Overlay for Membrane-Bound Protein Biochips

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The cell's membrane plays a fundamental role in the function and structure of an organism. It protects the inner part of the cell from the outside and allows chemical components such as food to get in or waste to be evacuated. Due to its complex mixture, based on several kinds of phospholipids and numerous proteins, its studies represent a major challenge for chemists, biologists, and biochemists about the function and role of each membrane's components. Studying a cell's membrane in vivo turns out to be challenging, but thanks to the development of the so called structure "Supported Bilayer Membrane[1]", it is now possible to reproduce in vitro and in a controlled way this bio-structure. It enables to study in an environment as close to their native one using tools as fluorescence microscopy or more recently Atomic Force Microscopy (AFM) [2]. The development of Atomic Force Microscope in liquid rendered the possibility to study behavior of membrane components at the micro and nano-scale level; for example the behavior of a mixed phospholipids membrane, proteins organized into 2D crystals, or in protein's complex [3]. More recently, the gathering between patterning methods and self-assembly of nature's elementary bricks has opened the way for the elaboration of complex surfaces, presenting attractive properties.

Since its discovery almost 15 years ago, MicroContact-Printing (μCP) represents a way to generate patterned surfaces in an easy and cheap way [4]. By mixing patterning methods and formation of Supported Bilayer Membrane, we can now generate Membrane Bound Proteins Biochips which can be analyzed using AFM, fluorescence microscopy or both at the same time. Based on these developments, we have elaborated two kinds of samples for studying membrane protein complexes. One sample based on a single pattern of repellent molecules coupled to the self-assembly of a Supported Bilayer Membrane[5] and a second sample with a more complex surface patterns[6]. The use of the overlay option, which mixes fluorescence to AFM imagery enables us to control and quantify the quality of each sample step. Figure 1 summarizes the formation and results of the first approach we call "Patterned Supported Bilayer Membrane", while figure 2 and 3 present fluorescence and AFM results.

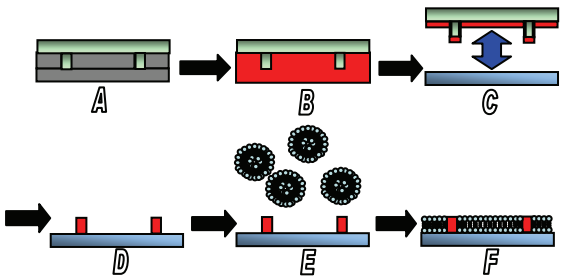

Figure 1: Principle of the formation of Patterned Supported Bilayer Membrane

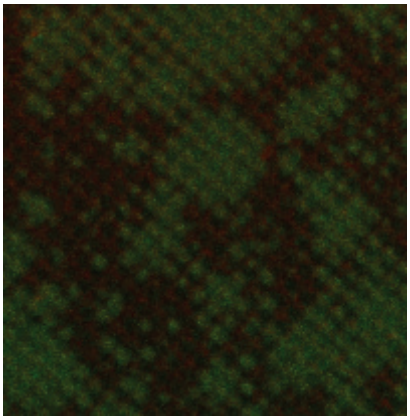

Figure 2: Fluorescence imaging of Patterned *E.Coli* Supported Bilayer Membrane. Scale bar 20µm

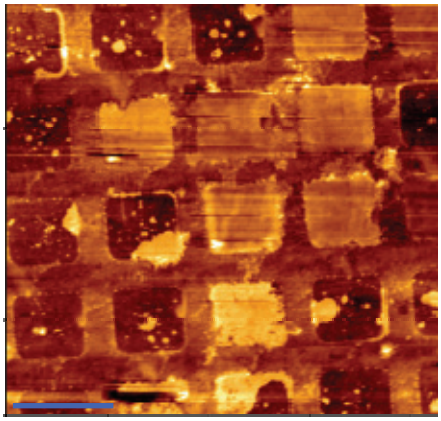

Figure 3: AFM imaging in liquid of *E.Coli* patterned membrane. Scale bar 5µm

Figure 4 and 5 show results obtained by OSM-µCP (One-Step-Multiple-µCP) coupled to the formation of a Supported Bilayer membrane. Both samples are currently being used for the study of a complex protein structure, the flagellum nano-motor. We will present the results we have obtained on both samples and show the significance of these approaches and also what kind of information can be extracted from an approach mixing AFM and Fluorescence imagery.

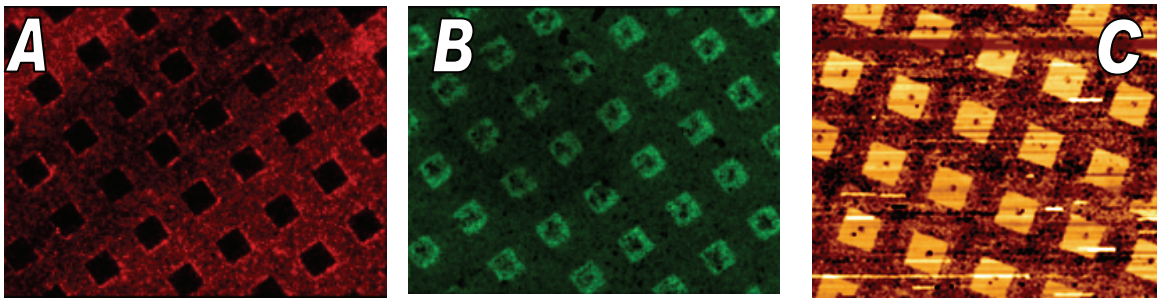

Figure 4: Fluorescence imaging of the different patterns. A: the Pll-g-PEG patterns, B: Green corresponds to the EggPC Supported Bilayer membrane, while the dark spot to antibodies anti-GST, C: AFM imaging of the surface in liquid media

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