

**Master 2 internship project
Year 2020-2021**

Laboratory/Institute: MEM/IBS
Team: Microscopie Electronique

Director: Winfried Weissenhorn
Head of the team: Guy Schoehn

Name and status of the scientist in charge of the project:

Wai Li Ling, Chercheuse **HDR:** yes no

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Program of the Master's degree in Biology:

- Immunology, Microbiology, Infectious Diseases Integrative Structural Biology
 Physiology, Epigenetics, Differentiation, Cancer Neurosciences and Neurobiology
 Planta International

Title of the project:

Cryo-electron microscopy structure of connexin26

Objectives (up to 3 lines):

The main objective of the internship is to acquire training in cryo-electron microscopy sample preparation and single particle reconstruction for resolving 3D protein structure. In particular, we aim to obtain 3D classes for the wild-type or recombinant Cx26 samples.

Abstract (up to 10 lines):

Membrane protein connexins (Cx) constitute gap junction channels formed by two connexons (hexamers of Cx) in adjacent cells. The gap junction channels allow intercellular communication but may also work as adhesion molecules supporting mechanical forces, a function largely ignored [1]. Resolving the Cx structure is fundamental to understanding gap junctions and mutant-related pathologies. For instance, abnormal expression of Cx26 can lead to deafness or cancer. In collaboration with the laboratory of Prof. Felix Rico at Aix-Marseille University, wild type and mutant Cx26 are expressed for single particle analysis to investigate the structural mechanisms of gap junction binding strength. Hexamers and dodecamers of connexins will be observed by negative staining and cryo-electron microscopy [2]. Structures will be complemented by force measurements using atomic force microscopy at Aix-Marseille University [3].

Methods (up to 3 lines):

Cx26 samples will be screened by negatively staining electron microscopy. Suitable samples will be vitrified on electron microscopy grids and cryo-electron microscopy images will be collected. Open source software RELION will be used to analyze the data for single particle reconstruction.

Up to 3 relevant publications of the team:

[1] Two-dimensional kinetics of inter-connexin interactions from single-molecule force spectroscopy. **Rico F**, Oshima A, Hinterdorfer P, Fujiyoshi Y, Scheuring S. *Journal of Molecular Biology* (2011) **412**, 72-79.

[2] Near-atomic cryo-EM structure of helical measles virus nucleocapsid. Gutsche I, Desfosse A, Effantin G, **Ling WL**, Haupt M, Ruigrok RWH, Sachse C, and **Schoehn G**. *Science* (2015) **348**, 704-707.

[3] **F. Rico**, L. Gonzalez, I. Casuso, M. Puig-Vidal, S. Scheuring, High-Speed Force Spectroscopy Unfolds Titin at the Velocity of Molecular Dynamics Simulations, *Science*. 342 (2013) 741–743.

Requested domains of expertise (up to 5 keywords):

computer skills, structural biology, transmission electron microscopy