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Obtaining 3 Å Resolution Structures of Biomedical Targets at 200 keV

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Increasingly, electron microscopes coupled with direct electron detectors are being used to determine the structures of macromolecular complexes at resolutions that were historically only attainable by X-ray crystallography. In order to maximize the efficacy of atomic models determined from cryo-electron microscopy densities in structure-based drug design, the resolution of the targeted structure should be such that coordinated ions and ordered water molecules are visible. Generally, such details become visible in the 2 to 3 Å resolution range. Despite the advent of high-speed direct detectors, which enable correction of beam- or stage-induced movements of the sample during acquisition while simultaneously mitigating the damaging effects of radiation [1], single particle cryo-EM structures in the 2 to 3 Å resolution range have thus far only been collected using high-end 300 keV microscopes. Unfortunately, access to these high-end instruments is not only expensive, but also limited at most institutes due to heavy usage. Often times, mid-range 200 keV instruments are also installed alongside high-end 300 keV microscopes, and these mid-range microscopes are primarily used to screen sample conditions and determine preliminary reconstructions to serve as initial models for higher resolution data collection at 300 keV. Since the purchase cost and service contracts for these mid-range 200 keV instruments are lower than 300 keV microscopes, the ability to solve high-resolution structures at 200 keV could lead to substantial savings in academia and industry, as well as lessening the demand on the 300 keV instruments.

We show that using a 200keV Talos Arctica microscope with a K2 direct detector, one can solve structures to resolutions that have until now only been attributed to 300keV instruments. Using the *Thermoplasma acidophilum* 20S proteasome as a test sample, we are capable of resolving structural features that are consistent with reconstructions that are better than 3 Å resolution, including glycerol and water molecules. We also show that such resolutions are not only attainable for the highly symmetric 20S complex, but can also be achieved for smaller ion channels and small asymmetric complexes. Notably, our data suggest that lower energy may be beneficial in resolving very small macromolecules, possibly due to slightly better contrast at the lower energy of 200 keV, as we have been able to resolve a 127 kDa polypeptide at better than 4 Å resolution using the Talos Arctica. We explain how the Talos Arctica is an efficient and cost-effective instrument that can be used as a workhorse for determining the high-resolution structures of biomedically important complexes. We will present the role that electron microscopy has played in the development of immunomodulatory drugs that bind to the Cul4-Rbx1-DDB1-Cereblon E3 ubiquitin ligase complex [2].

References:

[1] S Zheng *et al*, Nature Methods, submitted.
[2] M Matyskiela *et al*, Nature **535** (2016) p. 252.

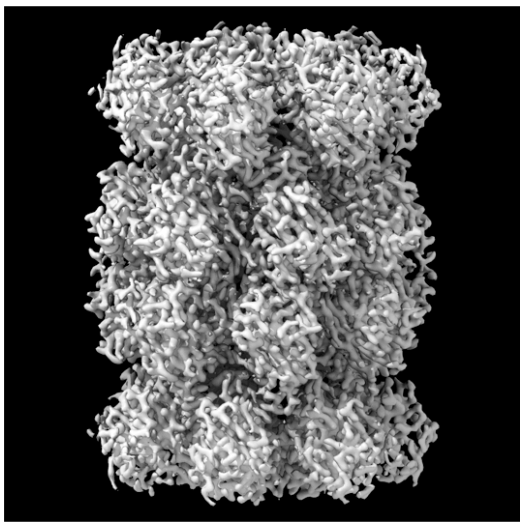

Figure 1. Structure of the 20S proteasome determined using a 200 keV Talos Arctica

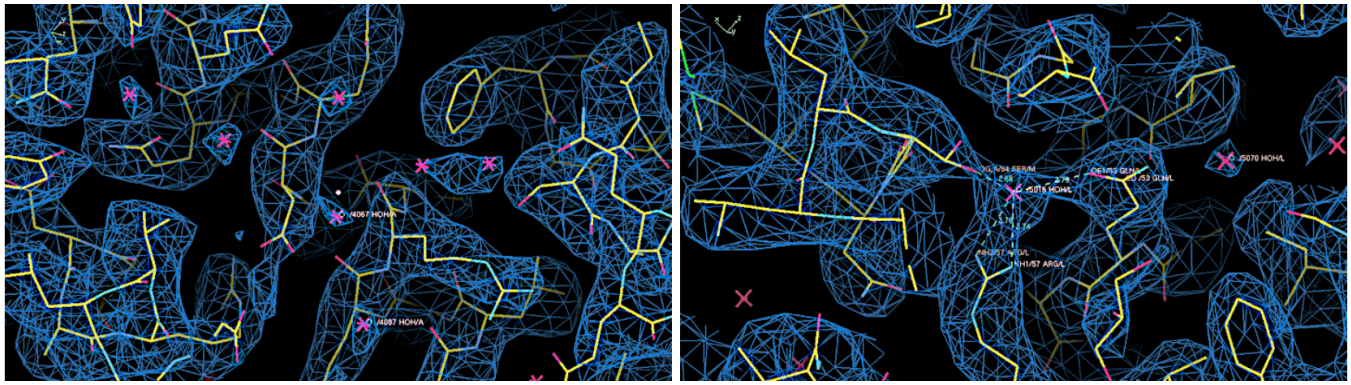

Figure 2. Detailed views of the 20S density, showing features that correspond to well-ordered water molecules.

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