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

*Micro and nanotechnologies for life sciences and medicine*

**NANOBIO TECHNOLOGIES A VISIT**

Christophe Vieu, INSA Toulouse, LAAS-CNRS
« Because technology provides the tools and biology the problems, the two should enjoy a happy marriage »


1- Nanotechnologies for Biology

- Devices
- Materials

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


2- Nanotechnologies from Biology

- Self-Assembly
- Bio-Nanomachines
3- New knowledge in biology

- Nanobiosciences
  - New experimental approaches
  - New theoretical approaches

Graph:
- Progress in oncology
- Technology
- Healthcare
- Knowledge
- Time

Nano-Impact?
Soft-Lithography as a clue for the visit


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
The complexity of Cell biology

- 500 000 proteins
- Data processing based on molecular interactions
- Data bus: stochastic
- Circuits: Adaptative

Nanotechnologies for Biology

Biopatterning
Microfluidics
Biodetection
Soft-lithography for Biopatterning

Printed DNA Micro-arrays exhibit improved Fluorescence emission


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 Peyrady, C Vieu, C Thibaut, C Séverac, Patent 16536, 13/09/07

Titration plate format
1536 wells

PDMS Macrostamps compliant with titration plates

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

MacroStamp® molding tool

MacroStamp format on a glass slide

0.5 μm

7 October 2008 - 40 ans du LAAS
How to print different probe molecules in one step?

The MacroStamp® concept


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

7 October 2008 - 40 ans du LAAS
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

Non specific: control experiment

Density < 1 nanoparticle/µm²

Specific: test of recognition

Density >10 nanoparticle/µm²

Biotin Labelled AntiBovin Rabbit IgG (A)

AntiBovin Rabbit IgG (B)

Anti Biotin/Streptavidin labelled Gold colloid

Biotin Labelled AntiBovin Rabbit IgG (A)

AntiRabbit Goat IgG (C)
Nano Immuno-Assay

Non specific : control experiment

- $R \approx 1$ TOhms
  - $U=1V$

Specific : positive test

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

7 October 2008 - 40 ans du LAAS
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

Ni islands (2-5 nm)

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
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

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

F1-FO ATP synthase
- Myosin
- Kinesin
- Dynein
- Flagellar nanomotor of bacteria
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: 1 000 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

Self-assembly or not?
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

7 October 2008 – 40 ans du LAAS
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
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

NanoBioSciences

-Single molecule investigations

Nature Reviews Molecular Cell Biology 4, 414 - 418

0.5 μm
NanoBioSciences

- Single molecule investigations

Biological signification ?
How to conciliate with statistical analysis ?

Yin, Hong et al 1995
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
NanoBioSciences

New methodologies: engineered surfaces for investigating fundamental 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
NANOBIO TECHNOLOGIES

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

![Graph showing progress over time with Technology, Healthcare, and Knowledge axes.]

Nano-Impact?
New Local Structures for Interdisciplinary research

- ITAV
- Canceropole Langlade
- InNaBioSanté Fundation
Existing Forces at Toulouse Campus

<table>
<thead>
<tr>
<th>Permanent positions</th>
<th>PhD, Post-doc</th>
<th>Running projects</th>
<th>International Publications 2000-</th>
<th>Patents 2000-</th>
</tr>
</thead>
<tbody>
<tr>
<td>139</td>
<td>82</td>
<td>38</td>
<td>326</td>
<td>21</td>
</tr>
</tbody>
</table>

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