

Post doctoral position

Title: Functionalization of AFM-tips for force measurements: study of the interaction *pseudomonas aeruginosa* bacterium-epithelial cell involved in cystic fibrosis

Key words: AFM, inter molecular interactions, Force measurements, Surface chemistry

Scientific field: Atomic Force Microscopy on biological objects

Supervisor: Prof Magali Phaner-Goutorbe

Team: Chemistry and Nanobiotechnology

Financial support: ANR Glycomime over 12 months starting in October 2013

Place : INL UMR CNRS 5270 – Ecole Centrale de Lyon (Ecully)

Details for the subject

Background, Context

The bacterium *Pseudomonas aeruginosa* is present in many natural environments such as soil, water and plants. For cystic fibrosis patients, it is the main cause of mortality due to severe infections in the lungs. Immuno-depressed or weaker patients such as badly burnt persons are also prone to these infections. Finally, this pathogen is responsible of 10% of nosocomial infections in hospitals. These bacteria have developed strains resistant to multiple antibiotics, and the treatment is often very long and grueling for patients. New therapeutic approaches, more effective, are currently under development.

As many viruses and pathogenic bacteria, *P. aeruginosa* use glycoconjugates (sugars) present on the surface of host cells as ligands for a first contact. The lectin-glycoconjugate interaction plays an important role in the adhesion process of the bacterium and on the creation of a pathogenic biofilm. The project developed by the team "Chemistry and Nanobiotechnology" consists in participating to the development of new drugs in order to avoid the cellular adhesion of the bacterium. The idea is to synthesize and to introduce synthetic glycoconjugates (glycomimetics) with higher affinity than the natural glycoconjugate present on the cells. A fundamental study of the interaction bacterium/cell and synthetic glycoconjugate/lectin is essential to understand the adhesion mechanism. In a previous work, we showed that Atomic Force Microscopy (AFM) is well-adapted to analyze the interaction at a nanometric scale (Fig.1). We showed that different arrangements could be obtained depending on the nature and the conformation of the synthetic sugar [1-3].

The objective of this Post-doctoral is to develop robust and reproducible chemical processes to functionalize AFM tips in order to measure the interacting forces involved in the adhesion process. The idea is to quantify the interaction in order to contribute with the whole Consortium (ANR Glycomime) at the identification of the most appropriate synthetic glycoconjugates.

These measurements will be performed by Atomic Force Microscopy (AFM) in the spectroscopic mode. Several studies have shown that it is possible to estimate the intermolecular interaction forces using an AFM tip functionalized with probe molecules in interaction with a surface functionalized with target molecules. Most of them were carried out on model system DNA/ DNA, Streptavidin/ Biotin, cell-cell [3]... This is the first one on this particular system with such application in Nanomedecine.

Research subject- work plan

Different AFM tips will be prepared, terminated by a bacteria or a cell, by lectins or glycoconjugates. The objective of the Post-doctoral work is to develop a robust and a reproducible chemistry to functionalize the AFM tip. Most of the work will be performed within the team "Chemistry and Nanobiotechnology" at INL, the surface chemistry and the test of the tip. The team is specialist in surface functionalization for biosensors and bio-arrays elaboration [4, 5]. The tips will be tested by different surface analysis such as ATR-FTIR, Fluorescence, SEM and AFM techniques.

Then, the tips will be used to interact with cells in partnership with the Institute of Physiology, University of Münster (Germany), tips terminated with a bacterium or a cell will be performed to study the cellular adhesion close to the real system. Then the interaction bacterium/cell will be done in presence of synthetic glycoconjugates to study the inhibition of the adhesion.

Scientific environment

The Post doc applicant will work in the team "Chemistry and Nanobiotechnology" supervised by Prof Magali Phaner-Goutorbe, in partnership with the Institute of Physiology, University of Münster (Germany), Dr. Hermann Schiller. This work is a part of the project ANR Glycomime involving different labs:

- Sébastien Vidal, Institut de Chimie et de Biochimie Moléculaires et Supra Moléculaires, Laboratoire de Chimie Organique 2 – Glycochimie, Université Lyon 1
- François Morvan and Jean Jacques Vasseur de l'Institut des Biomolécules Max Mousseron, Département des Analogues et Constituants des Acides Nucléiques (DACAN) Université de Montpellier 2,
- Olivier Vidal de l'Unité de glycobiologie structurale et fonctionnelle, Université de Lille.

Candidate skills

The candidate should have good knowledge in AFM in imaging and spectroscopic mode, have a taste for experimentation, surface chemistry and AFM measurements, eventually for modeling (for the forces curves) and be motivated by multidisciplinary research, especially by applications into Biology. Knowledge in Biology is not required.

References

- 1- *AFM investigation of Pseudomonas aeruginosa lectin LecA (PA-IL) filaments induced by multivalent glycoclusters*, D. Sicard, S. Cecioni, M. Iazykov, Y. Chevlot, S. E. Matthews, J.-P. Praly, E. Souteyrand, A. Imbert, S. Vidal and M. Phaner-Goutorbe, Chem. Commun., 2011, **47**, 9483–9485
- 2- *Caractérisation par microscopie à force atomique des arrangements protéine/sucre impliquant la lectine PA-IL de la bactérie Pseudomonas aeruginosa*, Delphine Sicard, Thèse de doctorat, 26 novembre 2012
- 3- *Atomic force microscopy : a nanoscopic window on the cell surface*, D. J. Müller, Y. F. Dufrêne, trends in Cell Biology, 2011, 21,8
- 4- *Silanization of silica and glass slides for DNA microarrays by Impregnation and Gas phase Protocols: A comparative study*, M. Phaner-Goutorbe, V. Dugas, Y. Chevlot, E. Souteyrand, Materials Science and Engineering, C31, 384-390, (2011)
- 5- *Immobilization of single-stranded DNA fragments to solid surfaces and their repeatable specific hybridization: covalent binding or adsorption?*, V. Dugas, G. Depret, B. Chevalier, X. Nesme, E. Souteyrand, Sensors and Actuators, B: Chemical 2004, 101, (1-2), 112-121