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Conformational heterogeneity of the AAA ATPase p97 characterized by single particle cryo-EM

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p97, a member of the AAA (ATPase Associated with various Activities) ATPase family, is essential and centrally involved in a wide variety of cellular processes. Single amino-acid substitutions in p97 have been associated with the severe degenerative disorder of Inclusion Body Myopathy associated with Paget disease of bone and Frontotemporal Dementia (IBMPFD) as well as amytropic leteral sclerosis (ALS). Current models propose that p97 acts as a motor transmitting the energy from the ATPase cycle to conformational changes of substrate protein complexes causing segregation, remodeling or translocation. Mutations at the interface between the N and the D1 domains impact the ATPase activity and the conformation of D2 on the opposite side of the protein complex, suggesting intermolecular communication. Because of limited structural information, the molecular mechanisms on how p97 drives its activities and the molecular basis for transmission of information within the molecule remain elusive. Structural heterogeneity is observed in vitro and is likely relevant for the in vivo biological function of p97. Single particle cryo-EM is the method of choice to study a flexible complex. The technique allows study in solution and also deals with sample heterogeneity by image classification. We have set-up the characterization of the conformational heterogeneity in WT and disease relevant p97 mutant using multi-likelihood classification and Hybrid Electron Microscopy Normal Mode Analysis HEMNMA. The multi-likelihood analysis shows a link between the conformations of the N and D2 domains. HEMNMA allows the analysis of the asymmetry of the conformational changes. Together these studies describe the structural flexibility of p97 and the coupling of the ATPase activity with conformational changes in health and in disease. Study of this model system also allows the development of new methods to understand the conformational heterogeneity of other protein complexes.

Keywords: Macromolecular complexes, Structural heterogeneity, Single particle cryo-EM