UFR de Chimie et de Biologie
UNIVERSITÉ Grenoble Alpes

Master's degree in Biology – Chemistry-Biology Department

Internship project Master 2
Year 2018-2019

Laboratory/Institute: Institut de Biologie Structurale
Director: W. Weissenhorn
Team: High Throughput Protein Technologies
Head of the team: Dr Darren Hart

Name and status of the scientist in charge of the project: **HDR: yes** ☒ **no** ☐
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Program of the Master's degree in Biology:

☐ Neurosciences and Neurobiology
☐ Immunology, Microbiology, Infectious Diseases
☒ Integrative Structural Biology
☐ Physiology, Epigenetics, Differentiation, Cancer

Title of the project:

Directed evolution of peptide inhibitors of influenza polymerase

Objectives (up to 3 lines):

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 (up to 10 lines):

This project combines structural biology, synthetic biology and protein engineering on an important human pathogen, influenza. The polymerase 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 inhibit polymerase assembly. Hit peptides will be characterized using biophysical methods including Biacore and isothermal calorimetry, then studied using X-ray crystallography and/or NMR.

Methods (up to 3 lines):

Large random libraries ($\leq 10^8$ variants) will be cloned into phage display plasmids. Affinity selections on immobilised enzyme domains will identify high affinity mimics of the original sequences, confirmed by ELISA. Characterisation will use biophysical methods, X-rays & NMR.

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. Thierry E, et. al (2016) Influenza Polymerase Can Adopt an Alternative Configuration Involving a Radical Repacking of PB2 Domains. Mol Cell 61:125-37
3. Delaforge E, et al. (2015) Large-Scale Conformational Dynamics Control H5N1 Influenza Polymerase PB2 Binding to Importin α. J. Am. Chem. Soc. 137:15122–15134.

Requested domains of expertise (up to 5 keywords):

Molecular biology, protein biochemistry, technology development