Near-atomic resolution structural biology enabled by cryo-EM.

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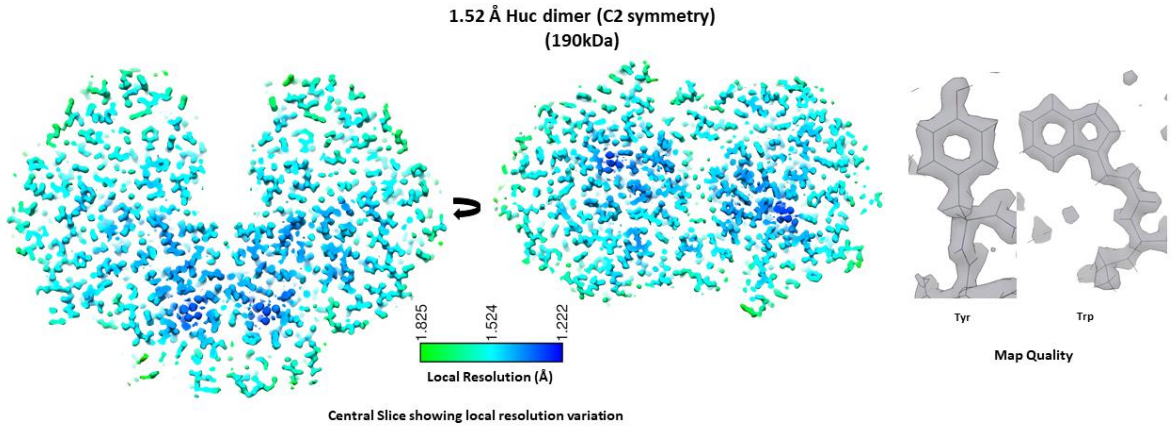
Cryo electron microscopy (cryo-EM) single particle analysis (SPA) is fast becoming a major tool in structure biology (1). Although high resolution transmission electron microscopy (HRTEM) is routinely used to image crystalline samples in material science to atomic/near-atomic resolution, achieving this resolution for biological macromolecules has been a major challenge in cryo-EM. Enabling this opens the door to critical applications like structure-based drug designing. The year 2020 heralded the age of cryo-EM SPA delivering atomic resolution (1.2 Å) on standard protein sample apoferritin (1). This was made possible using a generation 4 Titan Krios (Thermo Fisher) with a Cold FEG as an electron source, a new more stable energy filter (selctris-X) and the latest Falcon-4 camera. This prompted us to investigate the limitation of the more prevalent S-FEG system with conventional energy filter in achieving similar resolution. The present work details benchmarking and optimization of a generation 1 Titan Krios (Thermo Fisher) with S-FEG, Gatan Bio-Quantum energy filter to achieve 1.42 Å apoferritin structure using a Gatan K2 camera and later using a Gatan K3 camera to achieve a record breaking near-atomic resolution (1.52 Å) structure for real world sample (mycobacterial hydrogenase Huc) (3). The improved workflow now routinely generates sub 2 Å structures for variety of classes of protein including a standout result of 1.8 Å resolution for a 160 kDa asymmetric protein.



References:
1) Subramaniam, S., Kotecha, A. & Davis, J. H. (2022). IUCrJ 9, 713-714.
2) Nakane T, Kotecha A et al. Single-particle cryo-EM at atomic resolution. Nature. 2020 Nov;587(7832):152-156.
3) Grinter, R., Kropp, A., Venugopal, H et al. Energy extraction from air: structural basis of atmospheric hydrogen oxidation. bioRxiv 2022.10.09.511488; doi:https://doi.org/10.1101/2022.10.09.511488