Micro et nanotechnologies au service des sciences du vivant et de la médecine

Micro and nanotechnologies for life sciences and medicine

NANOBIOTECHNOLOGIES A VISIT

Christophe Vieu, INSA Toulouse, LAAS-CNRS

« Because technology provides the tools and biology the problems, the two should enjoy a happy marriage »

S. Fields, S. Proc. Natl. Acad. Sci USA 2001

1- Nanotechnologies for Biology

Devices

Materials

« Biology offers a window into the most sophisticated collection of functional nanostructures that exists. »

G.M Whitesides, Nature Biotech, The « right » size in Nanobiotech, 2003

2- Nanotechnologies from Biology

Self-Assembly

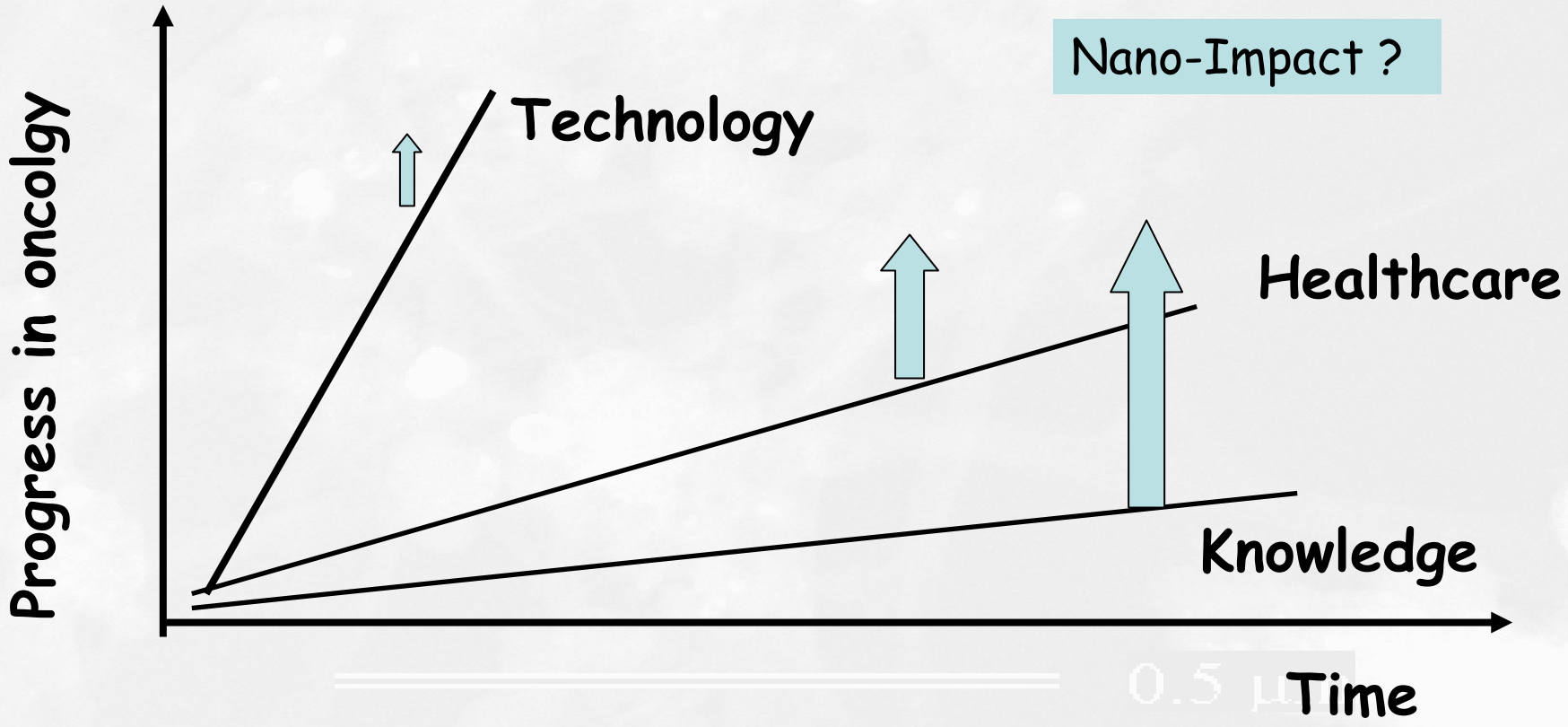
Bio-Nanomachines

3- New knowledge in biology

Nanobiosciences

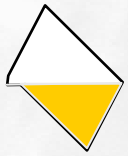
New experimental approaches

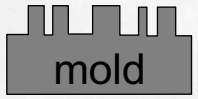
New theoretical approaches



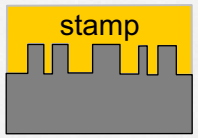
Soft-Lithography as a clue for the visit

Xia Y. and Whitesides G. M. (1998), "Soft Lithography," Angew. Chem. Int. Ed. 37, 550-575.

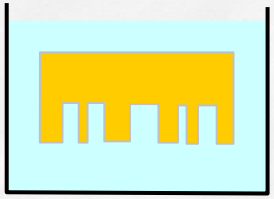




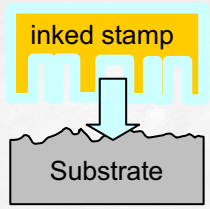
1. Elastomer is poured on the mold



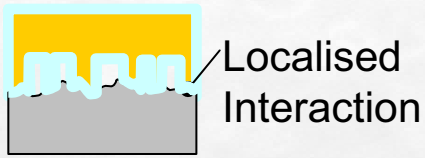
2. Elastomer cross-linking



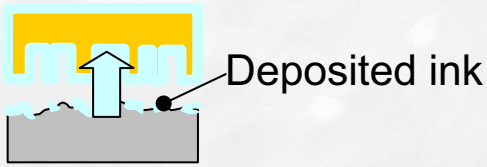
3 . Stamp inking



4 . Stamp approach



5 . Contact



6 . Stamp removal

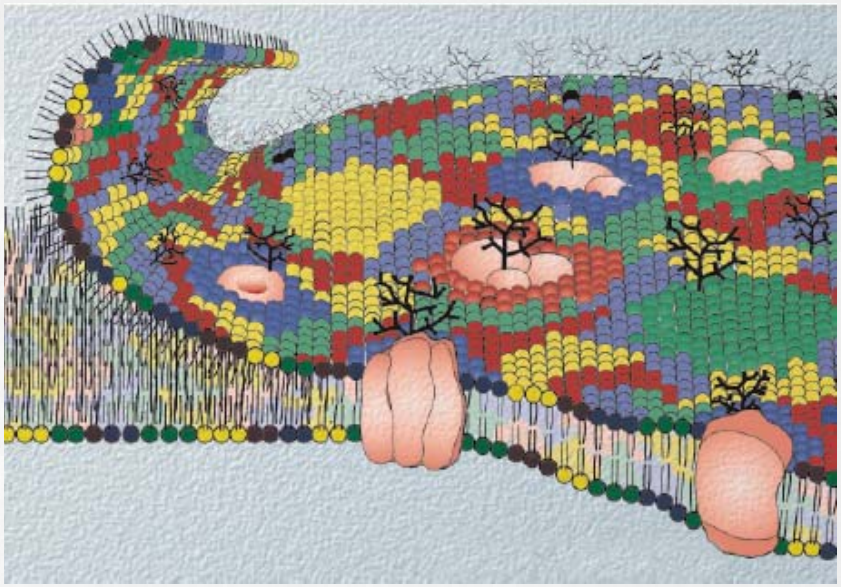
The complexity of Cell biology

-500 000 proteins

-Data processing based on molecular interactions

-Data bus : stochastic

-Circuits: Adaptative



Nature Reviews Molecular Cell Biology 4, 414 - 418

0.5 µm

The complexity of Cell biology

-500 000 proteins

-Data processing based on molecular interactions

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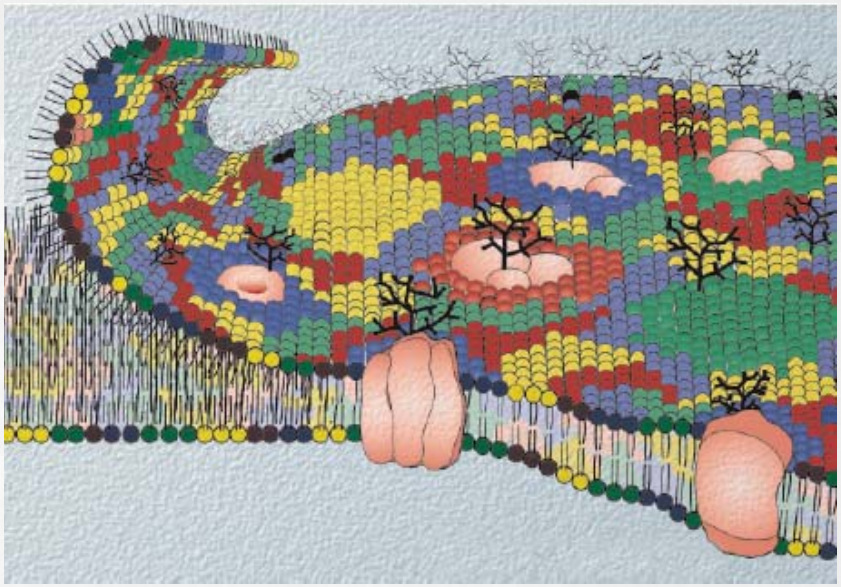


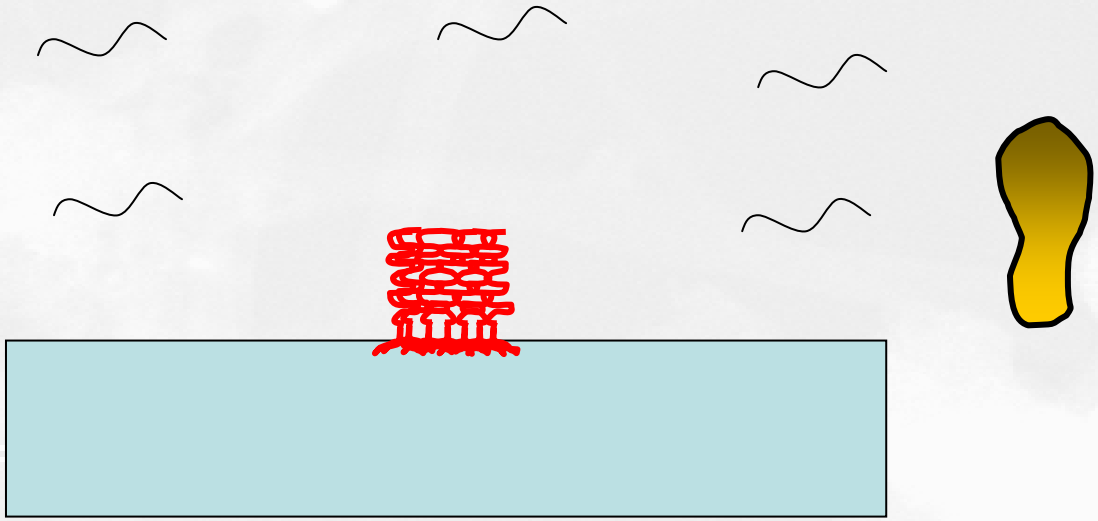
Nature Reviews Molecular Cell Biology 4, 414 - 418

Nanotechnologies for Biology

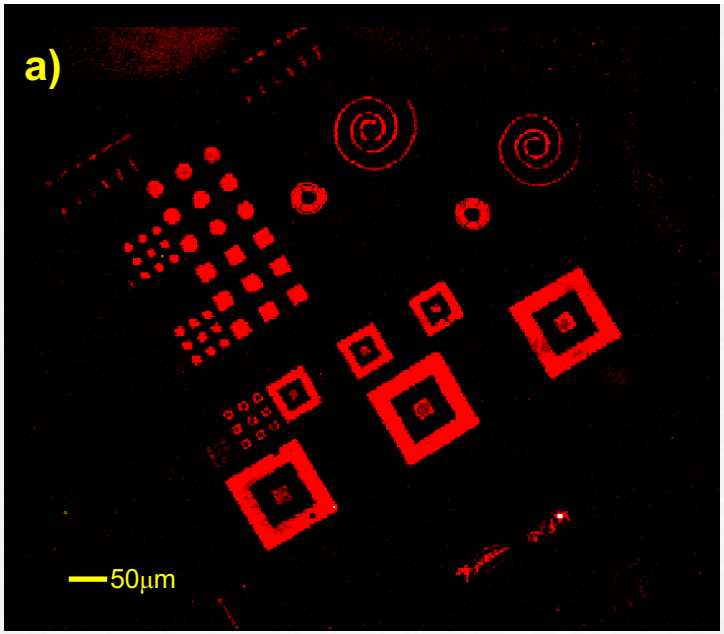
Biopatterning

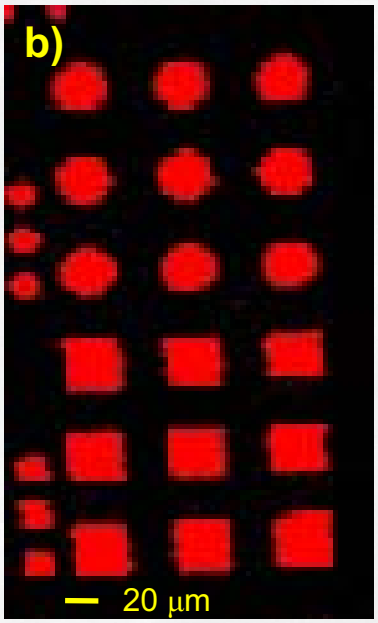
Microfluidics

Biodetection



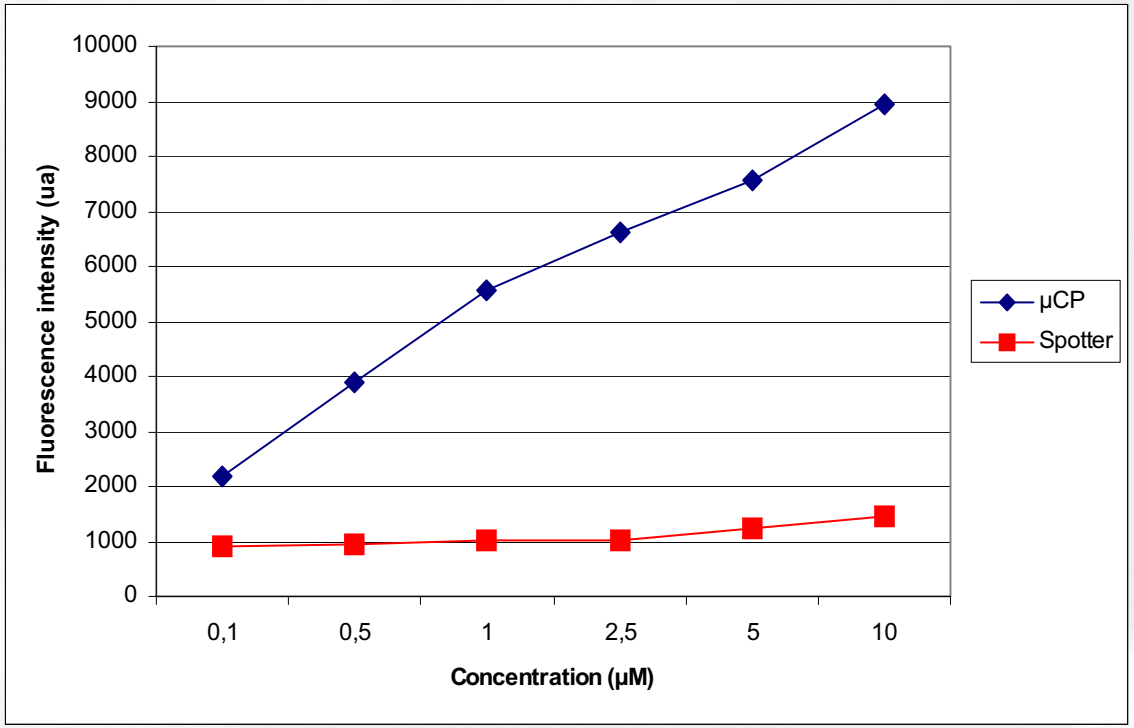
Soft-lithography for Biopatterning





Printed DNA Micro-arrays exhibit improved Fluorescence emission

C. Thibault et al, Journal of Nanobiotech (2005)

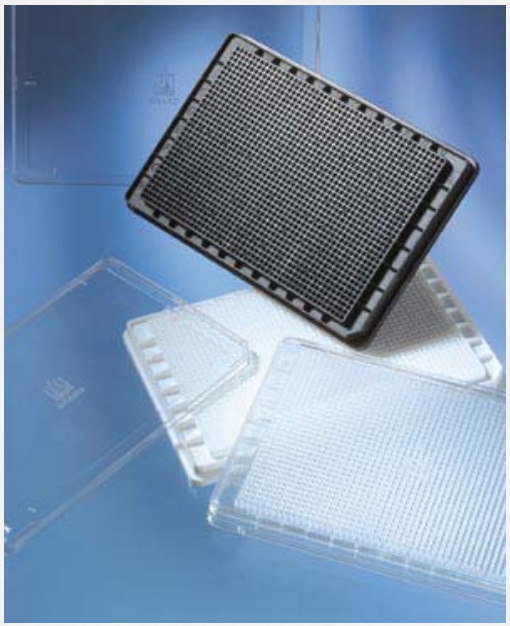


Inking time 30s, printing time 60s, unmodified PDMS stamps !

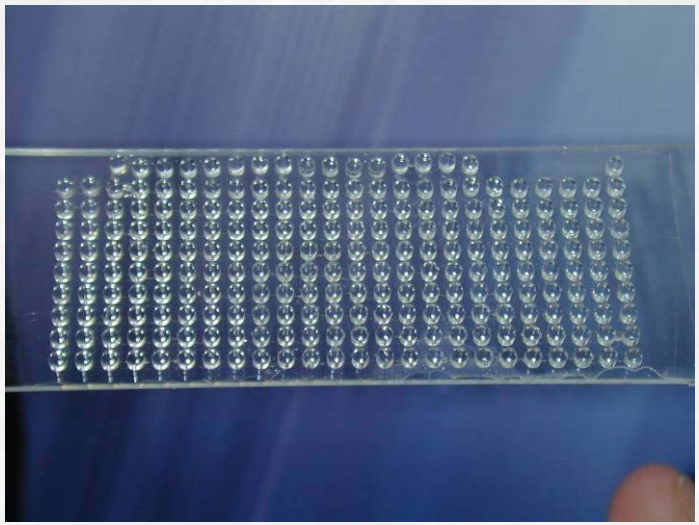
How to print different probe molecules in one step ?

The MacroStamp® concept

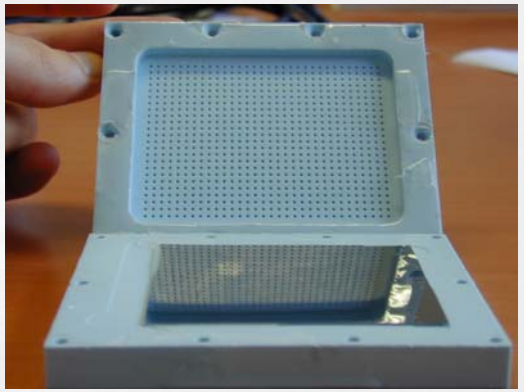
JC Cau, H. Lalo, JP Peyrade, C Vieu, C Thibaut, C Séverac, Patent 16536, 13/09/07



Titration plate format
1536 wells



MacroStamp format on a glass slide



MacroStamp
molding tool

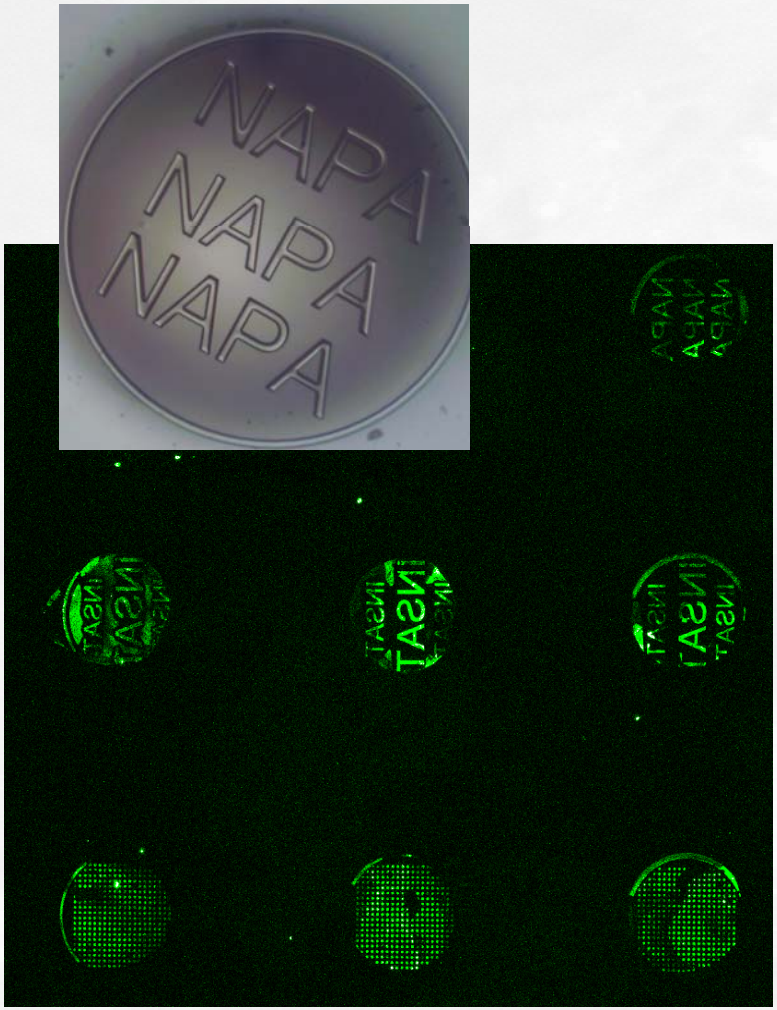
PDMS Macrostamps compliant with titration plates

800 different molecules on a glass slide – with Micro/Nano patterns

How to print different probe molecules in one step ?

The MacroStamp® concept

JC Cau, H. Lalo, JP Peyrade, C Vieu, C Thibaut, C Séverac, Patent 16536, 13/09/07

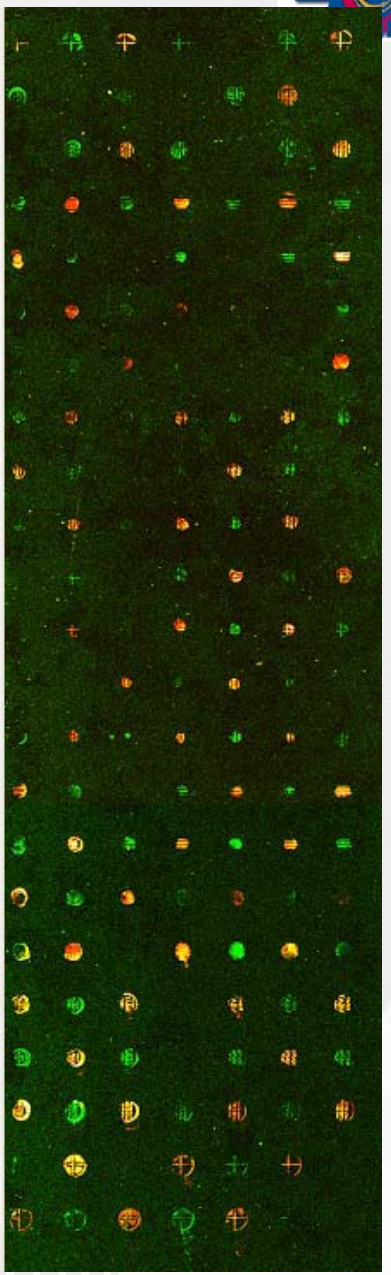


Micronic and

SubMicronic

(arrays 1µm pitch)

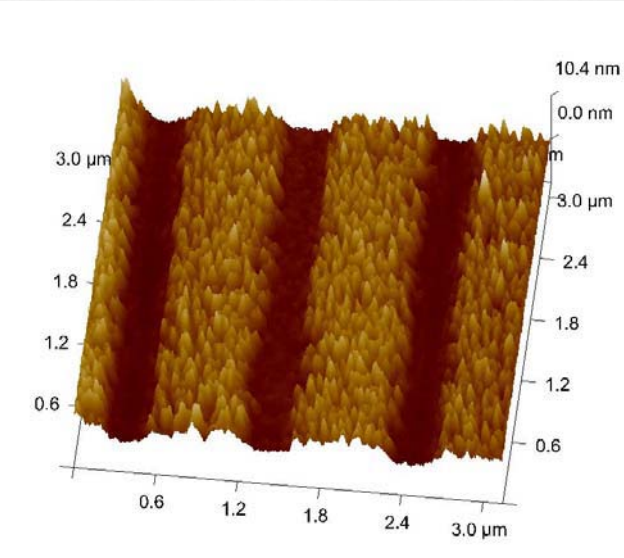
No cross contamination after hybridization

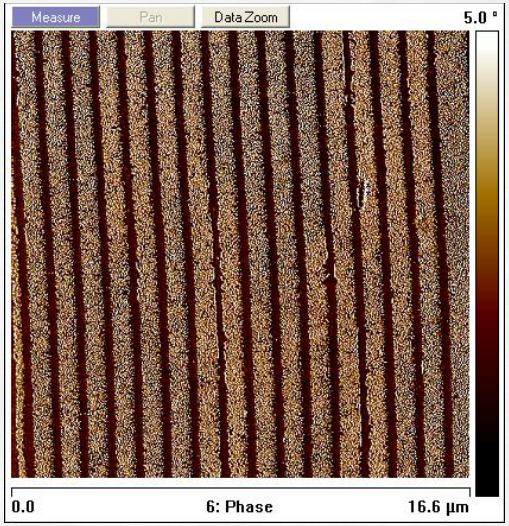


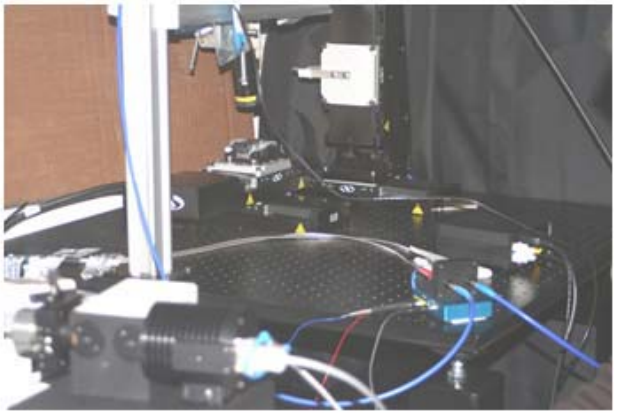
How to use nanoscale patterning capabilities for label-free detection ?

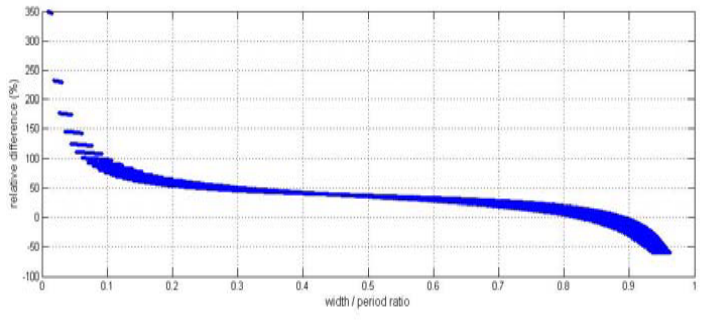
The Diffrachip® concept

JC Cau, H. Lalo, JP Peyrade, C Vieu, C Thibaut, C Séverac, Patent









Make use of diffractive arrays of probe molecules : Targeted sensitivity 100 pM

0.5 µm

High sensitivity detection using nanoscale devices

Basic idea : The active area of the sensor is miniaturized down to the size of the biomolecules to detect

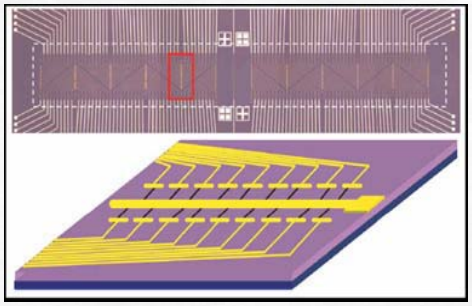
-Electrical sensing : Miniaturization of Transistors

-Mechanical sensing : Miniaturization of QCMicrobalances

-Optical sensing : Using concepts of photonics/plasmonics

High sensitivity detection using nanoscale devices

SoA : How far can we go ?



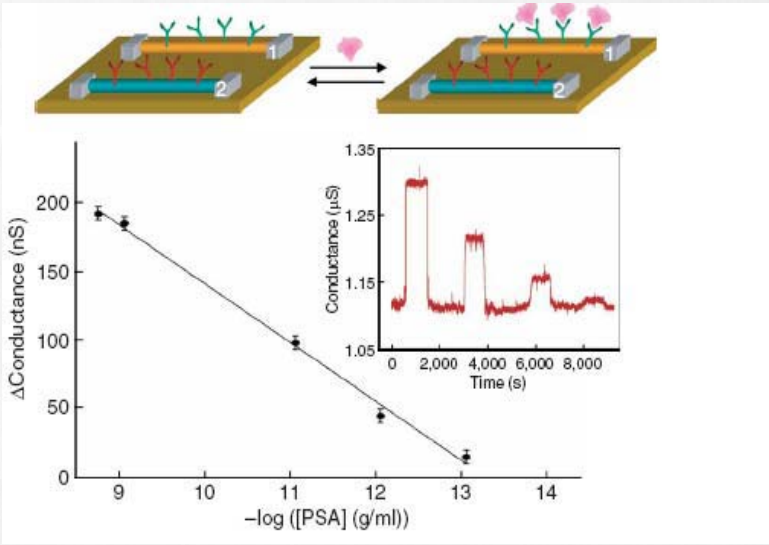
C.M. Lieber et al

Nature Biotech 2005

Nanowires FET

PSA detection inside undiluted serum

0.9 pg/mL (femtoMolar concentration)

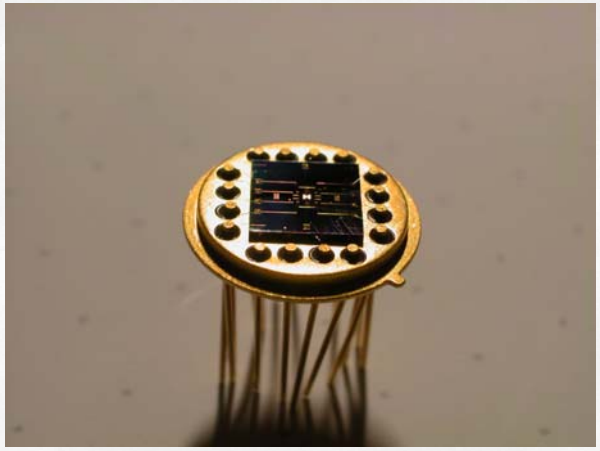


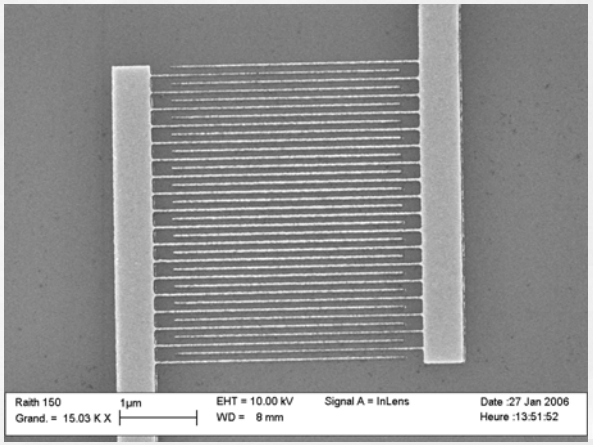
Detection of biomolecules using electrical nanodevices LAAS-CNRS Activity

Sense biomolecules using Conductance measurements

Make use of nanoelectrodes with gap close to biomolecule size

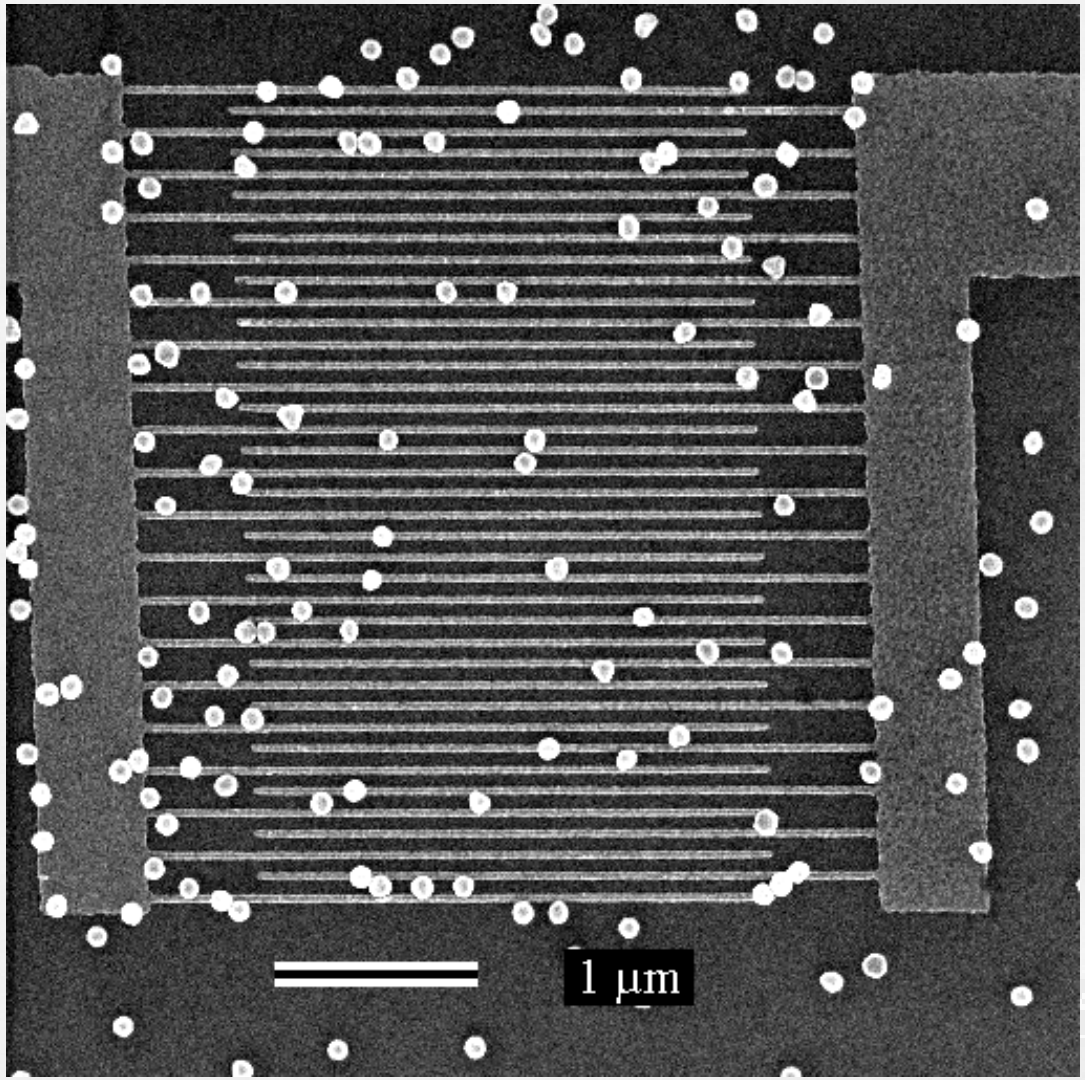
Ultra-high sensitivity



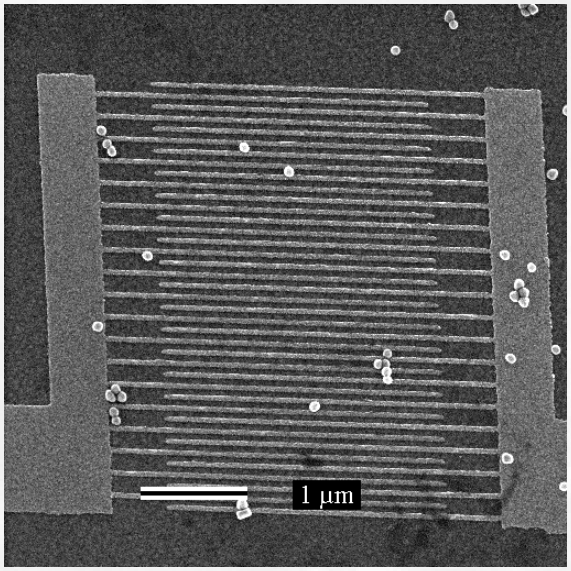


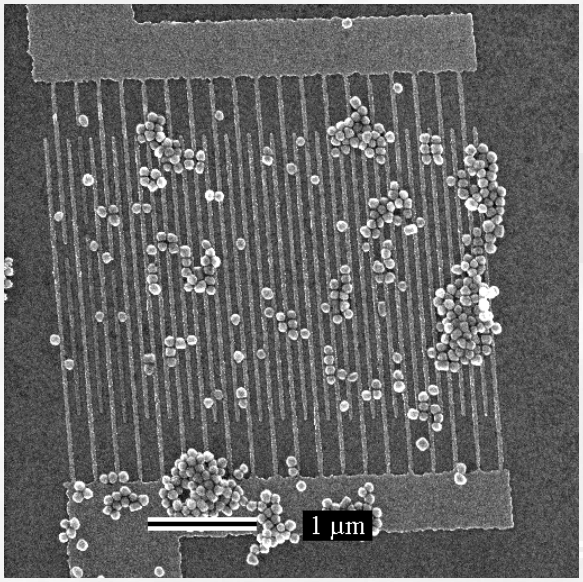
Capable to sense single nanoparticles

L. Malaquin et al, Nanotechnology 16(2005)



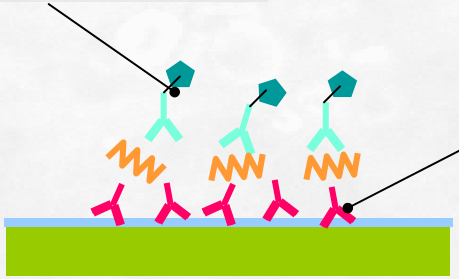
Nano Immuno-Assay





Biotin Labelled AntiBovin Rabbit IgG (A)

Non specific : control experiment

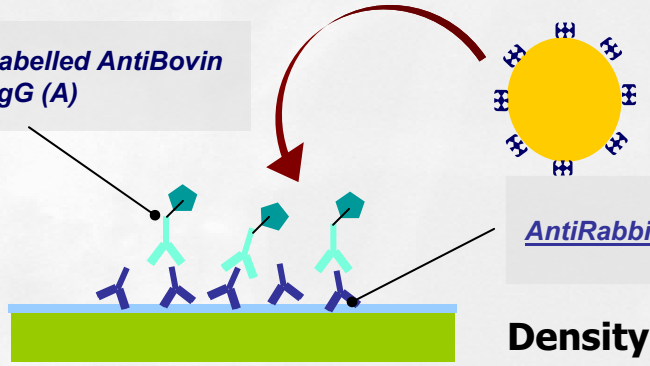


AntiBovin Rabbit IgG (B)

Density < 1 nanoparticle/µm²

Specific : test of recognition

Biotin Labelled AntiBovin Rabbit IgG (A)



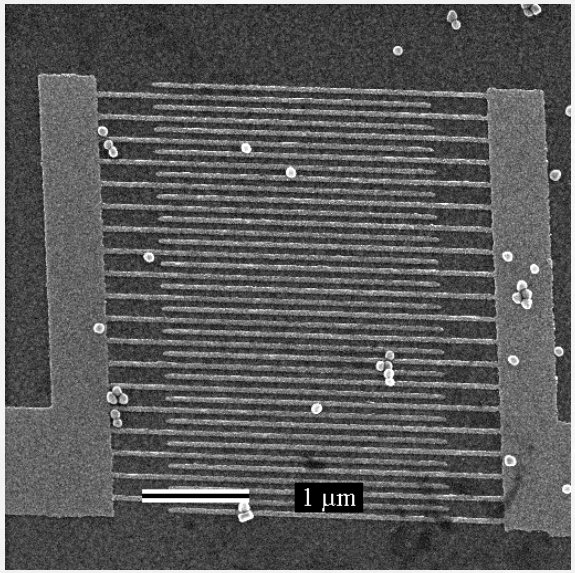
Anti Biotin/Streptavidin labelled Gold colloid

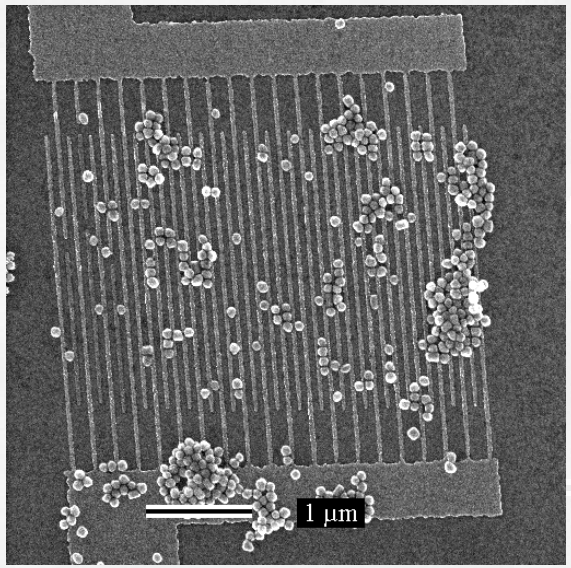
AntiRabbit Goat IgG (C)

Density >10 nanoparticle/µm²

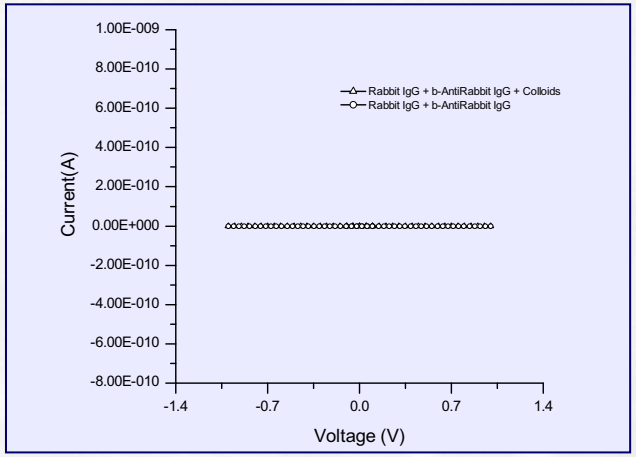
0.5 µm

Nano Immuno-Assay



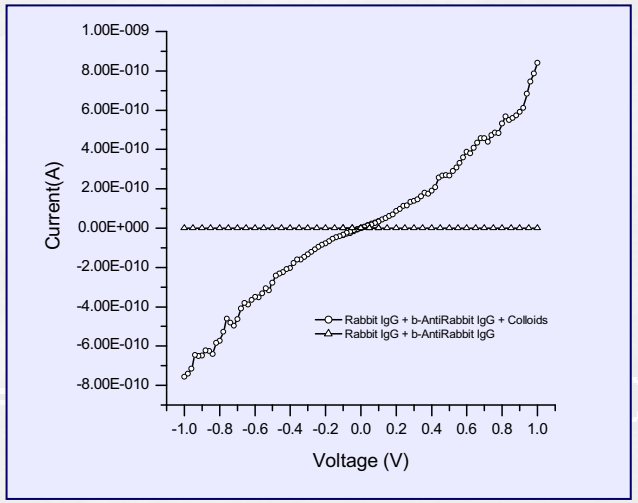


Non specific : control experiment



- **R ~ 1** TOhms
  U=1V

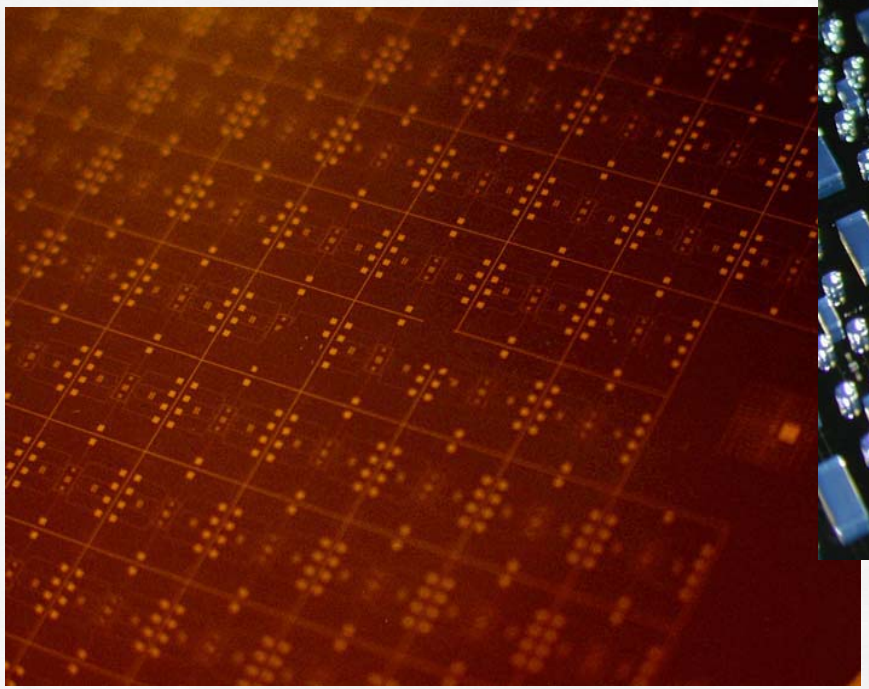
Specific : positive test

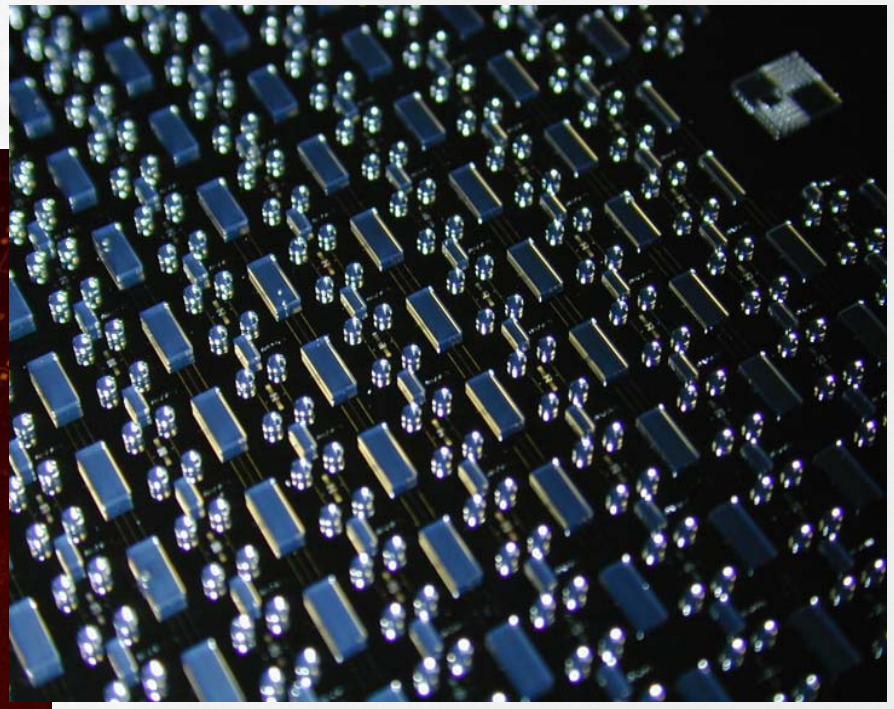


- **R < 1**
  GOhms
  U=1V

Wafer Scale integration of Nanoelectrodes arrays and microfluidic channels for medical diagnostics and high throughput screening

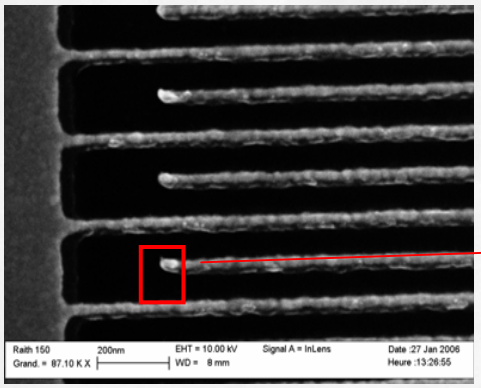
A. Martinez & C. Séverac

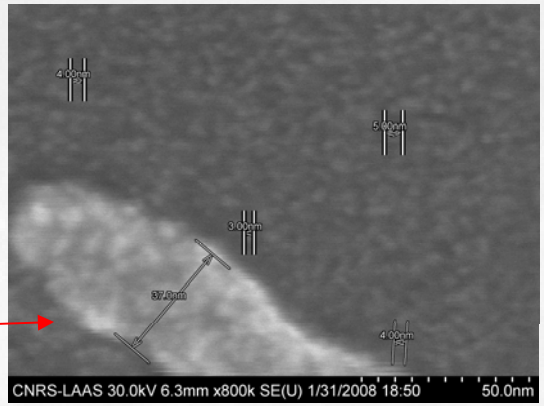


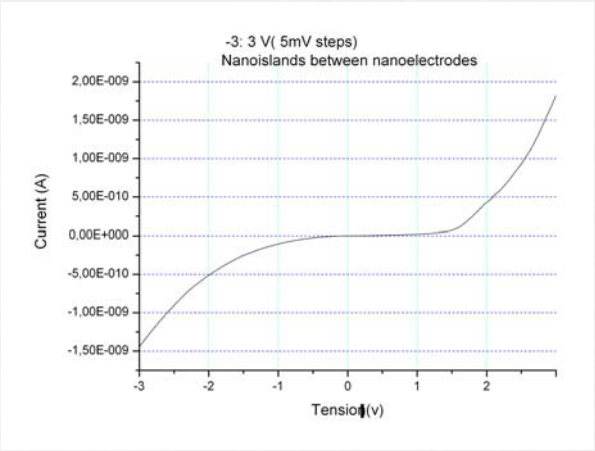


Label Free Electrical Sensing : Multiple Tunnel Junction devices

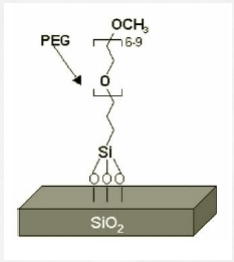
A. Martinez & C. Séverac

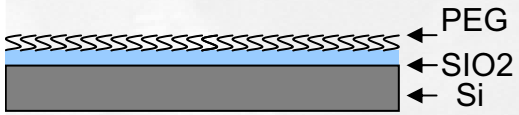






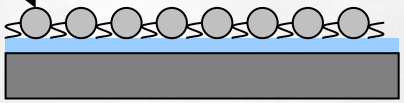
Deposition of Ni Islands between the nanoelectrodes





PEG coating

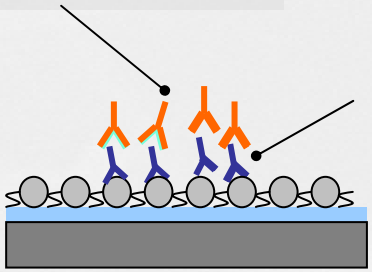
Ni islands (2-5 nm)



Ni Islands deposition

Rho A proteins

His-tagged Abs



Selective detection of activate form of RhoA – Coll Institut Claudius Regaud

High sensitivity Biodetection for medical analysis

Early diagnosis

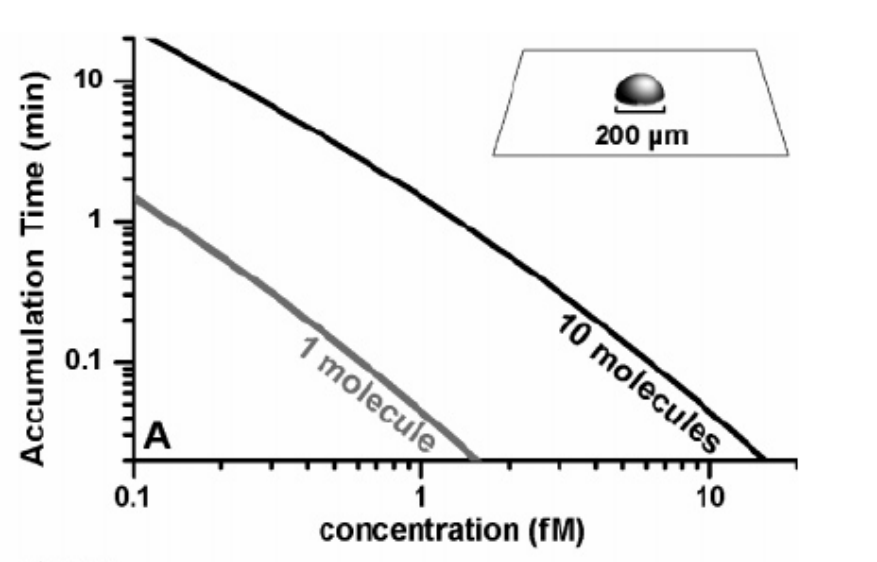
Technical issues of the fishing process:

1- TIME !

P.E. Sheehan et al

NanoLetters 2005

Limit around fM !

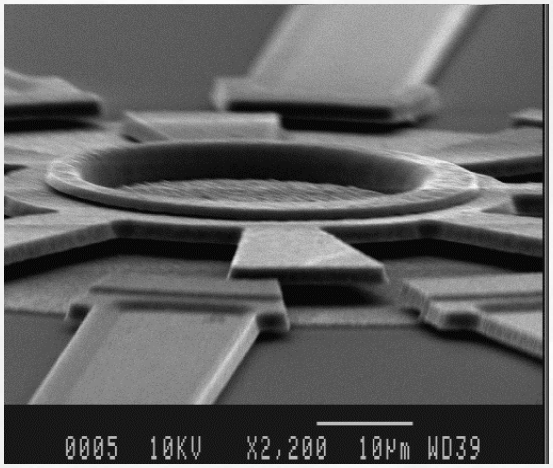


2- The good fish !

Discovery and validation of reliable Biomarkers

0.5 µm

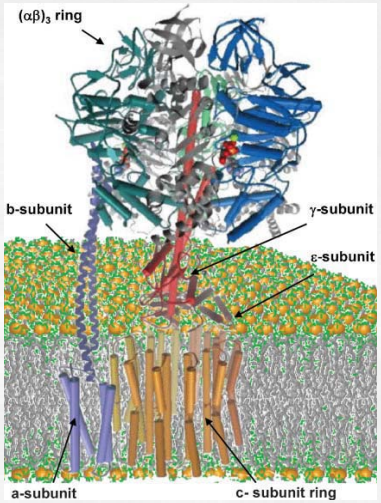
Nanotechnologies from Biology



LAAS Si Micromotor H. Camon et al 1999

- Size > 10 µm
- Hard material
- 2D
- Em actuation
- Poor efficiency
- Air or vacuum
- Fragile

•Techno-assembled : Alignment



D. Spetzler et al. Lab Chip, 2007, 7, 1633–1643

F1-FO ATP synthase

Myosin

Kinesin

Dynein

Flagellar nanomotor of bacteria

- Size : 5-50 nm
- Molecular material
- 3D
- Chemical actuation
- High efficiency
- Liquid
- Self-repairable

•Self-assembled : Stochastic

0.5 µm

PROJET FLANAMO – ANR 2006 PNANO

J. Chalmeau

Assembling the flagellar rotary nano-motor of *E-Coli* on a solid surface through Nanotechnologies

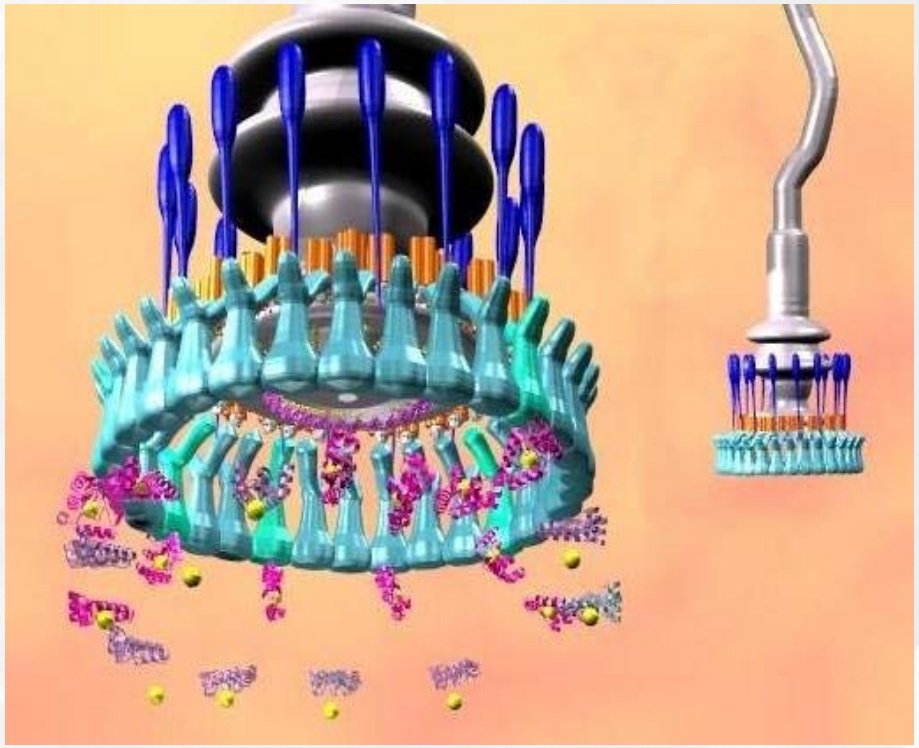
35 Proteins involved

Dimension : 45 nm

Speed : 20 000 rpm – 60 µm/s

Power : 1000 H^+/rotation

Reversible



Method: Engineering of a surface for

Re-creating the conditions of self-assembly

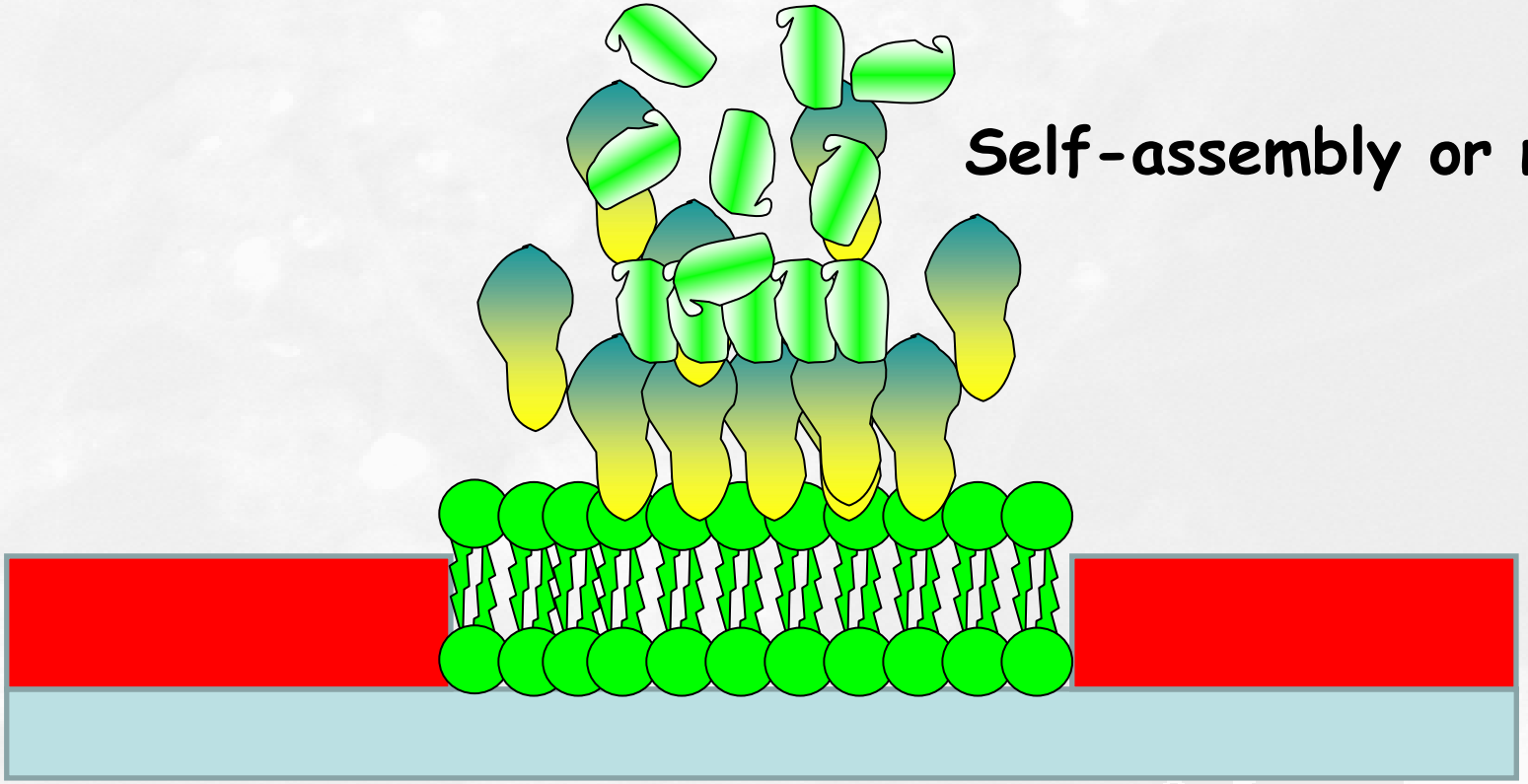
Observation using AFM

Objectives :

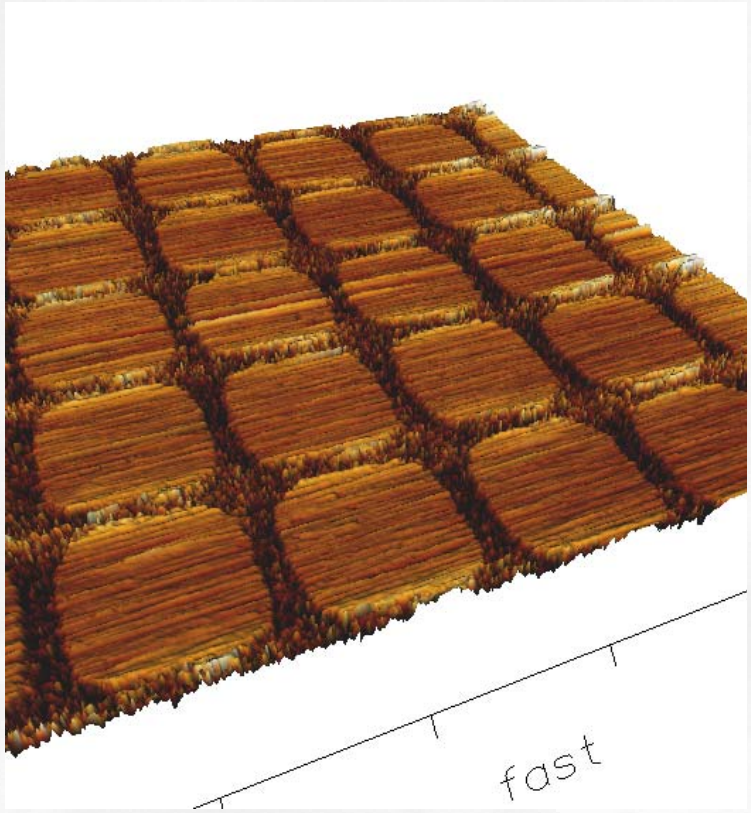
- **Understand the mechanism of the nano-motor**
- **Artificial Assembly of a bio-nanomachine from isolated proteins**

Assembling of purified proteins of the nanomotor on an artificial surface

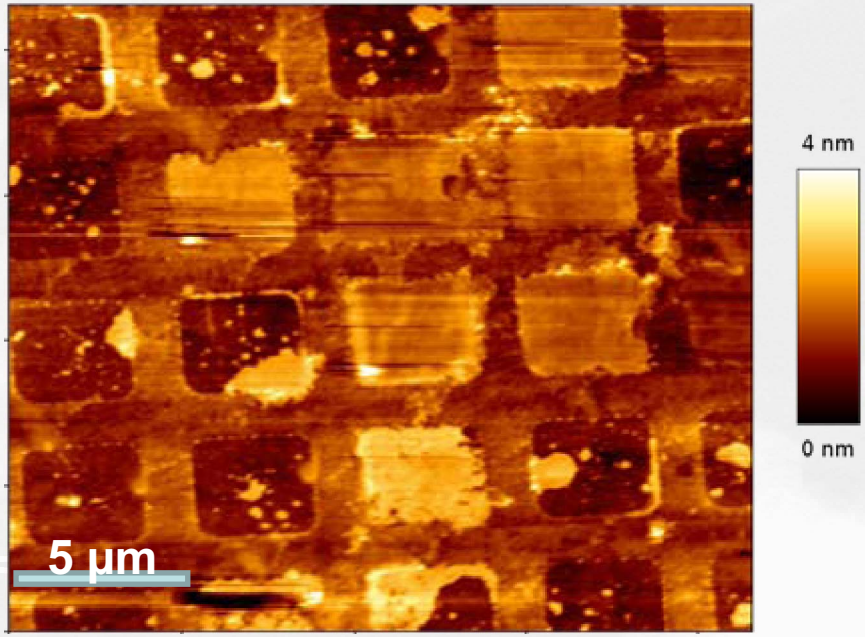
J. Chalmeau



Micro-domains of Supported Phospholipidic Membrane

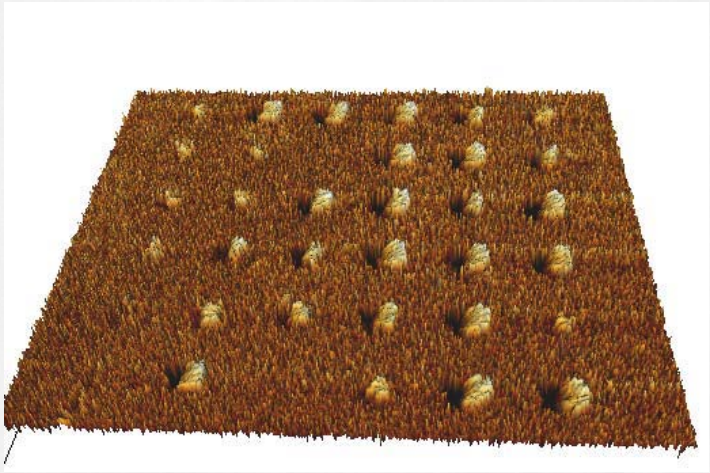


Micro-domains 4 µm Egg-PC

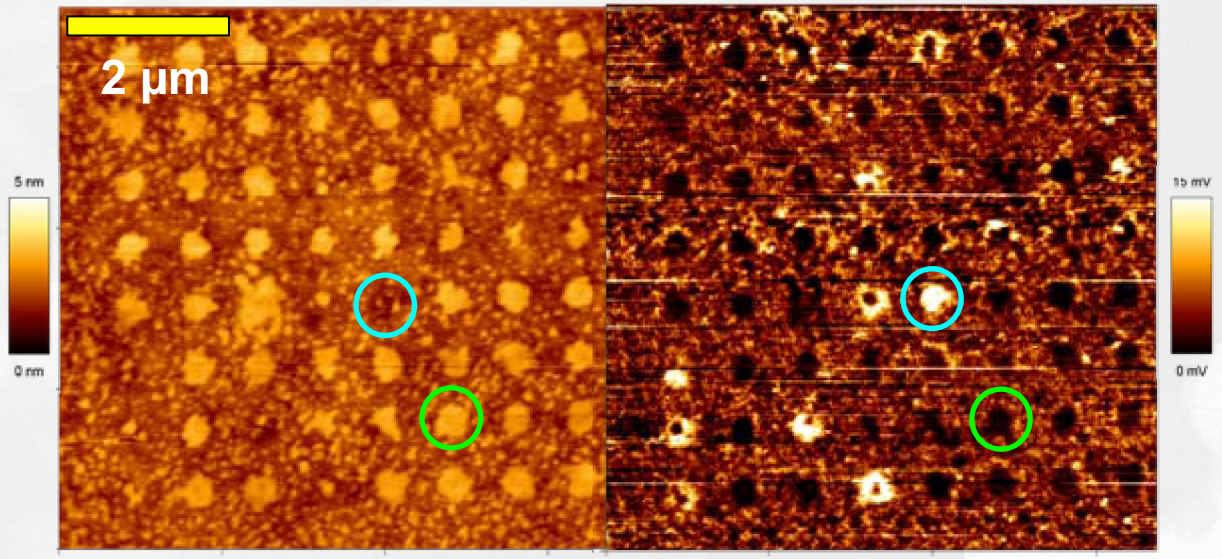


Micro-domains 4 µm E-Coli

Nano-domains of Supported Phospholipidic Membrane

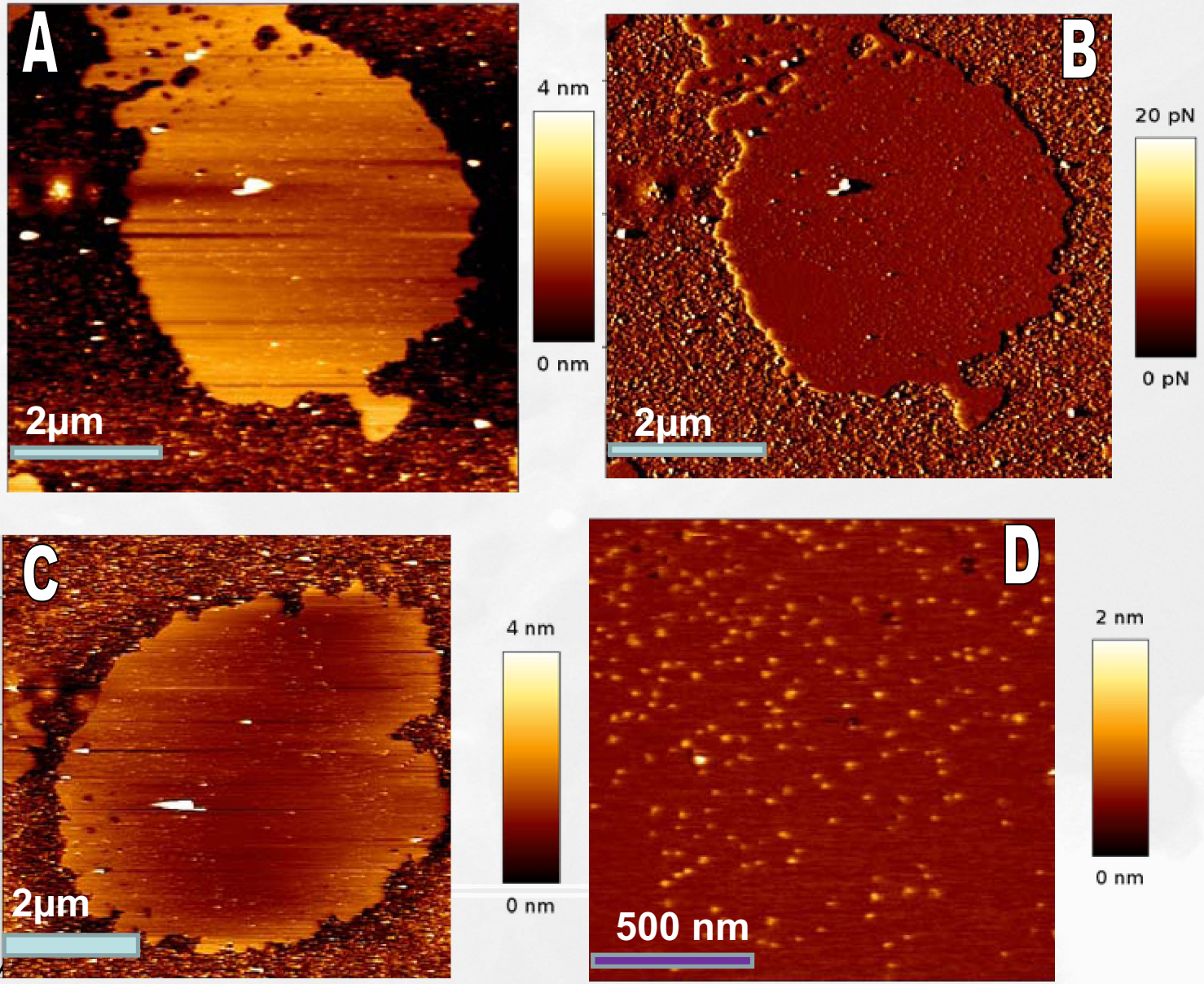


Nano-domains 200 nm Egg-PC

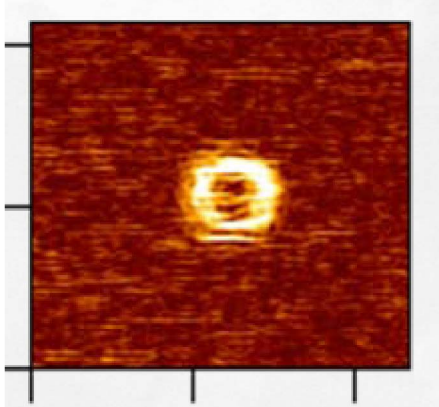


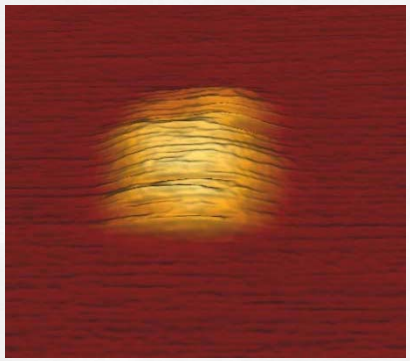
Nano-domains 400 nm E-Coli

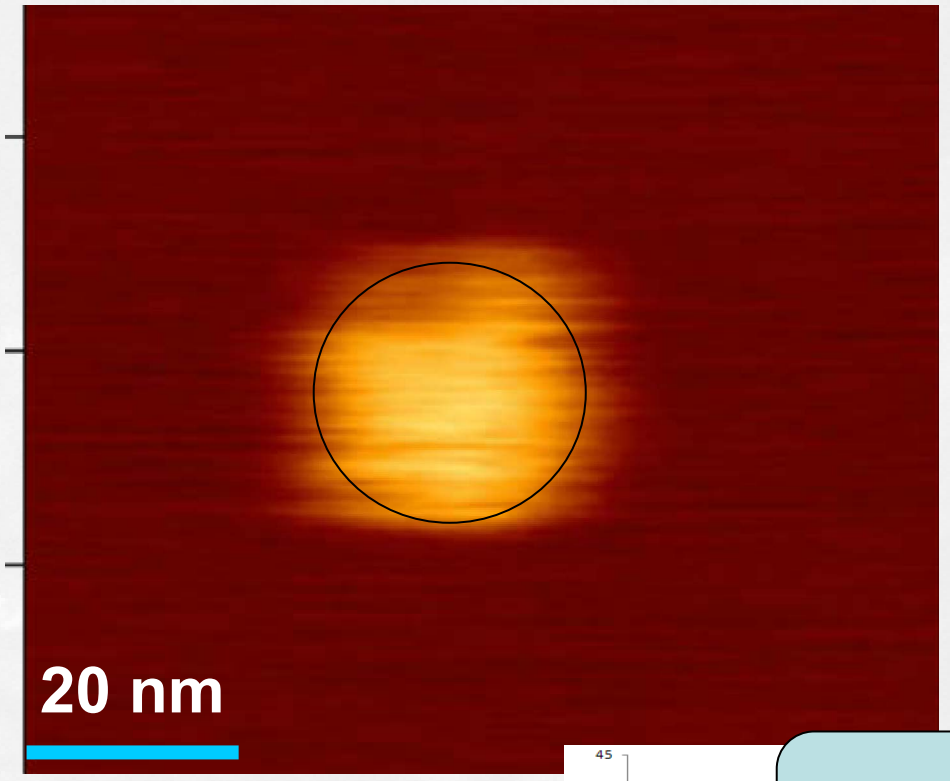
Assembly of the FliG protein on Microdomains of Supported Phospholipidic Membrane

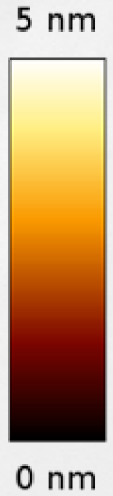


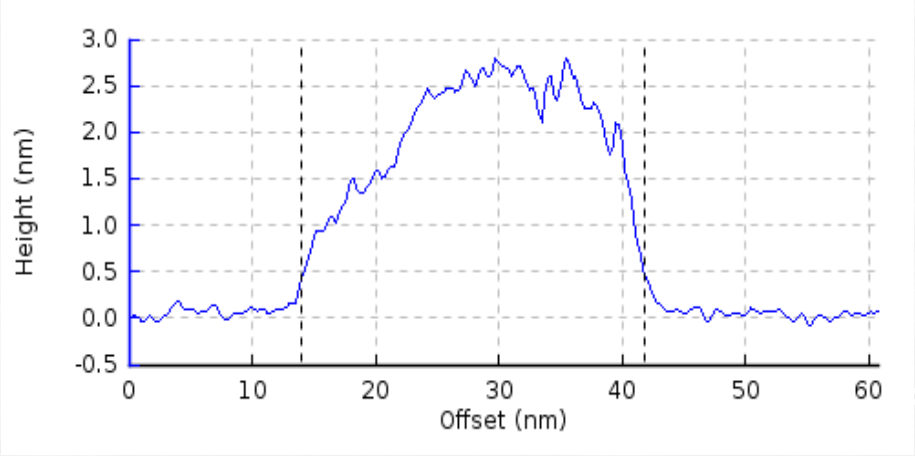
FliG assembly on Supported Phospholipidic Membrane

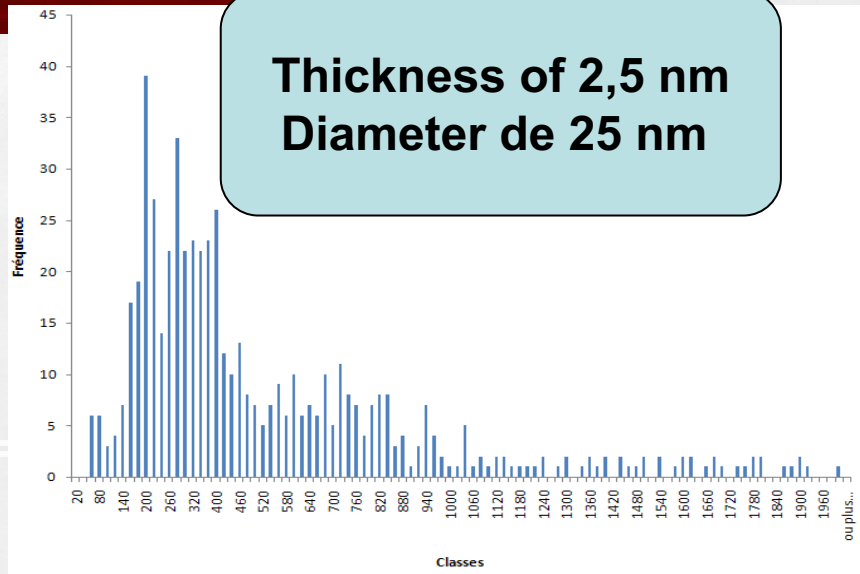






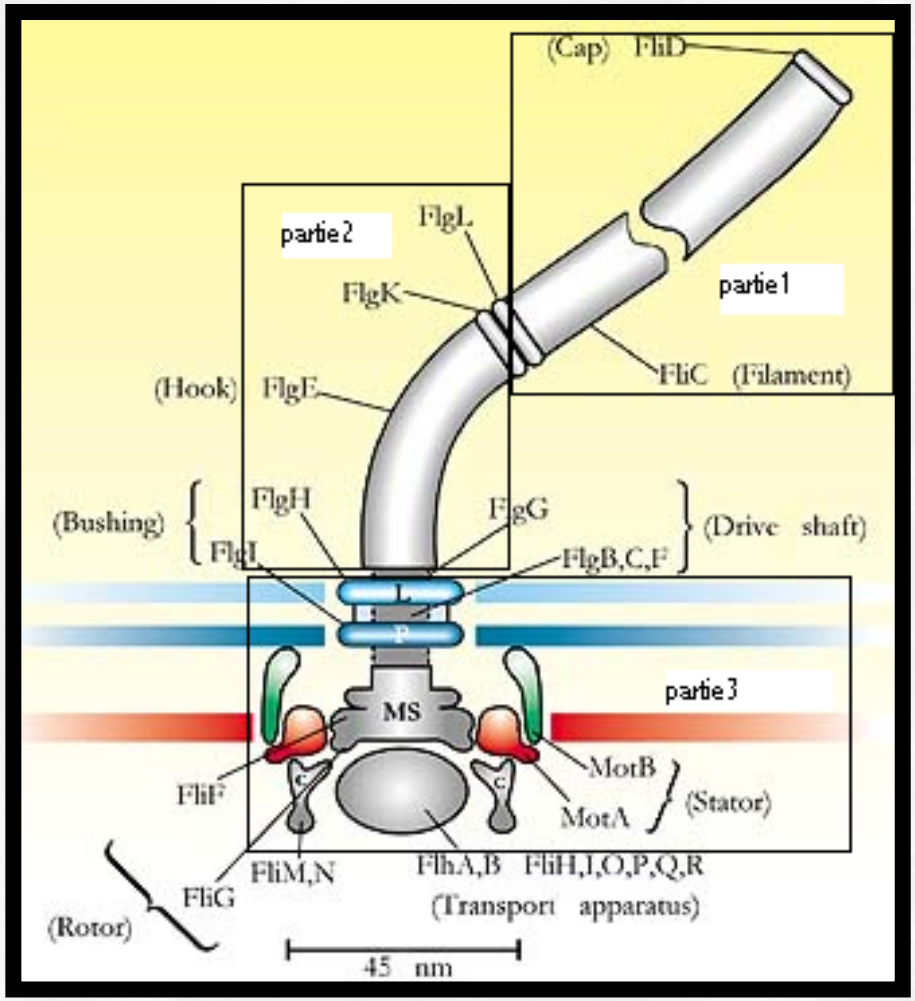


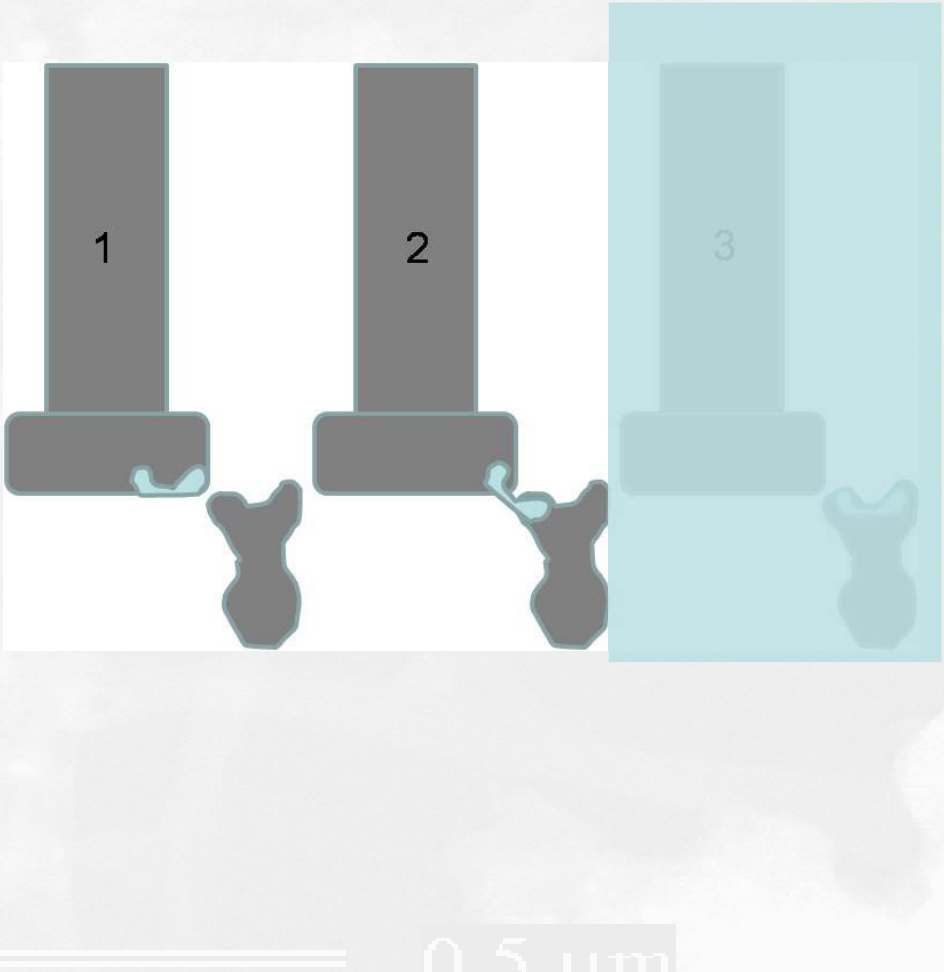




Thickness of 2,5 nm
Diameter de 25 nm

FliG position inside the nanomotor

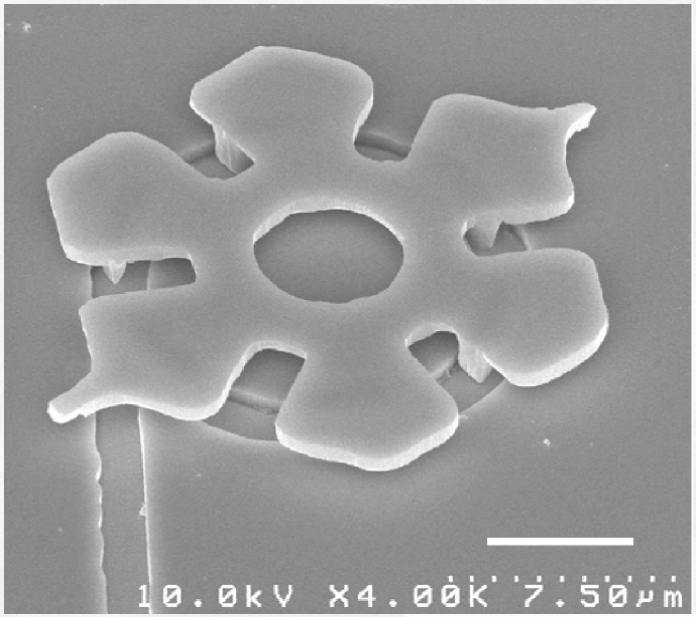




Nanotechnologies from Biology

-Assembling Bionanomachines on chip from purified proteins

-Using the assembled biomachine inside a device



A microrotary motor composed of a 20-µm-diameter silicon dioxide rotor driven on a silicon track by the gliding bacterium Mycoplasma mobile : 2 rpm

Y. Hiratsuka et al, 13618–13623 PNAS, 2006 vol. 103 no. 37

Video

0.5 µm

NanoBioSciences

- Single molecule investigations
- Single Cell investigations
- New methodologies

The complexity of Cell biology

-500 000 proteins

-Data processing based on molecular interactions

-Data bus : stochastic

-Circuits: Adaptative

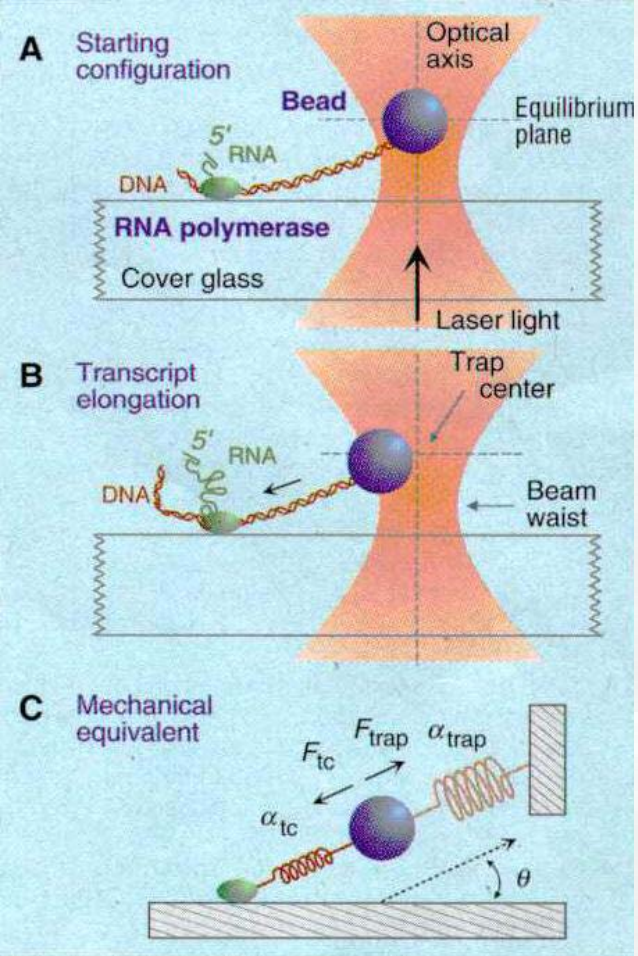
Nature Reviews Molecular Cell Biology 4, 414 - 418

NanoBioSciences

-Single molecule investigations

NanoBioSciences

- Single molecule investigations



Yin, Hong et al 1995

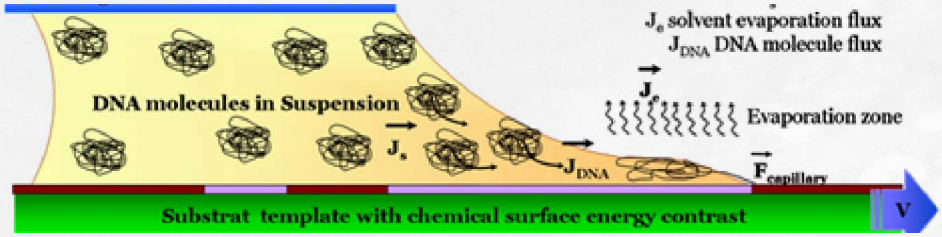
Biological signification ?

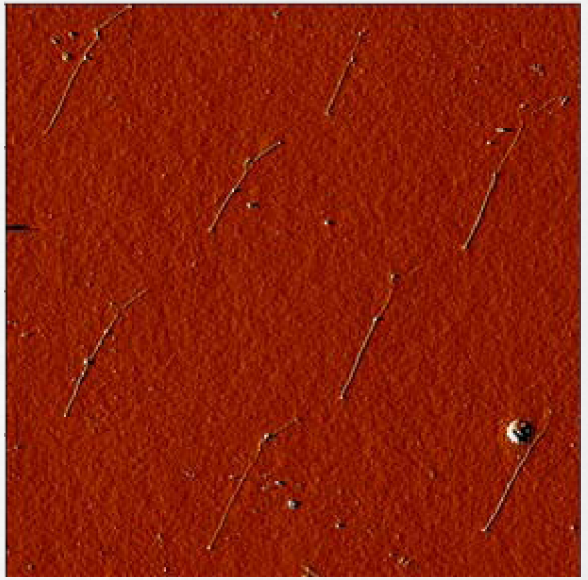
How to conciliate with statistical analysis ?

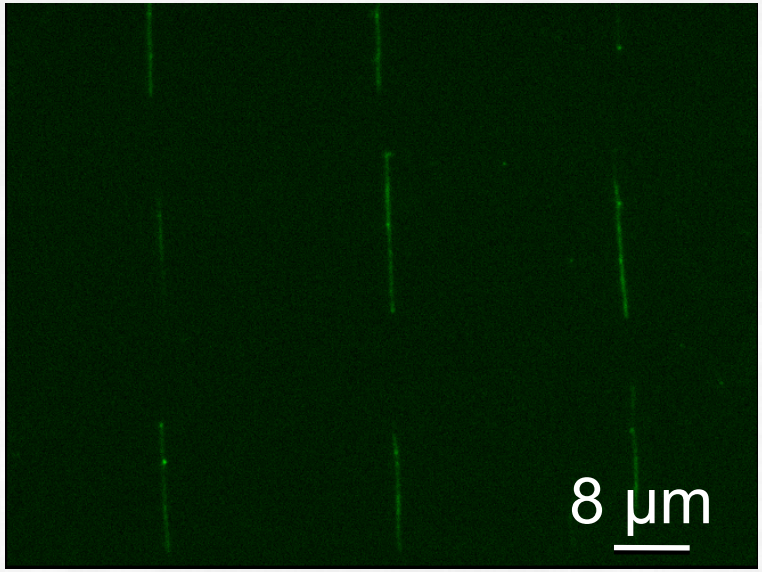
0.5 µm

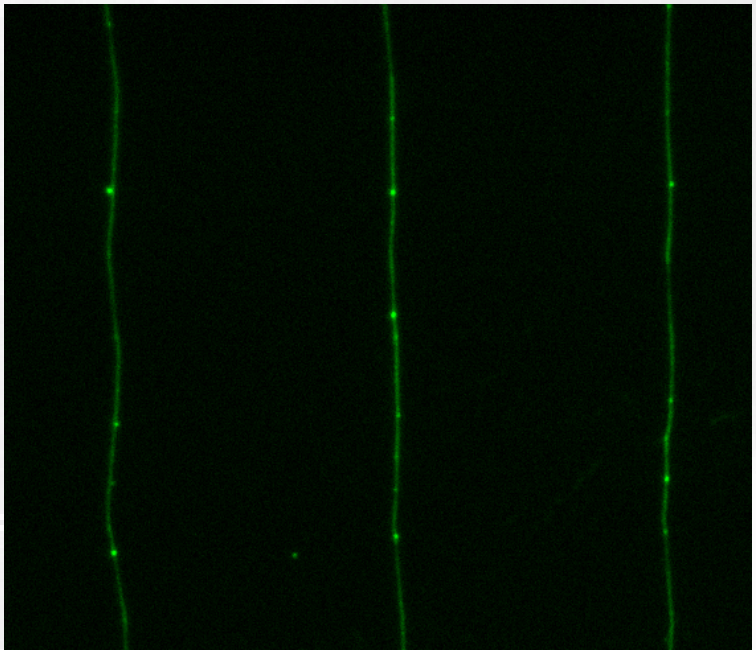
Ordered arrays of Single DNA molecules

M. Geneviève, A. Cerf





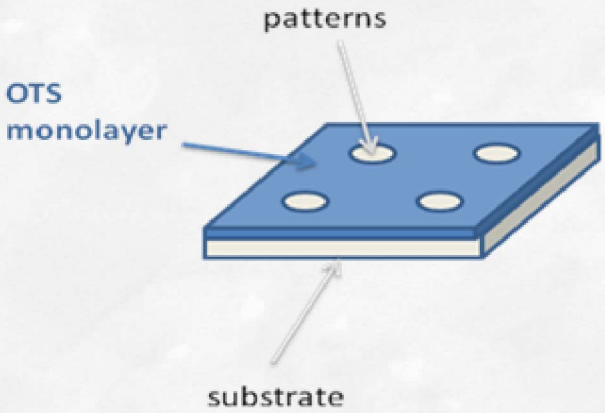


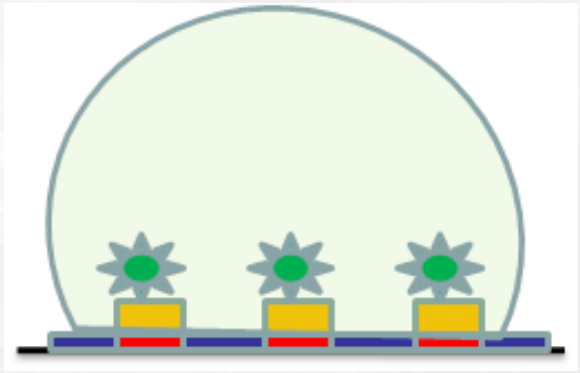


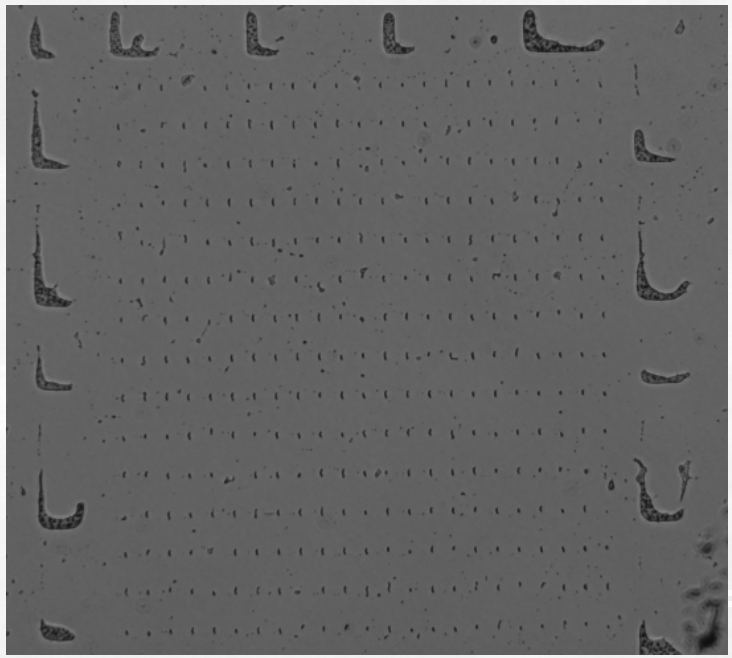
ITAV – Project - Nanomultiplex

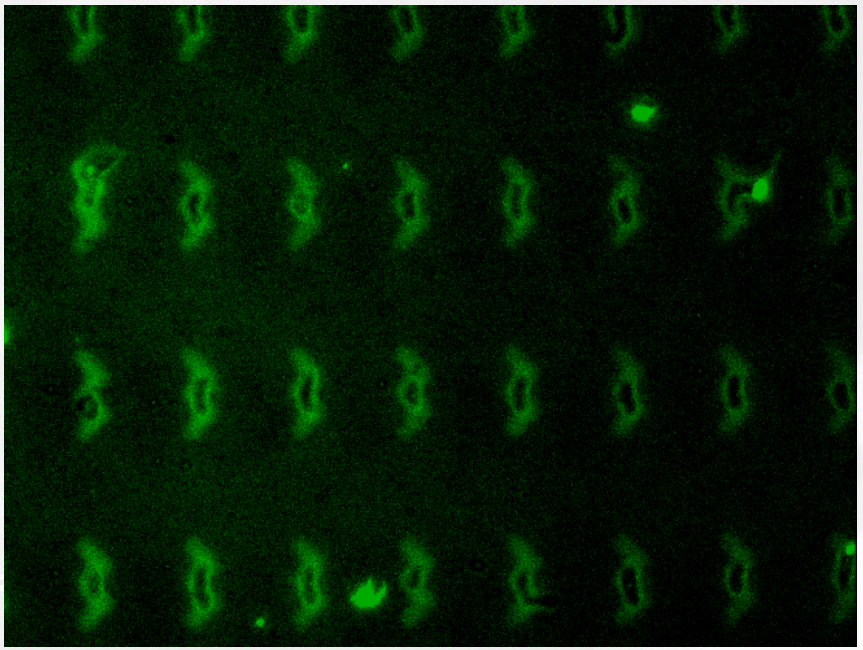
Single Cell investigations

A. Cerf et al, Colloids and Surfaces 2008



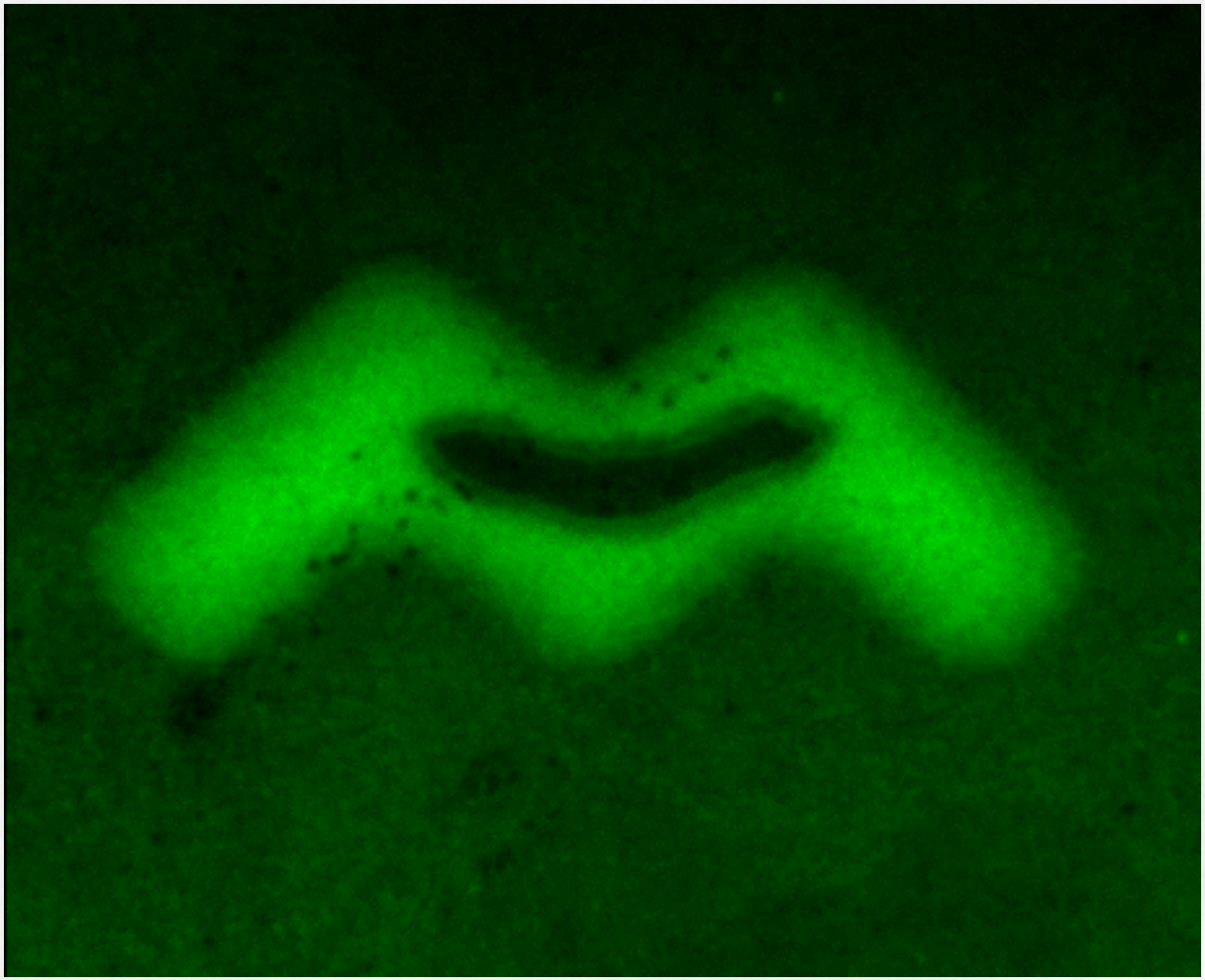






Single Cell investigations

A. Cerf et al, Colloids and Surfaces B, 1 September 2008, Pages 285-291



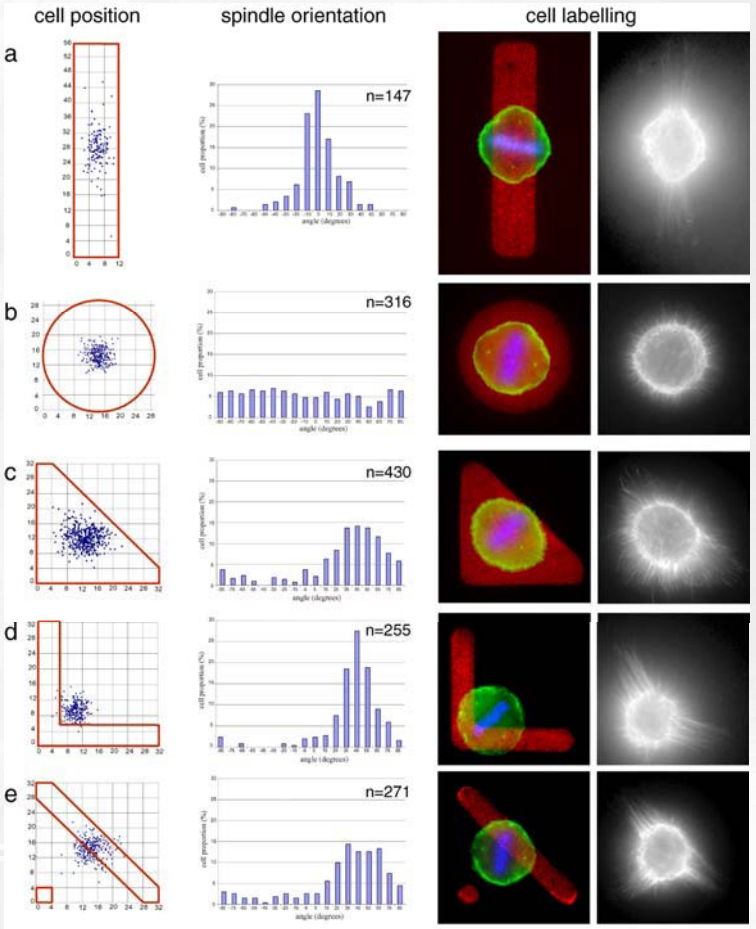
New methodologies: engineered surfaces for investigating fundametal mechanisms of biology

The extracellular matrix guides the orientation of the cell division axis
Manuel Théry, Victor Racine, Anne Pépin, Matthieu Piel, Yong Chen, Jean-Baptiste Sibarita and Michel Bornens
***Nature Cell Biology* 7, 947–953**

DNA Blue

Fibronectin Red

Centrosomes Green



NANOBIOTECHNOLOGIES

Conclusions

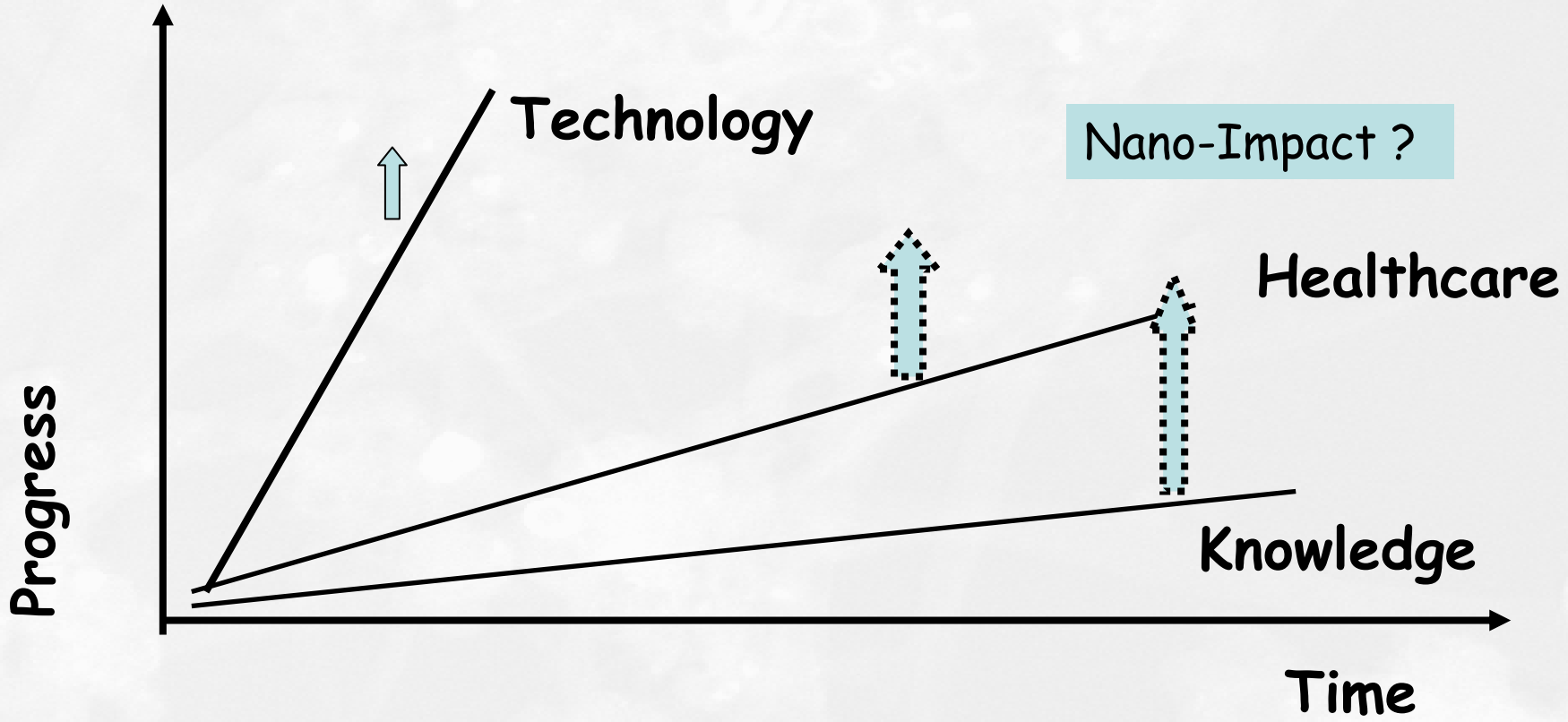
- Not a « Converging » Science

But A « divergent » cross-disciplinary field vector of progress for :

-fundamental knowledge

-Applications in Medicine and Environment

Nanobiotechnologies : The Future



New Local Structures for Interdiscplinary research

-ITAV

-Canceropole Langlade

-InNaBioSanté Fundation

Existing Forces at Toulouse Campus

Permanent positions	PhD, Post-doc	Running projects	International Publications 2000 -	Patents 2000-
139	82	38	326	21

+

Some examples of Industrial transfert (Dendris, Nanomeps, Innopsys, Nanobiochips ….)