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Thermo Scientific™ Glacios Cryo-TEM: A Versatile 200 kV Tool for Structure-Based Drug Discovery

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Advances in cryo-electron microscopy (cryo-EM) single-particle analysis (SPA) have led to an explosion in the number of near-atomic resolution structures being determined, with the atomic-resolution barrier being broken for the first time last year [1,2]. Improvements in resolution mean that SPA can be used to visualize bound ligands, enabling structure-based drug design (SBDD) for previously intractable proteins and protein complexes.

G-coupled protein receptors (GPCRs), the largest superfamily of cell surface receptor proteins, form one of the key drug target classes. While X-ray crystallography has played a major role in determining GPCR structures, cryo-EM is paving the way in the determination of agonist-bound GPCRs in the fully active state where they are coupled to canonical transducer proteins. Since the first cryo-EM GPCR structure in 2017, state-of-the-art 300 kV Cryo-TEM imaging technology allowed the determination of a number of GPCR complexes [3]. Here, we show that the 200 kV Glacios Cryo-TEM can also be used to support structure-assisted GPCR drug discovery at resolutions that allow modelling of the bound ligand.

The Glacios Cryo-TEM delivers a complete and compact cryo-EM solution at 200 kV. The powerful combination of aberration-free image shift (AFIS) and the Thermo Scientific Falcon™ 4 Detector significantly enhances SPA throughput, as demonstrated by 2.2 Å apoferritin reconstruction from 1 hr data collection. For highest resolution data acquisition and economical sample use, the 20 µm C2 aperture can be utilized to produce a 700 nm parallel beam which allows imaging six areas within a single 2 µm hole. This setup yielded a 1.9 Å apoferritin structure from 1h 45 min of data collection (Figure 1).

The glucagon-like peptide-1 receptor (GLP-1R) is a validated target for the treatment of type 2 diabetes and obesity, with numerous approved peptide therapeutics [4]. The emergence of small molecule GLP-1R agonists that can be taken orally has sparked renewed interest in the discovery and development of novel GLP-1R drugs, with PF 06882961 being among the most promising ones. A high-resolution structure of this compound bound to the active GLP-1R has been reported recently [5]. Here, we assessed the performance of the Glacios Cryo-TEM, using PF 06882961-bound GLP-1R as an example. A two-day data collection using the Falcon 4 Detector operated in Electron Event Representation mode yielded a 3.2 Å map with clear density for bound drug and multiple structurally ordered waters (Figure 2). Excitingly, while the global resolution was 0.2-0.4 Å lower compared to structures obtained from KriosCryo-TEM, the binding pocket density was effectively identical to that obtained from 300 kV data, demonstrating the potential utility of 200 kV cryo-EM for drug target structure determination [6].

Further improvements in resolution might be needed to enable the routine 200 kV SBDD on a wide range of pharmaceutical targets. Having an optimized sample, next-generation detector and thin ice might not always be sufficient to obtain high-resolution cryo-EM maps which permit modelling of the ligand-binding pocket. The Thermo Scientific™ imaging filters can be used to further improve the signal-to-noise ratio of TEM images resulting in improved resolution. To demonstrate the benefits of this new technology, we used Glacios

Cryo-TEM equipped with Selectris X and Falcon 4 detector to determine the cryo-EM structure of GLP-1R:GLP1 at 2.7 Å resolution. These technological advances provide a route towards the routine application of 200 kV cryo-EM in high-throughput screening of ligands and structure-based drug discovery.

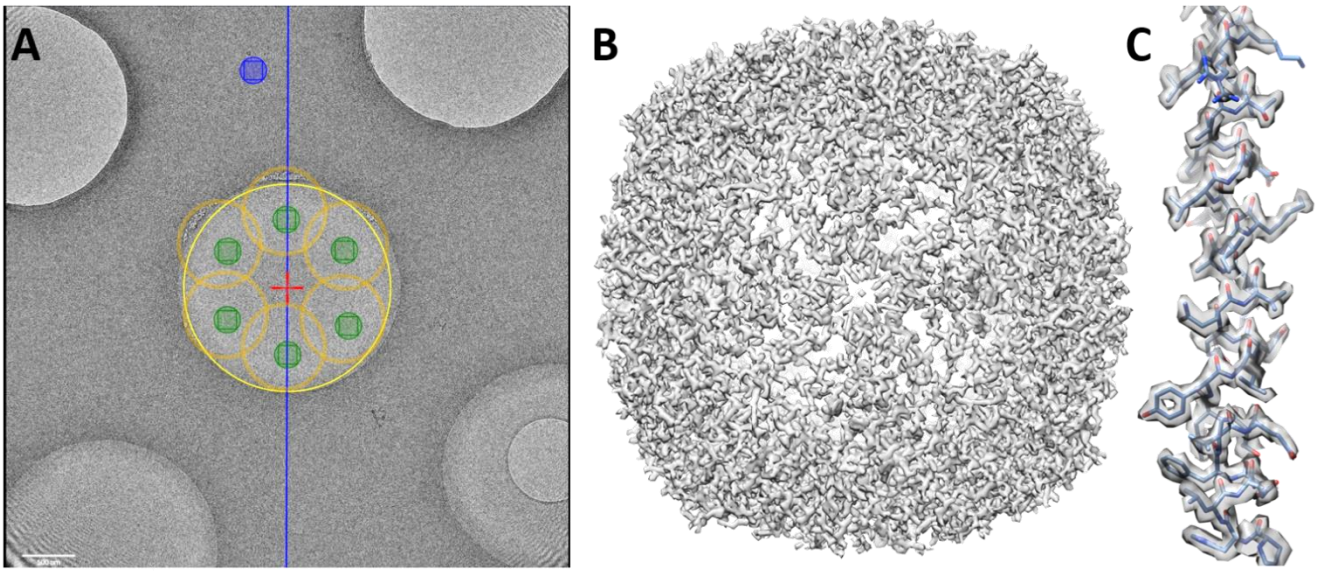

Figure 1. Apoferritin reconstruction using 20 μm C2 aperture setup. (A) During the template definition step, six acquisition areas were placed within a single 2 μm hole with a parallel beam size of 700 nm. (B) 1.9 Å apoferritin reconstruction from 1h 45 min of data collection. (C) Apoferritin atomic model fitted in the cryo-EM density map.

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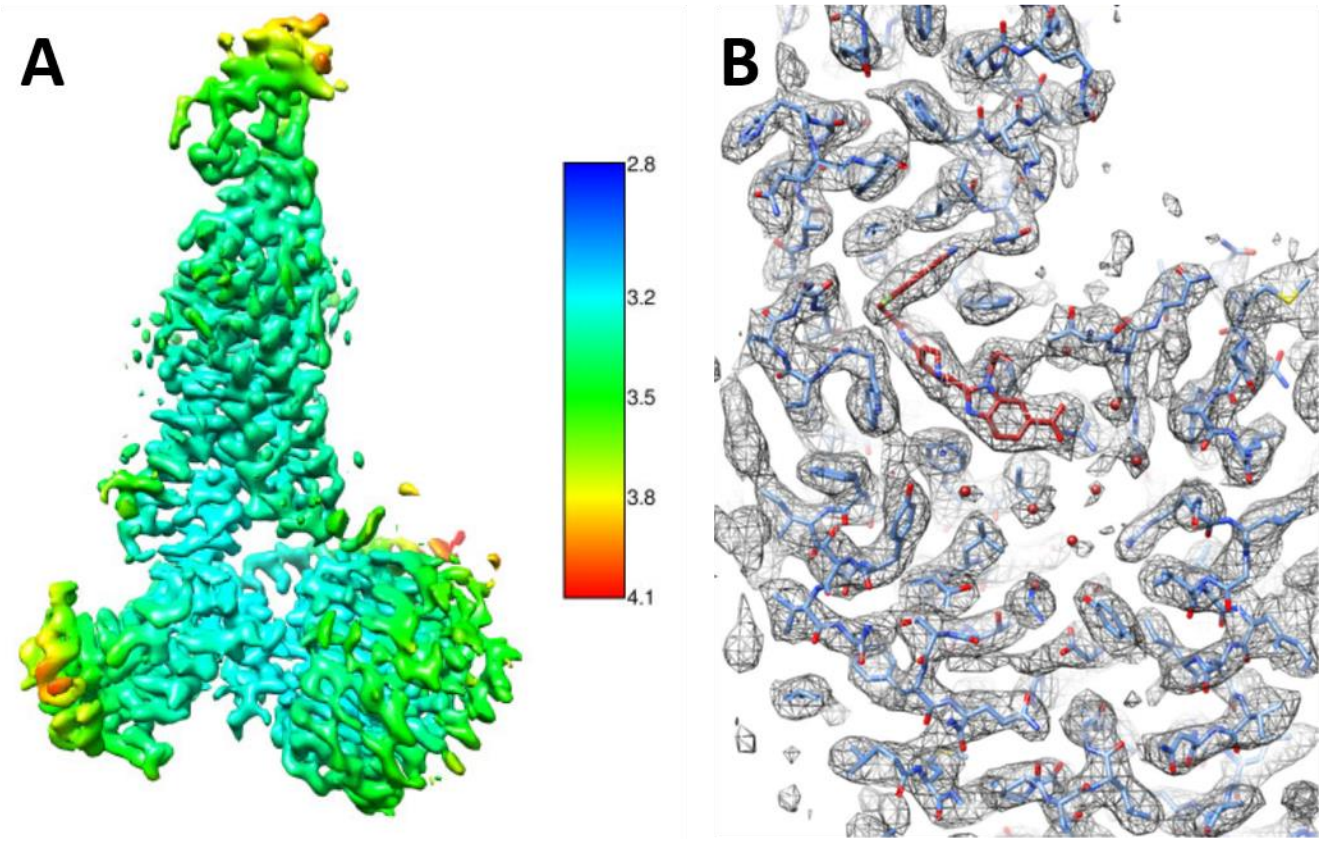

Figure 2. The cryo-EM structure of PF 06882961-GLP-1R-DNGs complex imaged using 200 kV Glacios cryo-TEM equipped with Falcon 4 direct electron detector. (A) Local resolution-filtered EM consensus map displaying local resolution (Å) coloured from highest resolution (dark blue) to lowest resolution (red). (B) Models of PF 06882961 (dark red) and the GLP-1R binding cavity (blue) built into the receptor-focused density map.

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