3D Flexible Refinement: Determining Structure and Motion of Flexible Proteins from Cryo-EM

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Proteins form the molecular machinery of the cell. They are inherently dynamic, often exhibiting a continuous landscape of conformations, with motion tightly coupled to function. Methods that uncover protein motion and the conformational landscape have the potential to illuminate fundamental questions in structural biology, and to enhance the ability to design therapeutic molecules that elicit specific functional changes in a target protein.

Single particle cryo-EM collects thousands of static 2D protein particle images that, in aggregate, may span the target protein's 3D conformational space. Cryo-EM therefore holds great promise for uncovering both the atomic-resolution structure and motion of biologically functional moving parts [1]. Nevertheless, methods for resolving continuous motion and structure from static 2D images have remained elusive. Established high-resolution cryo-EM refinement methods assume rigidity of the target molecule, and result in blurred, low- resolution density for flexible regions [2, 3, 4].

We introduce 3D Flexible Refinement (3DFlex), a motion-based deep neural network model of continuous molecular heterogeneity from cryo-EM data. 3DFlex directly exploits the knowledge that conformational variability of a protein is often the result of physical processes that transport density over space and tend to preserve local geometry. From 2D image data, 3DFlex enables the experimental determination of high-resolution 3D density and provides an explicit model of a flexible protein's motion over its conformational landscape. Experimentally, for large molecular machines (tri-snRNP spliceosome complex, translocating ribosome) and smaller flexible proteins (TRPV1 ion channel, αV β8 integrin, SARS-CoV-2 spike), 3DFlex can learn multiple highly non-rigid molecular motions while also resolving details of moving secondary structure elements. Further, we show that 3DFlex can improve 3D density resolution beyond the limits of existing methods because particle images contribute coherent signal over a wide range of conformations. These computational advances enable experimental determination of the structure and motion of dynamic proteins and complexes, aiding the study of biological mechanisms and function.

References

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