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High-Throughput Single Particle Cryo-Electron Microscopy Applied to the 26S Proteasome

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The 26S proteasome is a molecular machine with a key role in intracellular protein degradation in eukaryotes. The holocomplex is formed by a barrel-shaped core particle (CP) – the 20S proteasome - which is capped at both ends by a regulatory particle (RP) each. While the structure of the CP has been determined already a while ago by X-ray crystallography [1], structure determination of the RP or the entire 26S holocomplex poses a big challenge in structural biology. Some reasons hampering structural investigations are: (i) the sheer size of 2.5 MDa of the holocomplex. (ii) it's fragility – 26S proteasomes tend to dissociate during sample preparation and (iii) the structural variability of the intact complex. While X-ray crystallography is intrinsically depending on high levels of sample purity and homogeneity, cryo-electron microscopy of single particles can tolerate a fair degree of sample heterogeneity. This is achieved by applying smart image-classification strategies allowing a post-purification step in the computer. Prerequisite for such an *in-silico* sorting procedure is a large number of particle images to start with. Typically tens of thousands or even hundreds of thousands high-resolution particle images of consistent quality are required for classification and to calculate three-dimensional electron density maps.

In recent years we have gradually refined [2,3] the structure of the 26S proteasome to a current resolution of approximate 9 Å (at 0.5 Fourier shell correlation threshold), which allowed first insights into its molecular architecture. Since sample preparations of 26S proteasomes are quite heterogeneous a high-throughput single particle data acquisition approach was pivotal to generate large data sets for an exhaustive classification [4,5].

In this communication we demonstrate the capacity of our new data acquisition pipeline applied to the 26S proteasome. In conjunction with instrumentation combining the latest technological achievements in electron optics, cryogenics and robotics, namely a Titan Krios microscope (FEI Company, Eindhoven, The Netherlands) equipped with a robotic sample loading and transfer system and with a 8k (8192x8192 pixel-array) Tietz CCD camera (TVIPS GmbH, Gauting, Germany) - we were able to gather data sets of unprecedented size and quality under precisely controlled conditions. This is especially important for less well behaved samples such as the 26S proteasome, where the molecules of interest are so sparse that a single frame contains only a few of them.

References

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