



Projet de stage Master 2 (2018-2019)
Master 2 internship project (2018-2019)

Laboratoire : Institut de Biologie Structurale (Campus EPN, Grenoble)

Directeur: Winfried WEISSEHORN

Intitulé de l'équipe : Microscopie Électronique et Méthodes **Responsable :** Guy SCHOEHN

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Title of the project

Development of electron diffraction for 3D nano-sized crystals of proteins

Background: Electrons are much more strongly diffracted by protein crystals (4 to 5 orders of magnitude) than X-rays. Thus, 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 at at least 2 Å resolution, from 3D crystals that did not exceed 300 nm in each dimension. Since the first publication in 2013, about 15 structures solved by 3D electron diffraction (also called microED) have been deposited so far in the Protein Data bank, and the rapid evolution of direct electron detectors should enable the microED technique to become an essential and complementary tool in structural biology. Our group have worked on structural resolution by microED of peptides and proteins, including lysozyme and insulin crystals in collaboration with J.P Abrahams's group in Basel.

Objective: With the aim of installing a microED platform at the IBS, we would like to demonstrate the general potential of the technique to solve macromolecular structures from a few nano-crystals. To this end, we need to establish the best procedures for producing nano-crystals, and for preparing cryo-grids with the crystals. We also need to optimize strategies to collect electron diffraction data and process them for their use in structure refinement. This novel approach is very promising, but potential problems such as radiation damage, multiple scattering, and use of proper atomic form factors need to be investigated and taken into account.

Description: The work to be performed will include crystal preparation with different proteins available in our group, optimization of crystallization conditions and grid preparation, collection of diffraction patterns on our electron microscopes and assessment of diffraction quality. For suitable samples, full data sets will be collected and data processing will be carried out with software such as XDS or DIALS.

Relevant references

1. Shi, D., Nannenga, B. L., Iadanza, M. G. & Gonen, T. Three-dimensional electron crystallography of protein microcrystals. *Elife* **2**, e01345 (2013).
2. Clabbers, M. T. B. *et al.* Protein structure determination by electron diffraction using a single three-dimensional nanocrystal. *Acta Cryst D* **73**, 738–748 (2017).
3. Liu, S. & Gonen, T. MicroED structure of the NaK ion channel reveals a Na⁺ partition process into the selectivity filter. *Commun Biol* **1**, (2018).

Requested background and domain of expertise: Physicist with interest in structural biology, Bio-physicist or Biologist interested in structural biology and methods developments 