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:
Nuclear import of the HIV regulatory protein Tat: a structure/function study

5 Keywords to describe the project: HIV-1 Tat, importin β, X-ray crystallography, fluorescence polarization, fluorescence microscopy

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

The HIV-1 protein Tat is crucially required for the virus to replicate efficiently in the host cell. Tat binds to a specific RNA motif in the HIV-1 genome, the trans-activation response (TAR) element, resulting in the increased production of viral RNA transcripts. Deletion and mutagenesis studies have shown that viral replication is compromised when Tat function is impaired. In order to function, Tat must enter the nucleus of the host cell. This step is mediated by the human protein Importin β (Impβ), a 100 kDa protein with a snail-like shape that wraps around its macromolecular cargos and escorts them across the nuclear membrane. Although the recognition of Tat by Impβ holds great promise for chemotherapeutic intervention, the underlying molecular details are unclear.
Aims : This project aims to elucidate how Impβ delivers Tat from the cytosol to the nucleus by (i) characterizing the Impβ-Tat interaction using wildtype and mutant proteins (available in the lab) in *in vitro* binding assays and cell-based nuclear import assays, and (ii) pursuing the X-ray crystal structure of the Impβ/Tat complex.
Experimental techniques: recombinant protein expression/purification, crystallography, fluorescence polarization, cell culture, fluorescence microscopy
Recommended background: an interest in molecular biology, (bio) chemistry or structural biology

Relevant publications of the team (3 max):

1. Cingolani G, Petosa C, Weis K, Müller CW. (1999) Structure of Importin β bound to the IBB domain of Importin α. ***Nature*** 399:221-229.
2. Perrakis A, Musacchio A, Cusack S, Petosa C. (2011) Investigating a macromolecular complex: the toolkit of methods. ***J. Struct. Biol.*** 175:106-112.
3. 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