MS70.O03

Preparation of single-layered enzyme 2D crystals for electron crystallography

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Electron crystallography allows for a wide range of membrane proteins to be studied once conditions for two-dimensional (2D) crystallization have been identified. Two-dimensional crystallization is most frequently achieved via the dialysis approach, where the detergent-solubilized membrane protein is reconstituted into a lipid bilayer [1]. Vesicles, planar-tubular crystals, and sheets are the three most common 2D crystal morphologies. Vesicle and planar-tubular morphologies are observed for the largest percentage of 2D crystals of membrane proteins. Upon negative stain as well as electron cryo-microscopy (cryo-EM) grid preparation, each planar-tubular and vesicle 2D crystal will result in two ordered bilayers that can be analyzed separately by image processing. If any of these morphologies, however, contains a larger number of stacked crystals, data of tilted crystal stacks in particular can currently not be analyzed. Sheets constitute the most desirable morphology, allowing for the preparation of very flat samples for cryo-EM [2]. This is at present the only type of morphology that may be amenable to collection and analysis of electron diffraction data of highly ordered samples [3]. We could reproducibly induce single-layered sheet formation in the large majority of 2D crystals of two different enzyme samples and are working towards a general protocol applicable to other membrane protein 2D crystals.

[1] *Electron crystallography of soluble and membrane proteins. Schmidt-Krey, I. & Cheng, Y. (Eds.), Springer, New York, 586 pages, 2013.,* ***[2]*** *W Kühlbrandt and KH Downing , J Mol Biol , 1989, 207, 823.,* ***[3]*** *T Gonen, Meth Mol Biol, 2013, 955, 153.*

Keywords: Membrane proteins, Two-dimensional crystallization, Electron crystallography