**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

Department: **Virology**

**Title of the PhD project:** Study of the interplay between nucleoporins and chromatin factors to orchestrate HIV-1 replication.

**Keywords:** HIV-1, ChIP-sequencing, mass spectrometry, super-resolution microscopy, nuclear pore complex, chromatin.

**Department**: Virology

**Name of the lab:** Molecular Virology and Vaccinology

**Head of the lab:** Pierre Charneau

**PhD advisor:** Francesca Di Nunzio

**Email address**: dinunzio@pasteur.fr

**Web site address of the lab:** https://research.pasteur.fr/en/member/francesca-di-nunzio/

***Doctoral school affiliation and University*:** Biochimie, Biothérapies, Biologie Moléculaire et Infectiologie (B3MI) (Paris Diderot 7)

Presentation of the laboratory and its research topics:

Our lab, historically, has been deeply involved in studying the nuclear import step of HIV-1 life cycle, which is a key step for the replication of the virus[1](#_ENREF_1),[2](#_ENREF_2). New insights into the interaction between the nuclear pore complex (NPC) and HIV-1 pre-integration complex straighten our efforts in a better comprehension of the molecular mechanism underlying host-pathogens interaction. Recently, we showed that nuclear pore factors are essential for HIV-1 nuclear translocation and integration[3](#_ENREF_3). We distinguished the individual role of nucleoporins in the early steps of HIV-1 life cycle[4-7](#_ENREF_4). In particular, using high resolution imaging techniques in collaboration with the team of Christophe Zimmer (particularly FlAsH-PALM) [8](#_ENREF_8),[9](#_ENREF_9) we were able to set up an approach which will allow to investigate important questions about the early steps of HIV-1 life cycle, such as, where and when the uncoating (loss of the viral capsid) step happens and to zoom on the interaction among the viral components, the NPC and the host chromatin. The proposed project aims to unravel the involvement of specific nuclear pore factors in chromatin remodeling, which could play an important role in HIV-1 life cycle. For this project, we plan to combine approaches from virology, microscopy, transcriptomics, biochemistry, high-throughput pyrosequencing and cell biology, in close collaboration with several teams located in USA, in Italy and at the Pasteur Institute. Several French and European funding support the aforementioned projects.

Description of the project:

NPCs are stable structures with specific functions in nuclear transport, genome organization, genome stability and gene expression regulation[10](#_ENREF_10). Non dividing cells are the major target of HIV-1, thus its passage through the NPC is a key step for viral replication.

Several studies, including ours, investigated the mechanistic requirements of nucleoporins (Nups) in the HIV-1 life cycle. However, the study of the individual role of Nups in HIV-1 infection is complicated, because many Nups act as scaffold for others, thus their structural association is a major difficulty in determining the role of individual Nups in HIV-1 infection.

We previously identified the first cellular factor responsible for HIV-1 docking at the nuclear pore, Nup358/RanBP2, which interacts with assembled *in vitro* cores[6](#_ENREF_6).

Recently, we unraveled the distinct roles of two nuclear basket Nups, Nup153 and Tpr, in HIV-1 infection. We observe that Nup153 participates in HIV-1 nuclear import independently of the integrity of the nuclear basket using a complementation assay in Nup153 depleted cells. However, when we disrupted the integrity of the nuclear side of the NPC, which is mainly composed of Tpr, by knocking down Tpr, we dramatically reduced HIV-1 infectivity, but not the level of integration. Tpr organizes the chromatin underneath NPCs by excluding heterochromatin under pores. Therefore, we investigated whether these results could reflect the role of Tpr as regulator of chromatin organization underneath the NPC and of gene expression on the chromatin surrounding the integrated provirus. We report the first comparative study correlating the chromatin features surrounding the integration sites in Tpr depleted cells infected with HIV-1 and the spatial chromatin state underneath NPCs by super resolution microscopy. We showed that alteration of chromatin organization underneath the NPCs in Tpr depleted cells leads to viral silencing and HIV integration in less actively transcribed chromatin regions.

Our data support a model in which HIV-1 nuclear import and integration are concerted steps, and where Tpr maintains a chromatin environment favorable for HIV-1 replication.

Our research is currently focused on the interplay between HIV-1 components, NPC and chromatin. We aim to unravel the link between nuclear import, chromatin organization and transcriptional regulation. Interestingly, some nucleoporins interact with actively transcribed chromatin[10](#_ENREF_10) which is also the target of HIV-1 integration.

We aim to exploit Nups, to identify complexes composed by the target Nups, HIV-1 components, chromatin and nuclear cellular factors. These complexes may work in concert with Nups to orchestrate the viral replication underneath the nuclear pore complex.

In particular, Nup153 has a critical role in HIV-1 nuclear import, however it is still unknown how this Nup leads HIV-1 to use the pore. Besides, nuclear basket Nups bind particular chromatin regions and regulate genes activity, thus, our aim is to understand how Nups, chromatin factors and genes are concerted to orchestrate HIV-1 replication. Nuclear basket Nups may be another “cellular code” for specifying HIV-1 fate through their contacts with the underlying chromatin.

For this project, the PhD student will benefit of the expertise of the lab in early steps of HIV-1 replication cycle and of the development of innovative and trans-disciplinary approaches.

Overall, this study should add an important piece to our understanding of HIV-1 replication mechanisms and could serve in the development of new antiviral strategies.

References:

1 Zennou, V. *et al.* HIV-1 genome nuclear import is mediated by a central DNA flap. *Cell* **101**, 173-185, doi:S0092-8674(00)80828-4 [pii]

10.1016/S0092-8674(00)80828-4 (2000).

2 Arhel, N. J. *et al.* HIV-1 DNA Flap formation promotes uncoating of the pre-integration complex at the nuclear pore. *EMBO J* **26**, 3025-3037, doi:7601740 [pii]

10.1038/sj.emboj.7601740 (2007).

3 Lelek, M. *et al.* Chromatin organization at the nuclear pore favours HIV replication. *Nat Commun* **6**, 6483, doi:10.1038/ncomms7483

ncomms7483 [pii] (2015).

4 Valle-Casuso, J. C. *et al.* TNPO3 is required for HIV-1 replication after nuclear import but prior to integration and binds the HIV-1 core. *J Virol* **86**, 5931-5936, doi:10.1128/JVI.00451-12

JVI.00451-12 [pii] (2012).

5 Di Nunzio, F. *et al.* Nup153 and Nup98 bind the HIV-1 core and contribute to the early steps of HIV-1 replication. *Virology* **440**, 8-18, doi:10.1016/j.virol.2013.02.008

S0042-6822(13)00094-9 [pii] (2013).

6 Di Nunzio, F. *et al.* Human nucleoporins promote HIV-1 docking at the nuclear pore, nuclear import and integration. *PLoS One* **7**, e46037, doi:10.1371/journal.pone.0046037

PONE-D-12-15802 [pii] (2012).

7 Di Nunzio, F. New insights in the role of nucleoporins: a bridge leading to concerted steps from HIV-1 nuclear entry until integration. *Virus Res* **178**, 187-196, doi:10.1016/j.virusres.2013.09.003

S0168-1702(13)00295-5 [pii] (2013).

8 Lelek, M., Di Nunzio, F. & Zimmer, C. FlAsH-PALM: Super-resolution Pointillist Imaging with FlAsH-Tetracysteine Labeling. *Methods Mol Biol* **1174**, 183-193, doi:10.1007/978-1-4939-0944-5\_12 (2014).

9 Lelek, M. *et al.* Superresolution imaging of HIV in infected cells with FlAsH-PALM. *Proc Natl Acad Sci U S A* **109**, 8564-8569, doi:10.1073/pnas.1013267109

1013267109 [pii] (2012).

10 Capelson, M., Doucet, C. & Hetzer, M. W. Nuclear pore complexes: guardians of the nuclear genome. *Cold Spring Harb Symp Quant Biol* **75**, 585-597, doi:10.1101/sqb.2010.75.059

sqb.2010.75.059 [pii] (2010).

Expected profile of the candidate (optional):

We are looking for highly motivated and team player individuals with a strong motivation in the following fields:

* virology
* biochemistry
* cell biology
* molecular biology
* image and signal processing

We seek a PhD candidate to work on a Virology project based on the interaction between HIV-1, the NPC, chromatin, nuclear partners. The use of cutting edge technologies, such as super-resolution light microscopy in combination with Mass Spectrometry, ChIP-Sequencing will be required.

Please send your applications with full CV and motivation letter with 2-3 names of referees by October 20.

Before to apply please visit the guide for applicants on the website: <http://www.pasteur.fr/fr/enseignement/programmes-doctoraux/pasteur-paris-university-international-doctoral-program/application-information>

Contact: **dinunzio@pasteur.fr**