Structural and functional study of Biomphalysins, pore-forming toxins expressed in Biomphalaria glabrata: biochemical, biophysical and structural characterisation

Location:

IPBS (CNRS - UMR 5089) 205, route de Narbonne, Toulouse 31077

Structural Biophysics team (Lionel MOUREY) Structural Biophysics | IPBS

Duration:

Minimum 5 months

Responsible for:

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Summary:

Schistosomiasis (1) is one of the 20 neglected tropical diseases targeted in the WHO 2021-2030 guide goals. 200 million people are infected worldwide, and 280,000 deaths are reported annually in sub-Saharan Africa. Today, Praziquantel® is the most effective and widely used control method for this parasite (2).

Worms of the genus *Schistosoma* are the cause of this disease. Their reproductive cycle passes through an intermediate host, the freshwater snail Biomphalaria. The project specifically studies the *Schistosoma mansoni*/*Biomphalaria glabrata* pair (3).

The Molecular Mechanisms of Adaptation and Plasticity (2MAP) team at the Host-Pathogen-Environment Interaction Laboratory (IHPE) in Perpignan studies these organisms. David Duval and his colleagues recently discovered a new genetic family (4). The 23 genes identified code for proteins, the Biomphalysins, involved in the snail's immune system.

These β pore-forming-toxins, from the large family of aerolysin-like proteins (5, 6), are indeed capable of recognising different types of pathogen and their cytotoxic activity against *Schistosoma mansoni* is known (7).

The internship is part of the thesis project attached to the ANR AeroSnail, which aims at the biochemical, structural and functional characterization of Biomphalysins.

11 new Biomphalysins were added to the study in September. The conditions for recombinant expression in E. coli will be established next, in order to determine which ones are most suitable for recombinant expression in soluble form. For these proteins, the first purification tests will also be carried out during November/December 2021.

The objective of the internship is to finalise the purification protocols, then to characterise the Biomphalysins from a biochemical point of view and then from a biophysical point of view in solution, in order to set up structural approaches.

The team is specialised in X-ray crystallography. The main approach will therefore be by crystallisation and study by X-ray diffraction at the synchrotron (ESRF in Grenoble, SOLEIL in Paris-Saclay, ALBA in Barcelona). Other approaches may be considered depending on the results of the biochemical/biophysical characterisation, such as SAXS or cryoEM-TEM.

Details of the techniques addressed:

- Recombinant expression in E. coli

- Purification of proteins by affinity chromatography and gel filtration on FPLC systems

- Characterisation

  - biochemical by liquid chromatography, SDS-PAGE electrophoresis
  - biophysical by Differential Scanning Fluorimetry DSF, Dynamic Light Scattering DLS, Exclusion Chromatography coupled with multiple angle light scattering SEC-MALS, Native/top-down MS mass spectroscopy
  - Robotic crystallogenesis in microplates with Mosquito® and RockImager®

Knowledge/skills required:

- Knowledge of biochemistry and protein purification
- Knowledge of structural biology methods, especially X-ray crystallography
- Ability to work in a biochemistry laboratory
- Curiosity and dynamism, team spirit

References:

1. WHO. **Home/Newsroom/Fact sheets/Detail/Schistosomiasis** [online] (consulted the 9th April, 2021). https://www.who.int/fr/news-room/fact-sheets/detail/schistosomiasis
2. CIOLI D. *and al*., 2014. **Schistosomiasis control : praziquantel forever?** Molecular & Biochemical Parasitology, vol. 195, pp. 23-29 https://doi.org/10.1016/j.molbiopara.2014.06.002
3. McMANUS X.? *and al*., 2018. **Schistosomiasis**. Nature Reviews Disease Primers 4 (13). https://doi.org/10.1038/s41572-018-0013-8

4. GALINIER R. *and al*., 2013. **Biomphalysin, a New β Pore-forming Toxin Involved in *Biomphalaria glabrata* Immune Defense against *Schistosoma mansoni***. PLoS Pathog 9(3): e1003216. https://doi.org/10.1371/journal.ppat.1003216

5. SZCZESNY P. *and al.*, 2011. **Extending the Aerolysin Family: From Bacteria to Vertebrates**. PLoS ONE 6(6): e20349. https://doi.org/10.1371/journal.pone.0020349

6. PARKER M. W. *and al*., 1994**. Structure of the aeromonas toxin proaerolysin in its cater-soluble and membrane-channel states**. nature, vol. 367 https://doi.org/10.1038/367292a0

7. PINAUD S., POTEAUX P. *and al*., 2021. **New Insights Into Biomphalysin Gene Family Diversification in the Vector Snail Biomphalaria glabrata**. Frontiers in Immunology, 12:635131. https://doi.org/10.3389/fimmu.2021.635131