

## INTERNSHIP PROPOSAL

**Institute and Group:** IBS, Group Membrane

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**Research project title:** Expression, purification, crystallization and functional characterization of APT, a phosphate transporter of *Toxoplasma gondii*

**5 Keywords to describe the project:** Membrane protein, biochemistry and biophysical characterization, transport assays

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

In the long term, the project aims at deciphering the molecular mechanism of a membrane transport protein, namely the apicoplast phosphate transporter (APT) from *Toxoplasma gondii* by solving its atomic structure using X-ray crystallography, in order to pave the way towards the design of new drugs against *Toxoplasma* and *Plasmodium*. During the master internship, a new construct of APT will be expressed in yeast. The purification protocol will be adapted from the current construct, also expressed in yeast, and refined in order to get sufficient amounts of protein for crystallization. After being extracted from the membrane and solubilized in detergent, APT will be characterized by biophysical approaches in order to find the best stabilizing conditions (choice of detergent, buffer and additives). Functional assays already in place in our team, based on the transport of Pi into proteoliposomes will be used to characterize inhibitors and the transport properties of various mutants.

The host team has a long-standing expertise of membrane protein structural biology, and of transporters. Protocols for this project are already partly established as well as the transport assays. The project will thus give the opportunity to a master student to learn cutting-edge approaches in the very challenging field of structural biology of membrane proteins. The project is done in collaboration with Prof. K. Fischer from the university of Tromsø.

**Relevant publications of the team:**

1. E. Pebay-Peyroula, C. Dahout-Gonzalez, R. Kahn, V. Trézéguet, G.J.-M. Lauquin and G. Brandolin (2003) *Nature* 426, 39-44. Structure of mitochondrial ADP/AP carrier in complex with carboxyatractyloside.
2. S. Ravaut, A. Bidon-Chanal, I. Blesneac, P. Machillot, C. Juillan-Binard, F. Dehez, C. Chipot, E. Pebay-Peyroula (2012) *ACS Chem Bio* 7, 1164-9 (with cover page) Mutations of the mitochondrial ADP/ATP carrier causing genetic diseases impair the transport of nucleotides.
3. H. Mayerhofer, E. Sautron, N. Rolland, C., P. Catty, D. Seigneurin-Berny, E. Pebay-Peyroula, S. Ravaut (2016) *PlosOne* 11, e0165666 Structural insights into the nucleotide-binding domains of the P1B-type ATPases HMA6 and HMA8 from *Arabidopsis thaliana*