[MS3-02] Unravelling the structure of the human 26S proteasome: a hybrid approach.

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In eukaryotes the ubiquitin/proteasome pathway is responsible for the controlled targeting and degradation of a wide range of proteins, including key cellular regulators such as those controlling cell cycle progression and apoptosis. The 26S proteasome is a large multi-subunit ATP dependent protease complex of approximately 2.6 MDa that is responsible for the highly regulated proteolysis of proteins targeted for breakdown by ubiquitin conjugation [1]. It is a well recognised target for cancer therapy and its deregulation is associated with neurodegenerative conditions such as Alzheimer's and Parkinson's diseases. We have determined the structure of the human 26S proteasome by cryo-electron microscopy (cryo-EM) and single particle analysis to a resolution of 7-9Å [2]. Secondary structure elements are clearly identified throughout the 3D map. We describe in detail the conformational rearrangements on the 20S core induced by the binding of the regulatory particles.

We also directly identify the densities corresponding to the 6 ATPase subunits, the Rpt subunits, of the 19S regulatory particle. We have created structural models for the Rpt subunits that allow their tracing along the different domains of the Rpt hetero-hexamer and describe their structural relationship with both the 20S core and the non-ATPase subunits (Rpn subunits) of the 19S particles. Rpn1 and Rpn2 are the largest subunits of the 26S proteasome (~100 kDa). We have solved the structure of Rpn2 by x-ray crystallography, revealing a novel fold for its PC domain [3]. The fold of Rpn2 serves as signature for its assignment within the 26S proteasome map and provides a basis for the assignment of all remaining subunits of the complex. By combining our cryo-EM map with data from x-ray crystallography and structural modelling we reveal the organisation of the 19S regulatory particle subunits and present a molecular model for the complete human 26S proteasome. This data provides a strong basis to directly infer into the functional mechanisms of this fundamental molecular machine.

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