UFR de Chimie et de Biologie
UNIVERSITÉ Grenoble Alpes

Master Biologie – UFR Chimie et Biologie

Internship project Master 2 Recherche
Year 2017/2018

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

Name and status of scientist in charge of the project: D. Hart **HDR yes X no** ☐
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Specialty MASTER:

☐ Neurosciences and Neurobiology
X Integrative Structural Biology
X Immunology, Microbiology, Infectious Diseases
☐ Physiology Epigenetics Development Differentiation

Title of project:

Directed evolution of molecular decoys that block viral polymerase assembly

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. Testing of hits in assays will reveal if disruption of polymerase complex could become a therapeutic approach.

Abstract:

This 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. Hit peptides will be characterized using biophysical methods including Biacore and isothermal calorimetry, then studied using X-ray crystallography and/or NMR.

Methods:

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 of hits will use biophysical and structural methods (X-ray/NMR).

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:

Molecular biology, protein biochemistry, technology development