Laboratoire d'Analyse et d'Architecture des Systèmes

Coupling bio-assembly and surface patterning: partial reconstitution of a biological nano-machine the flagellar nano-motor of bacteria – FLANAMO ANR Project

Jerome Chalmeau a, Adilia Dagkessamanskaiab, Christian le Grimellec c,
Christophe Thibault a, Franck Carcenac a, Jean Marie Francois b, John Sternick d, Laurence Salomé e and Christophe Vieu a
a LAAS-CNRS ; Université de Toulouse ; 7, avenue du Colonel Roche, F-31077 Toulouse,
b ISBP, UMR CNRS 5504 & INRA 792 ,135 Avenue de Rangueil, F-31077 Toulouse Cedex 04.
c CBS-INSERM U554/CNRS UMR 5048, Universités Montpellier I and Montpellier II,29 rue de Navacelles 34090, Montpellier Cedex, France.
d Department of Biology, Mansfield University, Mansfield 16933 PA, USA
e IPBS, Université de Toulouse, IPBS, 205 route de Narbonne F-31077 Toulouse Cedex 04

Abstract

The Flagellum is a long thick filament which rotates at high speed and propels the bacteria through the media. A nano-machine, composed of assembled proteins embedded within the bacteria membrane, generates the rotation of the filament using as an energy source a chemical gradient between the outer and inner part of the cell. The nanoscale in-vivo investigation of this nanomachine remains quasi impossible, due to the invasiveness of the experimental methods which commonly alter or destroy the native configuration. We have thus developed a new challenging approach for studying in vitro the flagellar nano-motor, by coupling surface patterning and self assembly of proteins in liquid media. Thanks to the recent development of Atomic Force Microscopy (AFM) in liquid media, we are today on the way to study part of the Flagellar Nano Motor at the nanometric scale in environmental conditions as close as possible as the native ones encountered in the living organism. This work describes the technology associated to both the surface patterning required to create supported membranes and the nanoscale imaging of the assemblies of the proteins constitutive of nanomotor on these engineered surfaces.

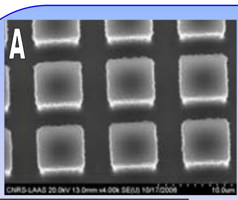
Grid patterns of Poly-Lysine-grafted-PolyEthylenGlycol Pll-g-PEG were achieved by µCP followed by an incubation of *E.Coli* phospholipids preformed into liposomes. A serie of patches of Supported Bilayer Membrane (SBM) of *E.Coli* phospholipids is generated on the surface and exhibit a repellent part (Pll-g-PEG patterns) and an attractive one (SPB). We overproduced high quantity of purified flagellum nanomotor proteins and incubated them on these patterns of SPB. The use of a patterned SPB into disconnected domains reduces the phospholipids and proteins diffusion into micrometric or nanometric patches and opens the possibility of a controlled protein assembly within each domain. By coupling fluorescence imaging for the membrane and PEG components with AFM imaging for the flagellar nanomotor proteins, we thus have a reliable process for investigating the possible assembly of natural nanomachines on artificial solid surfaces.

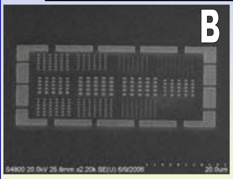
Step 1-3

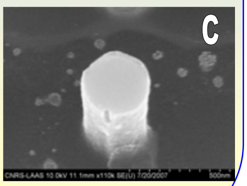
1. A silicon mold has been generated using Photo-lithography for micro patterns or Electron-lithography for nanopatterns. These molds have been treated using OTS as an anti-adhesive coating

2. A stamp is generated by curing a carefully mixed PDMS pre-polymer solution

3. The PDMS stamp is then inked by a Poly-Lysine-Grafted –PolyEthylenGlycol-tagged rhodamine (Pll-g-PEG-R) solution for 2 min at 100µg/ml in Hepes 10mM NaCl 150 mM

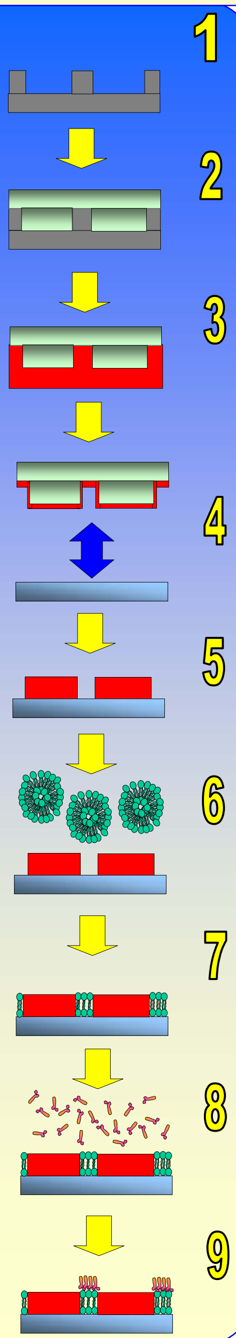




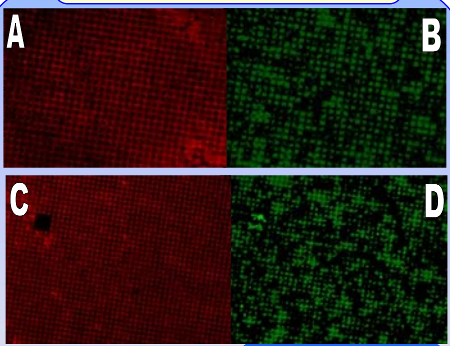


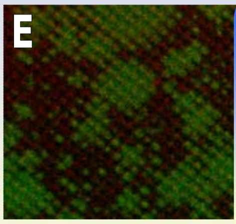
A. SEM view of the micronic silicon mold
B. SEM view of the nano patterns
C. exhibiting different sizes
D. Enlarged view of a single nano-pillar of 400 nm

Process

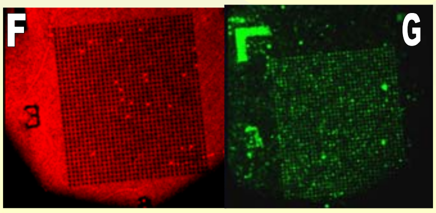


Fluorescence imaging Step 5 and 7





A: Fluorescence images of the Pll-g-PEG-rhodamine, 8 µm square
B: PEPG-tagged FITC SPB patches
C: Pl-g-PEG patterns 4 µm square
D: Patches of PEPG
E: Mismatches of both Fluorescence images
F:Nano patches of 400nm
G : PEPG SPB nano-patches



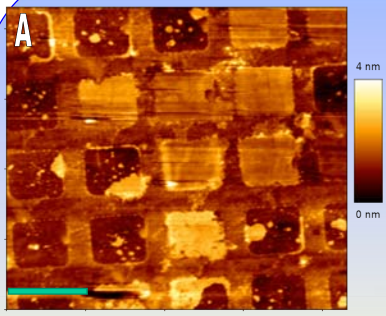
Step 4-7

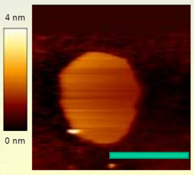
4. The inked stamp is brought into contact with a fresh and cleaned surface, regular glass slide or Mica sheet, presenting hydrophilic properties

5. The Pll-g-PEG-R molcecules are transferred from the stamp to the surface and created the desired patterns

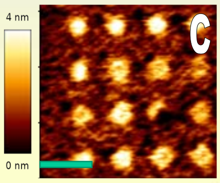
6. The prepared surface is then immersed into a solution with liposomes of E.Coli (73,5%PE, 25%PG, 1,5% FITC-DHPE) 100µg/ml in Hepes Nacl Cacl2 for 10 minutes

7.Lipsomes fuse into the patterns and form a Supported phospholipidic Bilayer (SPB). The samples are then rinsed several times using Hepes NaCl





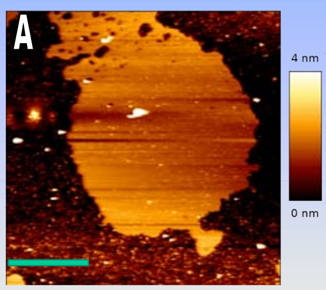
A: AFM image in liquid of the patterned micro-patches Pll-g-PEG-R/ PEPG-G Height signal, 10 µm scale bar
B: single micrometric patch of a SPB Height signal 4µmScale bar
C: AFM image in liquid of Nano-patches of EggPC Supported Bilayer Membrane Height signal 1 µm scale bar

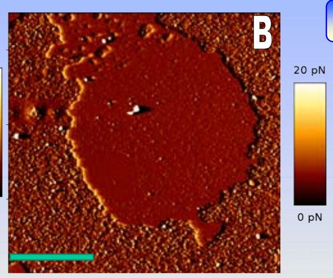


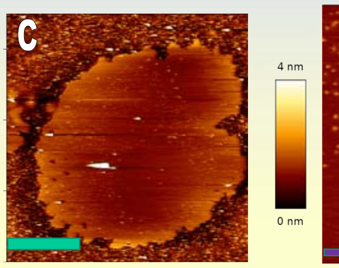
Step 8-9

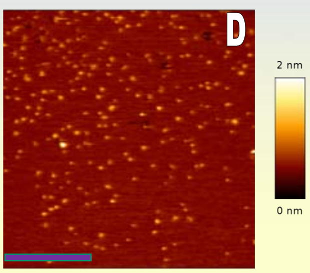
8. Purified proteins, here the FliG ,(40µg/ml in PBS) are then incubated on the engineered surface .

9. Surface is rinsed several time for removing unbound proteins and residues from the solution









A: AFM image in liquid of P-SPB Pll-g-PEG-R/ PEPG-G presenting FliG proteins Height signal, 2 µm scale bar
B: Vertical deflection signal of A 2µm scale bar
C: AFM image in liquid of the patterned micro-patches Pll-g-PEG-R/ PEPG-G presenting FliG proteins Height signal 2µm scale bar
D : Enlarged view P-SPB exhibiting FliG proteins Height signal , 500 nm scale bar

Conclusion

- We have created a reliable method for generating Partioned Supported Phospholipidic Bilayer (P-SPB) on a mica or glass slide surface.
- Fluorescence and AFM imaging in liquid media confirmed the formation of a E. Coli P-SBP on the desired location at the micro and nanometric scale
- By incubating a solution containing purified proteins we conclude that self-assembly of proteic aggregates takes place on the P-SPB

Perspectives

- Others proteins of the Flagellum nanomotor can be added and assembly of the different proteins can be observed on the P-SPB.
- Observation of proteins on a single nano-patche could opens the possibility of studying proteins on a surface in conditions very close to natural ones