Master 2 research internship in Integrated Structural & Cell Biology in Grenoble

Supervisor(s):
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Host laboratory:
IBS, https://www.ibs.fr/

Host group/team:
MEM: Methods and Electron Microscopy / Schoehn team

Title of the M2 research internship:

Development of 3D nano-sized protein crystal electron diffraction for structural biology

Project summary:
Since electrons are much more strongly diffracted by protein crystals (4 to 5 orders of magnitude) than X-rays, they constitute an ideal probe to study nano-sized crystals. Recent studies have shown that electron diffraction could be used to solve peptide or protein structures up to sub-atomic resolution, from 3D crystals that did not exceed 200 nm in at least one dimension. Since the first publication in 2013, about 70 protein structures solved by 3D electron diffraction (3D-ED) have been deposited in the PDB, and the rapid evolution of direct electron detectors should make 3D-ED an essential and complementary tool in structural biology. Our group has now a state of the art equipment, including a 200 kV cryo-electron microscope F20 and a Medipix 512x512 hybrid pixel detector that allow us to collect complete diffraction data on protein, peptide and small molecule nano-sized 3D crystals, at very low electron dose.
The present internship project aims at further developing the technique in order to broaden its use in structural biology. It includes sample preparation, in order to produce 3D crystals of suitable size by different approaches including optimized crystallization conditions or the use of either a cryo-microtome or a focused ion beam (FIB) for the machining of very thin crystal lamella. Automation of the data collection with SerialEM will also being developed. The main goal is to go towards serial electron diffraction, by locating crystals on an atlas of the grid and then collect rotation diffraction data for each identified crystals. Data processing will be carried out with software such as XDS or DIALS. If possible structure refinement will be carried out with the CCP4 suite of programs, including corrections for multiple diffraction and charge contribution. Protein samples either available in our group or provided by collaborators will be used during this training.
A background in structural biology or physics would be preferable. Some knowledge in Python programming may be a plus.

Keywords:
electron diffraction, 3D protein nanocrystals, structural biology

Relevant publications of the team:

Blum TB, Housset D, Clabbers MTB, van Genderen E, Bacia-Verloop M, Zander U, McCarthy AA, Schoehn G, Ling WL & Abrahams JP (2021) Statistically correcting dynamical electron scattering improves the refinement of protein nanocrystals, including charge refinement of coordinated metals. Acta Crystallogr Sect Struct Biol 77, 75–85.

Moriscot C, Schoehn G & Housset D (2023) High pressure freezing and cry-sectionning can be used for protein structure determination by electron diffraction. To be submitted to Ultramicroscopy