

INTERNSHIP PROPOSAL

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Research project title: Structure of a membrane protein complex by co-crystallisation with Lama Nanobodies.

5 Keywords to describe the project: bacteriophage infection, structural biology, membrane protein, co-crystallisation, crystallography

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

Bacteriophages are fascinating nanomachines that infect very specifically bacterial hosts. Infection of *E. coli* by the siphophage T5 is initiated by the irreversible binding of the Receptor Binding Protein of the phage, pb5, to the outer membrane transporter FhuA, which triggers the opening of the capsid and the perforation of the host cell wall. We aim at understanding the conformational changes that occur within the FhuA-pb5 complex. We have recently obtained well diffracting crystals (2.6 Å resolution) of the FhuA-pb5 complex interacting with Lama nanobodies (the FhuA-pb5 complex alone fails to produce well-diffracting crystals). The project is to reproduce the crystals of the FhuA-pb5-Lama nanobody complexes and determine the structure of the complex. Experimental techniques: protein over-expression, purification and characterisation, including a membrane protein, detergent exchange, crystallisation, diffraction assessment at the synchrotron and structure determination by molecular replacement. Recommended background: biochemistry and crystallography but above all enthusiasm and motivation.

Relevant publications of the team:

1. Noirclerc-Savoye M, Flayhan A, Pereira C, Gallet B, Gans P, Ebel C and Breyton C (2015) Screening the position of the His-tag: the example of tail proteins of phage T5 *Prot Expr & Purif* **109C**, 70-78
2. Flayhan A, Vellieux FMD, Lurz R, Maury O, Contreras-Martel C, Girard E, Boulanger P and Breyton C (2014) Crystal structure of pb9, the distal tail protein of bacteriophage T5: A conserved structural motif among all siphophages. *J. Virol.* **88**, 820-8
3. Breyton C, Flayhan A, Gabel F, Lethier M, Durand G, Boulanger P, Chami M and Ebel C (2013) Assessing the Conformational Changes of pb5, the Receptor-binding Protein of Phage T5, upon Binding to Its *Escherichia coli* Receptor FhuA. *J. Biol. Chem.* **288**, 30763–30772