

micro and nanoelectronics  
microsystems  
ambient intelligence  
image chain  
biology and health



# nanobiosystems for health and biology applications

**Pierre Puget**

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# CEA-LETI-MINATEC

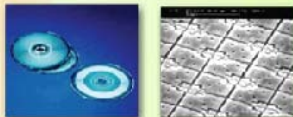
# CEA-LETI MAIN ACTIVITIES

**1000 employees**  
**1600 people working**  
**11,000 m<sup>2</sup> clean rooms**  
**43 M€ annual investment**  
**174 M€ annual budget**  
**(2/3 from external sources)**  
**200 industrial partners**  
**230 filed patents**



## Silicon microelectronics

60 %



## Optoelectronic devices

20 %



## Systems for biology & healthcare DTBS

20 %



## Systems for communications

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# microtechnologies for biology and healthcare

**150 permanent researchers**  
**25 theses post-doc**  
**7 external persons**



**facilities**

**Microfabrication**

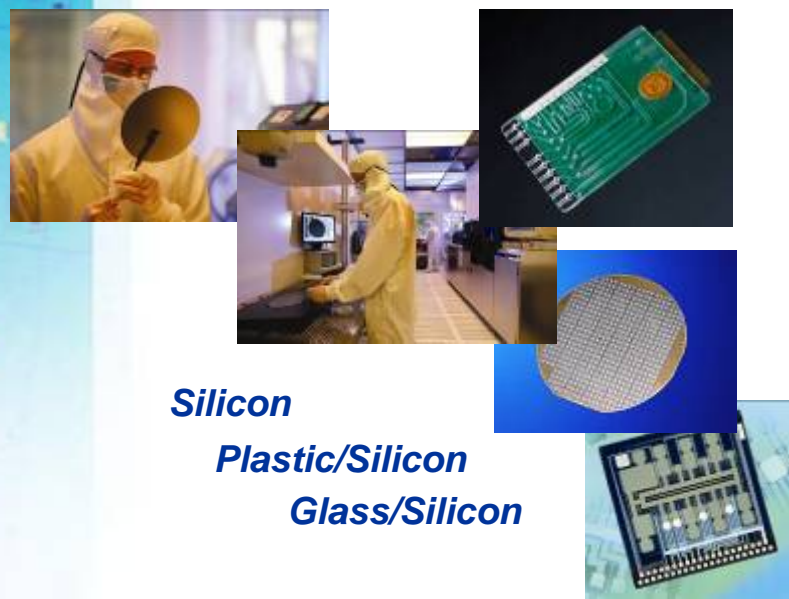
**Biotechnologies**

**Clean room 150 m<sup>2</sup>**

**Laboratories 1000 m<sup>2</sup>**

- ▶ spotting
- ▶ electropolymerisation
- ▶ in-situ syntheses
- ▶ fluorescence detection
- ▶ chemical engineering

- ▶ biology
- ▶ chemistry
- ▶ electrochemistry
- ▶ microfluidics
- ▶ packaging



**Silicon**  
**Plastic/Silicon**  
**Glass/Silicon**



# Main fields of application

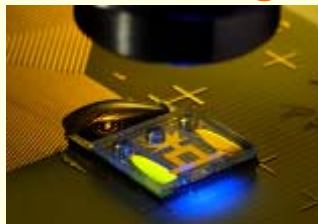
## Lab on a Chip

### IVD: In Vitro Diagnostics



microsystems  
for in vitro  
diagnostics

### Environment monitoring



$\mu$ systems for  
environment  
monitoring  
& homeland  
security

### Life Science - Chemistry



$\mu$ systems for in  
vitro  
pharmacology &  
chemistry

## In-vivo

### In vivo & Wearable



Implanted  
devices  
Nanobiopsies  
Smart textiles

### Molecular Imaging



Development  
of probes &  
instruments  
for in vivo  
optical  
imaging

# INDUSTRIAL PARTNERS

Joint team

Process control

**sanofi pasteur**  
La division vaccins du Groupe sanofi-aventis.



Chemical microreactor

*European Program*

- Intracom
- NTE
- Eurogentec
- Zeptosens
- Asper Biotech
- Smartex
- Sofileta
- Thuasne
- Silicon Biosystem

Cellchip



Liquid liquid extraction



Heavy metals analysis

**azbil Yamatake Group**

DNA Chip  
Oligosaccharide chip



Legionella detection



Point Of Care



# Main fields of application

## Lab on a Chip

### IVD: In Vitro Diagnostics



microsystems  
for in vitro  
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$\mu$ systems for  
environment  
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### Life Science - Chemistry



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## In-vivo

### In vivo & Wearable



Implanted  
devices  
Nanobiopsies  
Smart textiles

### Molecular Imaging

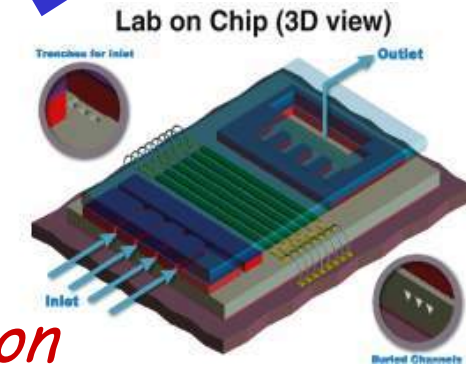


Development  
of probes &  
instruments  
for in vivo  
optical  
imaging

# The "lab on a chip" concept



*Miniaturization*

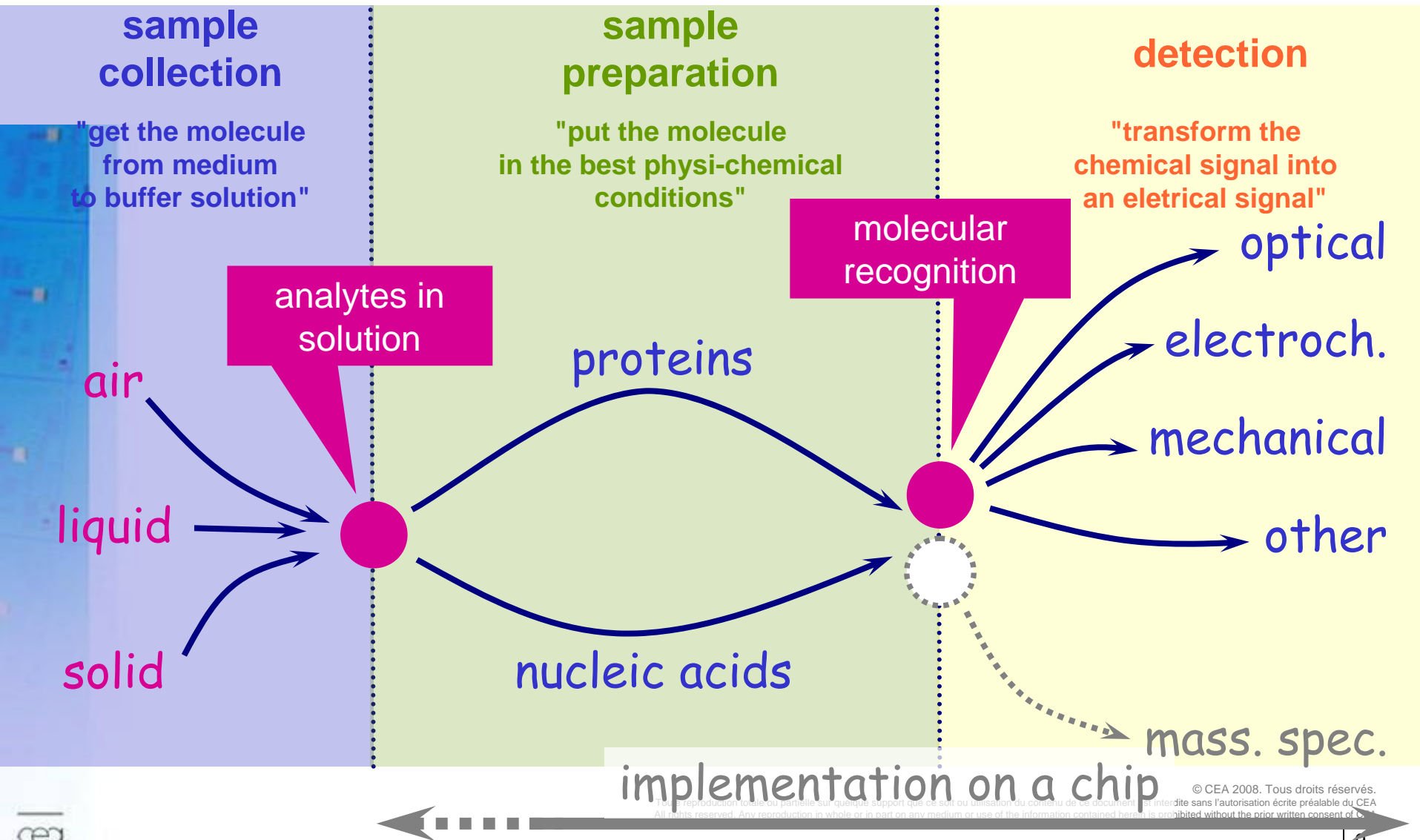


*Integration of several steps of a protocol*

- *increased performance*
- *ease of use and automation*
- *manufacturing costs (?)*

# From sample to results ... classical steps

a classical architecture of a complete bio-analytical system



# 4+1 specific competencies

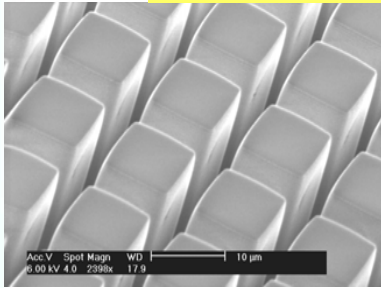
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- **SURFACE ENGINEERING FOR MICROSYSTEMS**
  - Control & reproducibility of surface functionalization protocols
  
- **(MICRO)FLUIDICS TOOL BOX**
  - Fluid handling in various ranges of volume
  
- **PACKAGING**
  - Bio compatibility & biostability
  
- **DETECTION**
  - performance and "system" criteria
  
- **ARCHITECTURE**
  - protocol implementation
  - integration of different functions/modules

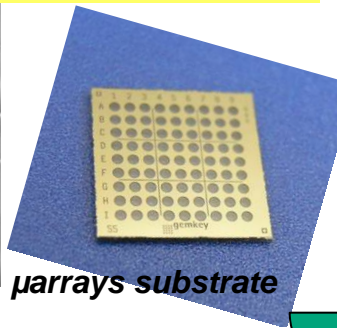
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# Surface engineering for microsystems

Microtechnologies fabrication



Microfluidics circuit for lab on a chip



Surface chemistry

Biochip



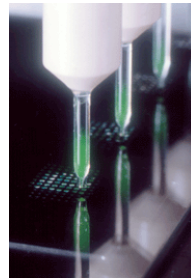
Silanisation step (silane grafting + spacer)

Functionalisation with biochemical compounds

Characterization



Liquid phase or Gas phase



Spotting or hydrodynamic flow



Fluorescence or mass spectrometry

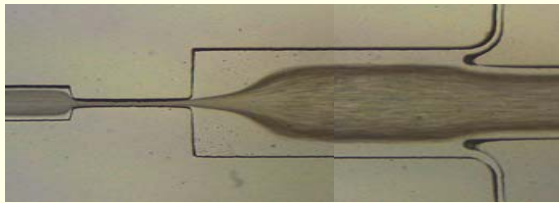


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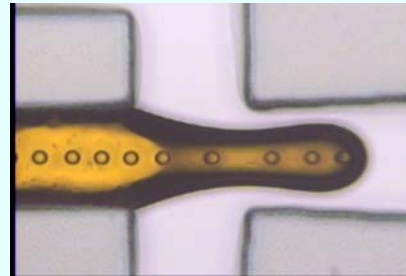
# Microfluidics toolbox for lab on chip applications

## 3 ways for fluid handling architecture:

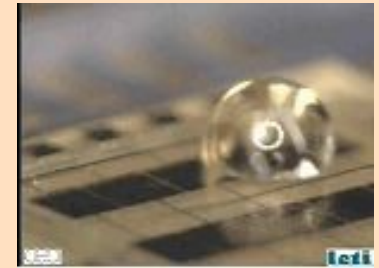
### Monophasic microfluidics



### Droplets in channel



### Digital microfluidics



**Technology**

Microfluidics circuits with etched channel. Specific packaging steps (low T°C)

Microfluidics circuits (glass or PDMS)

Microdroplets are actuated on microelectrodes pathway

**Microfluidics**

Mono-phasic

Di-phasic

diphasic (air/water; oil/water; air/ionic liquid)

**Fluid actuation**

Hydrodynamics force  
electro-osmotic force

Hydrodynamics

Electrostatic, SAW

**Valves or pumps architecture**

Thermopneumatic actuation;  
passive valves;  
mechanical;

External pump

No need

**Dispensing**

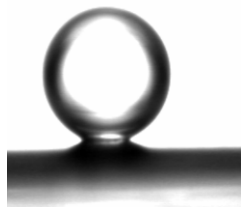
Capillaries, wells

Capillaries

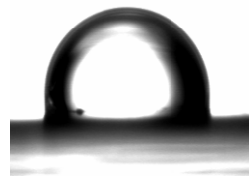
Integrated reservoirs

# Droplet microfluidics

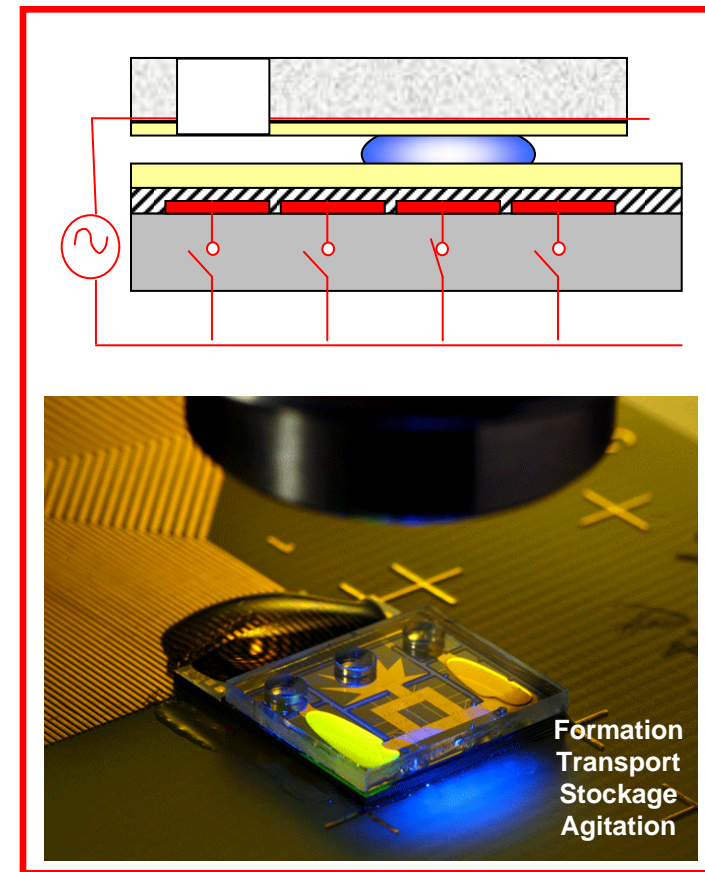
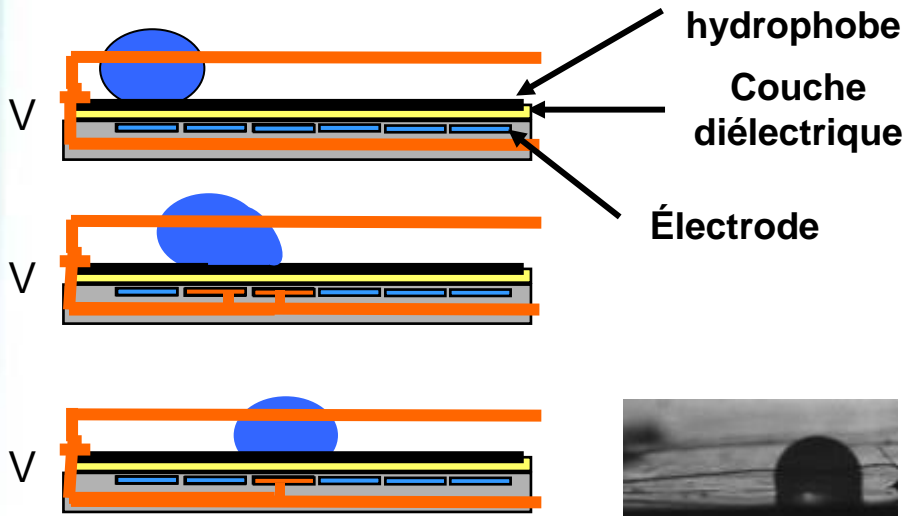
## physical principle



$V = 0\text{ V}$



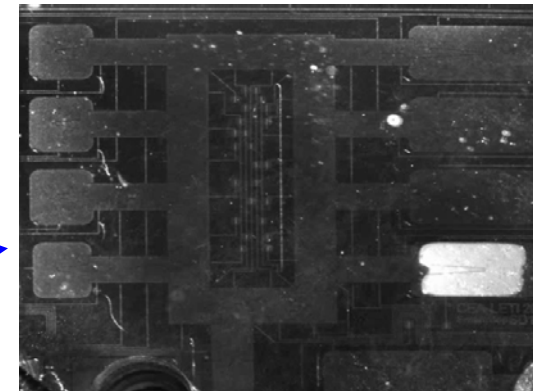
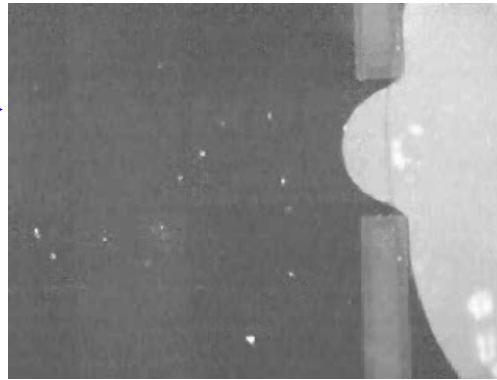
$V = 80\text{ V}$



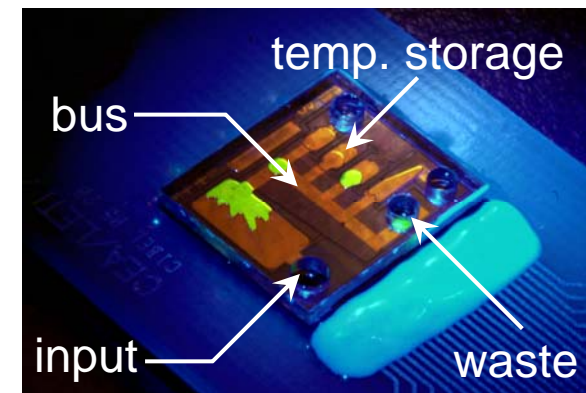
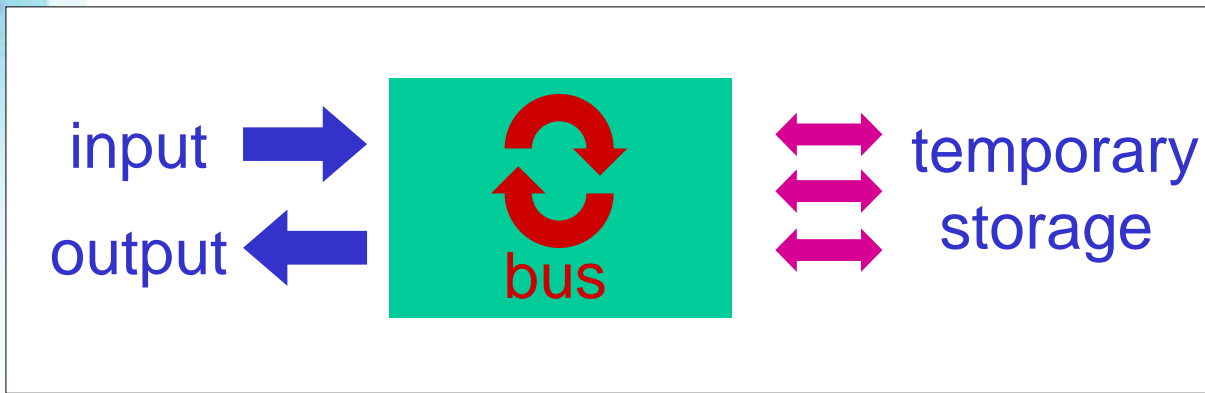
# Droplet microfluidics to integrate protocols

## ■ implementation of standard elementary functions

- dispensing
- mixing
- dilution



## ■ integration of several operations to implement a complete protocol



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# Specificities of « bio » packaging

## Microfluidic:

- Locally watertight packaging
- Inlet and outlet to connect the microsystem to the macroworld

## Biology:

- Grafting or labels sensitive to:
  - ◆ Solvent
  - ◆ Thermal conditions

## Biocompatibility: do not affect bio protocols and health

- target adsorption with inappropriate glue
- PCR inhibitors

## Biostability: do not being affected by bio!

- passivation layer consumption (especially in nano thickness)
- protein progressive deposition on sensing areas

WAFER LEVEL

- Med/ High temperature sealing
  - Silicon direct Bonding T=1000°C
  - Eutectic Si/Au T=300°C,
  - Anodic bonding T=350°C,

- Low temperature sealing
  - Photo resin
  - Glue for screen printing

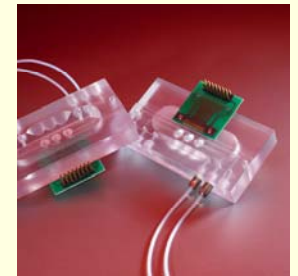


- Embedded reagents
  - lyophilisation (polymeric matrix)
  - dedicated assembling room (humidity, T°C)

CHIP LEVEL

- hybrid assembly
  - Pick and place
  - UV curing
  - Manual screen printing
  - Glue dispensing

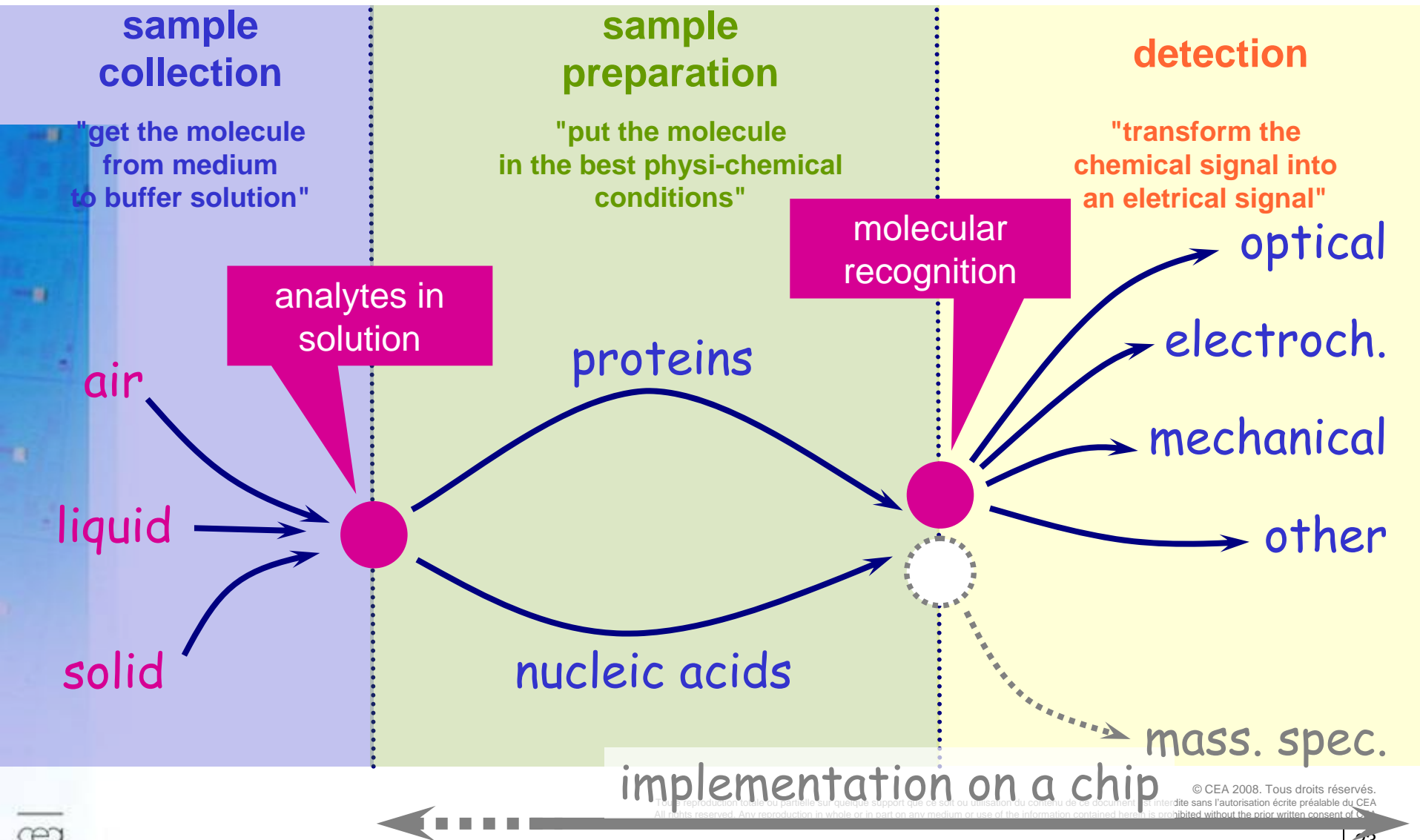
- Surface pretreatment
  - UV
  - Plasma O<sub>2</sub>



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# From sample to results ... classical steps

a classical architecture of a complete bio-analytical system



# Main requirements for the detection step

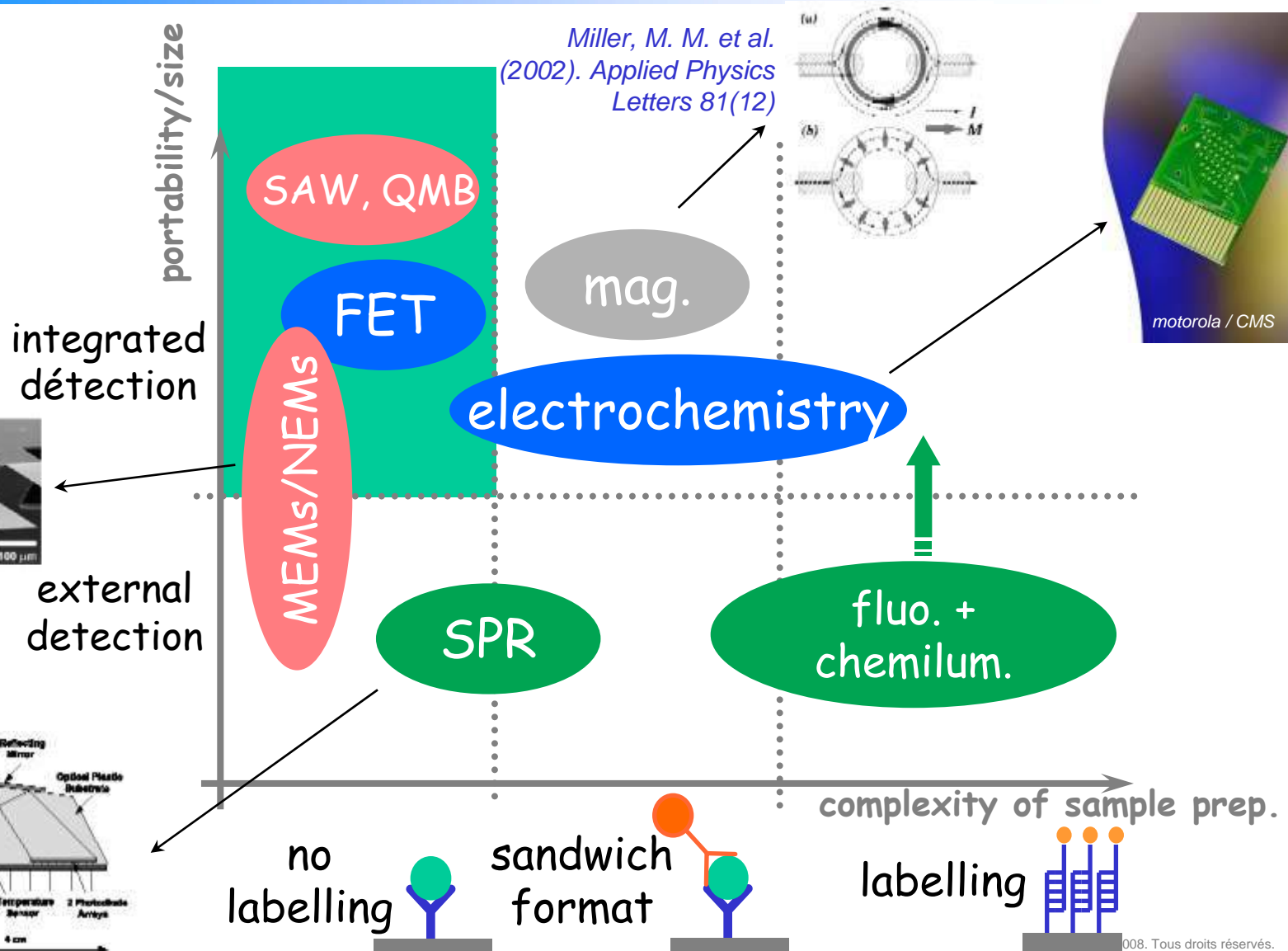
## ■ "System" criteria

- impact on the sample preparation protocol
- portability / "on chip" possible implementation
- cost issues, reuse of widely spread technologies

## ■ Performance criteria

- limit of detection, sensitivity, dynamic range
- simultaneous detection of several parameters (up to a few tens)

# Detection methods: "system" point of view



# Main requirements for the detection step

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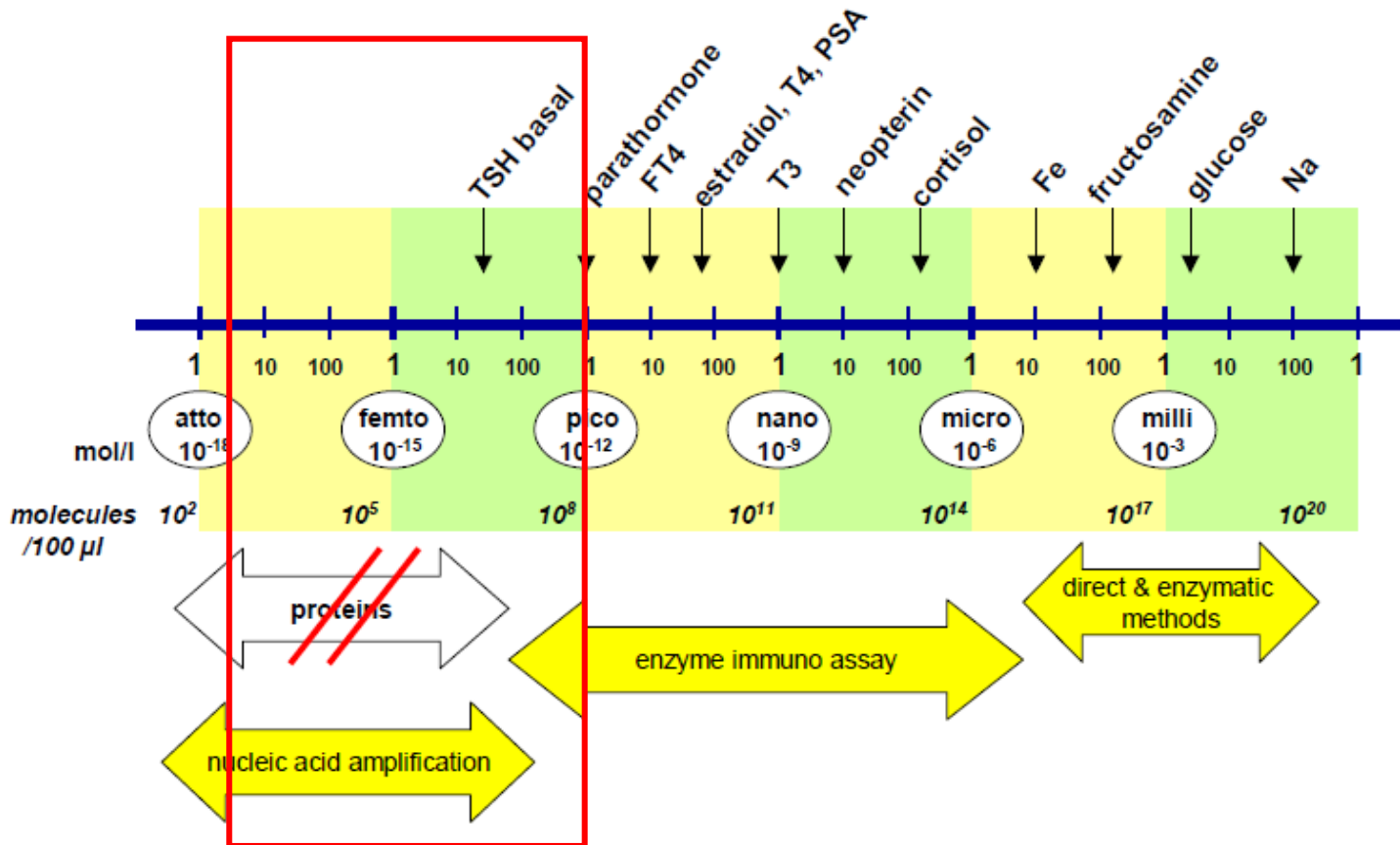
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what about the limit of detection ?

# Example of requirement (in vitro diag.)

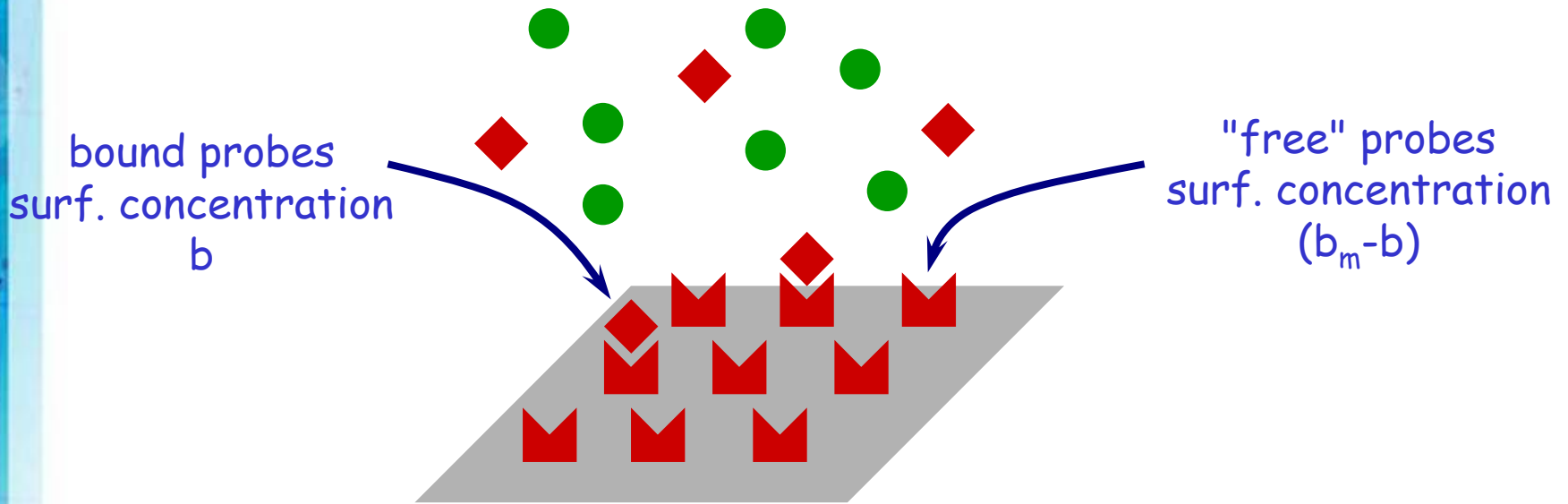
## Sensitivity

detection requirements for diagnostically relevant targets



ref: Kaspar, P. *Progress in Medical Diagnostics through Nanotechnologies*. in *LETI, 8th Annual Review*. 2006. Grenoble

# Capture of targets by immobilized probes



bulk concentration of targets

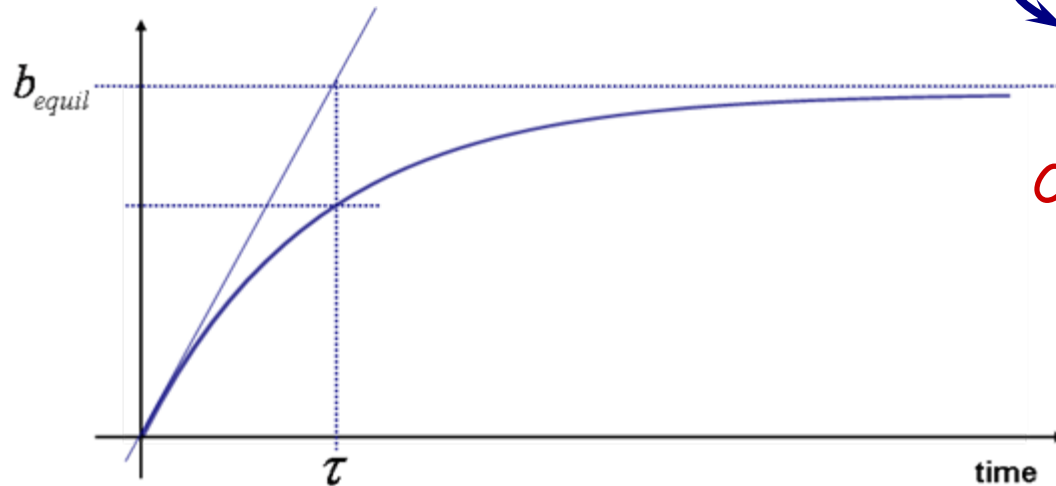
$$\frac{\partial b}{\partial t} = k_{on} C_t (b_m - b) - k_{off} b$$

initial surf. concentration  
of probes

surf. concentration of probes bound  
by targets

# A typical kinetic curve (typical assay)

$$b(t) = b_m \frac{C_t \cdot (k_{on} / k_{off})}{1 + C_t \cdot (k_{on} / k_{off})} \left(1 - e^{-(k_{on} C_t + k_{off})t}\right) = b_m \frac{C_t / C_0}{1 + C_t / C_0} \left(1 - e^{-t/\tau}\right)$$



$\sim 10^4 / \mu\text{m}^2$

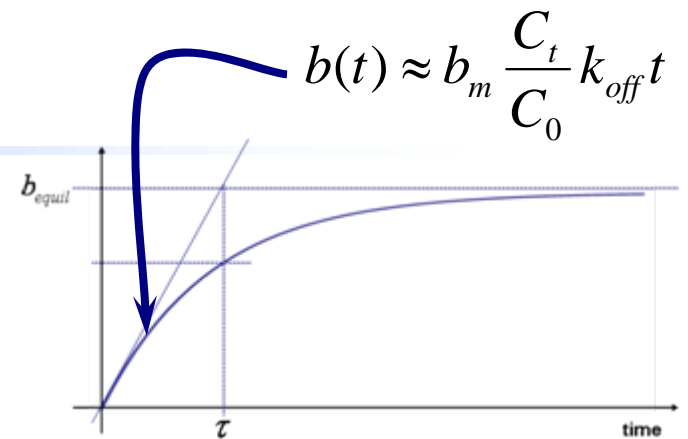
$C_0 = k_{off} / k_{on} \sim 1 \text{ nM}$

| $C_t$ conc. range  | # captured targets / $\mu\text{m}^2$ * | $\tau$     |
|--------------------|--|------------|
| pM ( $10^{-12}$ M) | $\sim 10$                              | 20-200 min |
| fM ( $10^{-15}$ M) | $\sim 1/100$                           | 20-200 min |

\* **ref:** Gervais T et al. 2006 Chem. Eng. Sc. 61(4) pp 1102)

# consequences

| $C_t$ conc. range  | # captured targets / $\mu\text{m}^2$ * | $\tau$     |
|--------------------|--|------------|
| pM ( $10^{-12}$ M) | ~ 10                                   | 20-200 min |
| fM ( $10^{-15}$ M) | ~ 1/100                                | 20-200 min |



- for "practical" time" of assay, equilibrium is not reached  
→ number of captured targets lower
- the sensor area should be large enough to capture at least one molecule!  
→ area of  $\sim \mu\text{m}^2$  for pM,  $1000 \mu\text{m}^2$  for fM ranges
- the criterion to compare the sensitivity of different sensing methods is

number of detectable molecules / area unit

for a given probe/target, it will give the lowest concentration that can be detected in a given amount of time

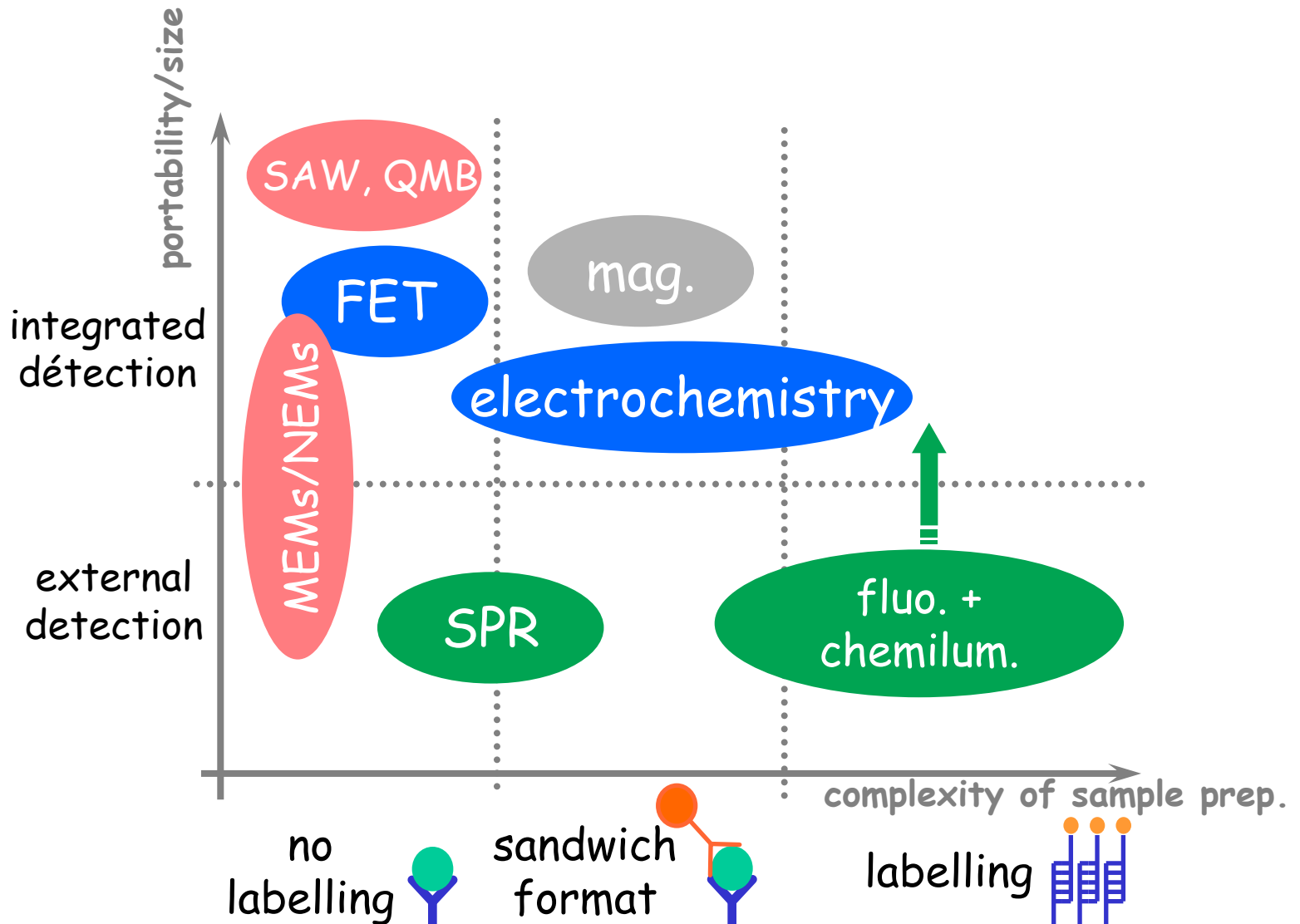
- and the above reasoning is assuming perfect mixing (neglecting diffusion time) !

# State of the art for a few methods

|                              | LOD *<br>(molec/ $\mu\text{m}^2$ ) | ref   | comments                                    |
|------------------------------|------------------------------------|---|---|
| fluorescence                 | 1                                  |   | commonly achieved on commercial instruments |
| SPR                          | 10                                 | Myszka D G 2004 <i>Anal. Biochem.</i> <b>329</b> (2) pp 316   | biAcore instrument                          |
| Quartz $\mu$ balance         | 700                                | <a href="http://www.q-sense.com/specifications_d300--31.asp">http://www.q-sense.com/specifications_d300--31.asp</a>           |   |
| $\mu$ cantilever in solution | 500 000                            | Braun T <i>et al.</i> 2005 <i>Physical Review E (Statistical, Nonlinear, and Soft Matter Physics)</i> <b>72</b> (3) pp 031907 |   |
| FBAR                         | 200                                | Zhang H and Kim E S 2005 <i>Journal of Microelectromechanical Systems</i> <b>14</b> (4) pp 699                                |   |

\* **order of magnitude for a target of 30 kD**

# Detection methods: "system" point of view



# Conclusion on detection

- analysis on non specific capture should be done to estimate "background biological noise"

$$b_T(t) \approx b_m \frac{C_{t,T}}{C_{0,T}} k_{off,T} t$$

$$b_N(t) \approx b_m \frac{C_{t,N}}{C_{0,N}} k_{off,N} t$$

- overall limit of detection is improved by:
  - improving the intrinsic sensitivity of the detector
  - optimising the density of grafted probes
  - selecting the highest affinity probes (and  $k_{off}$  ?)
  - specific concentration of the target during the sample preparation steps

# General conclusion on nanobiosystems

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- development of nanobiosystems requires
  - mastering a wide range of technologies
  - a necessary architecture/system approach
  
- to improve performances, research efforts should focus on:
  - (transduction/detection technologies are not the weak point anymore)
  - concentration processes
  - separation/purification methods for various ranges of volumes/concentration
  
- interest for collaboration on these subjects

# Acknowledgments

- Architecture: Claude Vauchier, Christine Peponnet, Frédéric Ginot
- Surface Chemistry: Françoise Vinet, Guillaume Delapierre
- Microfluidics: Yves Fouillet, Cyril Delattre
- Packaging and techno: Patrice Caillat
- Detection: François Perraut