Grenoble Alliance for Integrated Structural & Cell Biology

INTERNSHIP PROPOSAL

Institute and Group: IBS group VRM

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Research project title: Structure-Function studies of the orthoreovirus de novo protein Sigma One S

5 Keywords to describe the project: Virus evolution, protein structure, host-cell interaction, cell cycle control, *de novo* protein

Description of the project (aims, experimental techniques, recommended background):

The project aims to determine the 3D structure of the protein sigma 1s of mammalian Orthoreovirus and to provide new insights into the function and mechanisms of evolution of this protein. Sigma 1s has evolved by an extraordinary process where a new gene is created *de novo* by overprinting onto an existing (''ancestral'') open reading frame. Viruses notably use this trick in order to acquire novel functions without increasing the size of their genomes. So far, only a few *de novo* proteins have been structurally characterized and more information is needed to decipher the mechanisms underlying the interdependence between overlapping gene products.

Orthoreoviruses are non-enveloped, icosahedral viruses that contain 10 segments of double-stranded RNA (dsRNA). Although infection in human can be associated with mild respiratory and enteric symptoms, the mammalian orthoreovirus have been recognized as an anticancer agent (more than 30 viruses are in clinical trials). Sigma 1s has been shown to be involved in the host cell cycle regulation upon infection by mammalian Orthoreoviruses. Sigma 1s is known to block the cell cycle and therefore could be a key player in the oncolytic properties of Mammalian orthoreoviruses.

More specifically, the project will require to express, purify and crystalize Sigma 1s in order to solve its structure by X-ray crystallography and to characterize its structural properties by various biophysical methods (SEC-MALLS, AUC, DLS, SAXS,...). As, there is no homologue in the Protein Data Bank, the high-resolution structure of sigma 1s could reveal a new fold and highlight the evolutionary origin of the protein.

Justification that the internship's subject fits with the general theme of GRAL:

Structural characterisation will have a strong impact on the understanding of the evolution of these *de novo* proteins and will be the starting point to the identification host cell partners that could explain the oncolytic properties of orthoreoviruses. Hence, the project is conceived as an integrated study from the atomic structure to the roles of the protein in cell arrest cycle.

Relevant publications of the team:

1. Ensemble Structure of the Highly Flexible Complex Formed between Vesicular Stomatitis Virus Unassembled Nucleoprotein and its Phosphoprotein Chaperone. Yabukarski F, Leyrat C, Martinez N, Communie G, Ivanov I, Ribeiro EA Jr, Buisson M, Gerard FC, Bourhis JM, Jensen MR, Bernadó P, Blackledge M, Jamin M. J Mol Biol. 2016; 2. Structure of Nipah virus unassembled nucleoprotein in complex with its viral chaperone. Yabukarski F, Lawrence P, Tarbouriech N, Bourhis JM, Delaforge E, Jensen MR, Ruigrok RW, Blackledge M, Volchkov V, Jamin M. Nat Struct Mol Biol. 2014. 3. Procollagen C-proteinase enhancer grasps the stalk of the C-propeptide trimer to boost collagen precursor maturation. Bourhis JM, Vadon-Le Goff S, Afrache H, Mariano N, Kronenberg D, Thielens N, Moali C, Hulmes DJ. Proc Natl Acad Sci. 2013; 4.Production and crystallization of the C-propeptide trimer from human procollagen III. Bourhis JM, Mariano N, Zhao Y, Walter TS, El Omari K, Delolme F, Moali C, Hulmes DJ, Aghajari N. Acta Crystallogr Sect F Struct Biol Cryst Commun. 2012