

Master 2 internship project
Year 2022-2023

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 the increased virulence of SARS-CoV-2 nucleoprotein mutations

Objectives (up to 3 lines):

To investigate the impact of different adaptive mutations occurring in the intrinsically disordered region of the nucleoprotein of SARS-CoV-2 that are responsible for enhanced replication of the virus. The molecular basis of this phenomenon will be investigated using integrated structural biology (NMR, EM, SAXS).

Abstract (up to 10 lines):

The processes of replication and transcription of viral RNA represent important targets for inhibition of SARS-CoV-2, and the development of rational strategies to achieve this end requires a molecular understanding of the viral replication cycle. The nucleoprotein (N) is an essential cofactor of the replication machinery, encapsidating the viral genome, providing protection from the host cell environment, and playing an essential role in regulating gene transcription. N is also highly dynamic, with more than 40% of its primary chain being intrinsically disordered, regions that link the folded RNA-binding and dimerization domains. The disordered regions are nevertheless functionally active, comprising important mutations associated with variants of concern. The Blackledge group at the IBS used NMR spectroscopy to describe the structural and dynamic properties of N at atomic resolution for the first time. The Master internship will involve exploiting this expertise to investigate the impact of mutations associated with variants of concern such as *delta* and *omicron* on the molecular function of the protein.

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 at atomic resolution, the effect of the mutations associated with variants of concern on the interaction of N with viral and host partners and viral RNA.

Up to 3 relevant publications of the team:

- * The intrinsically disordered SARS-CoV-2 nucleoprotein in dynamic complex with its viral partner nsp3a. Bessa, Camacho-Zarco, Guseva, Salvi, Maurin, Perez, Botova, Malki, Nanao, Jensen, Ruigrok, Blackledge ***Science Advances*** 8, eabm4034 (2022)
- * Molecular basis of host-adaptation interactions between influenza virus polymerase PB2 subunit and ANP32A Camacho-Zarco, Kalayil, Maurin, Salvi, Delaforge, Milles, Jensen, Hart, Cusack, Blackledge ***Nature communications*** 11, 1-12 (2020)
- * NMR Provides Unique Insight into the Functional Dynamics and Interactions of Intrinsically Disordered Proteins. Camacho-Zarco, Schnapka, Guseva, Abyzov, Adamski, Milles, Jensen, Zidek, Salvi, Blackledge ***Chemical Reviews*** 122, 9331 (2022).

Requested domains of expertise (up to 5 keywords):

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