**PhD PROPOSAL FOR THE**

**PASTEUR - PARIS UNIVERSITY INTERNATIONAL DOCTORAL PROGRAM**

Time for applicants to contact host laboratories: September 13 – November 2, 2017

Deadline for full application: November 13, 2017

Interviews: January 30, February 2, 2018

Start of the Ph.D.: October 1, 2018

**Title of the Full Ph.D. project:** Structural and functional characterization of intact pestivirus non-structural membrane protein 2 (NS2).

**Keywords:** Pestivirus, uncleaved NS2-NS3, membrane protein expression and purification, x-ray crystallography, single-particle cryo-electron microscopy (cryo-EM).

**Department:** Virology.

**Name of the lab:** Structural Virology (Unité de Virologie Structurale).

**Head of the lab:** Prof. Dr. Félix A. Rey

**Ph.D. advisor:** M. Alejandra Tortorici (PhD, HDR)/ Prof. Félix A. Rey (PhD, HDR).

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**Web site address of the lab:** https://research.pasteur.fr/fr/team/structural-virology/

***Doctoral school affiliation and University*:** Ecole Doctorale MTCI (Médicament-Toxicologie-Chimie-Imageries). Université Paris-Descartes.

Presentation of the laboratory and its research topics:

Two of the main scientific goals of our research are:

1) to provide a structural basis for understanding the molecular mechanisms of membrane fusion used by enveloped viruses to enter into a target cell, and

2) to understand the molecular mechanism used by RNA viruses to replicate their genome by both structural and functional studies of viral RNA-dependent RNA polymerases (RdRp) and associated replication enzymes.

We study mostly viruses of global public health and/or of veterinary concern. The knowledge gained can be used for translational structure-based design of preventive or curative antiviral agents. Furthermore, these structural studies often provide crucial information about evolutionary relations between apparently unrelated viruses.

Our initial studies of the three-dimensional (3D) structure of the flavivirus and of the alphavirus envelope proteins introduced the concept of class II viral fusion proteins, which are formed by three globular domains -essentially constituted by -sheets- arranged in a rod-like shape as opposed to the first described class I viral fusion proteins, which are characterized by a central trimeric -helical coilded coil. We have also described the structures of rubi- and bunya-viruses envelope proteins, also belonging to the class II fusion proteins and more recently, we described the first structure of the class I coronavirus spike ectodomain in its pre-fusion form, determined using cryo-EM.

In addition, we have structurally and functionally characterized non-structural proteins such as the RdRp of the hepatitis C virus and more recently the pestivirus NS3 protease/helicase in complex with its cofactor, and enzyme that is also essential for viral genome replication and virus morphogenesis.

Description of the project:

Introduction: Pestiviruses infect a wide range of cloven-hoofed animals, wild and domestic, causing serious disease. The most studied are the classical swine fever virus (CSFV) ([1](#_ENREF_1)) and the bovine viral diarrhea virus, which impose important economic losses to the livestock industry worldwide ([2](#_ENREF_2)). Together with the closely related human hepatitis C virus (HCV) and flaviviruses like dengue or Zika viruses, pestiviruses belong to the *Flaviviridae* family of positive-sense RNA viruses. Their genome codes for a single polyprotein, which is processed by cellular and viral proteases to generate the individual mature viral proteins. The first proteolytic cleavage in the non-structural region is done by NS2, a 460 residues multi-pass trans-membrane cysteine protease. The remaining cleavages are done by NS3, which also has helicase activity. Both, NS2 and NS3 are essential for genome replication but also for virus morphogenesis. For pestiviruses, and in contrast to HCV, the cleavage between NS2 and NS3 in the polyprotein precursor is necessary for genome replication but abolishes particle morphogenesis. Upon infection, a host factor named Jiv, necessary for cleavage between NS2 and NS3, is available in the cytoplasm, but as replication ensues, Jiv becomes limiting and later rounds of polyprotein production lead to absence of cleavage thereby allowing virus morphogenesis.

State-of-the-art: This project follows our recent functional and structural characterization of the pestivirus CSFV NS3 ([3](#_ENREF_4), 4). By using structure-guided mutagenesis we have identified that NS4A appears to bind at a surface of NS3 that is occupied by NS2 in the uncleaved form (4). Thus, we identified a dual effect: while the NS2-NS3 junction is cleaved, NS4A can displace NS2 from the complex, and replication ensues. When NS3 is covalently linked to NS2, the NS4A factor, necessary for replication cannot bind, so replication cannot take place. This elegant control system, explains how pestiviruses temporally orchestrate the switch from genome replication to virion morphogenesis. Central to further understanding this process is determining the structure of its components, the main one missing being NS2, which has eluded structural studies so far (only its soluble cytosolic domain is known for HCV (5)).

Goals: 1. To determine the 3D-structure of the multi-pass trans-membrane NS2 protein. We have already identified promising conditions for protein production and detergent solubilization compatible with crystallization in collaboration with Nicolas Reyes on campus, who is an expert in membrane protein production for crystallization.

2. To understand the organization of the uncleaved NS2-NS3 protein on membranes, we are also preparing the ground to use cutting-edge single-particle cryo-EM to pursue this study, in combination with lipid nanodisc technology (6). The structural information obtained is expected to lead to the discovery of new strategies for antiviral research.

Step-wise research plan: 1) Optimization of constructs to produce high amounts of full-length CSFV NS2 in mammalian cells to be solubilized and inserted into nanodiscs to reconstitute the protein in its native environment. 2) Crystallization of CSFV NS2 using standard methods for membrane protein crystallization such as lipid cubic phase. 3) Determination of the 3D-structure of CSFV NS2 by X-ray crystallography or cryo-EM. 4) Development of functional studies to validate our structural data.

Optional project:

We recently described the first structure of the class I coronavirus (CoV) spike (S) ectodomain in its pre-fusion form, determined using cryo-EM ([7](#_ENREF_6)). There is an increasing evidence, however, that the transmembrane and the cytosolic regions of class I proteins modulate the antigenicity of the exposed ectodomain as shown for HIV-1 envelope trimer (8). Therefore, it is crucial to understand the conformation of these regions in order to identify conformational epitopes to be targeted for efficient vaccine design. Thus, our goal is to determine the 3D-structure of a CoV full-length S protein embedded in a native bilayer environment by cryo-EM combined with lipid nanodisc technology ([6](#_ENREF_8)).

**NOTE:** Both projects have a similar technical approach so the choice of the final project will depend on the particular interests of the selected student.

References:

*1. Rossi S, Toigo C, Hars J, Pol F, Hamann JL, Depner K, Le Potier MF. 2011. New insights on the management of wildlife diseases using multi-state recapture models: the case of classical swine fever in wild boar. PLoS One 6:e24257.*

*2. Lindenbach BM, CL, Thiel, HJ, Rice, CM. 2013. Flaviviridae, p. 712-746. In Knipe DaH, PM (ed.), Fields in Virology, 6th ed. Lippincot Williams & Wilkins, Philadelphia.*

*3. Tortorici MA, Duquerroy S, Kwok J, Vonrhein C, Perez J, Lamp B, Bricogne G, Rumenapf T, Vachette P, Rey FA. 2015. X-ray structure of the pestivirus NS3 helicase and its conformation in solution. J Virol 89:4356-4371.*

*4. Dubrau D\*, Tortorici MA\*, Rey FA, Tautz N. 2017. A positive-strand RNA virus uses alternative protein-protein interactions within a viral protease/cofactor complex to switch between RNA replication and virion morphogenesis. PLoS Pathog 13:e1006134. \*Equally contribution.*

*5. Lorenz IC, Marcotrigiano J, Dentzer TG, Rice CM. 2006. Structure of the catalytic domain of the hepatitis C virus NS2-3 protease. Nature 442:831-835.*

*6. Gao Y, Cao E, Julius D, Cheng Y. 2016. TRPV1 structures in nanodiscs reveal mechanisms of ligand and lipid action. Nature 534:347-351.*

*7. Walls AC\*, Tortorici MA\*, Bosch BJ\*, Frenz B, Rottier PJ, DiMaio F, Rey FA+, Veesler D+. 2016. Cryo-electron microscopy structure of a coronavirus spike glycoprotein trimer. Nature 531:114-117. \*Equally contribution. +co-corresponding authors.*

*8. Dev J, Park D, Fu Q, Chen J, Ha HJ, Ghantous F, Herrmann T, Chang W, Liu Z, Frey G, Seaman MS, Chen B, Chou JJ. 2016. Structural basis for membrane anchoring of HIV-1 envelope spike. Science 353:172-175.*

Expected profile of the candidate:

Applicants should have a high degree of motivation and a willingness to work in a very competitive field. A previous experience with DNA techniques (PCR, cloning, agarose gels, plasmid DNA purification etc), cell culture and protein expression and purification techniques (affinity columns, gel filtration, protein gels, etc) would be also greatly appreciated.

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