Structural disorder and conformational flexibility in single particle cryo-EM

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Single particle cryo-EM (cryo-electron microscopy) allows high-resolution imaging of macromolecular complexes in close-to-native state, at near-atomic resolutions. Cryo-EM has undergone several technological breakthroughs in microscopy, electron detectors, and image processing that have enabled its use recently in solving high-resolution structures of difficult proteins and complexes [1, 2]. As cryo-EM makes rapid progress, one of the key challenges that remains in the quest for higher resolutions on challenging targets is in dealing with conformational heterogeneity. Established methods for resolving discrete heterogeneity are ineffective in dealing with structural disorder and continuous conformational flexibility. Here, we describe two recent algorithmic developments in the cryoSPARC software system: non-uniform refinement for improving reconstructions in the presence of structural disorder, and 3D variability analysis for resolving continuous conformational motion and dynamics of protein molecules. We demonstrate that non-uniform refinement can improve the quality and resolution of 3D reconstructions for important targets such as membrane proteins, and that 3D variability analysis can capture significant aspects of molecular flexibility and conformational heterogeneity with the potential to reveal new biological insights into protein dynamics and function. [1] D Cressey and E Callaway, Nature 550 (2017), p. 167
[2] X Bai, G McMullan, and S Scheres, Trends in Biochemical Sciences 40 Issue 1 (2017), p. 49-57