Grenoble Alliance for Integrated Structural & Cell Biology

GRAL MSc RESEARCH SCHOLARSHIP 2020-2021

RESEARCH INTERNSHIP PROPOSAL

Institute / Group

IRIG / BCI - PBRC

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Research Project Title

Transmembrane pore formation by *Pseudomonas aeruginosa* toxin ExlA

Description of the project

Pseudomonas aeruginosa is a major bacterial nosocomial pathogen. The virulence of recently isolated clinical strains relies on the secretion of ExlA, a newly described pore-forming toxin (PFT). PFTs are produced as soluble monomers that assemble into an oligomeric transmembrane pore upon receptor binding and transmembrane insertion. ExlA-mediated pore formation can be reconstituted in model systems like liposomes and this project aims at documenting ExlA pore formation. Truncation experiments have spotted PFT activity to the last 300 residues of ExlA, that display no hydrophobic stretch and little sequence conservation. Still, a 30-residue segment is the single candidate to achieve membrane insertion. Cysteine mutants at each position will be characterized for function and for cysteine accessibility before and after pore formation in order to identify ExlA pore residues. Besides, ExlA derivatives with monomeric ascorbate peroxidase APEX2 or fluorescent protein muGFP attached will be used to image ExlA in negative stain electron microscopy and investigate ExlA oligomeric state by analytical ultracentrifugation in collaboration. Techniques: recombinant expression in *E. coli*, electrophoresis, immunodetection, fluorescent labeling, liposome preparation, cytotoxicity assay. Recommended background: biochemistry, microbiology or molecular biology.

Keywords

Pore-forming toxin, cysteine scanning, chemical labeling, negative stain electron microscopy, analytical ultracentrifugation

Relevant publications of the team

Basso, Pauline, Michel Ragno, Sylvie Elsen, et al. (2017) Pseudomonas Aeruginosa Pore-Forming Exolysin and Type IV Pili Cooperate To Induce Host Cell Lysis. MBio 8(1).

Reboud, Emeline, Pauline Basso, Antoine P. Maillard, Philippe Huber, and Ina Attrée (2017) Exolysin Shapes the Virulence of Pseudomonas Aeruginosa Clonal Outliers. Toxins 9(11).

Reboud, Emeline, Stéphanie Bouillot, Sabine Patot, et al. (2017) Pseudomonas Aeruginosa ExlA and Serratia Marcescens ShlA Trigger Cadherin Cleavage by Promoting Calcium Influx and ADAM10 Activation. PLoS Pathogens 13(8): e1006579.