Cryo-electron microscopy and 3D reconstruction of single, asymmetric biological molecules

Joachim Frank (joachim@wadsworth.org)

Howard Hughes ...

The methodology for 3D reconstruction of macromolecules in single-particle form from their EM projections (Frank, 1996) borrows from several fields, among these Diffraction Physics, Fourier Optics, and Signal Processing. Among the challenges posed by the specimens are (1) alignment and classification of images with extremely low signal-to-noise ratio (~0.5); (2) determination of orientation of molecules from their noisy projections; (3) 3D reconstruction from unevenly distributed projections; (4) heterogeneity of the molecule population. Although the method goes back to the end of the seventies, real breakthroughs were not achieved until the negative staining technique was replaced, in the mid-eighties, by embedment in vitreous ice. Results obtained with the ribosome exemplify the power of the technique: the currently highest-resolution density map, at 11.5A resolution (Gabashvili et al., 2000), allows individual proteins to be fitted and RNA helices to be recognized. Binding of ligands and the dynamic behavior of the ribosome (see Frank and Agrawal, 2000) can be captured in “snapshots,” from which animations are derived. [Supported, in part, by NIH R37 GM29169 and R01 GM55440]

References

J. Frank (1996)
J. Frank and R.K. Agrawal (2000)
Gabashvili et al. (2000)