

Innovative bioelectronic noses based on olfactory receptors

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Neurobiologie de l'Olfaction et Modélisation en Imagerie
INRA Jouy-en-Josas



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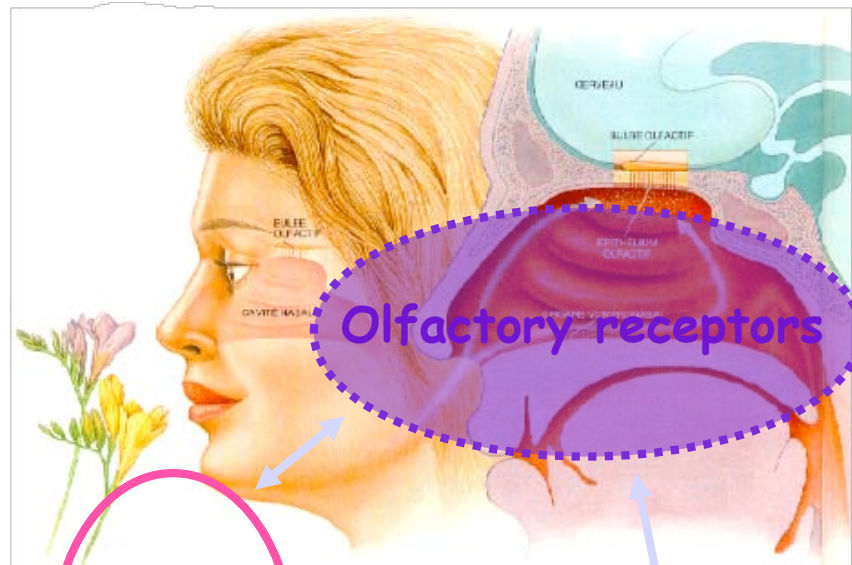
1> Animal olfactory detection

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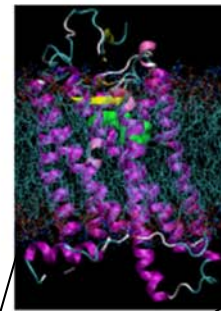


> Olfactory detection

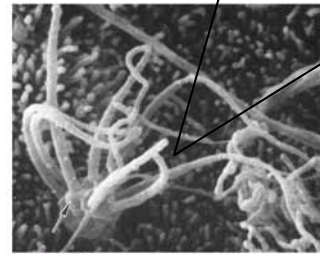


Odorants

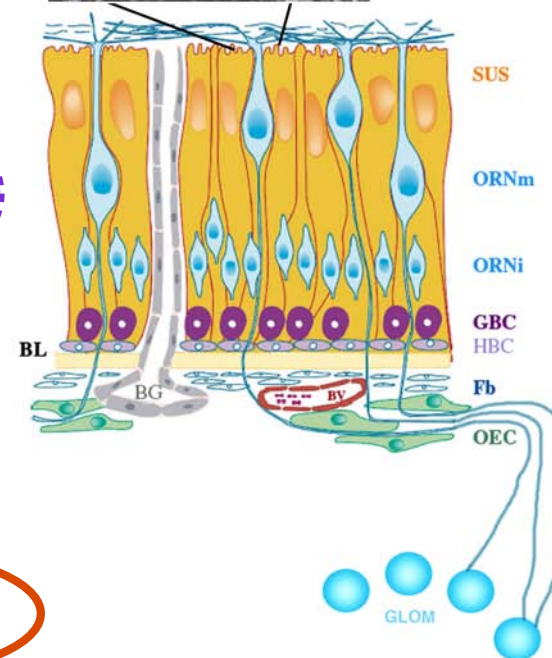
OBPs



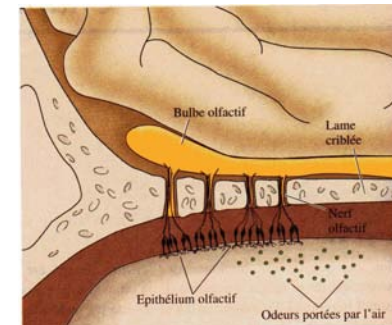
~ 10^3 different types of receptors



1 single type of receptor expressed / sensory neuron



~ 10^7 olfactory sensory neurons



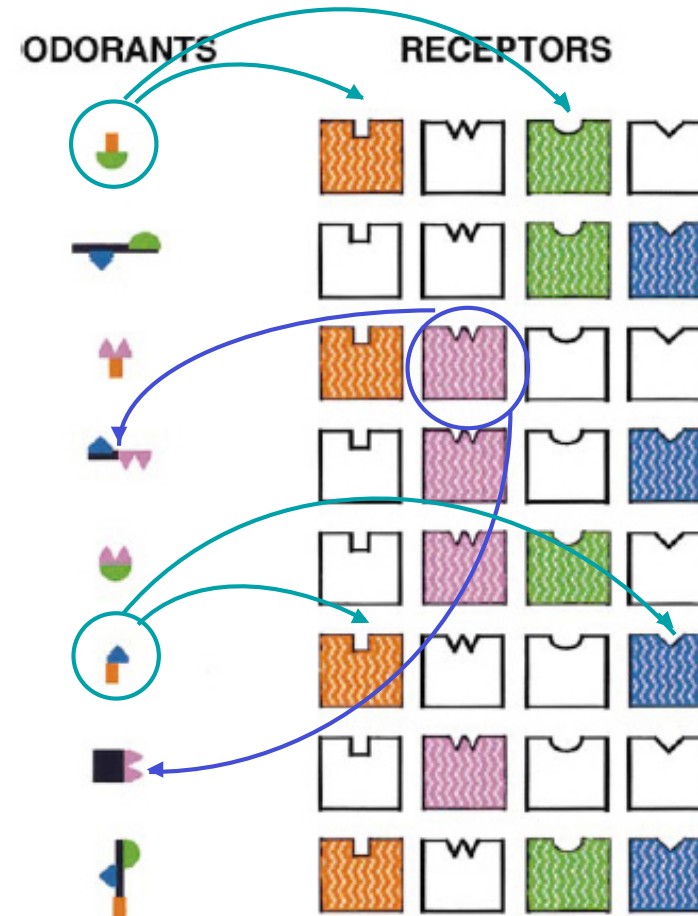
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> Olfactory detection

> combinatorial odorant coding by receptors



[from Malnic et al. Cell 1999]

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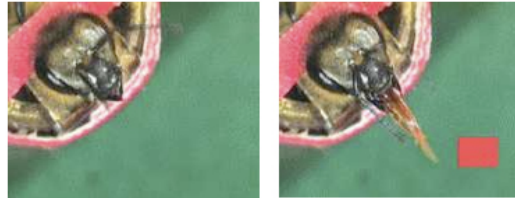


> Animals efficiently detect and discriminate odors

Trained dogs, rats, bees

detect explosives and drugs

--> security



**> 2008 : squads of explosives-sniffing rats in Colombia,
to detect landmines**



[revue Habib Biosens. Bioelectron. 2007]

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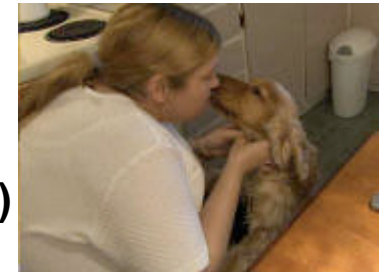
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Dogs diagnose cancer from associated odors

(melanoma, prostate, bladder, breast, lung, leukemia, lymphoma, ...)

or other pathologies (diabetes, schizophrenia, asthma, tuberculosis, ...)



Rats sniff out tuberculosis from sputum samples

--> health care, large-scale diagnosis in Tanzania



Rats detect females oestrus odors

--> control of reproduction in livestock



[Rampin et al. Behav. Brain Res. 2006]

D'Amico et al. Sensors and Actuators B 2008
Turner & Magan Nature Rev. Microbiol. 2004

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> Other potential applications

Environmental care (air or water quality, contaminants, smokes, obnoxious odours)



Quality control (foodstuffs or cosmetics : origin, purity, degradation, toxicity)



On-line monitoring of environmental (ripening, fermentations, ...) **or industrial processes**



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2 > Development of nanobiosensors based on the electrical properties of olfactory receptors

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> **SPOT-NOSED : Single PrOTein NanObioSEnsor griD array**

European project 2003-2006

Purpose :

Development of a **nanobiosensor** based on the **electrical properties** of **single olfactory receptors** anchored between **nanoelectrodes**

Principle :

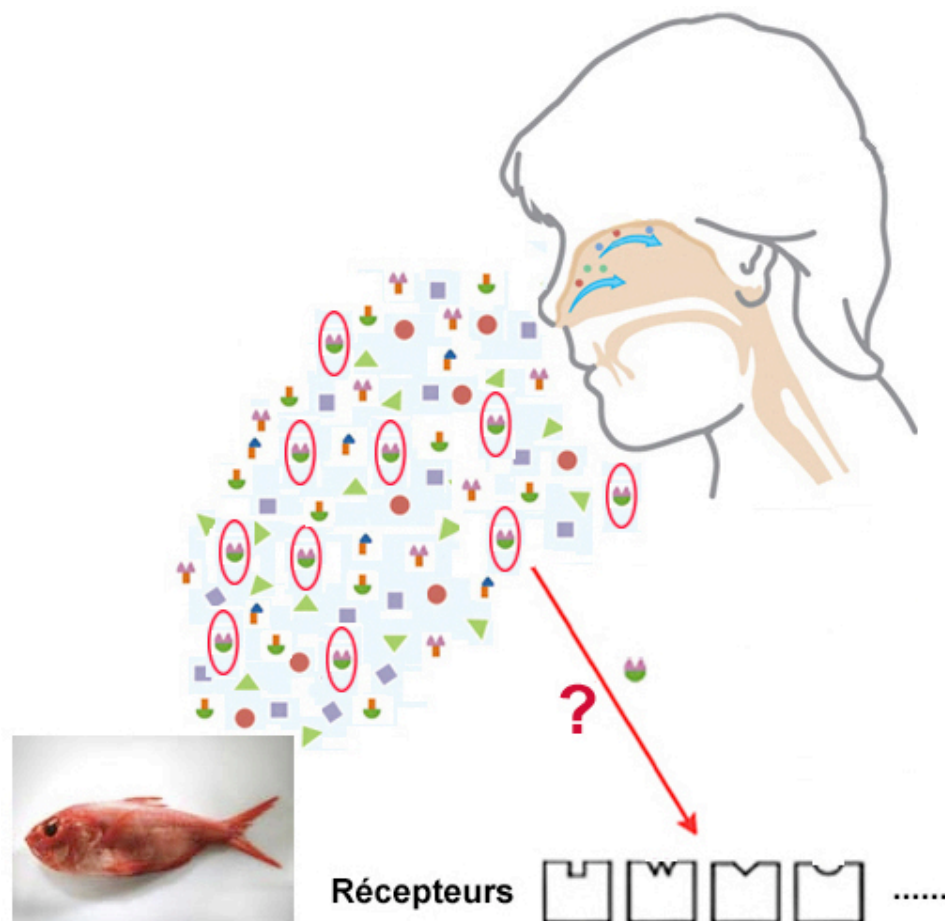
ligand binding --> **conformational change** of the receptor -->
modification of electrical impedancemetric properties of the receptor

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> Biosensors based on olfactory receptors

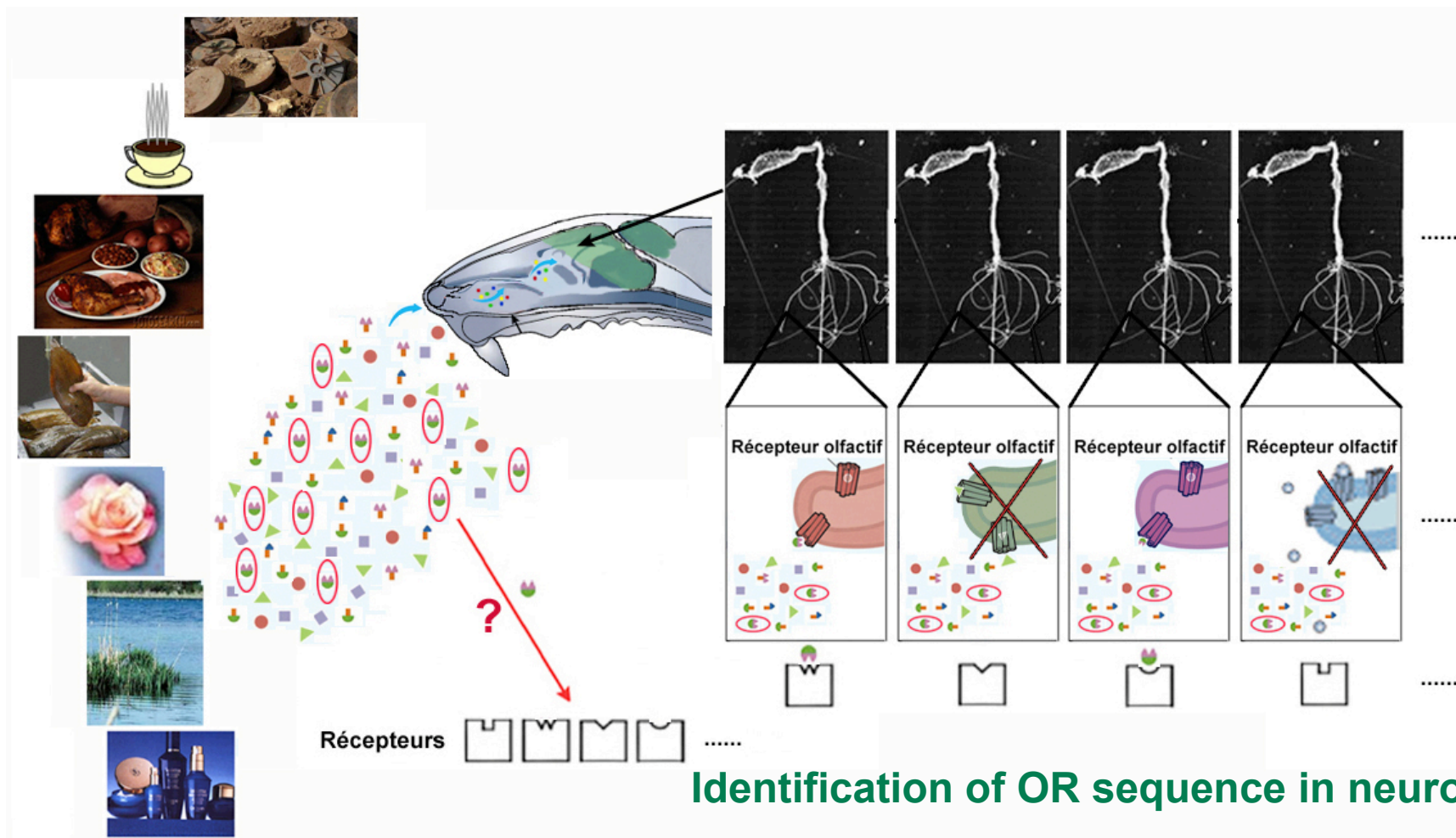


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> Identify the OR relevant to detect an odorant of interest



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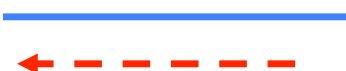




RNA preparation



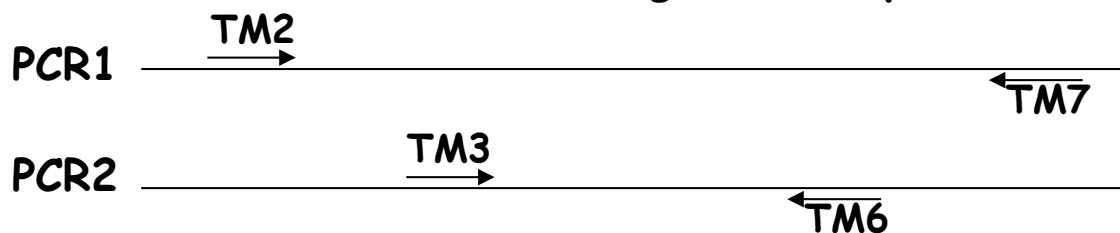
Reverse transcription



cDNA



Nested PCR, degenerated primers



PCR product sequencing

Identification of the olfactory receptor present

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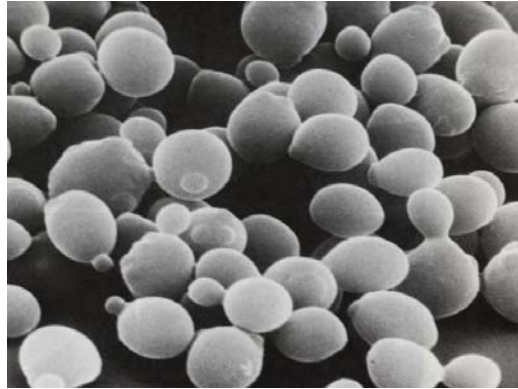
3 > An efficient heterologous expression system for olfactory receptors : the yeast *S. cerevisiae*

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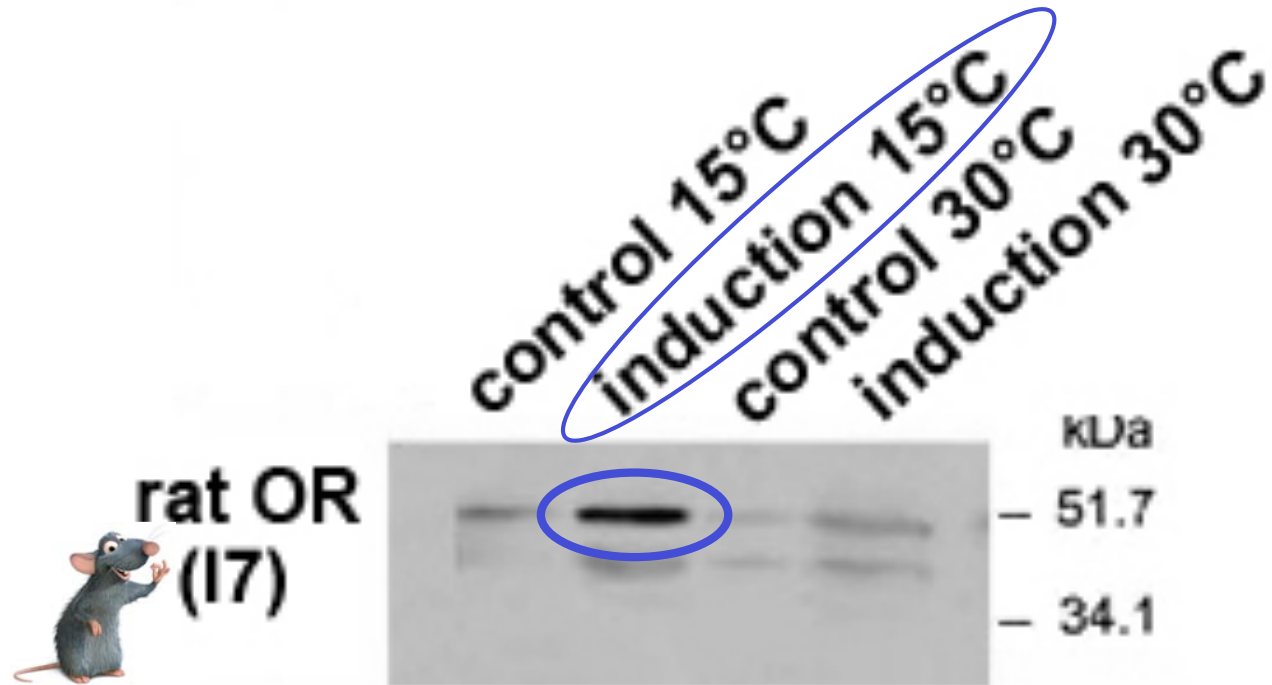
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> Quantitative expression of olfactory receptors in yeast



Saccharomyces cerevisiae

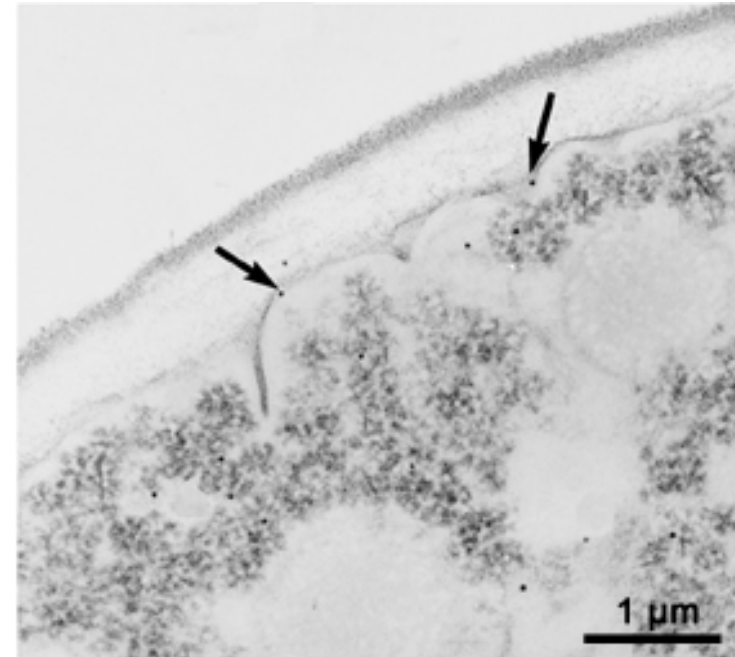
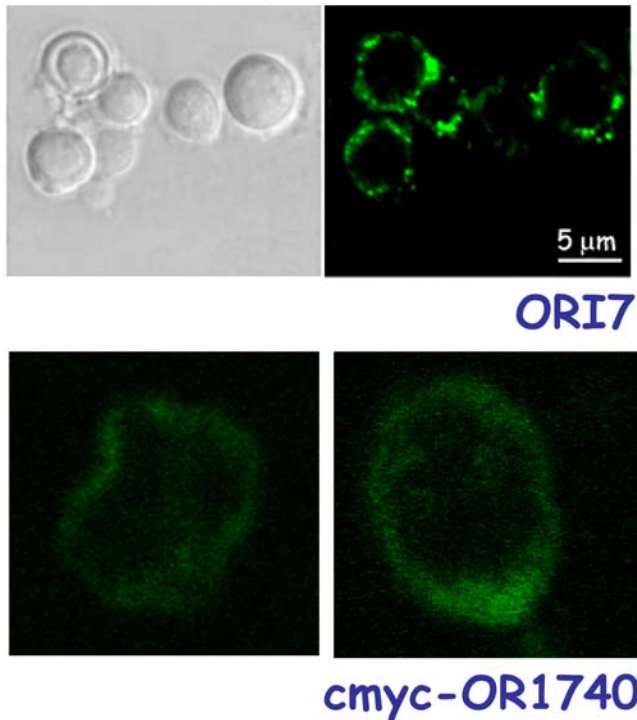


> low temperature allows

- ↳ correct folding
- ↳ membrane trafficking of the receptors

[Minic et al. FEBS J. 2005]

> Presence of olfactory receptors at the plasmic membrane



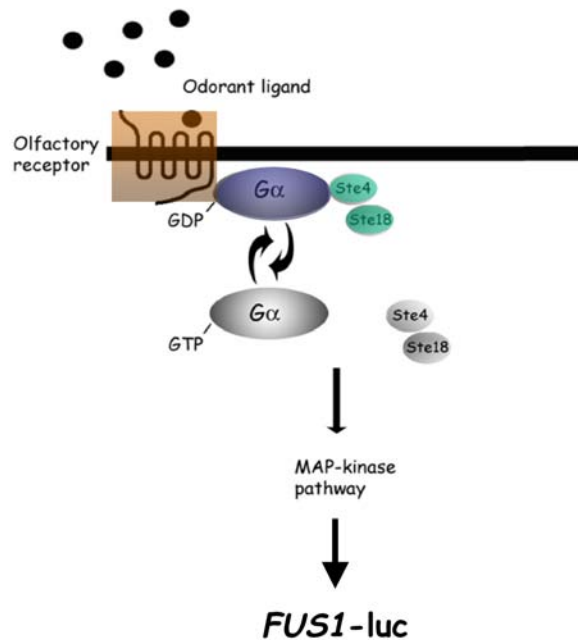
[Minic et al. FEBS J. 2005]

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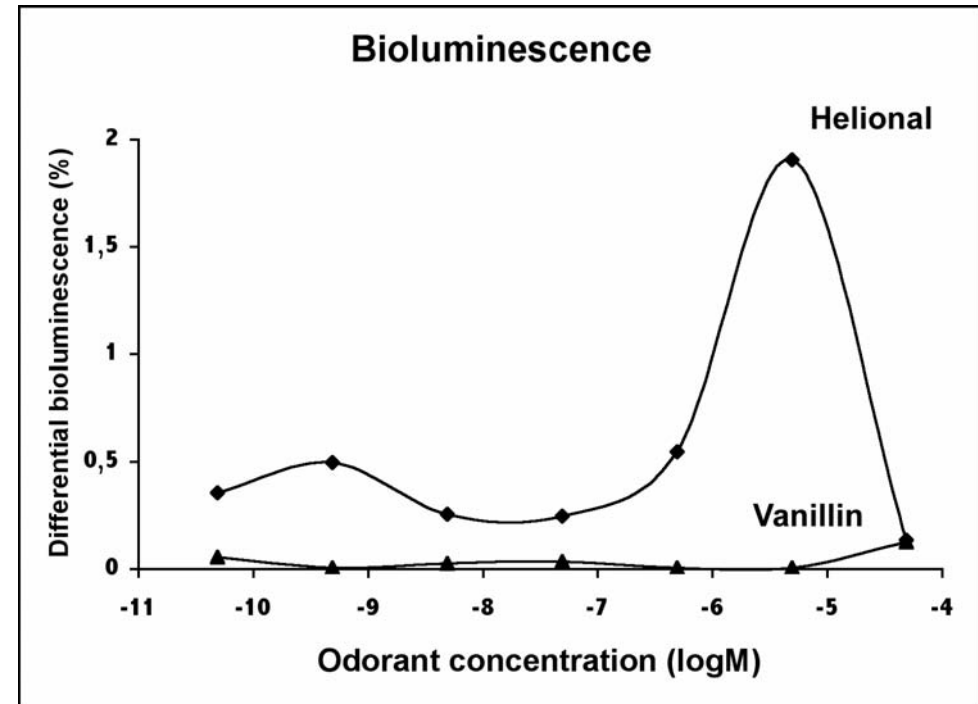
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> Cellular system of odorant detection by its receptor



**> opens the possibility
of yeast-based
olfactory biosensors**



hOR17-40 receptor

[Pajot-Augy et al. JRSTR 2003]

[Minic et al. FEBS J. 2005]

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4 > Nanosomes preserving olfactory receptors functionality

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> Acellular structures carrying olfactory receptors...



AFM (Atomic Force Microscopy)

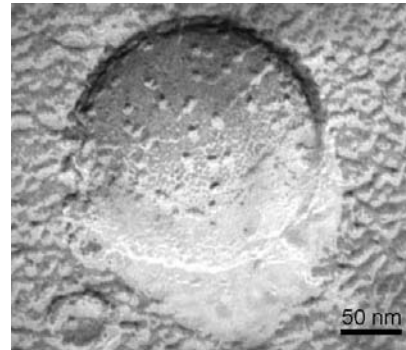
**sonication of yeast
membrane fraction**

Ø~50nm

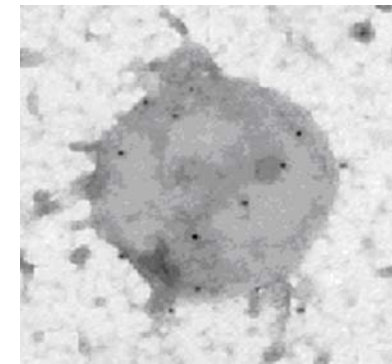
1-10 olfactory receptors

[Vidic et al. Anal. Chem. 2007]

[Casuso et al. Mat. Sci. Eng. C 2008]



**Electron Microscopy
Cryo-fracture**



**Electron Microscopy
Gold immunolabeling**

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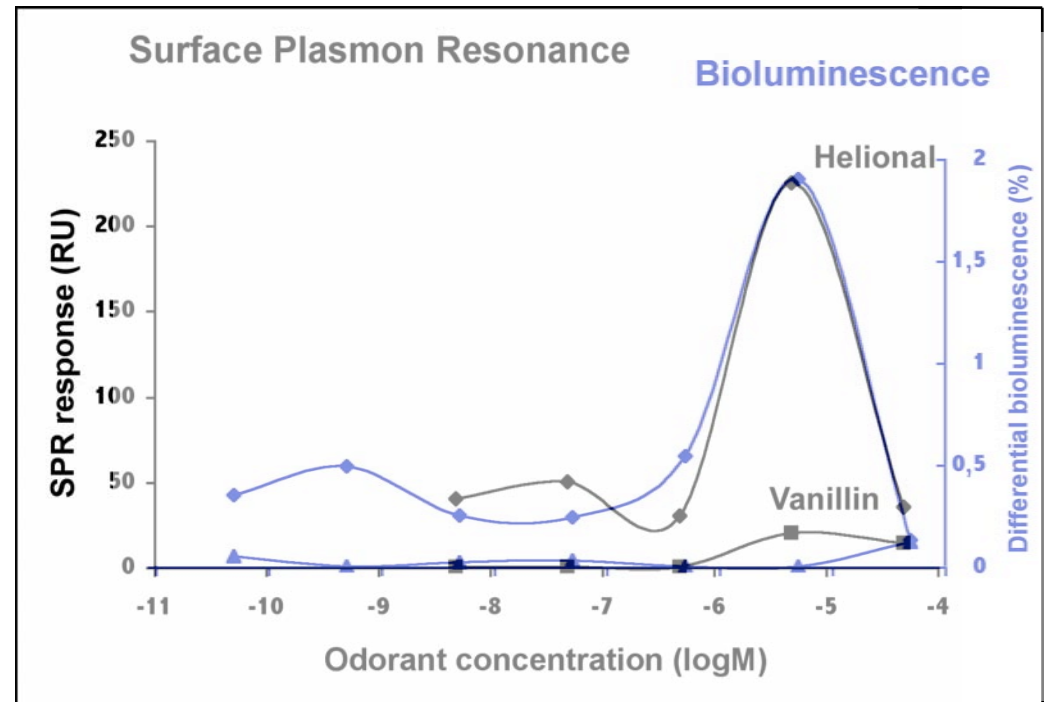
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> ... with a functionality similar to that in whole yeast cells

> evaluated by Surface Plasmon Resonance

> functional sensing elements for olfactory biosensors at the micro-nano-scale



Signature of the receptor behavior in response to odorant stimulation

[Minic et al. Lab on a Chip 2006]

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5> SPOT-NOSED Consortium achievements

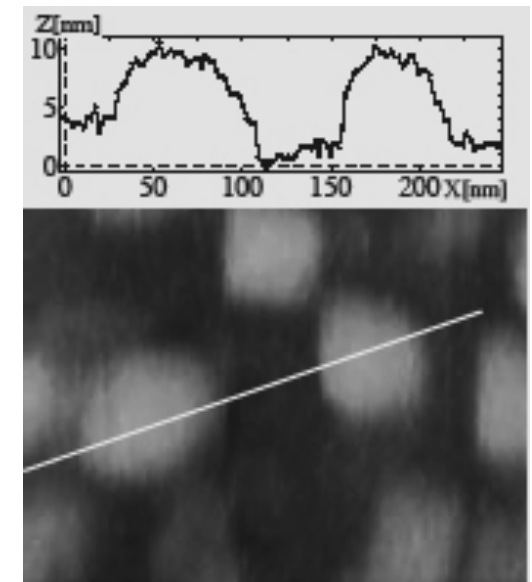
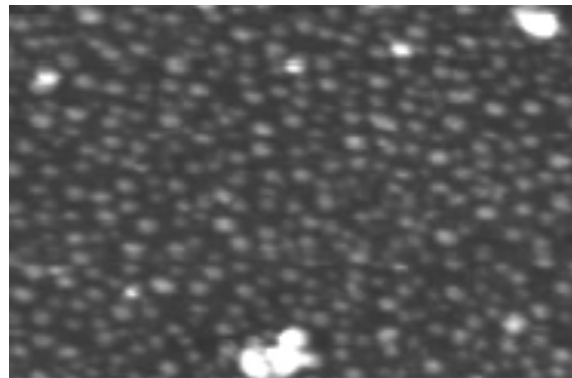
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> Immobilization of nanosomes ...

- electrodes or chips
- for Electrochemical Impedance Spectroscopy or Surface Plasmon Resonance measurements



AFM

[Casuso et al. Mat. Sci. Eng. C 2008]

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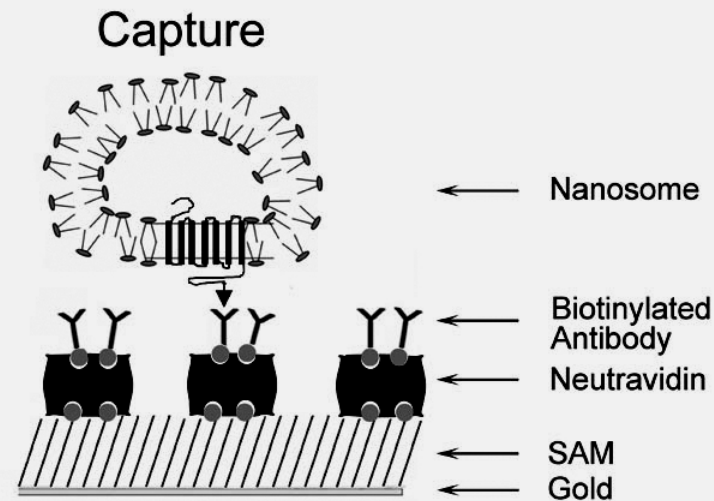
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> ... on functionalized gold...

Capture via a specific antibody grafted on a mixed SAM

Localization/orientation/density of
receptors, stabilization of the assembly



[Hou et al. Biosens. Bioelectron. 2005]

[Rodriguez Segui et al. Analytical Letters 2006]

[Vidic et al. Anal. Chem. 2007]

[Casuso et al. Mat. Sci. Eng. C 2008]

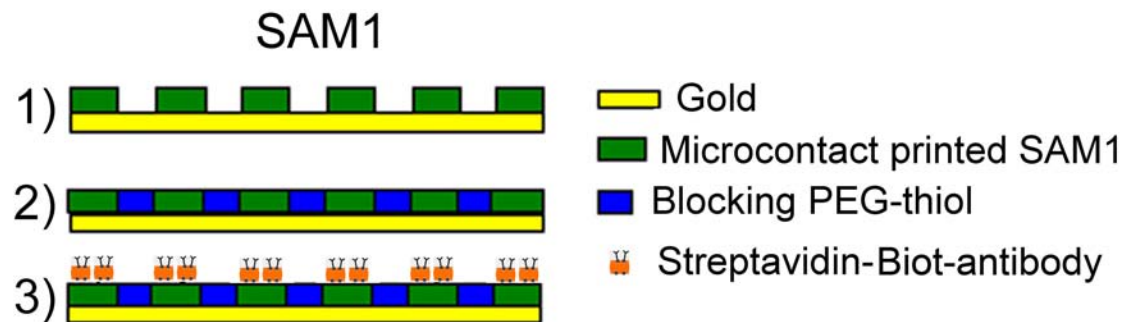
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> ... using microcontact printing

SAM1 : **BAT** (biotin-terminated alkyl thiols)/
PEGAT (PEG-terminated alkylthiols)

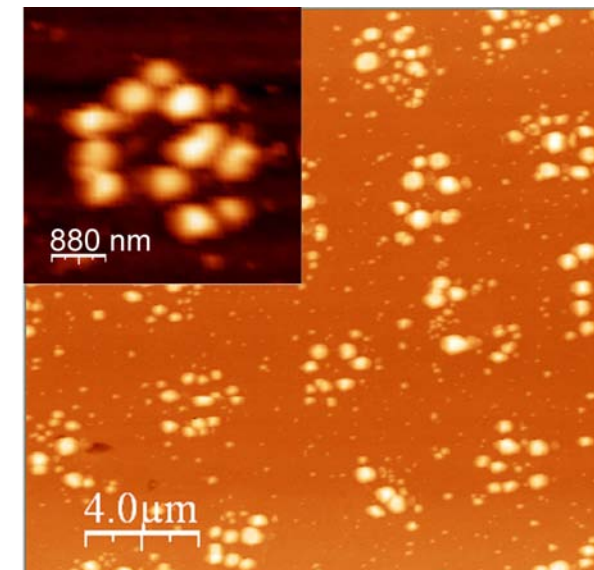


- **PDMS stamps** with posts Ø2.5mm protruding 800nm
- **Patterning by inking procedures** after oxygen plasma hydrophilization

- grafting of **specific biotinylated antibodies** onto streptavidin

- **nanosomes deposition**

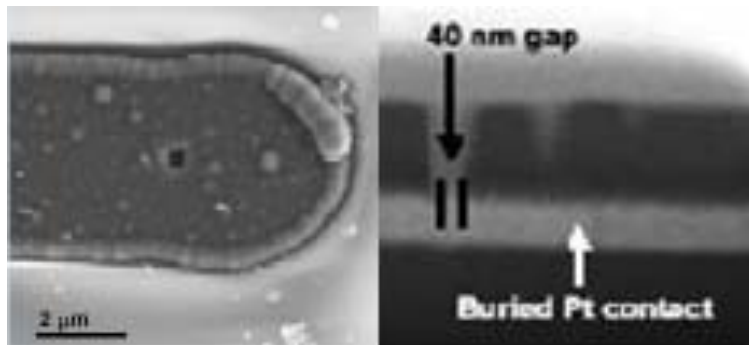
[Vidic et al. Anal. Chem. 2007]



AFM

(inset : High-resolution topography)

> Nanoelectrodes fabricated by micro + nano-techniques...



Focused Ion Beam milling : aperture 40 nm

> ... High-gain low-noise electronic instrumentation with extended bandwidth to probe the electrical response with Scanning Probe Microscopy + user-friendly interface

[Gomila et al. Sensors & Actuators B 2006]

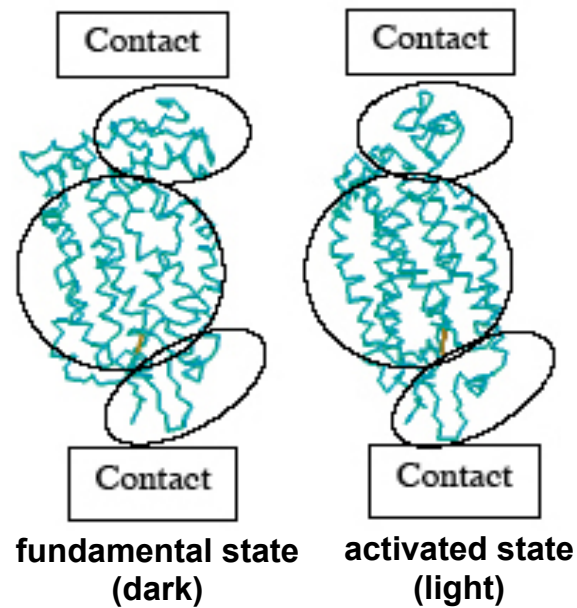
[Ferrari et al. Rev. Sci. Instr. 2004]
[Italian Patent n° MI2003A002543]

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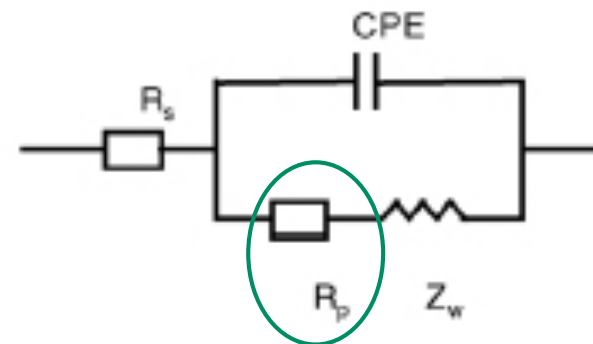


> Modelization of single receptor electrical behavior



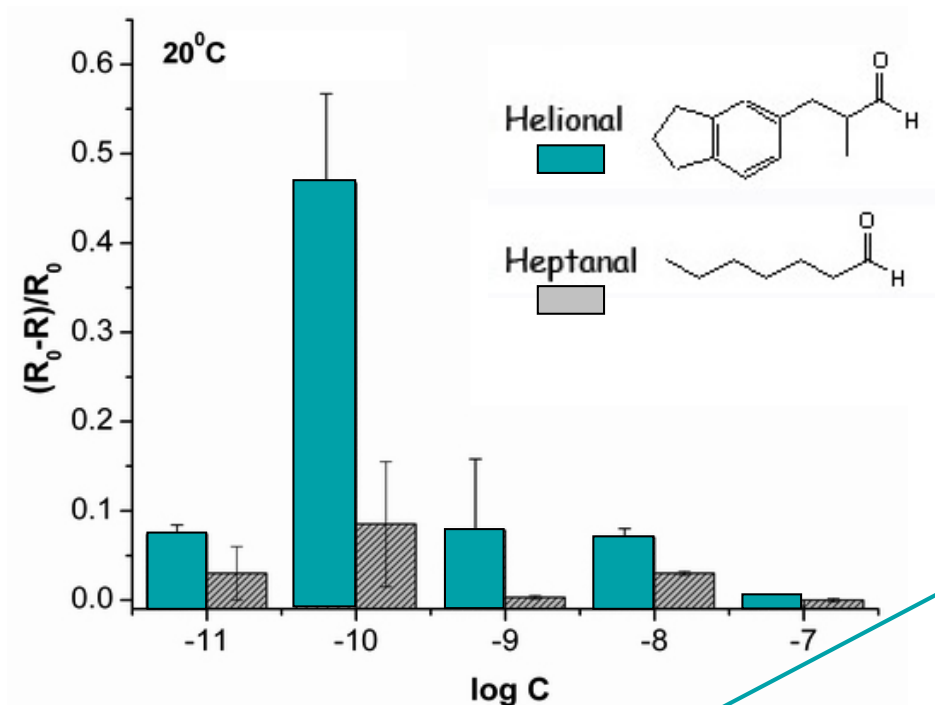
prototype = rhodopsin

Equivalent electrical circuit, mimicking the electrical properties of the receptor



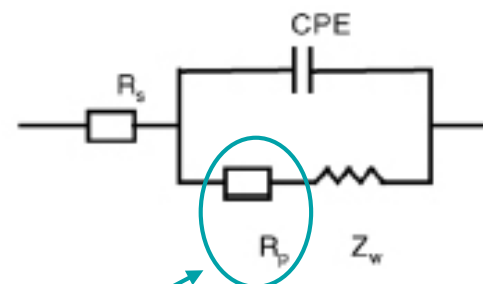
[Pennetta et al., in "Nanodevices for the Life Sciences" 2006]
[Pennetta et al. Noise and Information in Nanoelectronics 2004]

> Electrochemical detection (EIS) of functional response of ORs on immobilized nanosomes



Relative variation of polarization resistance

Data processing using electrical model



Immobilized olfactory receptors

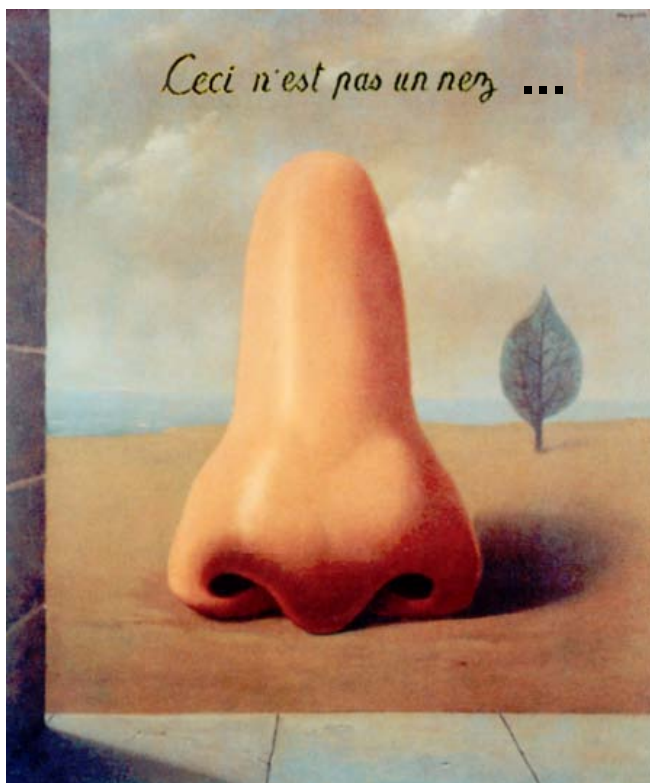
--> discriminate between odorants

--> concentration-dependent response

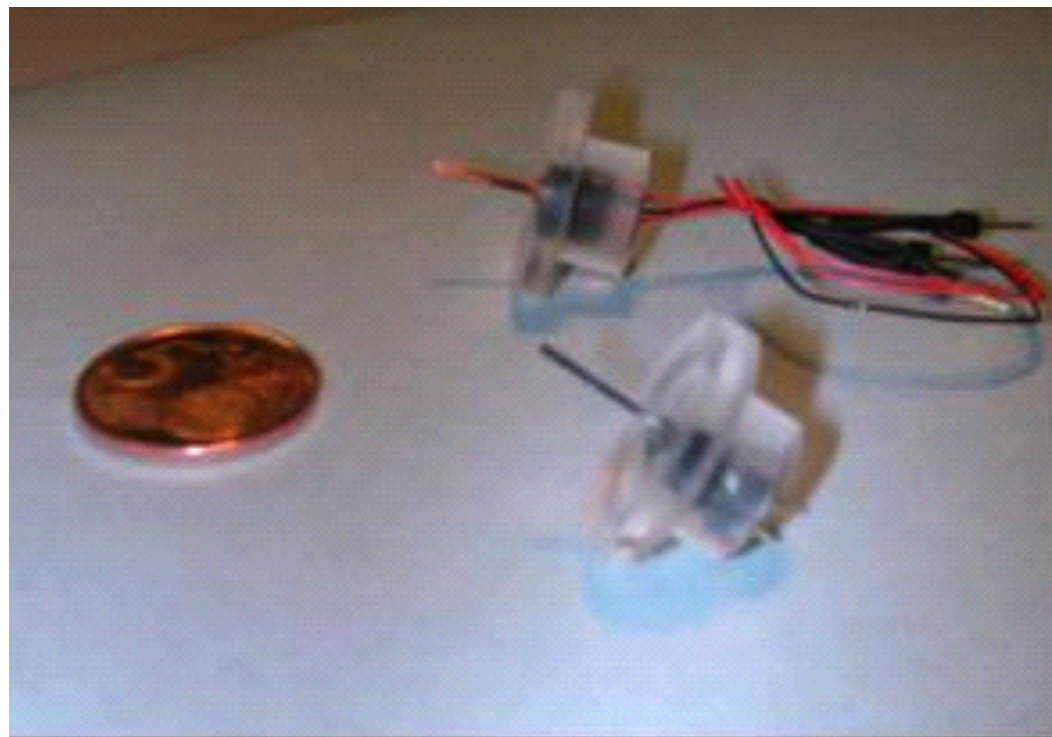
[Hou et al. Biosens. Bioelectron. 2006]

[Benilova et al. Mat. Sci. Eng. C 2008]

[Pennetta et al., in "Nanodevices for the Life Sciences" 2006]



... but this device could come close to it!



SPOT-NOSED olfactory nanobiosensor prototype (2006)

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6> BOND European project [2009-2012]

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www.bondproject.org

BOND : Bioelectronic Olfactory Neuron Device

**Development of a portable olfactory system for the
detection of odorants from complex mixtures**

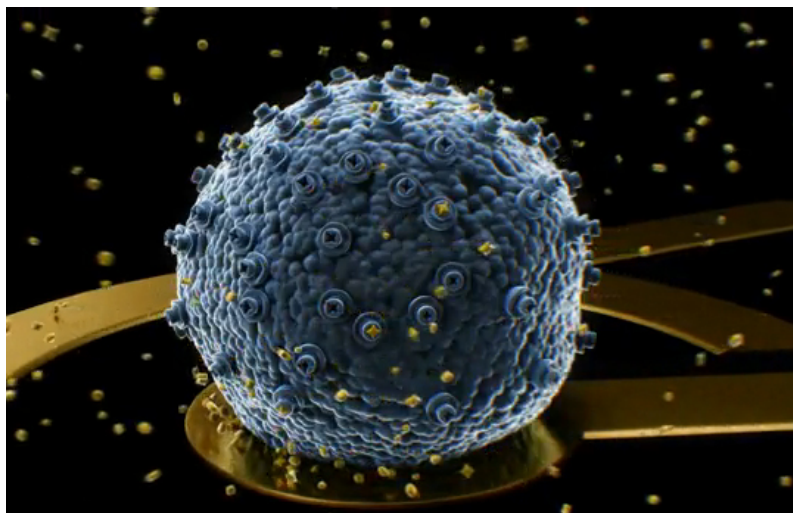
**Nanosciences, nanotechnologies, materials
& new production technologies (NMP)**



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Sensitive part of the sensor based on liposomes carrying olfactory receptors to mimick the natural olfactory system

An array of nanoelectrodes will be integrated in the electronic chip that will detect the current from the olfactory receptors



Sanmarti et al., EuroNanoForum XXIV, Linz, Austria, 2010

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Jasmina Vidic

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(Jeanne Grosclaude VIM)

Jean-François Gibrat MIG

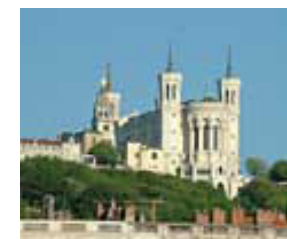
(Stéphane Téletchéa MIG)

Christine Péchoux GPL

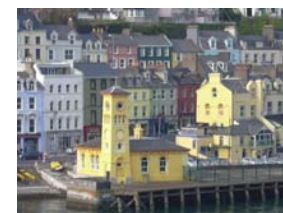
Ian Connerton U. Nottingham, UK

Francis Galibert CNRS Rennes

SPOT-NOSED European Consortium (Spain, Italy, France)



BOND European Consortium (Spain, Italy, England, Ireland, France)



Thank you for your attention!

Merci!

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