Grenoble Alliance for Integrated Structural & Cell Biology

GRAL MASTER 2 RESEARCH SCHOLARSHIP - Program 2017 - 2018

INTERNSHIP PROPOSAL

Institute and Group:

IBS, Viral Infection and Cancer Group

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Research project title: Native mass spectrometry as a tool to characterize ribonucleoprotein complexes

5 Keywords to describe the project:
Native Mass Spectrometry (MS), ribonucleoprotein complex, stoichiometry, RNA, transient transfection.

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

Besides their role in translation, RNA molecules are key players in genetic and epigenetic regulation. To fulfill its various functions, RNA interacts with RNA-binding proteins to form diverse ribonucleoprotein complexes (RNPs). Thus, knowing the composition and stoichiometry of RNPs is of great importance to better understand their function. This could be achieved by native MS (nMS), a powerful and emerging technology we apply to RNA. Recently we set up robust *in vitro* protocols to prepare RNA in a way compatible with nMS analysis (unpublished results). Using this approach, we also successfully established the stoichiometry of an *in vitro* assembled HIV export RNP, containing the cellular export factor Crm1 and its cofactor Ran, the viral Rev protein, and the viral Rev-responsive RNA element (RRE) of 350 nucleotides.
The proposed project aims to characterize the HIV export RNP which will be assembled *in vivo* by cell transient transfection, purified and studied by nMS. Our long term goal is to apply these technologies to any type of RNP complex to better understand the function of RNA molecules.

Experimental techniques: The student will set up 1) the transient transfection conditions 2) the purification of the RNP in a manner compatible with nMS.

Recommended background: an interest in biochemistry, cellular and structural biology.

Relevant publications of the team:

1. Boeri Erba E, Petosa C.The emerging role of native mass spectrometry in characterising the structure and dynamics of macromolecular complexes. Protein Science, 2015, 24(8):1176-92. doi: 10.1002/pro.2661.
2. Dian C, Bernaudat F, Langer K, Oliva MF, Fornerod M, Schoehn G, Müller CW, Petosa C. (2013) Structure of a truncation mutant of the nuclear export factor CRM1 provides insights into the auto-inhibitory role of its C-terminal helix. ***Structure.*** 21:1338-1349.

3. Langer K, Dian C, Rybin V, Müller CW, Petosa C (2011). Insights into the function of the CRM1 co-factor RanBP3 from the structure of its Ran-binding domain. ***PLoS ONE***. 6(2):e17011.