Title

Robust membrane processing for automatic protein sampling in cryo-electron tomograms with TomoCHAMPS

Cyan Ching[1], Julien Maufront[1], Aurelié Di Cicco[1,2], Manuela Dezi[1,2], & Daniel Lévy[1,2]

Affiliations for all authors

1. Institut Curie, Université PSL, Sorbonne Université, CNRS UMR 168, Laboratoire Physico Chimie Curie,75005 Paris, France.
2. Sorbonne Université, Paris, France

Abstract

Cryo-electron tomography (cryo-ET) allows visualisation of molecular structures in their native states and context at near-atomic resolution. Due to technical restrictions in image acquisition such as the low intrinsic contrast of biological materials and minimised electron dose to prevent radiation damage in exposed sample, sophisticated computational methods are required to accurately extract and enhance signal of target objects. In particular, small membrane proteins (< 150 kDa) exhibit weak signal. To obtain high-resolution structural information of such proteins, increasingly large datasets are acquired and mined. While existing softwares demand extensive manual curation in handling membrane protein cryo-ET data, we present TomoCHAMPS (**tomo**graphy-based **ch**aracterisation and **a**nalysis of **m**embranes for **p**rotein **s**ampling): an automatic image processing workflow dedicated to cryo-ET based characterisation and analysis of membranes for resolving membrane protein structures. By integrating improved open-source tools/methods and in-house scripts in a configurable manner, TomoCHAMPS is expected to support efficient processing of large *in vitro* and cellular transmembrane and membrane-binding protein cryo-ET datasets up until subtomogram averaging with minimised manual input required. Main features of TomoCHAMPS include pre-processing, tilt series alignment, 3D reconstruction, membrane segmentation within user-defined regions of interest, geometric analysis of membranes, and membrane protein sampling. Here, we showcase the application of TomoCHAMPS to three *in vitro* reconstituted membrane contact site cryo-ET datasets aiming at resolving small membrane protein structures (present as dimers, size range: ~55-80 kD).