Structure of membrane proteins in action by subtomogram averaging

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Membrane proteins perform key functions in human body and are prominent drug targets. Functions of membrane proteins are intrinsically based on the presence of lipid bilayers. Membrane composition and concentration gradients across membranes modulate the action of channels and transporters from bacteria to humans. Versatility of cryo-EM enables a hybrid approach of obtaining structures at near-atomic resolution by single particle cryo-EM and combining it to the structures *in situ* such as membrane proteins in membrane vesicles under concentration gradients, although at lower resolution.

During my talk I will demonstrate the power of this hybrid approach, applied to bacterial secretion systems [1] and the parametric serotonin-gated ion channel 5HT-3 [2]. Importantly, in order to obtain biologically relevant information about function of ion channels, it is required to achieve high(er) resolution for *in situ* structural analysis. I will discuss progress on *in situ* structure determination of ion channels at high(er) resolution by subtomogram averaging made by my group and as a part of the Dynamo software [3,4].

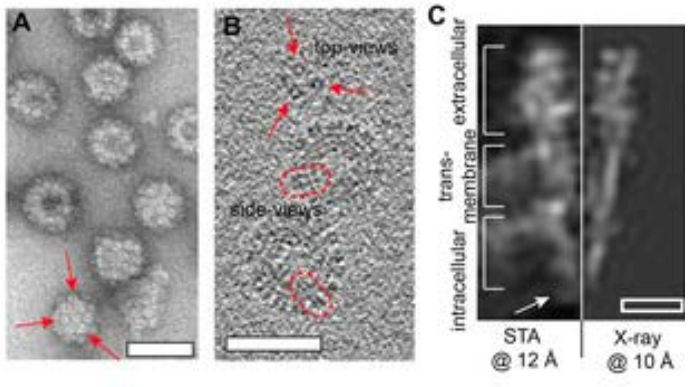

Figure 1: Approach of determining membrane protein structure under a concentration gradient. A,B) Purified proteins are re-constituted into closed lipid vesicles buffer exchange allows generating concentration gradients followed by cryo electron tomography (in B). C) Structure of the mouse serotonin receptor 5HT-3 in lipid vesicles at a resolution of 12 Å by subtomogram averaging compared to an X-ray structure filtered to 10 Å. Figure adapted from [2].

References

[1] - Kudryashev et al, Structure of the Type VI Secretion System Contractile Sheath, Cell, 2015, 5:952-962.
[2] - Kudryashev et al, The Structure of the Mouse Serotonin 5-HT3 Receptor in Lipid Vesicles, Structure, 2016, 24:165-170.
[3] - Castaño-Díez et al, Dynamo Catalogue: Geometrical tools and data management for particle picking in subtomogram averaging of cryo-electron tomograms, J. Struct. Biol., 197 (2017) 135-144.
[4] - Castaño-Díez et al, Dynamo: A flexible, user-friendly development tool for subtomogram averaging of cryo-EM data in high-performance computing environments, 178 (2012) 139-151.