Protein dynamic analysis by the image processing of cryogenic electron microscopy.

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Since a protein exerts the function by changing its conformation, it is desirable to visualize the continuous change of conformational states at atomic resolution as a movie. Recent technological advancements in cryogenic electron microscopy (cryo-EM) make it possible to determine the structure of biological macromolecules at atomic resolution. A unique strength of cryo-EM is that it does not require researchers to separate and purify each structural state of a protein at the sample preparation step; the structural states can be separated at the image processing stage. At present, there is a high expectation that this advantage can be used to reveal the individual structural states of equilibrium reaction intermediates over several steps with a sample in silico. For this reason, protein dynamics is currently a hot topic in cryo-EM and various approaches to protein dynamics have been proposed.

Recently there are attempts to solve continuous dynamics use computational algorithms that can map a single particle image to a single structural state. Representative examples are CryoSPARC 3D Variability Analysis (3DVA) (based on 3D principal component analysis (PCA)) and CryoDRGN (based on deep learning). Using these new tools, we conducted a continuous dynamics analysis of V-ATPase. Since V-ATPase is a large membrane protein (600-800kDa) and is expected to be in continuous rotational motion, the overall structure of V-type ATPase is an extremely challenging target for protein dynamics analysis. The VO part of V-ATPase transports ions through a membrane using its molecular rotary motor using the energy generated by ATP hydrolysis in the V1 parts. So far, our analysis, combining local 3D refinement and Cryo-EM dynamics analysis with the image processing, successfully captured motions in the VO and V1 parts of V-ATPase separately at high resolution, including the continuous rotational motion of the central axis part (D-F-d) relative to the intramembrane c-ring.