Experiment Brief

K2 Camera

Title

Breaking the molecular weight barriers for cryo-electron microscopy: High-resolution structure determination of <100 kDa protein complexes at 200 keV.

Gatan Instrument Used

The K2® camera contains a direct detection transmission complementary metal-oxide-semiconductor (CMOS) detector; the real-time electron counting capability of this camera continues to break barriers in cryo-electron microscopy (cryo-EM).

Background

Technical advances in the field of cryo-EM have allowed high-resolution structure determination of biological complexes in near-native environments. Cryo-EM has been shown to obtain structural information on large molecular complexes, however it has been more challenging to obtain high-resolution information on <100 kDa protein complexes. Typically, very high-performance 300 keV transmission electron microscopes (TEMs) equipped with a direct electron detector have been used for most high resolution structure determination in cryo-EM. Although TEMs operating at 300 keV offer reduced specimen charging and reduced inelastic electron scattering as compared to those at lower voltages, the benefits are largely restricted to imaging thicker specimens. The cryo-EM community is slowly embracing the fact that more modest 200 kV TEMs with good microscope alignments and real-time electron counting detectors are good enough for high-resolution structure determination, and even more interestingly can be used to enable structure determination of sub 100 kDa proteins. This is well exemplified in a recent article (Herzik Jr. et. al. Nat. Comm. 10:1032) where the authors show the use of conventional TEM at 200 keV with a Gatan K2 camera, determine high-resolution structures of proteins with masses <100 kDa. This paves the way to a more economical and accessible solution for cryo-EM facilities worldwide.

Materials and Methods

A small aliquot (3 µL) of horse liver alcohol dehydrogenase, human hemoglobin and catalytic subunit of protein kinase A were loaded on freshly discharged UltrAuFoil R1.2/1.3 300-mesh grids (Electron Microscopy Services), followed by plunge freezing in liquid ethane cooled by liquid nitrogen. Cryo-EM data was collected on a Thermo Fisher Scientific Talos Arctica TEM operating at 200 keV. Movies were collected using a K2 Summit® camera operating in counting mode (0.556 Å pixel^{-1}) at a nominal magnification of ×73,000 and using a defocus range of –0.5 to –1.6 µm. Movies were collected over an 11 s exposure with an exposure rate of ~1.95 e^{-}pixel^{-1} s^{-1}, resulting in a total exposure of ~69 e^{-}Å^{-2} (1.57 e^{-}Å^{-2} frame^{-1}). Motion correction and dose-weighting were performed using the MotionCor2 frame alignment program.

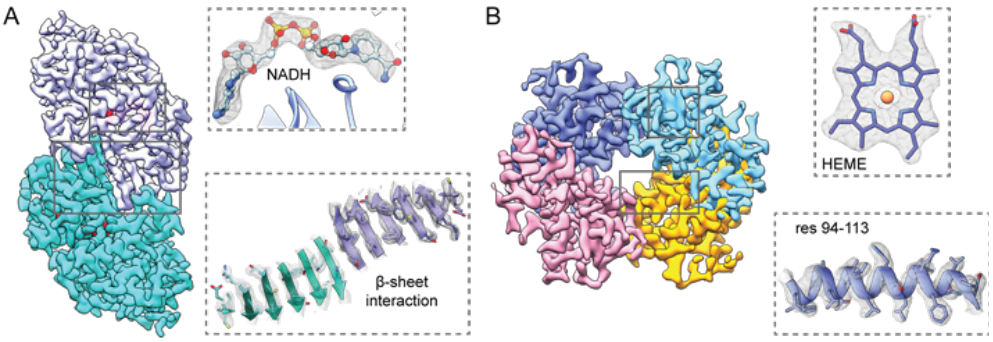

Figure 1. (A) Cryo-EM reconstruction of 82 kDa horse liver alcohol dehydrogenase at 2.7 Å resolution. Zoomed-in views of the EM density (gray mesh) for reduced form of nicotinamide adenine dinucleotide (top) and the β-sheet interaction between the two monomers (bottom). (B) 2.8 Å resolution cryo-EM reconstruction of ~64 kDa human methemoglobin. Good map to model correlation is demonstrated by the segmented EM density of the heme cofactor (top) and a representative α-helix encompassing residues 94-113 (bottom).

Summary

These results break the previously defined barriers for target sizes that can be resolved by cryo-EM without the need for a 300 keV TEM. Proteins with molecular weights <100 kDa can readily be solved at near-atomic resolution with a Gatan real-time counting direct electron camera.

Reference

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EB-K2-CryoWeight-FL1-CA-SEP19