Master 2 research internship in Integrated Structural & Cell Biology in Grenoble

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Title of the M2 research internship:
E. coli outer membrane recognition by Colicin N: evaluation of the contribution of lipopolysaccharides and OmpF.

Project summary:
The outer membrane of Gram- bacteria is asymmetrical, the inner and outer leaflets being formed by phospholipids and lipopolysaccharides (LPS), respectively. The LPS extends from the cell surface, and plays important structural roles, is involved in host pathogen interactions,
and affects sensitivity to external stressors, including antibiotics. Here we suggest studying the interaction of Escherichia coli-specific bacteriocins with different components of the outer membrane.
Colicins are produced by E. coli cells and are released in the environment to reduce competition from other E. coli strains. After binding to receptor molecules on the bacterial surface, colicins translocate across the outer membrane via porins before exerting their toxic activity within the targeted compartment.
The pore-forming group A colicin N (ColN) uses the outer membrane porin OmpF as a receptor and translocator. However, it is not clear whether the LPS, that is closely associated to OmpF, plays a distinct role in the recognition event. There are controversial reports in the literature: some studies suggest a specific ColN-LPS interaction whereas others claim that LPS only slightly enhances interaction with OmpF.
The aim of this internship is the comparative study of the interaction of a ColN construct with different model-outer membrane systems from different E. coli strains. In particular, we will study the influence of the presence of O-antigens (surface sugar polymers that form the distal portion of LPS) and OmpF on ColN – outer membrane interaction. To do so we will use different biophysical techniques, like Isothermal Titration Calorimetry (ITC), BioLayer Interferometry (BLI), and NMR. The ColN construct we will use lacks the pore-forming domain and is amenable to solution state NMR. The internship will also give the opportunity to the student to get familiar with protein expression and purification, protein reconstitution in model membranes and biomolecular NMR spectroscopy.

Keywords:
lipopolysaccharide, colicin, NMR

Relevant publications of the team:
Bersch B, Dörr JM, Hessel A, Killian JA, Schanda P. Angew Chem Int Ed Engl. 2017 Feb 20;56(9):2508-2512. Proton-Detected Solid-State NMR Spectroscopy of a Zinc Diffusion Facilitator Protein in Native Nanodiscs. doi: 10.1002/anie.201610441. Epub 2017 Jan 27.

Baeta T, Giandoreggio-Barranco K, Ayala I, Moura ECCM, Sperandeo P, Polissi A, Simorre JP, Laguri C. The lipopolysaccharide-transporter complex LptB2FG also displays adenylate kinase activity in vitro dependent on the binding partners LptC/LptA. J Biol Chem. 2021 Dec;297(6):101313. doi: 10.1016/j.jbc.2021.101313. Epub 2021 Oct 19. PMID: 34673027

Kurauskas V, Hessel A, Ma P, Lunetti P, Weinhäupl K, Imbert L, Brutscher B, King MS, Sounier R, Dolce V, Kunji ERS, Capobianco L, Chipot C, Dehez F, Bersch B, Schanda P. How Detergent Impacts Membrane Proteins: Atomic-Level Views of Mitochondrial Carriers in Dodecylphosphocholine. J Phys Chem Lett. 2018 Mar 1;9(5):933-938. doi: 10.1021/acs.jpclett.8b00269. Epub 2018 Feb 8. PMID: 29397729