

**Master 2 internship project
Year 2019-2020**

Laboratory/Institute: IBS

Director: W. Weissenhorn

Team: High Throughput Technologies (VRM)

Head of the team: Darren Hart

Name and status of the scientist in charge of the project: HDR: yes no

Address: IBS, 71 avenue des Martyrs, 38000 Grenoble

Phone: 04 57 42 85 86

e-mail: darren.hart@ibs.fr

Program of the Master's degree in Biology:

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

Title of the project:

Directed evolution of Influenza polymerase inhibitors

Objectives:

The aim is to identify interacting regions of the influenza polymerase trimeric complex from X-ray structures and evolve them using phage display technology into high affinity inhibitors of enzyme assembly. This represents a possible future therapeutic approach.

Abstract:

This new project combines structural biology, synthetic biology and protein engineering on an important human pathogen, influenza. The mature polymerase enzyme of influenza virus is a heterotrimeric complex. The monomers first bind through interacting regions and then fold into the active trimeric conformation that has recently been described by X-ray crystallography. We will identify several important regions (20-30 amino acid regions) responsible for inter-subunit association and evolve these into high affinity binders using directed evolution by phage display. We expect that, when added to infected cells or cells expressing polymerase recombinantly, these in vitro evolved high affinity peptides will bind competitively and inhibit polymerase assembly.

Methods:

Phage Display, Directed Evolution, Molecular Biology, Protein Purification, Interactions using biophysical techniques, crystallisation.

Up to 3 relevant publications of the team:

1. Hart DJ & Waldo GS (2013) Library methods for structural biology of challenging proteins and their complexes. *Curr. Opin. Struct. Biol.* 23:403-408.
2. Reich S et al. (2014) Structural insight into cap-snatching and RNA synthesis by influenza polymerase. *Nature* 516:361-366.
3. Thierry E et al. (2016) Influenza Polymerase Can Adopt an Alternative Configuration Involving a Radical Repacking of PB2 Domains. *Mol. Cell* 61:125-137.

Requested domains of expertise (up to 5 keywords):

DNA handling, SDS-PAGE, protein affinity purification