CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE
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PhD position : **Mechanisms of action of polymyxins : cyclic and peptidic antibotics**	
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Context and objectives :

Discovered in 1949, colistin is a powerful antibacterial peptide of the polymyxin family and is well known for its important reactivity on the outer membrane of Gram-negative bacteria. Its use has been limited until recently because of the risk of occurrence of neuro-and nephrotoxic effects [1]. The recent renewed interest of clinicians for this molecule in the framework of multi-drug treatment of infections caused by Gram-negative bacilli resistant has resulted in the emergence of resistant strains, for which treatment options are limited or nonexistent [1, 2]. Due to the quick stop development of colistin, its mechanism of action at the molecular scale remains to be elucidated and its use can be improved. The results published in the literature indicated that the antibiotic is associated with the lipopolysaccharides (LPS) [3-6] and phospholipids of the outer membrane of Gram-negative bacteria, leading to a local perturbation of the membrane followed by a leak cytoplasmic content and bacterial death [7].

In order to better understand the mechanism of action of the polymyxin family, we propose to conduct a study of the physico-chemical interactions between polymyxin E and model lipidic membranes consisted of purified lipids and bacterial lipid extracts. Furtermore, polymyxins A, B, C and D will be used in our experiments. To this end, we will prepare the model membranes and supported membrane using the technique of Langmuir [8] and the method of liposome fusion on solid supports. In particular, we will use the lipid A-Kdo2 [9] representing the highly conserved part of LPS, capable of interacting with polymyxins.

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Experimental measurements will be to use the Brewster angle microscopy (BAM), the Infrared Reflection Absorption Spectroscopy by polarization modulation (PM-IRRAS) measurements of surface pressure and surface potential [10, 11]. Monomolecular films and lipid bi-and multi-layers transferred or deposited onto solid supports will be studied by vibrational spectroscopy (FTIR, IRRAS) and atomic force spectroscopy (AFM) [12-14], and by electrochemical methods. Polymyxins functionalized with redox groups will be used as probes for electrochemical detection of LPS [15]. The AFM measurements (topography, kinetic activity of colistin on the membrane structure [16]) will be implemented by force measurements between colistin and membrane lipids. Polymyxins be changed or not, and then chemically grafted onto the AFM probes to determine the nature (hydrophobic interaction / hydrophilic effect of the charge and cycle effect) and to quantify the intermolecular interactions responsible for the activity of colistin and particularly its surfactant properties [17-19].

These studies allow us to identify and quantify the groups involved in the interaction between polypeptides and lipids, the precise nature and the kinetics of membrane-polymyxin interactions. On the other hand, microscopic and spectroscopic methods will evaluate the impact of polymyxin on the morphology and distribution of lipid domains. These key structures for the dynamic organization of bacterial membranes may play a role in the activity of polymyxin. Finally, electrochemical studies will provide information on the impact of polymyxin on transport across lipid membranes.

Funding and duration:

Graduate 36-month contract - Doctoral SESAMES (University of Lorraine).

The salary amounts to € 1,685 gross. A teaching load is possible that brings the salary € 2025 gross. For more information: http://www.sesames.uhp-nancy.fr/

Terms:

Applicants should contact the supervisor and the director of the Graduate School (Xavier ASSFELD by email Xavier.assfeld@univ-lorraine.fr).

They must provide a file containing:

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- One complete CV (curriculum mentions)
- Notes the theoretical examinations of Master 2 or equivalent
- The opinion of the clinician M2 or equivalent (such notice shall be sent directly by the training supervisor to the Director of the Graduate School)
- The opinion of the head of the Master or equivalent (such notice shall be sent directly by the head of the Master Director of the Doctoral School)

Submissions must be received in electronic form only, **before Friday 10th of May 2013 at 11:59 pm**. The selection of candidates (on record) to hearing will be for **31st of May 2013**. **The hearings are scheduled for 26th and 27th of June 2013**. Interviewed the candidates will be ranked in order of merit and choose, in order, the subject of their choice until all allowances (currently unknown number).

References :

[1] L.M. Lim, *et al.*, Pharmacotherapy, 30 (2010) 1279-1291.
[2] A. Antoniadou, *et al.*, J Antimicrob Chemother, 59 (2007) 786-790.
[3] M.M. Domingues, *et al.*, Biopolymers, 98 (2012) 338-344.
[4] C. Mestres, *et al.*, Int. J. Pharm., 160 (1998) 99-107.
[5] S. Roes, *et al.*, Langmuir, 21 (2005) 6970-6978.
[6] H.S. Chung, C.R.H. Raetz, Biochemistry, 49 (2010) 4126-4137.
[7] R.A. Dixon, I. Chopra, Antimicrob. Agents Chemother., 29 (1986) 781-788.
[8] B. Korchowiec, *et al.*, Colloids and Surfaces, B: Biointerfaces, 103 (2013) 217-222.
[9] S.O. Opiyo, *et al.*, BMC Evol. Biol., 10 (2010) No pp given.
[10] G. Sautrey, *et al.*, J. Phys. Chem. B, 115 (2011) 15002-15012.
[11] B. Korchowiec, *et al.*, Biochim. Biophys. Acta, Biomembr., 1808 (2011) 2466-2476.
[12] N.P. Mortensen, *et al.*, Langmuir, 25 (2009) 3728-3733.
[13] R.L. Soon, *et al.*, Antimicrob. Agents Chemother., 53 (2009) 4979-4986.
[14] R. Brasseur, *et al.*, Surf. Interface Anal., 40 (2008) 151-156.
[15] S. Iijima, *et al.*, Biosens. Bioelectron., 26 (2011) 2080-2084.
[16] A. Berquand, *et al.*, Biochim. Biophys. Acta, 1664 (2004) 198-205.
[17] M. Eeman, *et al.*, Langmuir, 25 (2009) 3029-3039.
[18] O. Domenech, *et al.*, Biochimica Biophysica Acta, 1778 (2009) 1832–1840.
[19] G. Francius, *et al.*, Biochim. Biophys. Acta, 1778 (2008) 2058-2068.