

GRAL MSc RESEARCH SCHOLARSHIP 2020-2021

RESEARCH INTERNSHIP PROPOSAL

Institute / Group

IRIG / IBS – MEM

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Research Project Title

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

Description of the project

Since electrons strongly interact with biological matter, they constitute an ideal probe to study nanosized crystals. Recent studies have shown that electron diffraction enables to solve peptide or protein structures at at least 2 Å resolution, using 3D crystals that did not exceed 200 nm in each dimension. Since the first publication in 2013, about 40 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 is developing a 3D-ED platform at the IBS, with the aim of demonstrating the general potential of the technique to solve macromolecular structures from a few nano-crystals. To this end, we will establish the best procedures for producing nano-crystals, preparing cryo-grids with the crystals and collecting and processing electron diffraction data 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. The work to be performed will include crystal preparation with different proteins available in our group or provided by collaborators, optimization of crystallization conditions and grid preparation, 3D-ED data collection on the F20 cryo-electron microscope equipped with a dedicated direct detector. Serial-EM will be used to optimize data collection. 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. A background in structural biology or physics would be preferable

Keywords

Electron diffraction, protein crystallography, nanocrystals, structure détermination, refinement

Relevant publications of the team

Ohayon, D., De Chiara, A., Dang, P.M.-C., Thieblemont, N., Chatfield, S., Marzaioli, V., Burgener, S.S., Mocek, J., Candalh, C., Pintard, C., et al. (2019). Cytosolic PCNA interacts with p47phox and controls NADPH oxidase NOX2 activation in neutrophils. PCNA controls NOX2 activation in neutrophils. *J. Exp. Med.* 216, 2669–2687.

Vassal-Stermann, E., Effantin, G., Zubieta, C., Burmeister, W., Iseni, F., Wang, H., Lieber, A., Schoehn, G., and Fender, P. (2019). CryoEM structure of adenovirus type 3 fibre with desmoglein 2 shows an unusual mode of receptor engagement. *Nat. Commun.* 10, 1181.
