Tomodrgn: Resolving Structural Heterogeneity in Situ

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Compositional and conformational dynamics are integral to the assembly and function of macromolecular complexes. Fueled by deep learning, new single-particle cryo-EM image analysis tools have revealed these structural dynamics in isolated samples. However, a key goal of structural biology is to interrogate these dynamic structures in their native cellular environment, which would reveal how distinct structural states are partitioned throughout the cell, how they uniquely interact with other cellular components, and how they respond to genetic and environmental perturbations.

Cryo-electron tomography (cryo-ET), which has the potential for high-resolution imaging directly in flash-frozen cells, represents a promising path toward achieving this goal. Indeed, modern cryo-ET workflows have revealed molecularly interpretable, sub-nm structures of key complexes, including the ribosome. To date, most cryo-ET processing algorithms aim to increase resolution by relying on expert-guided classification of structures into a discrete set of approximately homogeneous classes. Such discrete classification models scale poorly to highly heterogeneous ensembles and are inherently ill-match to molecules undergoing continuous motion. To analyze such complex structural ensembles in situ, we developed tomoDRGN, which employs a modified variational autoencoder to embed individual particles in a continuous latent space and to reconstruct unique volumes informed by the latent.

Here, we describe the tomoDRGN model architecture, which was purpose-built for tomographic datasets; we detail its performance on simulated and exemplar experimental datasets, and we highlight tools built to aid in interpreting tomoDRGN outputs in the context of a cellular tomogram. Additionally, we showcase its application to the process of bacterial ribosome biogenesis - specifically comparing the structural ensembles observed in situ with those observed in isolated samples.