M2 Master internship project - call 2025-2026:

Title: Functional Specificities of Bacterial ExoY-like Nucleotidyl Cyclase Toxins as Purinyl and Pyridylyl Cyclases: Biochemical and Structural Studies.

Keywords : Life and health science, biochemistry, structural biology, enzymatic mechanism, nucleotidyl cyclases (NC), cUMP/cCMP-dependent signaling, host-pathogen interactions, actin cytoskeleton.

Doctoral School : Structure and Dynamics of Living Systems (SDSV), Université Paris-Saclay, F. **Specialty:** Life and Health Sciences, biochemistry and structural biology.

Research unit: Institute for Integrative Biology of the Cell (I2BC), Université Paris-Saclay, CEA, CNRS, Gif-Sur-Yvette, F;

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lab. website: www.i2bc.paris-saclay.fr/structural-biochemistry-of-microtubules-kinesins-and-their-cargos-gigant-menetrey/

Abstract:

ExoY-like nucleotidyl cyclase (NC) exotoxins are virulence factors produced by pathogenic β- and γ-proteobacteria. Inactive in bacteria, these enzymes become potent NCs once injected into eukaryotic cells, where they hijack the host's actin as an atypical cofactor for activation **(Fig. 1)**. Upon activation, ExoY toxins produce supraphysiological levels of purine (cAMP, cGMP) and pyrimidine (cCMP, cUMP) cyclic nucleotides, thereby disrupting host signaling pathways **[ref. 1]**. The signaling pathways and functions of the non-canonical cyclic nucleotides cCMP and cUMP remain poorly understood, especially in eukaryotic cells **[ref. 2]**. ExoY homologs can exhibit significant sequence diversity, use either monomeric (G-actin) or filamentous (F-actin) actin as cofactors, and differ in substrate preference **(Fig. 1)**. Their effects on the actin cytoskeleton also remain unclear.

During this internship, you will biochemically and structurally characterize distinct ExoY homologs. Understanding their catalytic and substrate specificities, and how these relate to structure–function relationships, is key to deciphering their cytotoxic and virulence mechanisms, uncovering new signaling pathways, and exploring possible inhibition strategies.

Enzymatic Characterizations: During this internship, you will express and purify recombinant ExoY-like NCs using optimized HPLC-based purification protocols for in vitro studies. A fluorescent Terbium-Norfloxacin (TBN) assay will be optimized to monitor the conversion of purine and/or pyrimidine NTPs into cNMPs in solution. This assay will assess how cofactors (e.g., metals, actin-ATP/ADP), chemical compounds, and conditions (pH, ionic strength) influence enzyme activity and readout. Once optimal conditions are established, you will compare the enzymatic specificities and efficiencies of *P. aeruginosa*, *V. vulnificus*, and/or *A. schubertii* ExoYs. If the assay proves sufficiently sensitive and stable, further work may determine kinetic parameters (Michaelis–Menten constants) and nucleotide-binding affinities to better define enzymatic behavior in cellular contexts. A long-term goal is to use this assay to screen small-molecule libraries and identify potential inhibitors of ExoY-like toxins.

Structural Studies: Building on these biochemical results, you will take part in structural analyses of ExoY enzymes from *P. aeruginosa* and/or *A. schubertii*. The objective is to elucidate their conformational dynamics when complexed with F- or G-actin and purine or pyrimidine nucleotide analogs or products, using X-ray crystallography or cryo-EM, to gain atomic-level insights into their catalytic mechanisms and substrate specificities. Chimeric proteins developed in the team **[ref. 3]** will be used to stabilize ExoY–actin complexes with ligands, facilitating these studies. This work will be initiated during the M2 traineeship and could lead to a PhD project aimed at elucidating the signaling by pyrimidine cyclic nucleotides.

Selected References:

1. Seifert R & Schirmer B (2022) cCMP and cUMP come into the spotlight, finally. *Trends Biochem Sci*. 47(6):461-463. doi: 10.1016/j.tibs.2021.12.008.

2. Teixeira-Nunes M*, et al.* (2022) Bacterial Nucleotidyl Cyclases Activated by Calmodulin or Actin in Host Cells: Enzyme Specificities and Cytotoxicity Mechanisms Identified to Date. *Int J Mol Sci* 23(12). (team publication)

3. M. Teixeira-Nunes, et al. (2023) Functional and structural insights into the multi-step activation and catalytic mechanism of bacterial ExoY nucleotidyl cyclase toxins bound to actin-profilin. **PLoS Pathogens** 19(9):e1011654. doi: 10.1371/journal.ppat.1011654. (team publication)

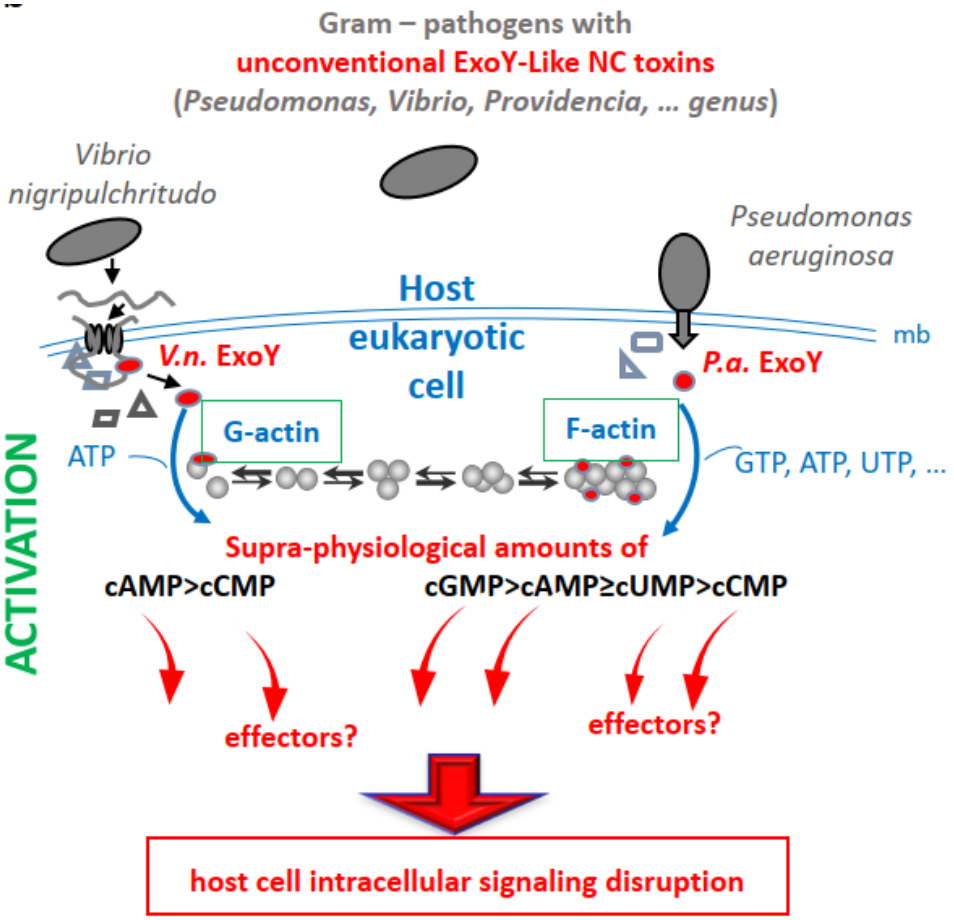

Fig. 1: The catalytic properties of bacterial ExoY nucleotidyl cyclase (NC) toxins remain incompletely understood at the enzymatic, structural, and cytotoxic levels. ExoY homologs from β- and γ-proteobacterial pathogens differ in their use of actin, either monomeric (G-actin; *Vibrio* ExoY) or filamentous (F-actin; *Pseudomonas* ExoY), as cofactors, as well as in their substrate preferences, suggesting distinct modes of host cell toxicity. The eukaryotic targets and cytotoxic mechanisms driven by the overproduction of cUMP or cCMP remain to be elucidated **[ref. 1-2].**