Thesis subject:

Characterization of the nanomechanical properties of biological lipid membranes with a new Atomic Force Microscopy mode: the Circular mode AFM.

PhD Advisors:

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Context of the thesis:

The thesis is part of the project activities of the Laboratory of Excellence (LABEX) at the Université de Technologie de Compiègne (UTC) in France on the Control of Technological Systems of Systems (MS2T) (www.utc.fr/labexms2t).

Nanobiomechanics concerns the characterization of the mechanical properties of biomaterials or of biological systems performed by combining techniques derived from Nanosciences and Physics. The main techniques permitting to obtain mechanical quantifications on biological samples on the nanometer scale are atomic force microscopy (AFM), optical tweezers, magnetic cytometry and micropipette. Among these techniques, AFM largely proved its uniqueness in providing both mechanical and topographic information on living samples under physiological conditions with a high resolution. The (nano)mechanical characterization of biological specimen is essential to understand the structure, interactions and processes of living organisms and to the development of biotechnological/biomedical applications. AFM is a scanning probe technique where the surface of a sample is scanned while the interaction forces with a sharp cantilever tip are recorded. The (x, y, z) positioning of the sample is adjusted by a piezoelectric scanner ensuring high-precision movements. A laser beam is reflected on top of a soft AFM cantilever and it is collected by a photodiode to record tip deflection. While the specimen is moved by the scanner, the deflection signal measures the forces resulting from the interaction between the AFM tip and the surface with piconewton sensitivity. AFM is particularly well-suited for the characterization of biological samples because it can operate in aqueous solutions under physiological conditions, in real-time and with (sub)nanometer resolution [Muller and Dufrene, 2008; El Kirat et al., 2010].

Biological membranes separate biochemical/cellular reactions within specific compartments and protect the cell interior from the outside environment. They consist of lipid bilayers in which proteins are ensuring the selective transport of nutrients, wastes, ions, etc. The organization of biomembranes has attracted much attention in the last decade [Bagatolli et al., 2010], and some of the most important issues were addressed with the help of AFM [El Kirat et al., 2010; Garcia-Manyes et al., 2010; Morandat et al., 2013]. The nanomechanics of biological lipid membranes can be measured with the AFM by approaching the tip to the surface, which increases the vertical force it applies on the membrane [Morandat et al., 2013]. As a result, the lipid membrane is progressively compressed between the AFM tip and the solid support until the membrane is suddenly pierced and the tip jumps to contact with the support. With this method, AFM determines the force required to punch through the membrane, which measures the thickness and the mechanical resistance of SLBs at the same time on the approach portion of the force curve [Butt and Franz, 2002].

So, for the moment AFM can only measure forces under normal stress, thus giving access to the punch-trough resistance or to the adhesive forces (bioaffinity) of living samples. Some published studies report the measurement of lateral forces related to the friction force of biomembranes but the usual AFM equipment is very limited in terms of speed to permit the rapid and efficient measurement of friction forces (it takes at least 4h to obtain a friction profile for a single sample) [Morandat et al., 2013]. Most importantly, with classical AFM modes the sample is scanned line by line from left to right

then back from right to left, so the speed of the AFM tip is changing all the time making very difficult the exploitation of the friction force (friction requires a constant speed scanning process). By contrast, the circular mode AFM is well-suited to friction force measurements (scanning speed is kept constant) and since the movement of the tip is going in the same direction (turning clockwise or anti-clockwise), the speed of scan can be increased up to (1mm/s compared to 0.1mm/s with classical AFM modes). This friction profile can give access to the viscosity and diffusion rates of lipids in lipid membranes, which are essential parameters to monitor the health status of living cells (cancer, hypertension, stress...).

PhD thesis description:

In this project, we propose to apply a new circular AFM mode developed by P.E. Mazeran (Roberval Lab) and O. Noel (Univ. du Maine) [Nasrallah et al., 2010] to measure the friction force of biomimetic lipid membranes in order to demonstrate the usefulness of this new mode in biomechanics. We will first characterize the friction force of simple models of biomembranes (mono- and bilayers of lipids with up to two lipid components). Then the models will be completed with other lipids to obtain lipid membranes mimicking the natural envelope of cells. As shown recently with our unpublished pioneering results, these characterizations with the AFM in circular mode offer the possibility to measure the friction and the punch-through forces at the same time, with the complete friction curve obtained within 15 minutes. Punch-through forces are thoroughly described in the literature for biomembranes. Since friction forces on biomembranes on the nanometer scale are not known yet, we will verify the reliability of our results via the punch-through forces measured simultaneously.
Experiments will be scheduled as follows: Year 1: characterization of lipid monolayers (2 components models) by circular mode AFM; Year 2: characterization of lipid bilayers (2 components models) by circular mode AFM; and Year 3: characterization of complex biomimetic models of lipid membranes by circular mode AFM.
During the three years, the experimental procedures and the treatment/analysis of force/friction curves will be improved to have well-defined recipes and tools dedicated to circular mode AFM. This new mode of AFM is already patented, and all the biological applications are the most promising ways of exploitation by industrials (AFM companies such as Bruker are already interested if applicable to Biology).

Candidate's profile: M.Sc in Physics, BioPhysics or Mechanics with notions of Biology.

Financial Support requested: 36 months salary of Ph D student. Equipment (Langmuir-Blodgett trough 25 keuros, hardware upgrade of multimode AFM for circular mode 32 keuros) and 24 keuros of consumables (lipids, surfaces, AFM tips, mica surfaces, tweezers…) and missions/congress fees (6 keuros).

Adequation to Labex MS2T scientific fields: Optimized design of technological SoSs // Multi-level and multi-physical optimization of a set of complex systems. Indeed, circular mode AFM will require a multi-disciplinary optimization of the biological sample preparation, of AFM hardware upgrade and of treatment/analysis procedures.

Other related projects submitted: one project submitted to the Région Picardie (circular mode AFM on organic thin layers and biologic layers), and one project submitted to the ANR (circular mode AFM on biomolecules to measure bioaffinity).

References:

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