Harnessing the power of cryo-EM for development of cancer therapeutics: High-resolution structures of the human CDK-activating kinase bound to inhibitors

Basil Greber[1], Victoria Cushing[2], Junjie Feng[3], Adrian Koh[4], Abhay Kotecha[5], Matthew Fuchter[6], Simak Ali[7]

[1]The Institute of Cancer Research [2]The Institute of Cancer Research, [3]The Institute of Cancer Research, [4]Materials and Structural Analysis Division, Thermo Fisher Scientific, [5]Thermo Fisher Scientific, [6]Imperial College London, [7]Imperial College London

basilgreber@gmx.net

The human CDK-activating kinase (CAK) is a trimeric protein complex that acts as a master regulator of cell growth and division. It regulates transcription initiation and the cell cycle by phosphorylating RNA polymerase II and cyclin-dependent kinases, respectively. Due to this central role in cellular physiology, the CAK has been identified as a promising target for cancer drugs, with multiple inhibitors undergoing clinical trials, and as a possible target in anti-viral therapy. Structural data are of great importance for the rational, structure-based design of next-generation therapeutics. Harnessing the power of cryo-electron microscopy (cryo-EM) for this task requires the development of workflows that enable structures of small, asymmetric complexes such as CAK to be determined at high resolution and with high throughput.

We previously determined structures of the human CAK bound to nucleotide analogues and two inhibitors using a 200 kV-electron microscope with a K3 electron detector, achieving up to 2.5 Å resolution for the 85 kDa CDK-cyclin core module of the complex [1,2]. These structures elucidated the structural basis of the activity, regulation, and inhibition of the human CAK. One of the inhibitor-bound structures revealed interesting conformational differences between the clinical inhibitor ICEC0942 bound to CAK, its cellular target, and the same inhibitor bound to CDK2, an off-target complex with the potential to cause side effects in patients [2], hinting at the potential of using structural data to guide the development of next-generation inhibitors in this system.

We therefore set out to structurally characterise CAK complexes using high-end cryo-EM instrumentation, aiming to uncover the structural basis of inhibitor selectivity in order to enable the rational, structure-based design of more specific compounds that cause fewer side-effects in patients. We have now obtained several structures of the 85 kDa CDK-cyclin-module of the human CAK bound to inhibitors and nucleotides at up to 1.9 Å resolution using a Titan Krios microscope with a cold-FEG, a Selectris X energy filter, and a Falcon 4i electron-counting detector. In addition to achieving high resolution from large datasets, this setup combined with live processing also enabled structure determination of this small, asymmetric, ligand-bound complex at 3-4 Å within one hour from the start of data collection.

References

[1] Greber et al. (2020), PNAS 117 (37): 22849-57

[2] Greber et al. (2021), Biophys J. 120 (4): 677-686