Laboratoire Physico-Chimie Curie
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TITRE DU STAGE :

Reaching sub-nanometric resolution of membrane bound proteins by sub-tomogram averaging and cryo-electron tomography

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Ce stage peut être poursuivi en thèse : OUI
Si oui, la thèse est-elle financée : possible
SUJET du stage de M2 :

Electron cryo-microscopy is revolutionizing structural biology and protein structure determination. It allows to determine the structure of proteins purified and homogeneous at atomic resolutions. Since the award of the Nobel Prize in Chemistry in 2017, computational instrumental and analytical developments have been carried out and opened up new fields of application. One of these development axes is the analysis of complex, multicomponent and heterogeneous systems in terms of conformation and flexibility. The aim is to determine the structure of proteins in situ within cells or of multicomponent machinery reconstituted in vitro. For this purpose, computational analysis developments are in progress for 3D reconstruction that take into account the heterogeneity and flexibility of biological objects.
In this context, our team is interested in the 3D architecture of machinery composed of proteins associated with membranes and involved in major cellular functions (see https://science.institut-curie.org/research/multiscale-physics-biology-chemistry/umr168-physical-chemistry/team-levy.). To do this, we designed in vitro systems and obtained cryo-tomography data sets of two types of samples on state of the art microscopes. The first, made up of protein filaments assembled on membranes, must allow sub-nanometric resolutions to be achieved. The challenge is to sort out the variability of filament organization. The second is made of proteins that bridge two membranes. The challenge is to sort out the flexibility of the assemblies. In both cases, this requires image analysis approaches called subtomogram averaging. Briefly, sub-volumes extracted from cryo-tomograms are compared and aligned with a template by applying a basic algorithm of iterative refinement, consisting in a refinement loop in which geometrical transformations are applied to every particle, and a selection process followed by averaging. The most common procedure is iterative refinement based on cross correlation optimization, specifically the Roseman's fast scheme (Roseman, 2003) to restrict the computation to a selected region of interest when comparing particles with the template. Structural heterogeneity analysis is derived from 3D Classification based in principal component analysis (PCA) and multireference alignement (MRA).

The student must have training in computational analysis, bioinformatics or physics and an interest in cryo-electron microscopy and structural biology. Knowledge of (*or at least familiar with*) Matlab or Python will be a plus.