CEA - INSTITUT RAYONNEMENT MATIÈRE DE SACLAY

Post-Doctoral position (2013) of 12 months

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Study of protein ligand-receptor interactions with self-sensing force probes

(Etude des interactions protéiniques *ligand-récepteur* avec sondes de force à capteur intégré)

Keywords : AFM, Ligand/Protein interaction, Self-sensors, Nanotechnology, Surface functionalization

Mots clés : AFM, Interaction Ligands/Protéines, Capteurs intégrés, Nanotechnologies, Fonctionnalisation de surface

ABSTRACT

Atomic force microscopy (AFM) becomes an unavoidable technique to analyze at the molecular level biological matter in its living environment. Initially designed for passive analysis, AFM probes become active tools for nanolithography and nano-fabrication in the field of materials. The latest generation of bio-functionalized tips are now investing biology such as news tools for cell manipulation, drug injection in cells, toxicology detection or medical diagnosis.

The aims of this post-doctoral are: 1) the development of innovative chemistry between a functionalized probe and biological materials, 2) characterization of the assembly with a “single-biomolecule” and finally 3) the analysis of protein interaction of specific receptors with innovative self-sensing force probes. This multidisciplinary subject is focus on the assembly of nanomaterials, on new surface chemistry for bio-functionalization, on AFM force spectroscopy and biochemistry. Techniques for samples characterization such as AFM, FTIR spectroscopy, Electron Microscopy, Fluorescence, Electrochemical spectroscopy and XPS will be used.

RESUME (French)

Le développement des microscopes à force atomique (AFM) pour des applications en milieu liquide est en pleine expansion. Conçues dans un premier temps comme passive pour analyser les surfaces, les sondes AFM deviennent des outils actifs de nanolithographie ou de nanofabrication dans le domaine des matériaux. Les dernières générations de sondes bio-fonctionnalisées investissent aujourd'hui la biologie comme outils de manipulation cellulaire, d'injection intra-cellulaire, de détection moléculaire d'agents toxiques ou de diagnostic médical.

Ce post-doctorat a pour objectif : 1) le développement d'une chimie innovante entre une sonde fonctionnalisée et des matériaux biologiques, 2) la caractérisation de l'assemblage « pointe-molécule biologique unique » et enfin 3) l'analyse de l'interaction protéinique de récepteurs spécifiques avec des sondes de force à capteur intégré innovantes. Le sujet, de nature pluridisciplinaire, portera sur de l'assemblage de nanomatériaux, sur de la chimie de fonctionnalisation de surface, sur la maîtrise de la technique AFM et sur la biochimie. Des techniques telles que l'AFM, la spectrométrie FTIR, la microscopie électronique, la fluorescence, la spectroscopie électrochimique, l'XPS seront utilisées pour la caractérisation des échantillons.

PROBLEMATICS

With its capabilities to "observe", to handle and to explore the functional components of the cell with the sub-nanometric resolution, Atomic Force Microscopy (AFM) revolutionized research in biotechnologies, to become an instrument impossible to circumvent. For the first time, the cellular machinery can be scanned with a space resolution of less than one nanometer in its medium of survival. The intra-proteinic forces of interaction can be analyzed with a molecular sensitivity reaching the picoNewton [1]. The system based on the local scanning probes for force measurement with such a sensitivity offer ultrafine analysis suitable for nanomedicine and the personalized diagnosis [2]. For example, AFM technique makes it possible to refine our comprehension of the destabilization of proteins implied in the neuro-degenerative diseases and the mechanisms by which some molecules bind and modulate the transmembrane receivers of the cells. The characterization of the cell-cell interactions implied in pathologies like cancer, and also the interactions with the disease-causing agents are other research fields where the measuring instruments of force using local scanning probes can bring answers [3]. These force nanosensors allow the diagnosis by recognition of ligands-proteins interactions molecules, in applications concerning pharmacological engineering, toxicology analysis or detection of pathogens agents.

The last generations of functionalized AFM tips invest today biology with tools for cellular handling or medical diagnosis. However, it remains many technological challenges and issues to solve in instrumentation, signal treatment and materials and functionalization chemistry to open new horizons of research. This post-doctoral work will be the opportunity to explore in details a hard point of the AFM force spectroscopy which still remains to be controlled taking into account the current state of the art [1-4]. Indeed, the control of the chemical functionalization of AFM probes will allow the selectivity and reproducibility necessary in the process of analysis and nanomanipulation of proteins present on the substrate.

POST-DOCTORAL WORK

The objective of this post-doctoral work is the functionalization by an innovative surface chemistry of novel scanning probes, based on self-sensors we proposed recently [5-6], to obtain toxic agent molecular detection. The analysis of this assembly of nanomaterials with biomolecules and the application of such a system for the development of a diagnosis tool for cells will be also treated in this work. This work will start by the fabrication of this new AFM probes. Then, these new tips will have to be functionalized by a chemistry developed in our lab [7]. Hence, this chemistry gives spontaneous chemical grafting properties between the probe and the biomolecules such as peptides or proteins. This strategy will allow to covalently fix on the tip apex only one biomolecule. Furthermore, this technological breakthrough will give the possibility to graft in biocompatible conditions without previous necessary modification of the protein or without the use of the standard multi-steps chemical techniques exposed in the state of the art [4]. This latter point is an important benefit contributing to largely limit the usual risks of artefacts. That is particularly the case for the repetitive force spectroscopy measurements by AFM conducted during long period [4] dependant of the stability problems of the tip apex grafted with chemical material.

The assembly will be characterized by different techniques of the physico-chemistry (XPS, EIS (Electrochemical Impedance Spectroscopy), Infrared spectrometry, fluorescence, …). It will be also study on a reference biological system in order to obtain the minimal detection sensitivity of the AFM characterizations, and by the same to validate this new approach for the development of a new tool for toxic agent molecular detection.

For this post-doctoral position with an experimentalist profile, the candidate will have to apprehend different surface analysis techniques for thin layers with the ATR-FTIR (Attenuated Total Reflection Fourier Transform Infrared), the XPS (X-Ray photoelectron spectrometry), Electrochemical grafting, fluorescence, and SEM (Scanning Electron Microscopy), the synthesis techniques of organic chemistry, of surface chemistry and irradiation chemistry, but also AFM technique. Previous skills of the candidate for several of these techniques will be greatly appreciated.

This interdisciplinary project will be jointly performed between a surface chemistry lab and a biology lab of CEA:

_ The “Interfaces and Surfaces Chemistry Lab” (CEA/DSM/IRAMIS/SPCSI, Dr Jérôme Polesel Maris, Dr. Thomas Berthelot, Dr. Pascal Viel)

_ The “Immunoanalysis studies and research laboratory (LERI)”, (CEA/DSV/iBiTec-S/ Department of pharmacology and immunoanalysis (SPI), Dr. Hervé Volland, Dr. Cécile Féraudet-Tarisse).

Financial support: CEA program over 12 months.

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