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Building, refining and validating atomic models in cryo-EM density maps

The interpretation of the cryo-EM density by atomic models is typically the last step of the structure determination process. Atomic models are worth much more than making for a pretty picture. Frequently, important mechanistic insight is based on those models and producing reliable and accurate atomic models is therefore of great importance. This lecture provides an introduction to strategies and tools developed for the building, refinement and validation of atomic models in cryo-EM density maps. We start with a discussion of resolution of three-dimensional cryo-EM reconstructions and will treat post-processing procedures for map representations such as sharpening, blurring and filtering. We will consider the fundamental challenges for model building in cryo-EM maps, introduce available methods and tools that help address them, work out the similarities and differences to related procedures in X-ray crystallography and discuss some strategies and 'best practices' with challenging examples. We will further explore basic aspects of coordinate refinement strategies in real and reciprocal space and briefly touch upon more advanced issues such as posed e.g. by local variations in effective resolution of cryo-EM maps. Equivalently important are methods for validation of the built atomic models. We will discuss various metrics that assess agreement of the models with the experimental data or rank and validate model geometries based on prior knowledge and expectations from high-resolution structures deposited in community databases.

Selection of recommended reading:

1. Brown, A. *et al.* Tools for macromolecular model building and refinement into electron cryo-microscopy reconstructions. *Acta Crystallogr D Biol Crystallogr* **71**, 136–153 (2015).
2. Murshudov, G. N. Refinement of Atomic Structures Against cryo-EM Maps. *Meth. Enzymol.* **579**, 277–305 (2016).
3. DiMaio, F., Zhang, J., Chiu, W. & Baker, D. Cryo-EM model validation using independent map reconstructions. *Protein Sci* **22**, 865–868 (2013).
4. Falkner, B. & Schröder, G. F. Cross-validation in cryo-EM-based structural modeling. *Proc Natl Acad Sci USA* **110**, 8930–8935 (2013).