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# Structure of the Human 26S Proteasome Revealed by Cryo-Electron Microsocpy

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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]. The 26S proteasome is a well-established target for cancer therapy and its deregulation is associated with neurodegenerative conditions such as Alzheimer's and Parkinson's diseases.

The 26S proteasome consists of a 20S core associated with two 19S regulatory particles. Four stacked rings of seven highly homologous α or β subunits, arranged as a αββα barrel-like structure, form the 20S core [2]. The proteolytic active sites, formed by the *N*-terminal Thr residues of the subunits β1, β2 and β5, are located within the central cavity of the 20S core barrel. The 19S regulatory particles are located at each end of the 20S core and are responsible for the recognition, unfolding and translocation of substrate proteins into the proteolytic core. Despite its fundamental role in eukaryotic homeostasis, the structural organisation of the 26S proteasome regulatory subunits and its overall functional and regulatory mechanisms are still largely unknown. High resolution structural information is required to address these issues.

We have determined the structure of the human 26S proteasome by cryo-electron microscopy and single particle analysis (Figure 1). Secondary structure elements are clearly identified throughout the 3D map. With the improved resolution we can now describe in detail the conformational rearrangements on the 20S core subunits induced by the binding of the regulatory particles, which we previously observed by analysis of negatively stained samples [3]. We can also directly identify the densities corresponding to the six ATPase subunits of the 19S regulatory particle, the Rpt subunits, and describe their structural relationship with the 20S core. Finally, our map allows us to explore the organisation of the remaining non-ATPase subunits of the 19S regulatory particle.

References:

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[3] P.C. da Fonseca & E.P. Morris, *J. Biol. Chem.*, 283 (2008) 23305.

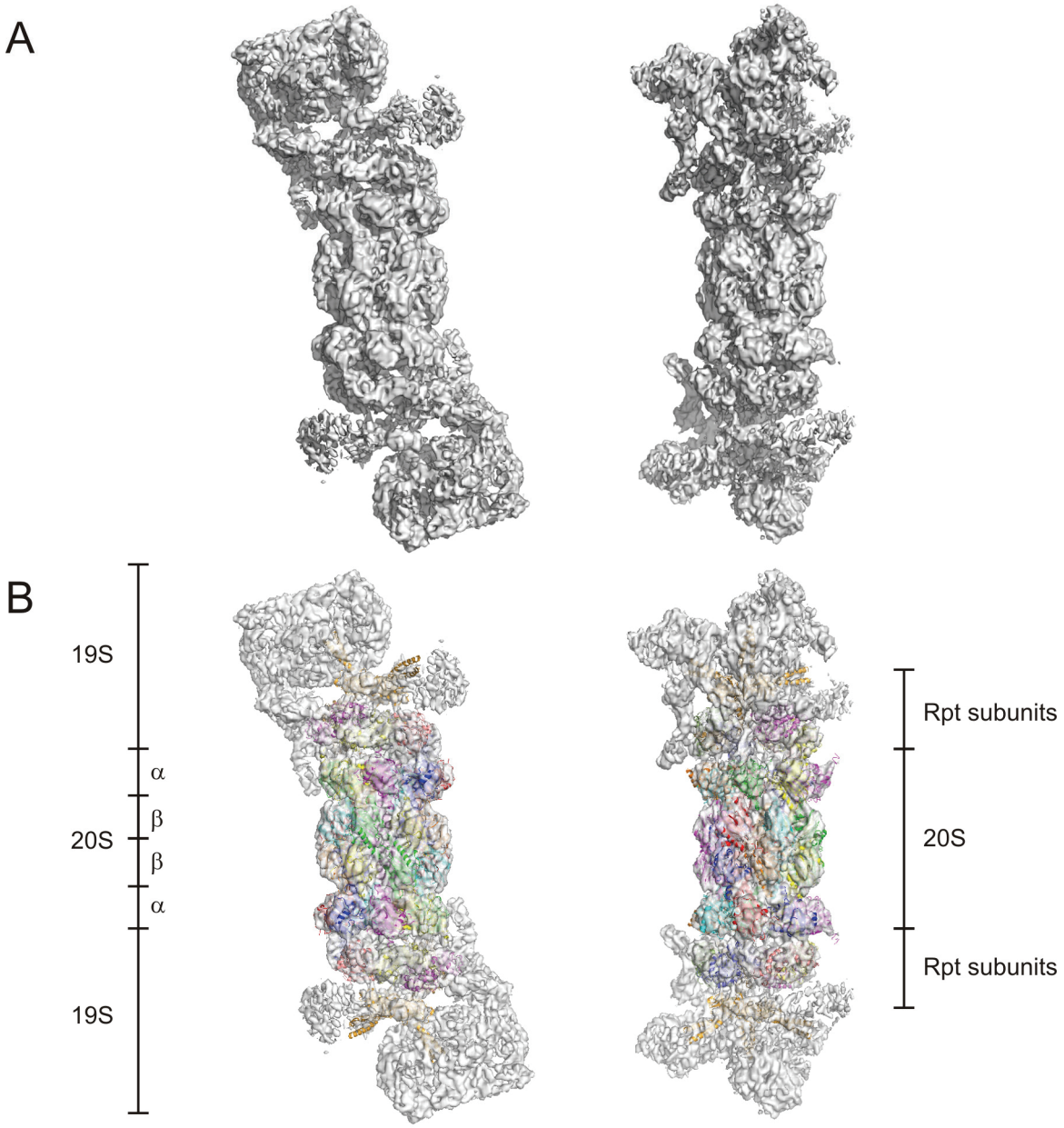


**Figure 1. Structure of the 26S proteasome.** **A:** Surface representations of the 3D map of the double-capped 26S proteasome, viewed along (left) and normal (right) to its two-fold axis. **B:** The map of the 26S proteasome is represented as transparent surfaces, oriented as in A, showing the docked coordinates for the 20S core (coordinates from the bovine 20S core, PDB accession number 1IRU) and a model for the 19S Rpt subunits determined using the coordinates of the different sub-domains of their archaeal homologue, the PAN complex (PDB accession numbers 3H4M and 2WG5). The approximate location of proteasome components is indicated.

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