

## MS11 Hybrid approaches and validation (X-ray and electron microscopy)

Chairs: Felix Rey, Mathieu Botte

### MS11-O1 How reliable are atomic models based on cryo-EM reconstructions? Improvements in model fitting and validation.

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Recent developments in the field of single particle electron cryo-microscopy (cryo-EM) made it possible to obtain 3D reconstruction based atomic models with the overall quality comparable to X-ray crystal structures. However in comparison to X-ray crystallography, the level of structural details is often less uniformly spread across the entire cryo-EM reconstruction, what causes the major difficulty in obtaining reliable atomic models for not well defined or flexible regions. This raises the need of developing new tools for “multi-resolution” fitting of atomic models and their validation or customization of the usage of existing programs.

Our analysis of local Real Space Correlation Coefficient (RSCC) of over 50 near-atomic resolution structures deposited in the Electron Microscopy Data Bank (EMDB) revealed that only a few of those fit the deposited cryo-EM map at a level commonly accepted for X-ray crystal structures (Figure 1). Among the best fitting atomic models is the structure of 70S ribosome from *Escherichia coli* determined at 2.65-2.9 Å resolution (PDB ID: 5AF1<sup>[1]</sup>) obtained by cryo-EM reconstruction and a pseudo-crystallographic refinement approach employing manual model adjustments, density-guided modeling and energy optimization using the Rosetta package and efficient conformational sampling with the usage of customized “distance-slacked chemical” restraints. Testing the newly established refinement protocol employing customized chemical restraints on the set of analyzed cryo-EM based atomic models will be discussed. Interestingly, re-refinement of analyzed structures revealed significant discrepancies in the highest resolution limit determined by cryo-EM gold-standard FSC and X-ray crystallography derived criteria indicating the need of employing validation techniques assessing to which resolution limit reliable structural features extend.

<sup>[1]</sup> Niels Fischer, Piotr Neumann, Andrey L. Konevega, Lars V. Bock, Ralf Ficner, Marina V. Rodnina & Holger Stark, Structure of the *E. coli* ribosome–EF-Tu complex at <3 Å resolution by Cs-corrected cryo-EM, (2015), Nature 520, 567–570.

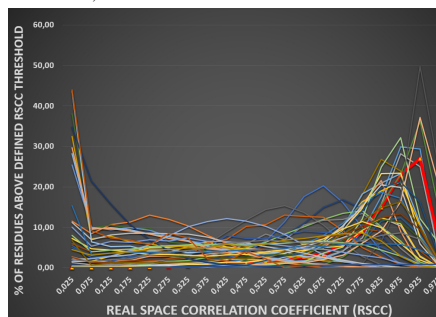


Figure 1. Histogram of RSCC of analyzed cryo-EM structures (5AF1 structure is highlighted in red).

**Keywords:** cryo-EM, model fitting and refinement, validation