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Title of the M2 research internship:

Transmembrane pore formation by *Pseudomonas aeruginosa* toxin ExlA.

Project summary:

Pseudomonas aeruginosa is a major bacterial nosocomial pathogen. The virulence of recently isolated, deadly clinical strains relies on the secretion of ExlA, an ill-characterized 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 membranes 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 strong 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 cysteine accessibility, before and after pore formation, in order to identify pore residues. Besides, ExlA derivatives covalently coupled to monomeric ascorbate peroxidase APEX2 or fluorescent protein mGFP will be used to image ExlA in negative stain electron microscopy and investigate ExlA oligomeric state by analytical ultracentrifugation (coll.). Techniques: recombinant expression in *E. coli*, electrophoresis, immunodetection, fluorescent labeling, liposome preparation, cytotoxicity assay. Recommended background: biochemistry, microbiology or molecular biology.

Keywords:

chemical labeling, pore-forming toxin, biochemical coupling

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* doi: 10.1128/mBio.02250-16

Reboud, Emeline, Pauline Basso, Antoine P. Maillard, Philippe Huber, and Ina Attrée (2017) Exolysin Shapes the Virulence of *Pseudomonas Aeruginosa* Clonal Outliers. *Toxins* doi: 10.3390/toxins9110364

Bertrand Q, Job V, Maillard AP, Imbert L, Teulon JM, Favier A, Pellequer JL, Huber P, Attrée I, Dessen A. (2020) Exolysin (ExlA) from *Pseudomonas aeruginosa* Punctures Holes into Target Membranes Using a Molten Globule Domain. *J Mol Biol.* doi: 10.1016/j.jmb.2020.05.025