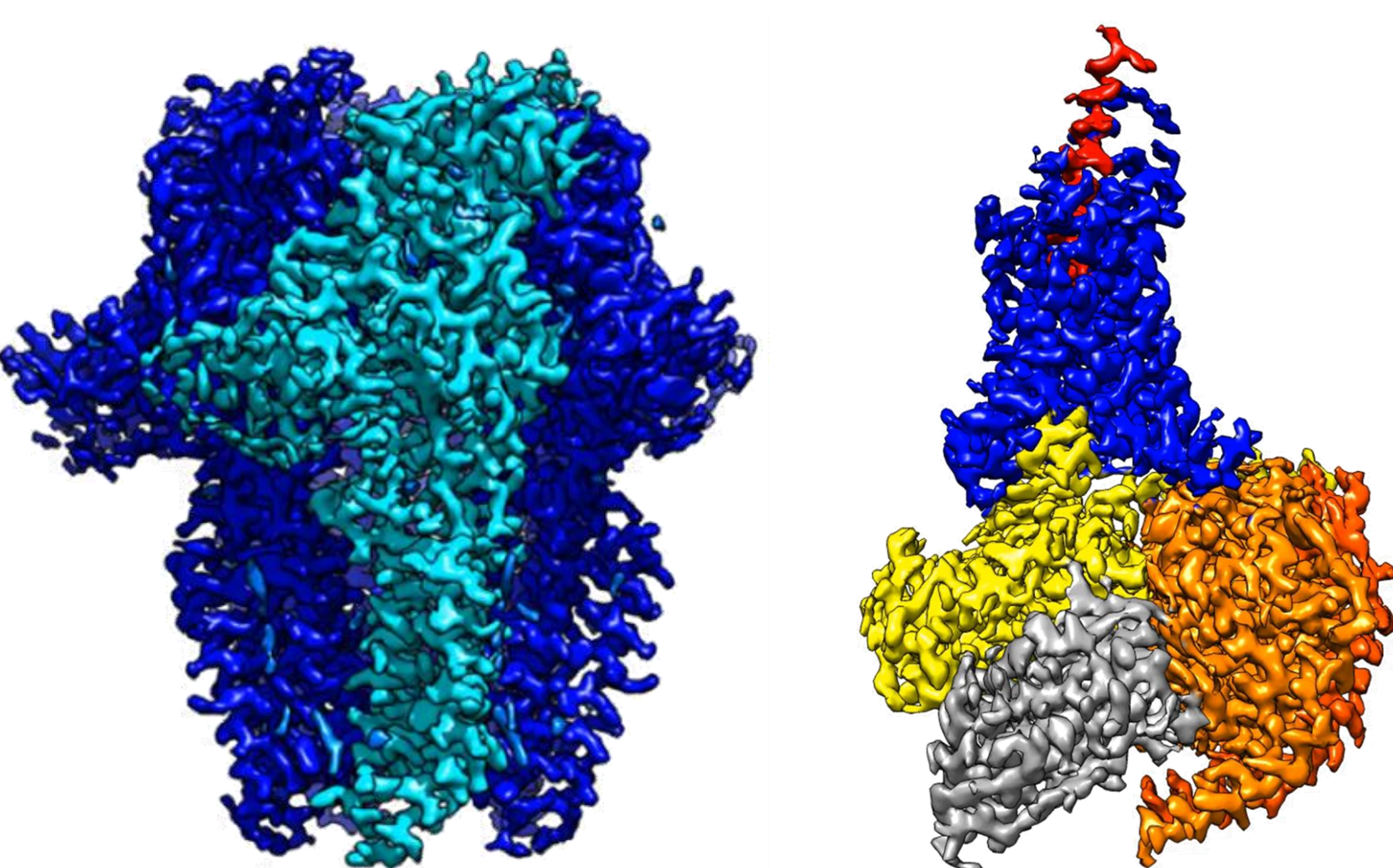
GABA_A and GLP-1 Receptor

(Collaboration: MRC-LMB and Monash Univ.)

Both are popular drug targets, GABA_A for neuronal diseases and GLP1R for type 2 diabetes and obesity. Using just the Glacios, we achieved resolutions of 2.4\AA in 12hrs and 2.6\AA in 20hrs respectively, water molecules and side chain rotamers can be identified which are important to structure based drug design.

Human GABA_A @ 2.4\AA

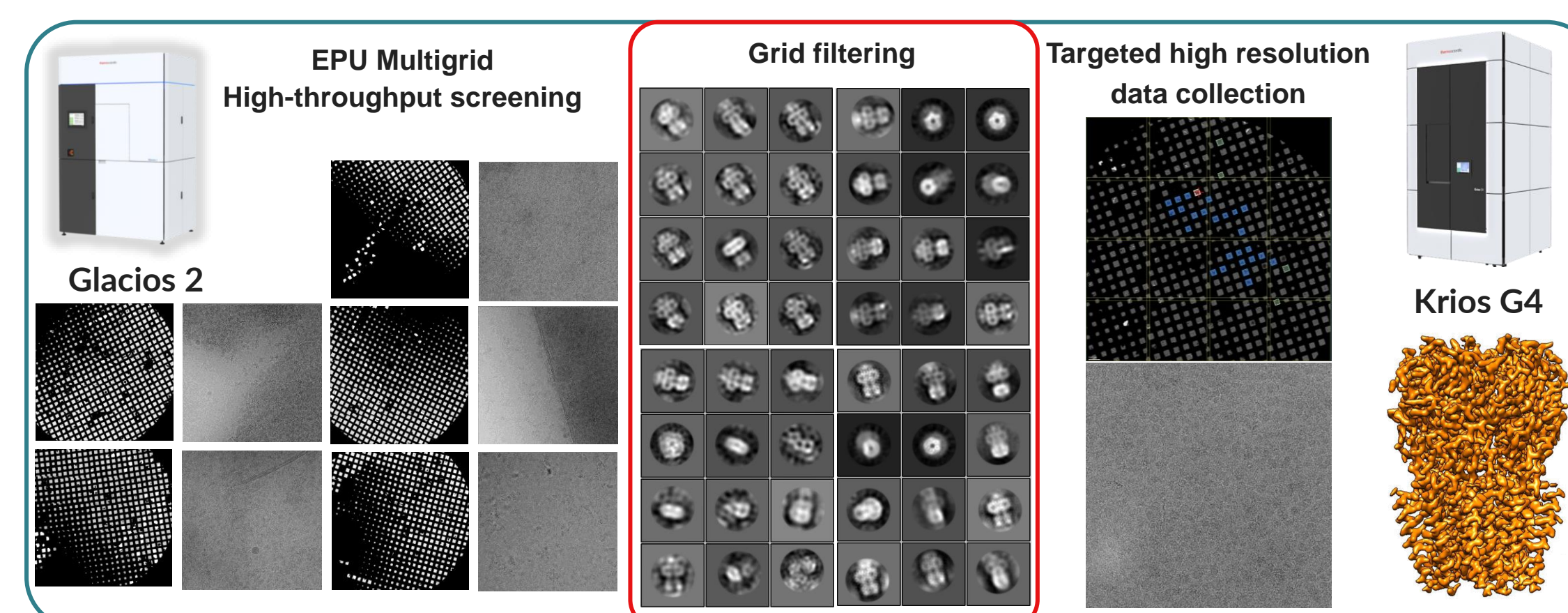
GLP1R @ 2.6\AA



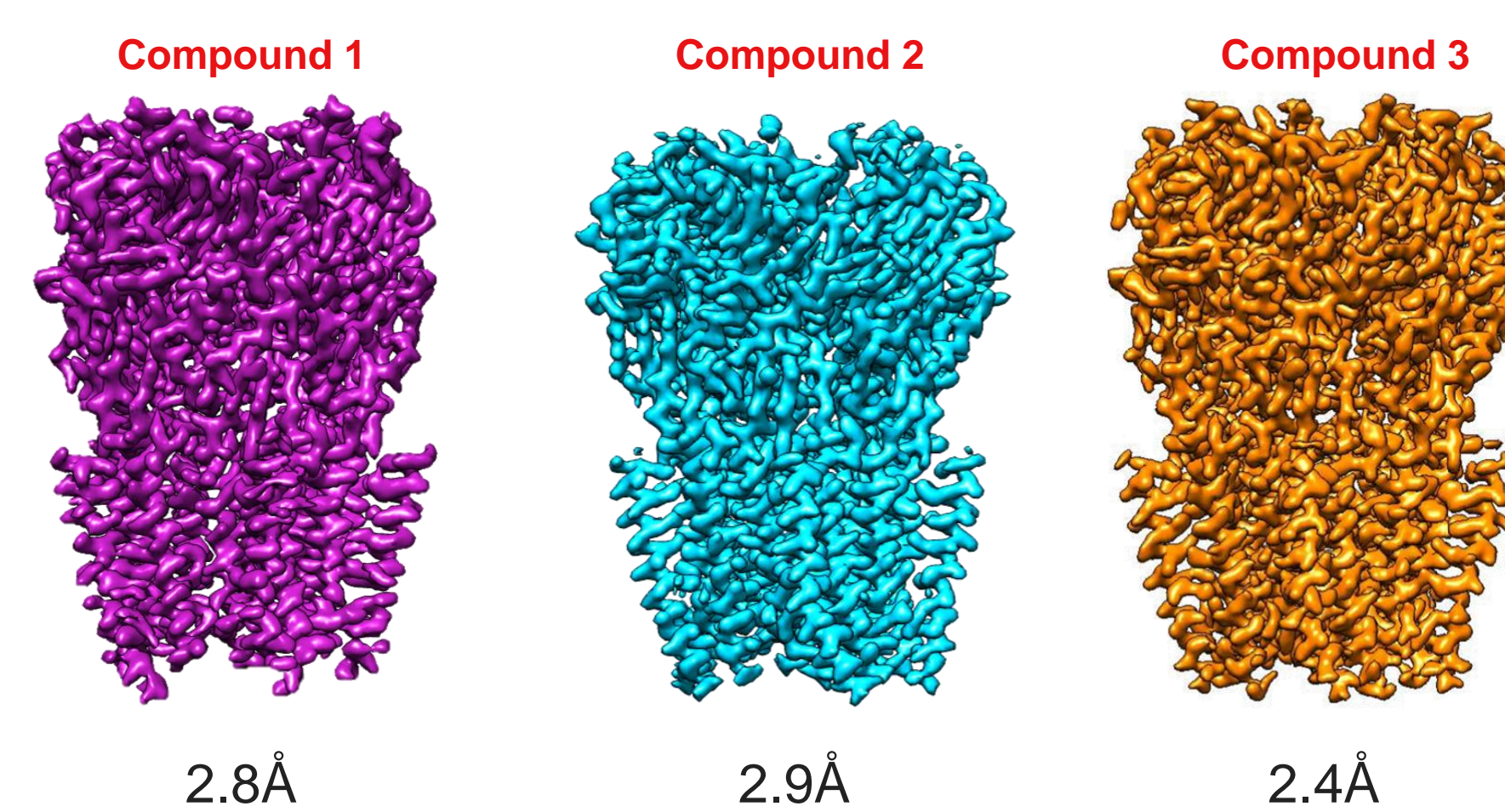
Glycine receptor (GlyR)

(Collaboration: Sudha Chakrapani, CASE Western University)

High-resolution reconstruction from cryoEM data requires samples of good quality as judged by optimal ice thickness, good range of particle orientations, high particle density and homogeneity. In this example, we collected dozens of small datasets of GlyR samples with different ligands using EPU-MG on the Glacios to screen for grids with potential to produce high resolution reconstructions. These grids then used for full data collection on a Krios for high-resolution reconstructions.



So far, this approach has resulted in 3 maps at high-resolution showing compounds of interest bound.

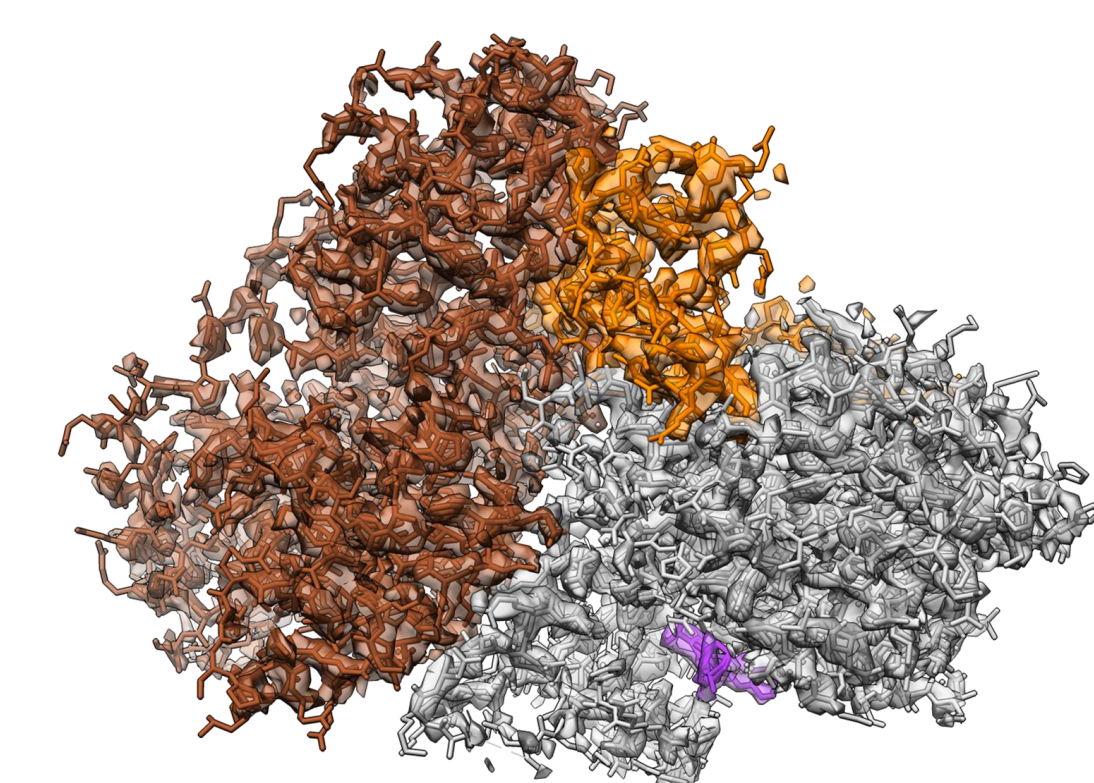


Cyclin activating kinase (CAK)

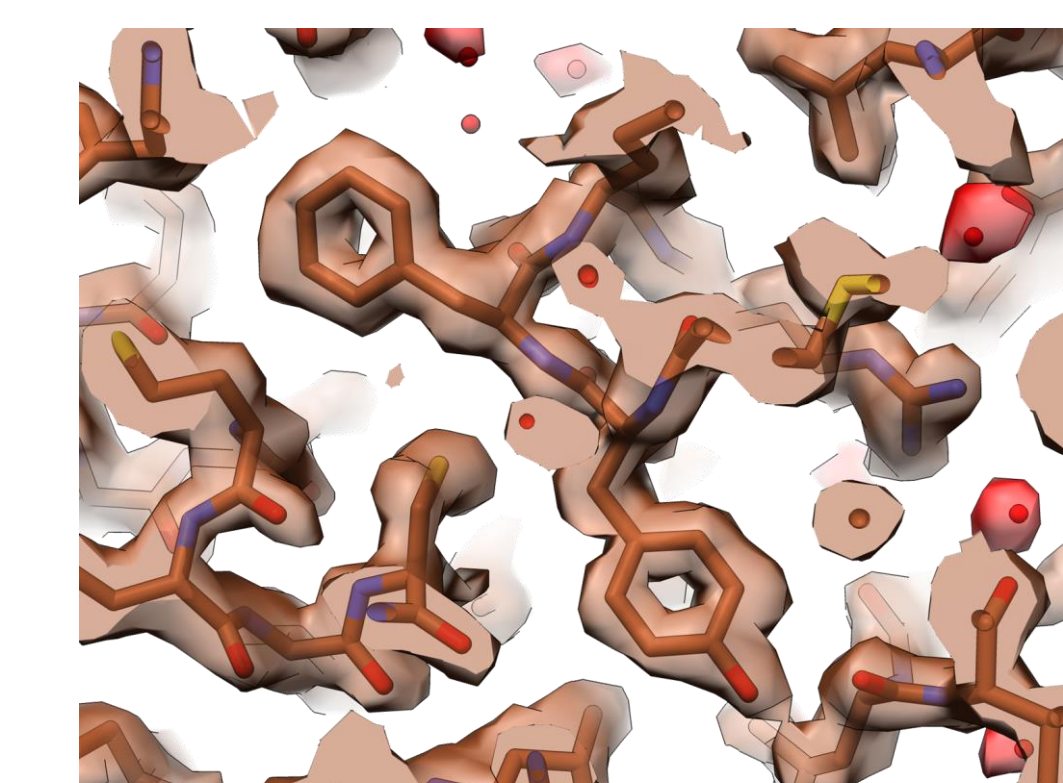
(Collaboration: Basil Greber, ICR London.)

The human cyclin activating kinase (CAK) complex is an interesting target for cancer drugs due to its involvement in transcription initiation control and the cell cycle. At 85kDa in size, this complex is challenging to study via cryoEM. Despite that, using the Glacios 2 with Selectris-X and Falcon 4i, this complex was resolved to 2.3\AA with an overnight data collection. The ligand in magenta at the bottom-right of the map, was clearly resolved with its surrounding ordered waters, providing valuable lead optimization clues for structure-based drug design.

Human CAK complex @ 2.3\AA with Glacios 2



Human CAK complex @ 1.9\AA with Krios

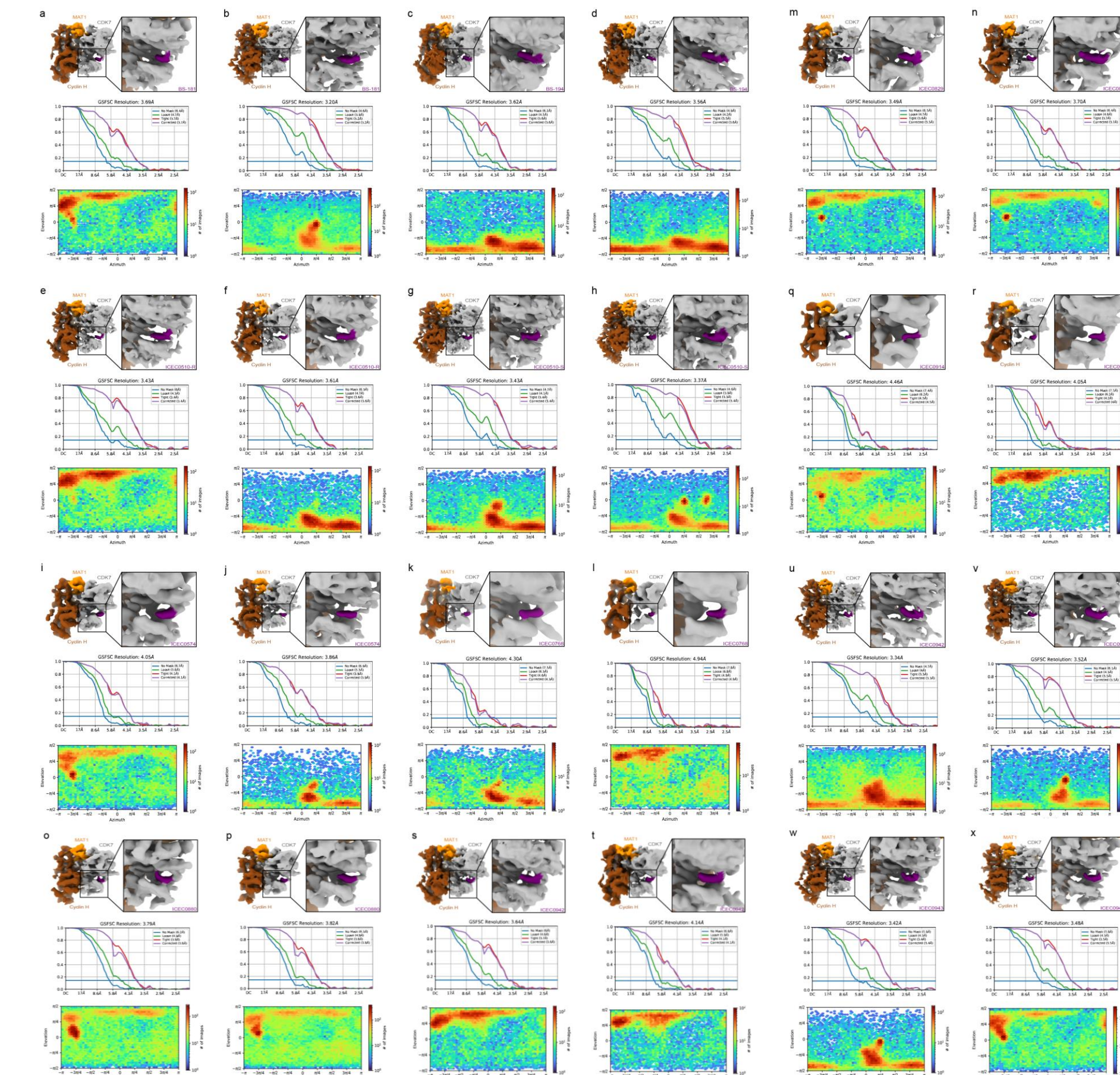


Moving to the Krios with the same grid, this sample achieves 1.9\AA resolution, leading to more ordered waters to be visible and also greater confidence in the orientations of side-chains and compound conformation.

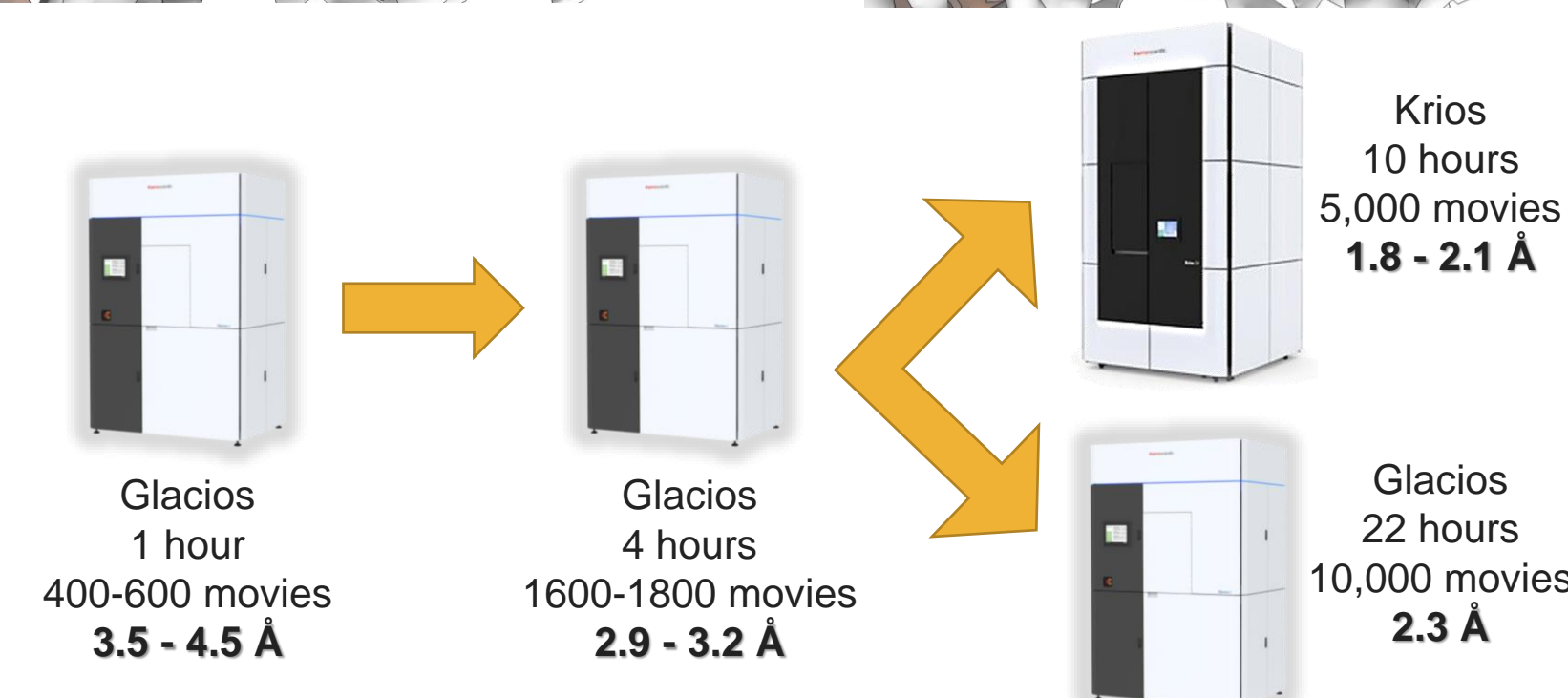
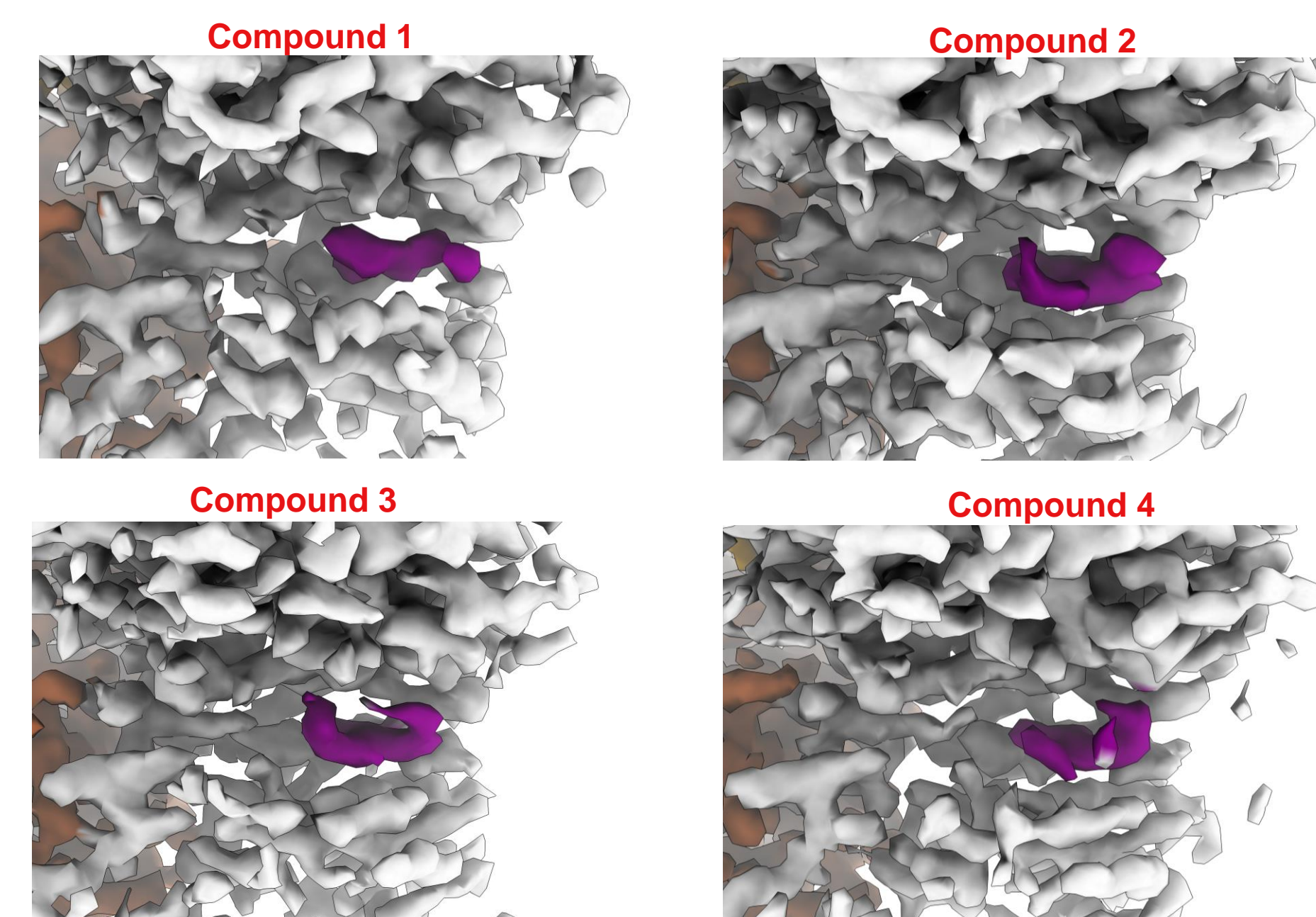
CAK compound screening

(Collaboration: Basil Greber, ICR London.)

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



After 4 hours, with just $\sim 1,600$ images per dataset, we achieved $2.9\text{-}3.2\text{\AA}$ resolution on most grids. We were able to determine with strong confidence that the ligand was bound to the complex.



Conclusion

The more accessible workhorse 200kV Glacios 2 can, with the help of a Selectris energy filter, provide high resolution cryoEM data or a high-throughput screening instrument to feed the best possible grids to a 300kV Krios for the highest possible resolution.

References

• Xin Zhang et al, DOI:10.1016/j.str.2021.04.008.