

**Master 2 internship project  
Year 2020-2021**

**Laboratory/Institute:** IBS UMR 5075 CEA-CNRS-UGA **Director:** Winfried Weissenhorn  
**Team:** Flexibility and Dynamics of Proteins **Head of the team:** Martin Blackledge

**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:**

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

**Title of the project:**

**Molecular basis of avian influenza polymerase adaptation to human hosts**

**Objectives (up to 3 lines):**

To investigate the impact of different adaptive mutations (for example those present in the flu pandemics of 1918 and 2009) on the essential interaction of the 627-NLS domains of influenza polymerase with ANP32A recently characterized by NMR spectroscopy and to eventually aid the conception of viral inhibitors.

**Abstract (up to 10 lines):**

Avian influenza represents a human pandemic threat, especially via adaptive mutations that facilitate human to human infection, resulting in highly pathogenic strains. Mutations linked to human adaptation are concentrated in the 627-NLS domains of the PB2 subunit of its polymerase. This subunit is imported into the nucleus, where viral replication and transcription occurs. Once in the nucleus, interaction of the 627-NLS domains with the host protein ANP32A plays an essential species-specific regulatory role on infection. The 627-NLS domains show a high level of conformational flexibility that is essential to their function. In addition, ANP32A possesses an intrinsically disordered domain that plays a critical role in the interaction with 627-NLS and in species adaptation in general. This high level of flexibility requires NMR-based technology to study the impact of different mutants on this interaction.

**Methods (up to 3 lines):**

Expression in *E. coli* and purification of proteins by affinity and size exclusion chromatography. High resolution NMR spectroscopy will be used to characterize the effect of the adaptive mutations on the interaction of ANP32A with the 627-NLS domains at atomic resolution.

**Up to 3 relevant publications of the team:**

\* Delaforge E. et al. Large-scale conformational dynamics control H5N1 Influenza polymerase PB2 binding to Importin alfa. *J Am chem Soc.* (2015) doi: 10.1021/jacs.5b07765

\* Camacho-Zarco AR. et al. Molecular basis of host-adaptation interactions between influenza virus polymerase PB2 subunit and ANP32A. (2020) Preprint doi: 10.1101/2020.03.18.996579

**Requested domains of expertise (up to 5 keywords):**

Molecular biology and protein biochemistry. Interest in NMR spectroscopy as a biomolecular tool.