MS42 New approaches to structure solution by crystallography and CryoEM: computational features and new algorithms

Chairs: Prof. Isabel Usón, Dr. Tom Burnley

MS42-O1

Solving X-ray crystal structures with cryo-EM reconstructions

Airlie McCoy[1], Randy Read[1], Robert Oeffner[1], Andrea Thorn[2]

1. Department of Haematology, Cambridge Institute for Medical Research, University of Cambridge, Cambridge, United Kingdom
2. Rudolf-Virchow-Zentrum, Würzburg, Germany

email: ajm201@cam.ac.uk

Electron density from electron microscopy reconstructions has been used for molecular replacement phasing of x-ray crystal structures since the pioneering work on icosohedral viruses [1]. Both negative stain and cryo-EM images have been used. The method is useful when the EM reconstructions are lower resolution that the X-ray data, so that phase extension is required after molecular replacement. The refined, higher resolution structure may then be docked back into the electron microscopy reconstruction, where it may form part of a larger macromolecular complex. The methods for using electron microscopy reconstructions for models for molecular replacement have much in common with the so-called cross-crystal averaging methods standard to X-ray crystallography. However, there are some issues particular to their use, chief amongst which is accommodating scale factor errors in the reconstruction. Marked differences in the low resolution Fourier terms, and the presence of high internal model symmetry can also present challenges for molecular replacement. Recent optimizations of the Phaser [2] algorithms to improve molecular replacement with electron microscopy reconstructions will be discussed.

References:

[1] Wynne SA, Crowther RA & Leslie AGW (1999), Mol. Cell. (3) 771-780

[2] McCoy AJ, Grosse-Kunstleve RW, Adams PD, Winn MD, Storoni LC, & Read RJ. (2007) J Appl Cryst. (40) 658-674.

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MS42-O2

Cryo-EM and X-ray crystallography as complementary methods for structure determination

Holger Stark[1]

1. Department of Structural Dynamics, Goettingen, Germany

email: hstark1@gwdg.de

Single particle cryo-EM has recently gained a lot of attention because numerous structures of large biological macromolecular complexes have been determined at high resolution. The number of map depositions in the EMDB database is constantly increasing and structures of complexes are being published that represented evasive targets for X-ray crystallography for a long time. It is therefore timely to ask what are the differences in maps obtained by cryo-EM and X-ray crystallography? Are the quality standards the same for both methods? What are the requirements on the sample for successful structure determination either by X-ray or cryo-EM? Which technique works best for a given complex and how does the information content differ? These questions will be addressed in the presentation based on our studies of large macromolecular complexes like the proteasome, ribosome and spliceosome.

Keywords: cryo-EM, macromolecular complexes