

# Outstanding Challenges for Conformational Heterogeneity Analysis in Single-Particle Cryo-EM

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Since the resolution revolution in cryo-EM the method has become a dominant technique for high resolution structure determination of biological macromolecules. However, it is often still treated as a method for obtaining individual high resolution structures, as had been the necessity for decades with X-ray crystallography, despite the potential access it gives to the conformational ensemble of any given molecule. Here we delve into existing techniques for the analysis of conformational heterogeneity of single particle cryo-EM datasets, discussing the caveats of different methodologies, difficulties that arise when analyzing certain types of real datasets (e.g. membrane proteins), and the importance of realistic protocols for fake cryo-EM data generation for the production of ground truth conformational ensembles to benchmark these methods.