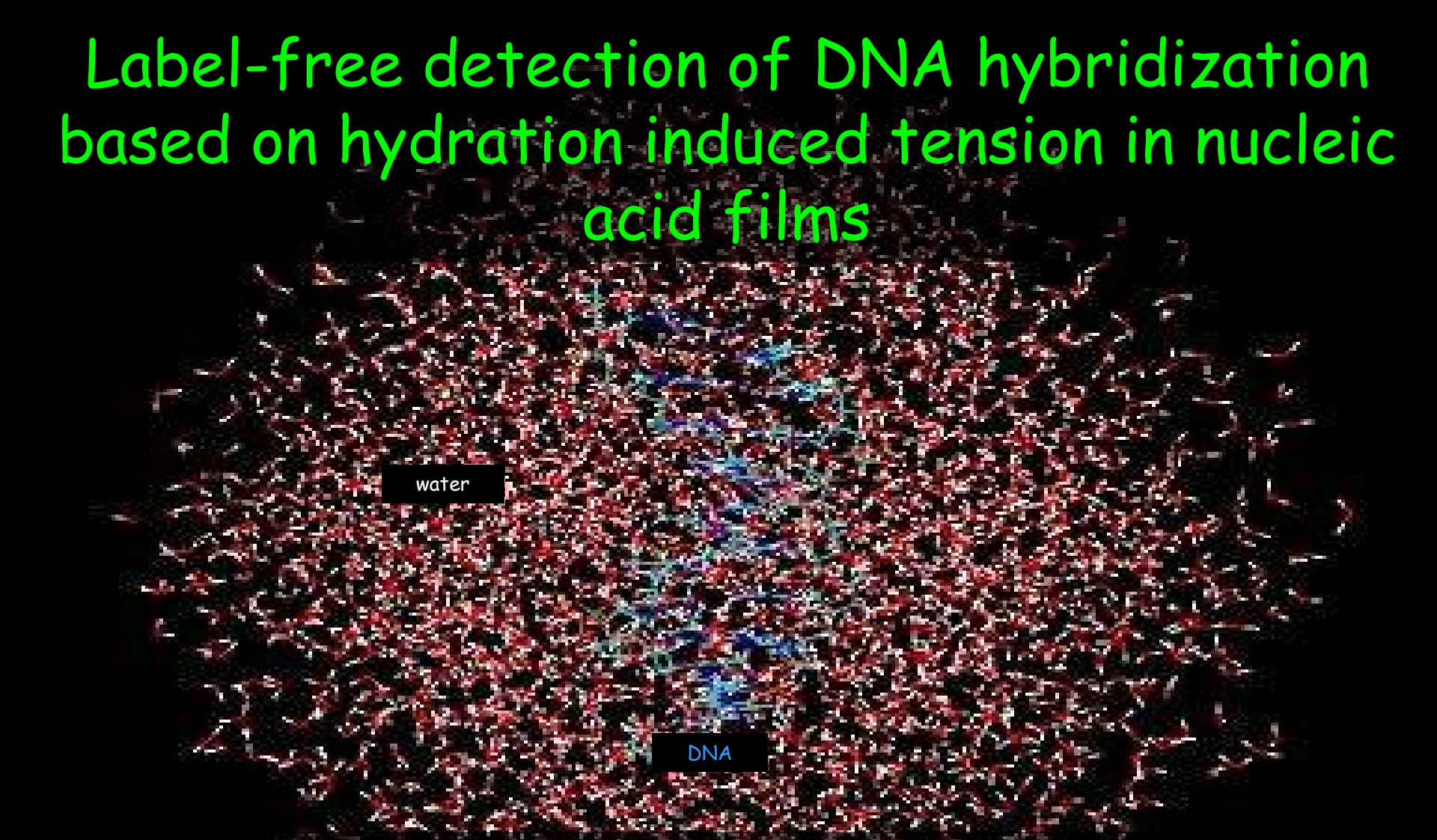
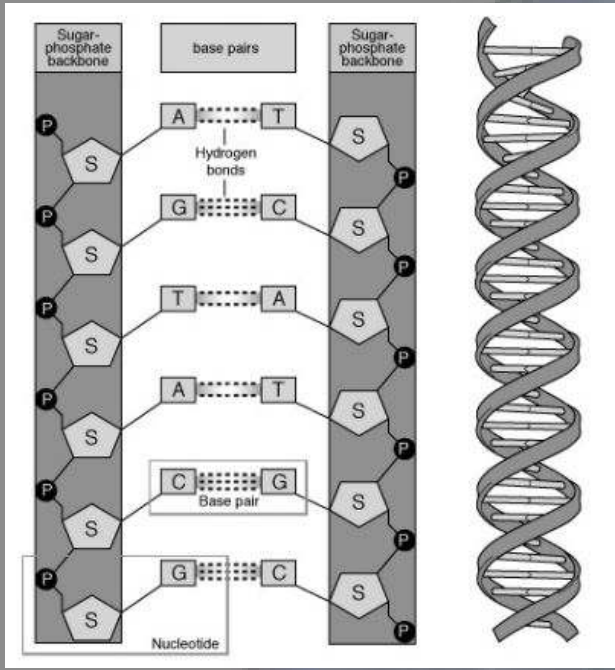
Label-free detection of DNA hybridization based on hydration induced tension in nucleic acid films



Johann Mertens, Maria Arroyo-Hernández, Montserrat Calleja, Daniel Ramos, and Javier Tamayo
Bionanomechanics Lab (IMM-CNM), CSIC. Madrid, Spain
Celia Rogero, Jose Angel Martín-Gago, Carlos Briones
Astrobiology Center (CSIC-INTA). Madrid, Spain.

nanobio europe
Barcelona (Spain)
June 9-13, 2008

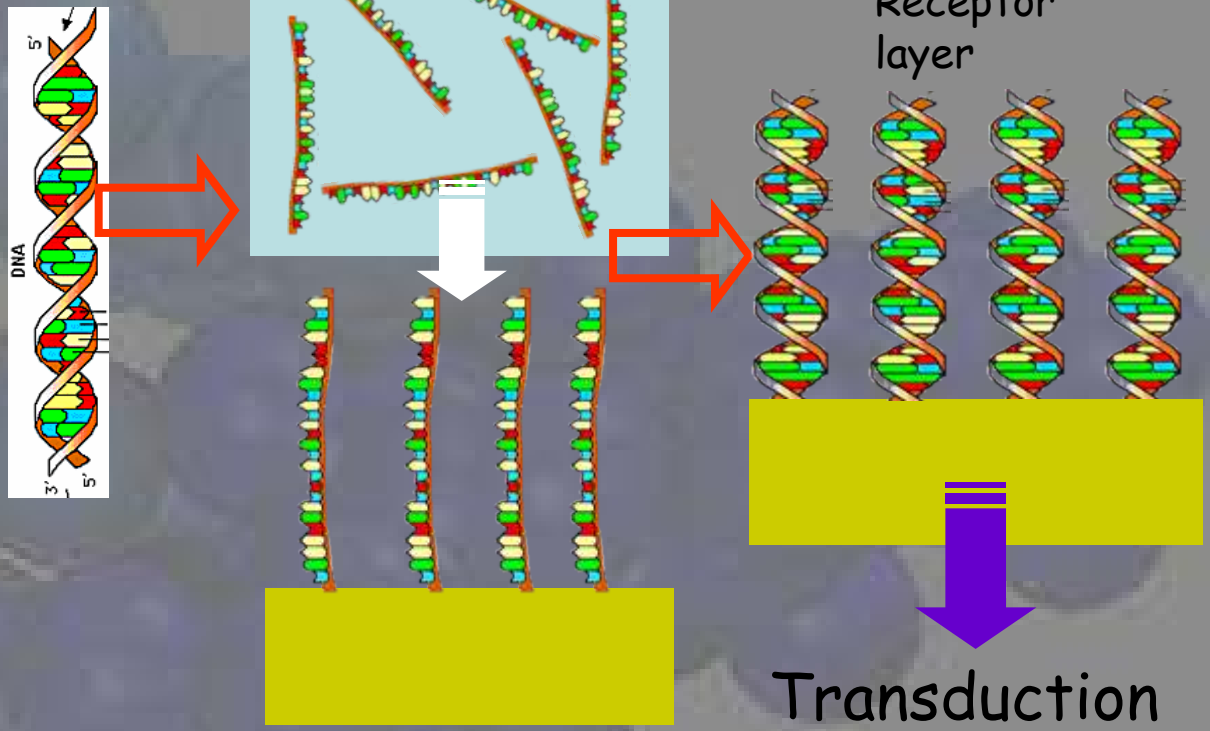
DNA structure



Specificity and weak strength of base pairing is the base of life and…

- DNA nanotechnology
- Sensors

DNA sensor

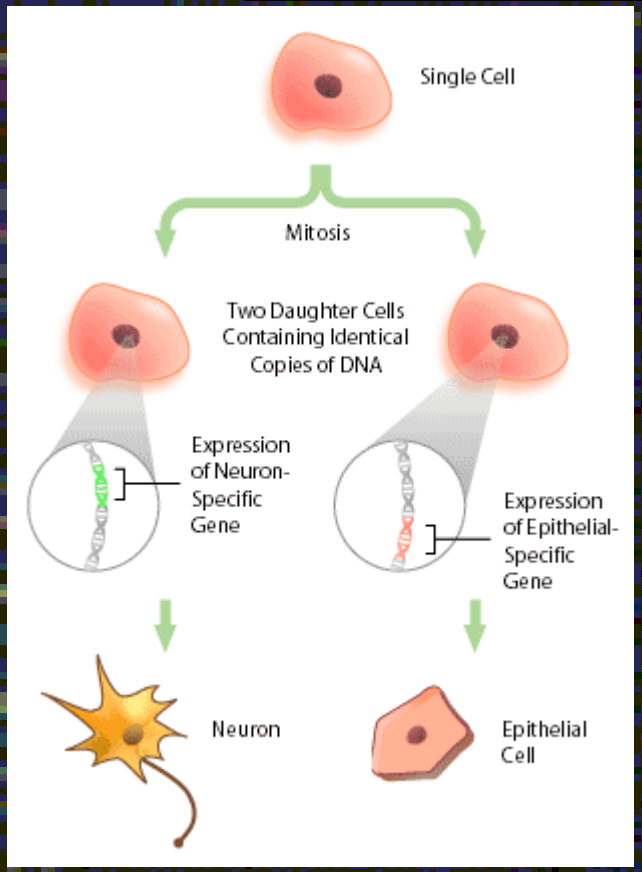


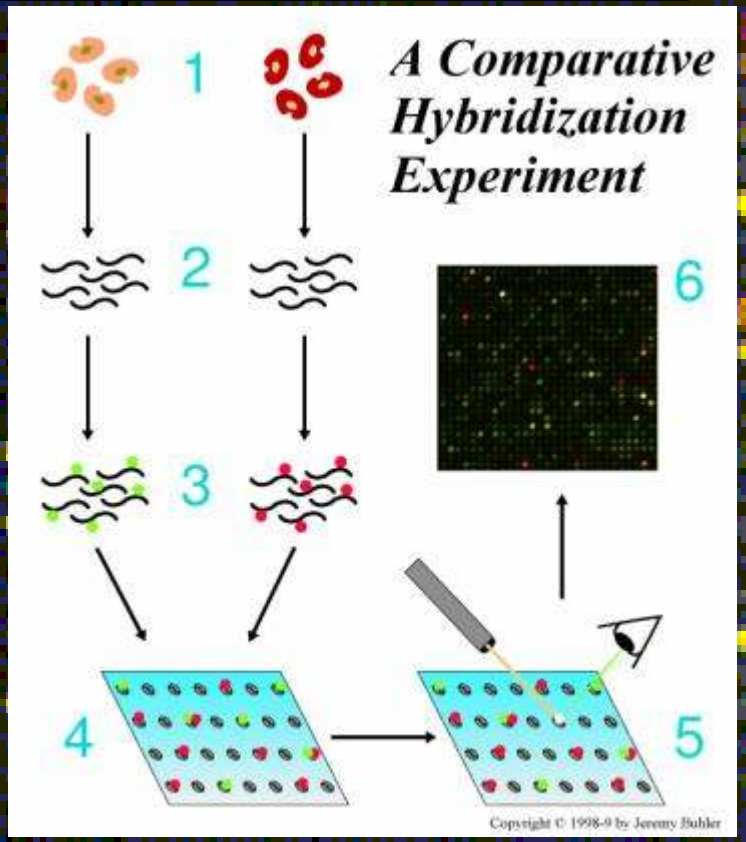
Transduction

- Optical
- Electrical
- Mechanical

- Meaning to the genomes
- Gene function
- Early detection of disease

DNA arrays: concept and applications





- ✓Expression of thousands of genes
- ✓Genes related to disease
- ✓Pharmacogenomics

- ☒Sample labelling: time-costly, background signal, steric hindrance
- ☒Low sensitivity: PCR or large number of cells=>low genetic purity, change of the sample stecheometry

MOLECULAR RECOGNITION DETECTION BY SURFACE STRESS BIOSENSORS

CANTILEVER PREPARATION

20-50 nm Gold

~100μm

<1μm

ANTIGEN (PESTICIDE)/ANTIBODY RECOGNITION (real time!!)

25 nM Antibody

ANTIBODY

STICIDE

- Label free
- Sensitive
- Scalable to large arrays
- Capability for integration
- Semiconductor technology:batch prodution and low cost

HORMONE/ANTIBODY RECOGNITION

Polymer cantilever

MOLECUL

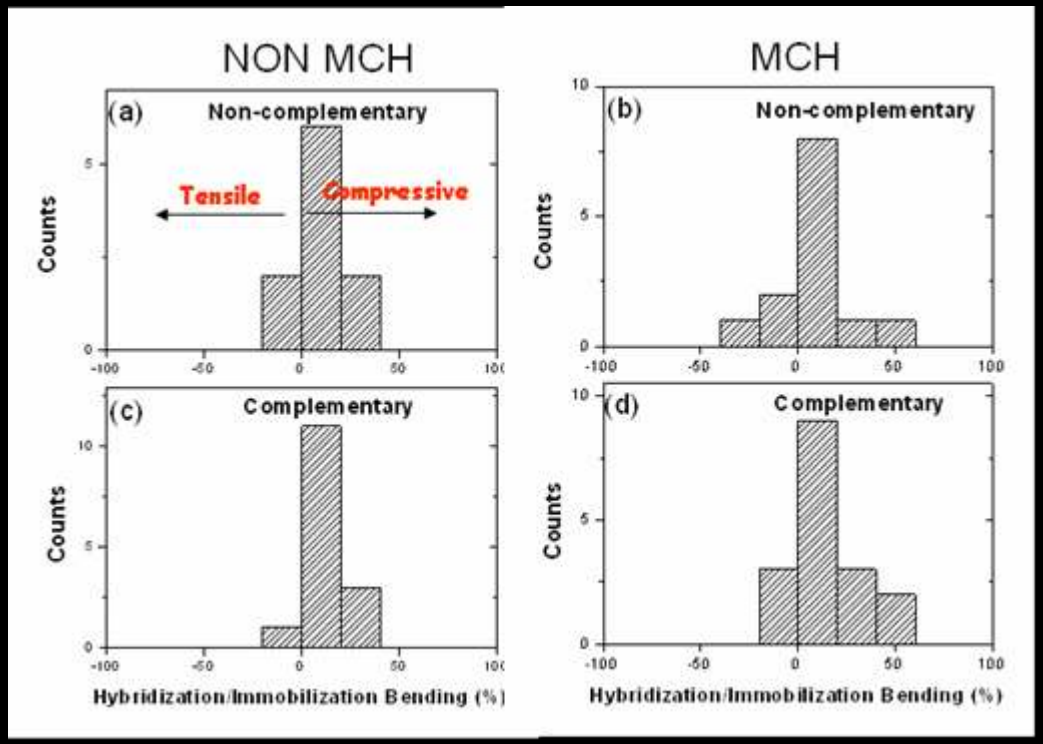
Candidate for next generation of DNA arrays?

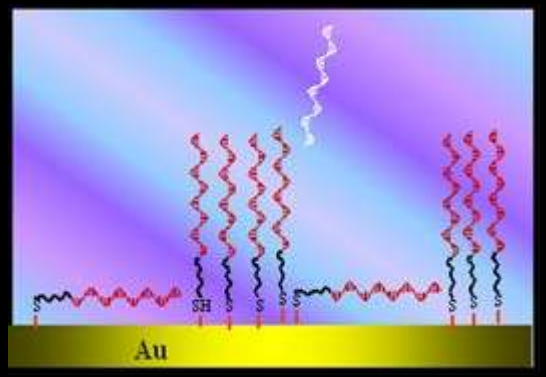
10 minutes

100 μm

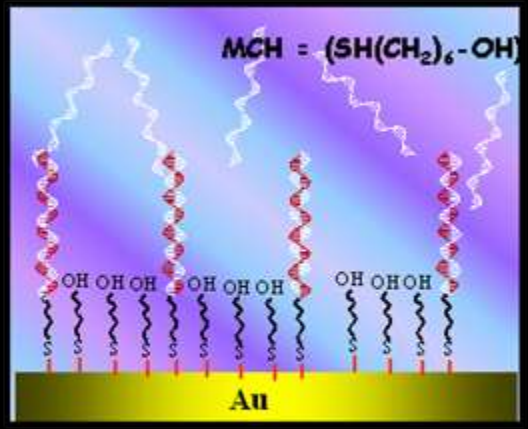
M. Calleja, J. Tamayo, M. Nordstrom and A. Boisen, Applied Physics Letters 88, 113901 (2006)

HYBRIDIZATION-INDUCED SURFACE STRESS





MCH treatment



- Remove loosely bound DNA probes
- Enhance accesibility of DNA probe for hybridization

The hybridization can not be infered from the cantilever bending whereas parallel characterization by fluorescene and SPR experiments give significant hybridization signals

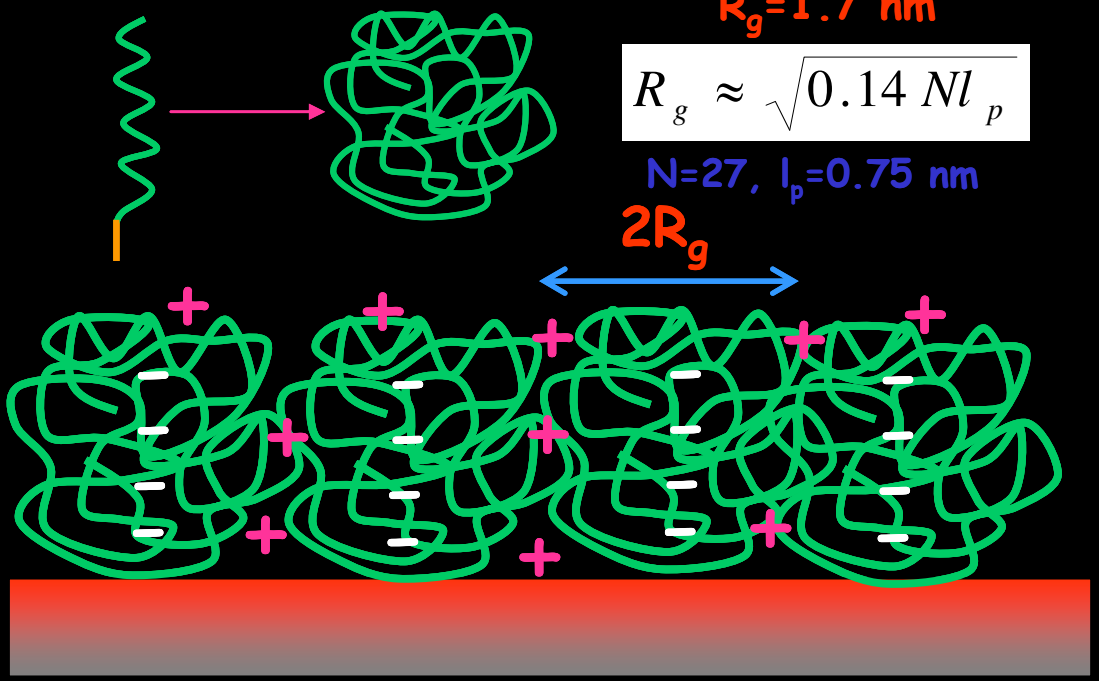
M. Álvarez, L.G. Carracosa, M. Moreno, A. Calle, A. Zaballos, L.M. Lechuga, C. Martínez-A and J. Tamayo, *Langmuir* 20, 9663 (2004)

- DNA hybridization produces a surface stress below the detection sensitivity for a single microcantilever≈5 mN/m. It is neccesary the use of differential signals with a reference cantilever (remove temperature, turbulences, ion fluctuations). See Hegner, McKendry and Gerber work
- This is in contrast with the surface stress generated by the antigen/antibody recognition ≈100-200 mN/m

PARADOX: GOOD CONDITIONS FOR DOUBLE HELIX FORMATION ARE THE WORST TO OBTAIN SURFACE STRESS

DNA flexibility gives an additional channel for intermolecular energy release, in addition to the surface stress mechanism

ssDNA is not a straight rod



Electrostatic repulsion

$$F_E = \sqrt{2\pi}\,\frac{k_B T}{\left(l_e \frac{a}{\lambda_D} K_1\left(\frac{a}{\lambda_D}\right)\right)^2} \times \frac{\exp\left(-\frac{d}{\lambda_D}\right)}{\sqrt{\frac{d}{\lambda_D}}}$$

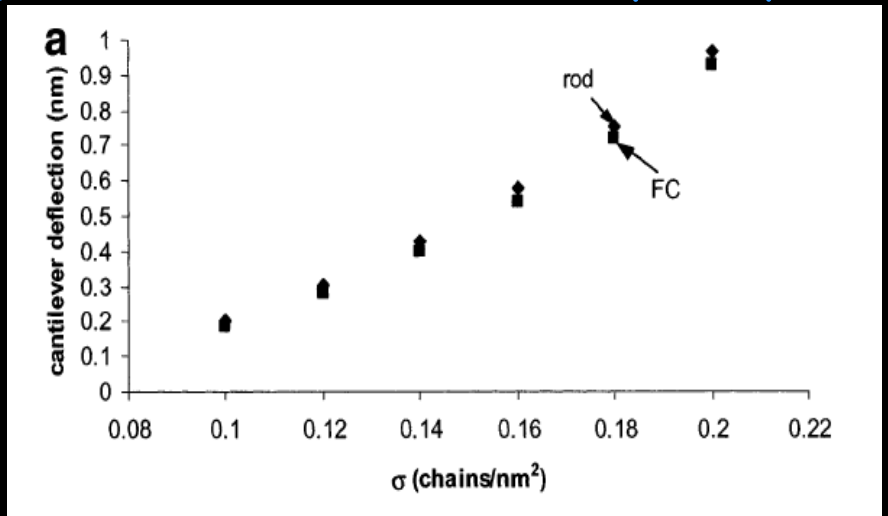
$\lambda_D \approx 0.3$ nm

Hydration force

$$F_H = b\sqrt{\frac{\pi}{2}} \times \frac{\exp(-d/\lambda_H)}{\sqrt{d/\lambda_H}}$$

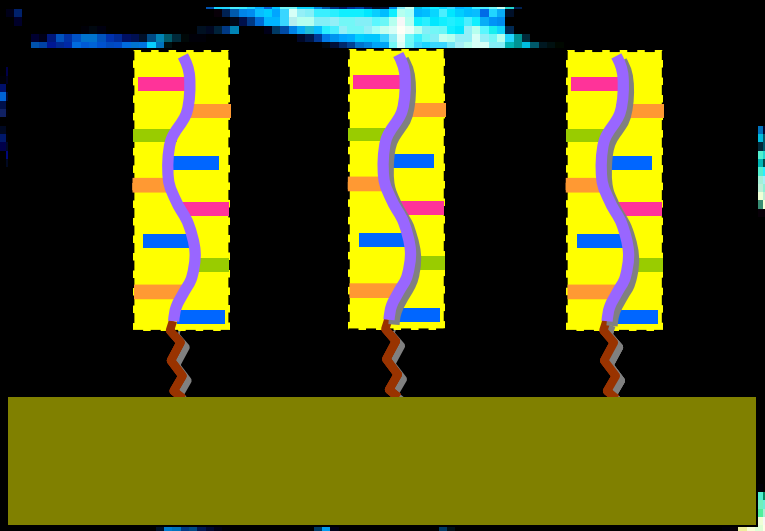
$\lambda_H \approx 0.3$ nm

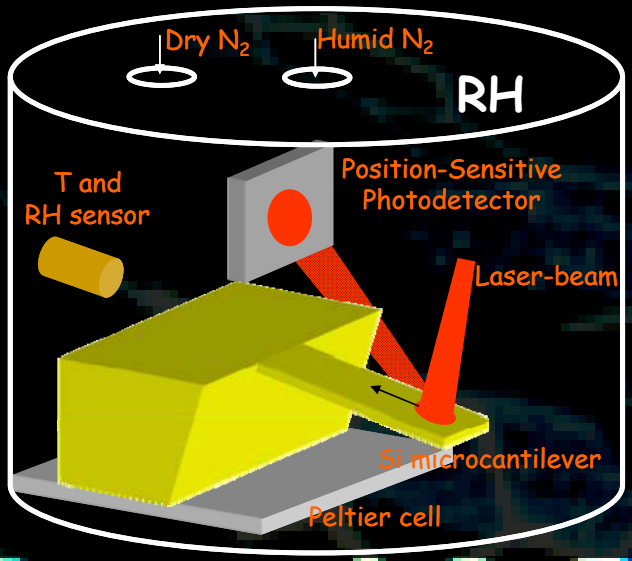
At usual surface densities most of the interactions are weak to obtain significant surface stress upon hybridization

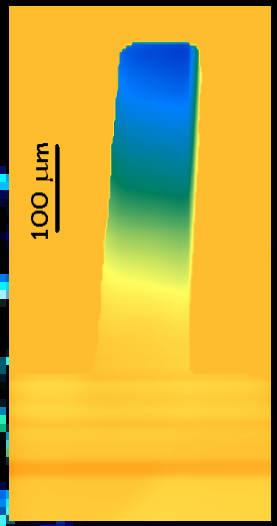


Hagan, Majumdar and Chakraborty, J. Phys. Chem. B, 106, 10163 (2002)

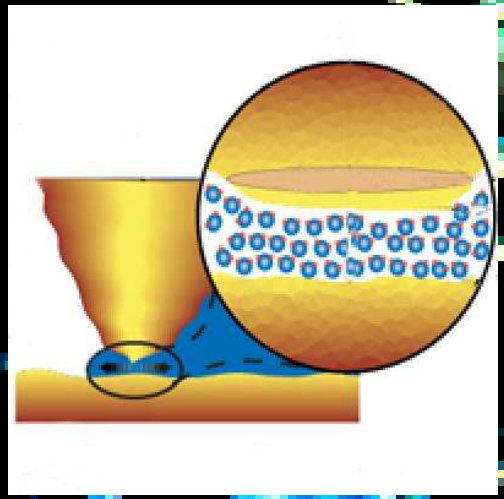
We have built-up highly packed ssDNA SAMS and studied the effect of adsorption of water in the intermolecular channels







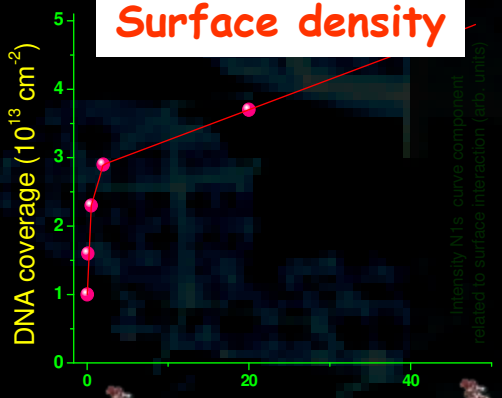
Exploit the high forces generated when water is confined in subnanometer channels: Disruption of the hydrogen bond network



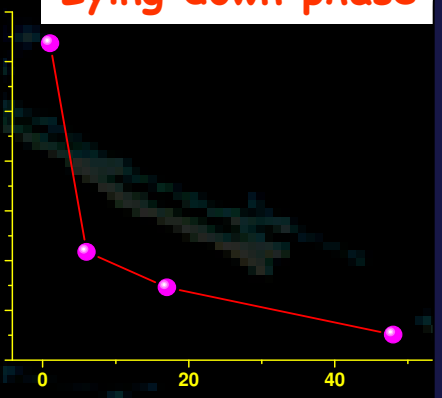
- Water confined in pores with diameters between 1 and 3 nm: water is in a liquid phase, short range order and a strong decrease of the solidification temperature by several tens of degrees.
- When the pore diameter is below 1 nm, the water is in an intermediate state between liquid water and crystalline water.

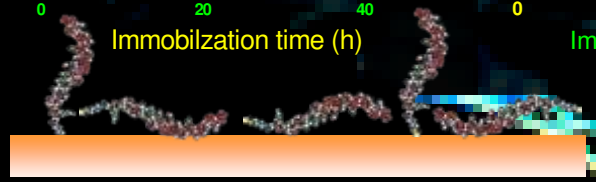
XPS analysis

Surface density

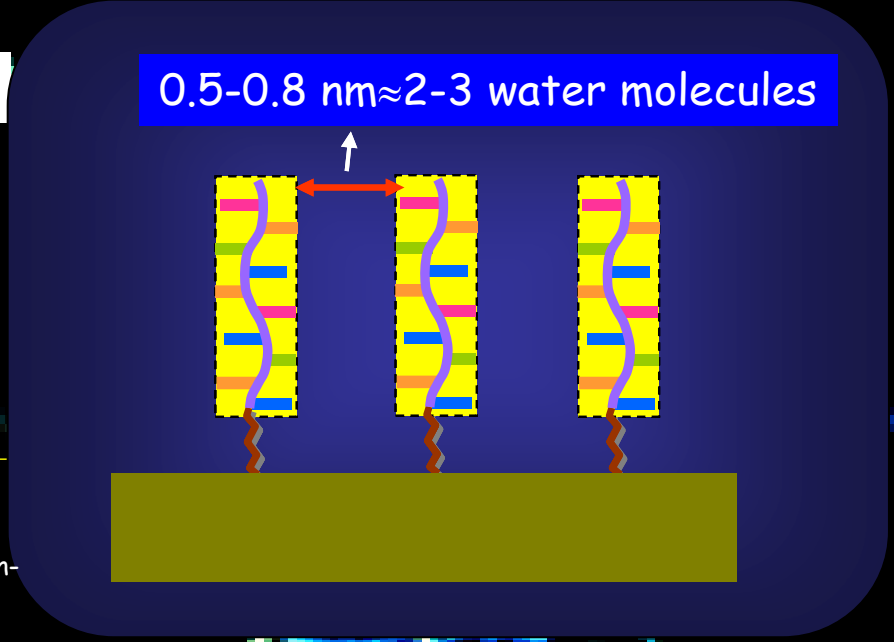


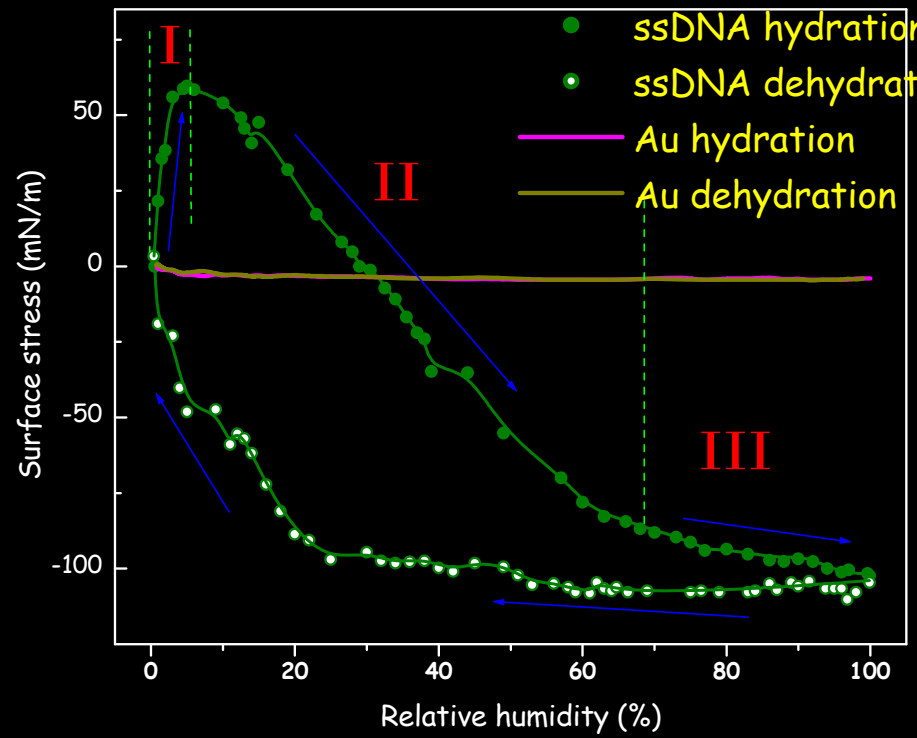
Lying down phase

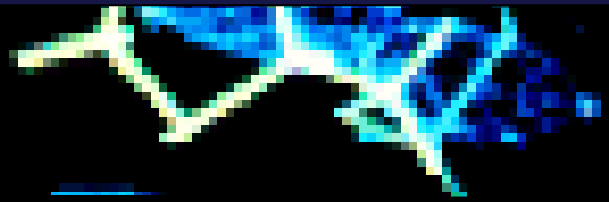


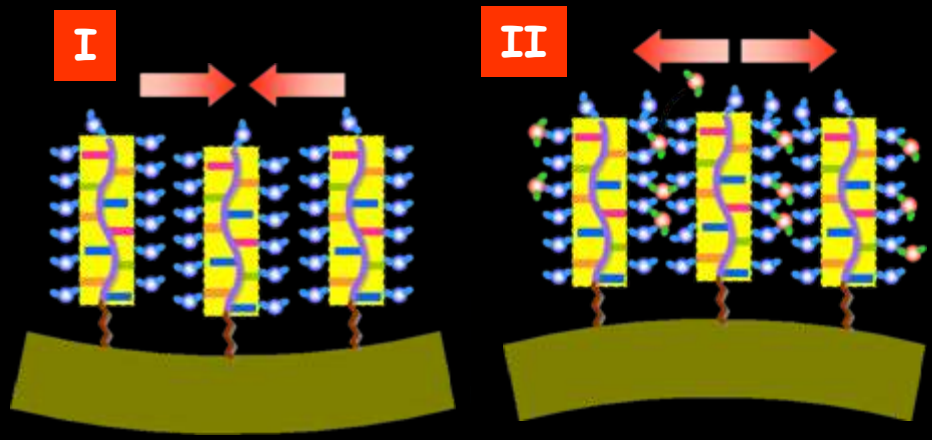


C. Rogero, J. A. Martín-Gago, C. Briones. C. Astrobiología CSIC-INTA.





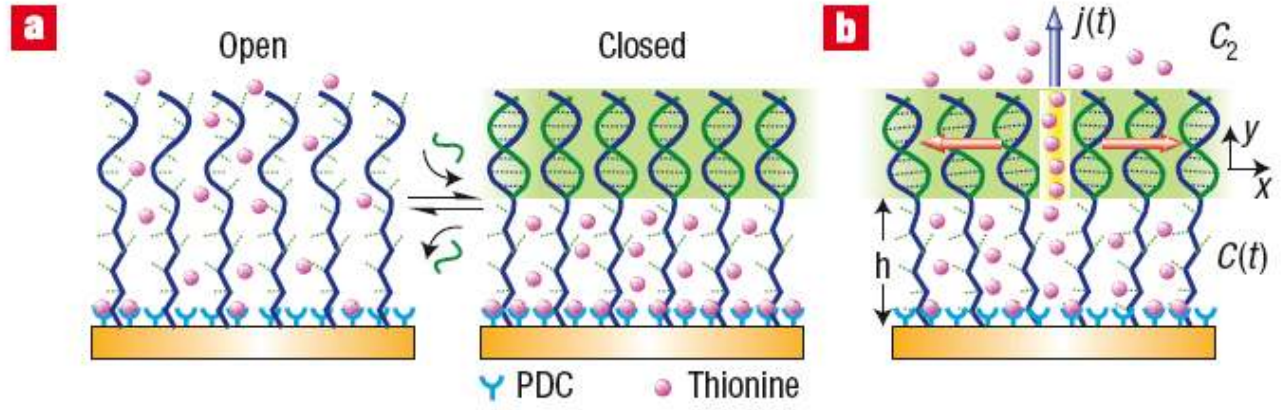




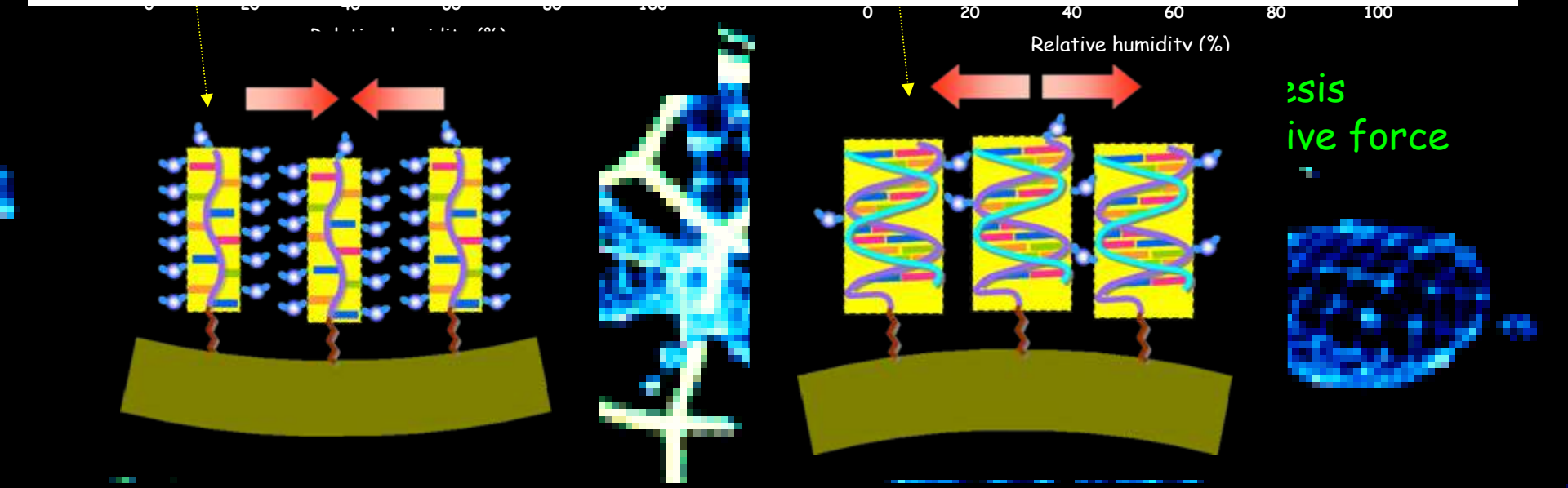
Dipole/Dipole forces

Steric Hydration forces

Molecule store (release) gated by DNA hybridization



Y. Mao, S. Chang, S. Yang, G. Ouyang and L. Jiang, Nature Nanotech.2, 366 (2007).



WATSON-CRICK PAIRING OF THE ssDNA ON THE CANTILEVER WITH A NUCLEIC ACID SAMPLE GATES THE WATER ADSORPTION IN THE INTERMOLECULAR CHANNELS

What is the nature of the intermolecular forces in the DNA membrane?

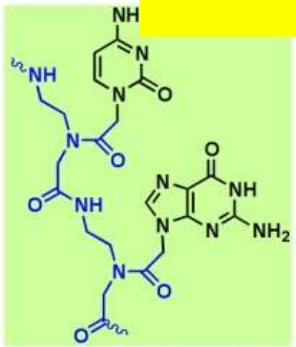
Electrostatic phenomena play a fundamental role in the DNA structure

DNA has an per e⁻ per 0.17 nm length. Depending on the electrolyte content, the counter-ion cloud can make the DNA strongly repel each other or condense in tightly packed large molecular complexes.

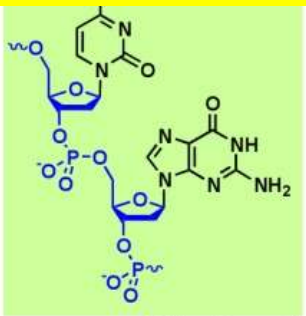
PNA v

PNA is a
charged
by a pep

Electrostatic interactions are not the key ingredient to understand the observed phenomena



PNA Peptide Nucleic Acid

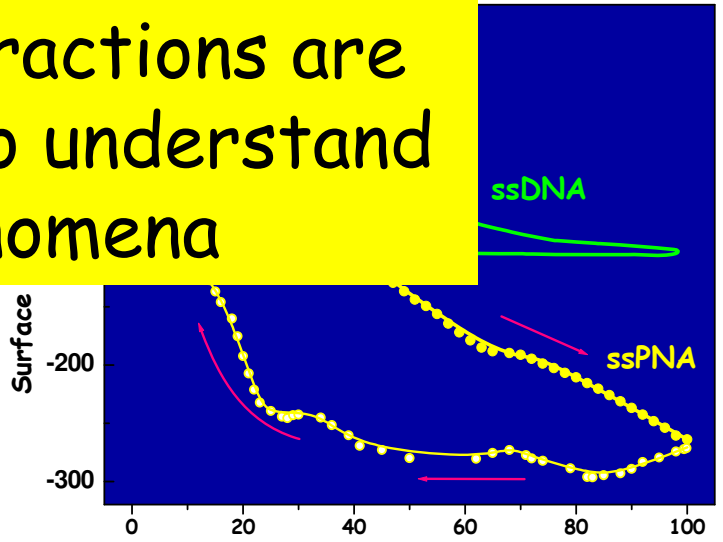


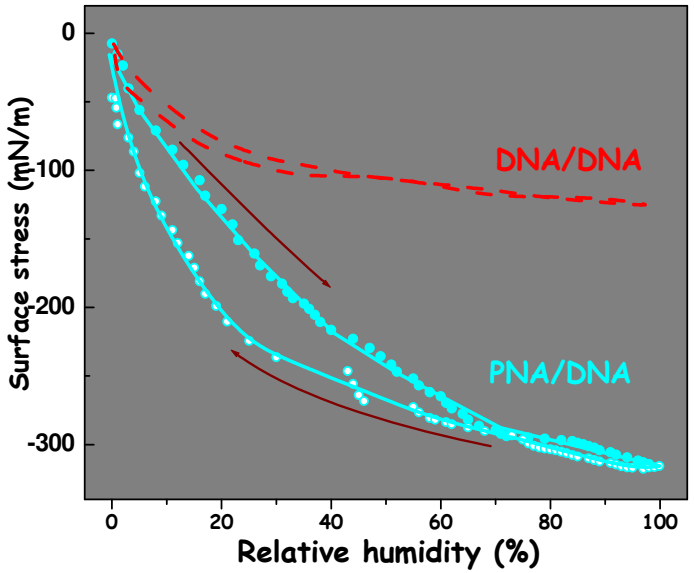
DNA Deoxyribo Nucleic Acid

- no nucleic acid ⇒ no degradation by nucleases
- no peptide ⇒ no degradation by proteases
- forms extremly stable complexes with DNA and RNA
- high sequence selectivity

Nielsen, Egholm, Berg, Buchardt, *Science* **1991**, *254*, 1497

C. Rogero, J. A. Martín-Gago, C. Briones. C. Astrobiología CSIC-INTA.

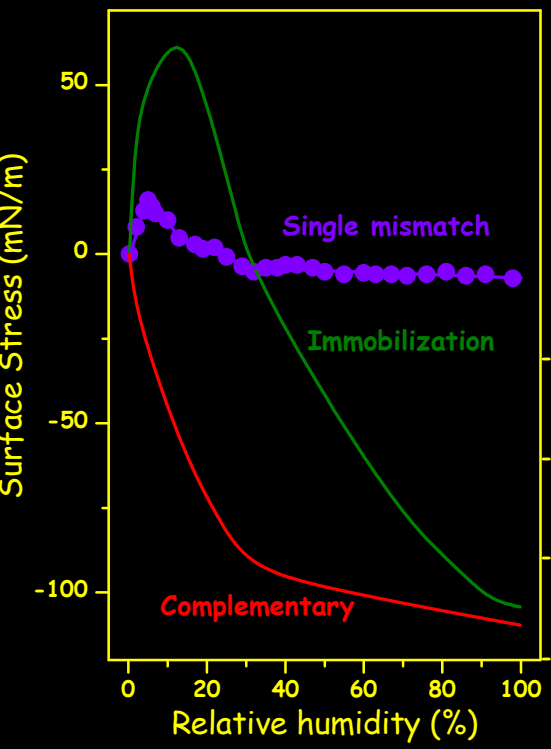


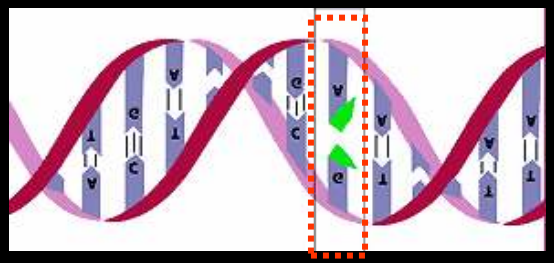


PNA is uncharged and the self-assembled monolayers are formed in ultrapure water!!!

Towards DNA biochips

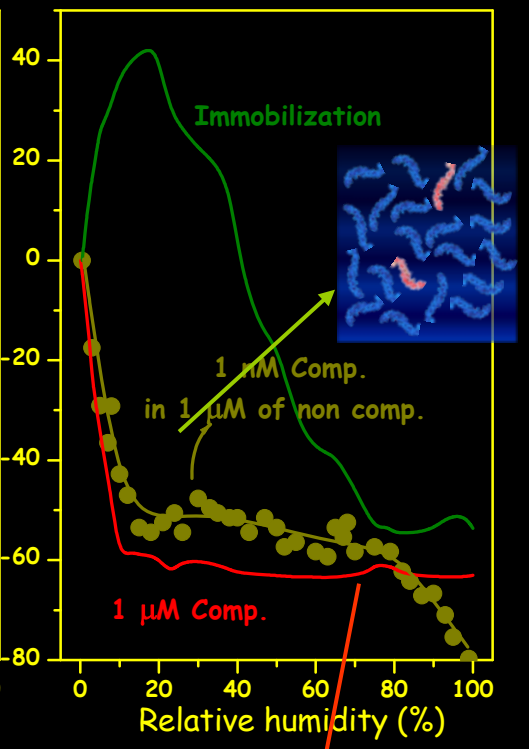
HIGH SPECIFITY

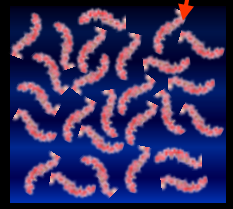




Sensitivity to a single mismatch

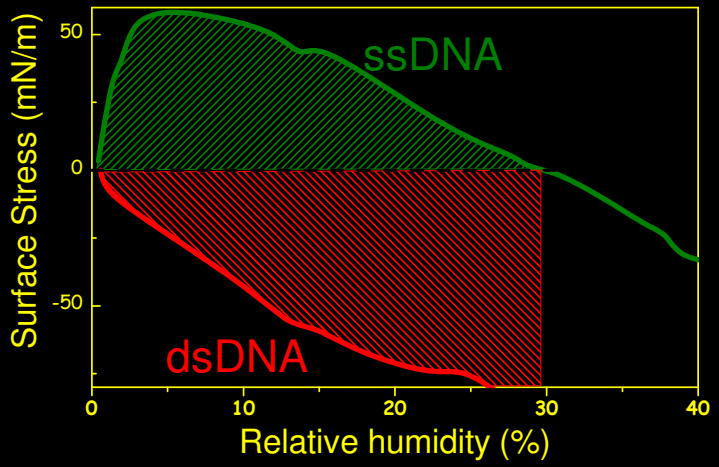
HIGH SENSITIVITY

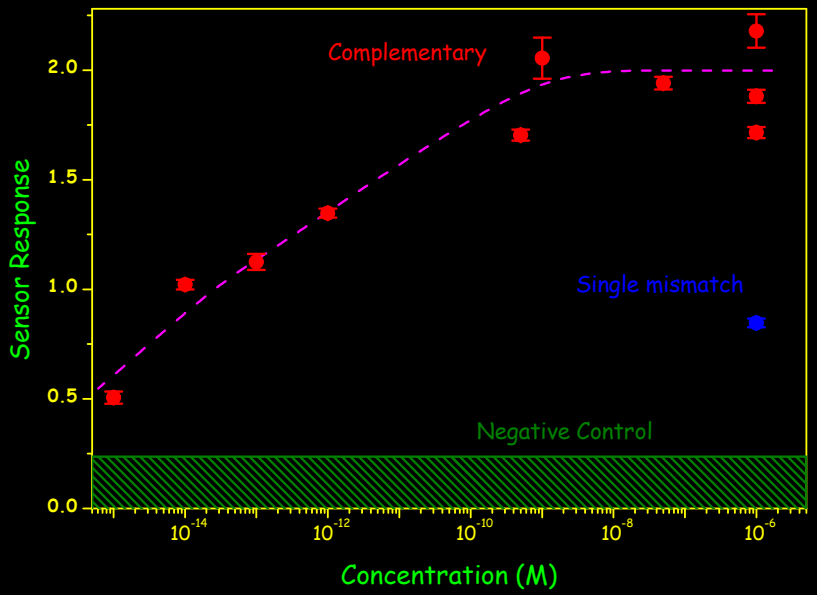




Fishing small amounts of target in 1000-fold excess of non matching DNA

ROBUSTNESS



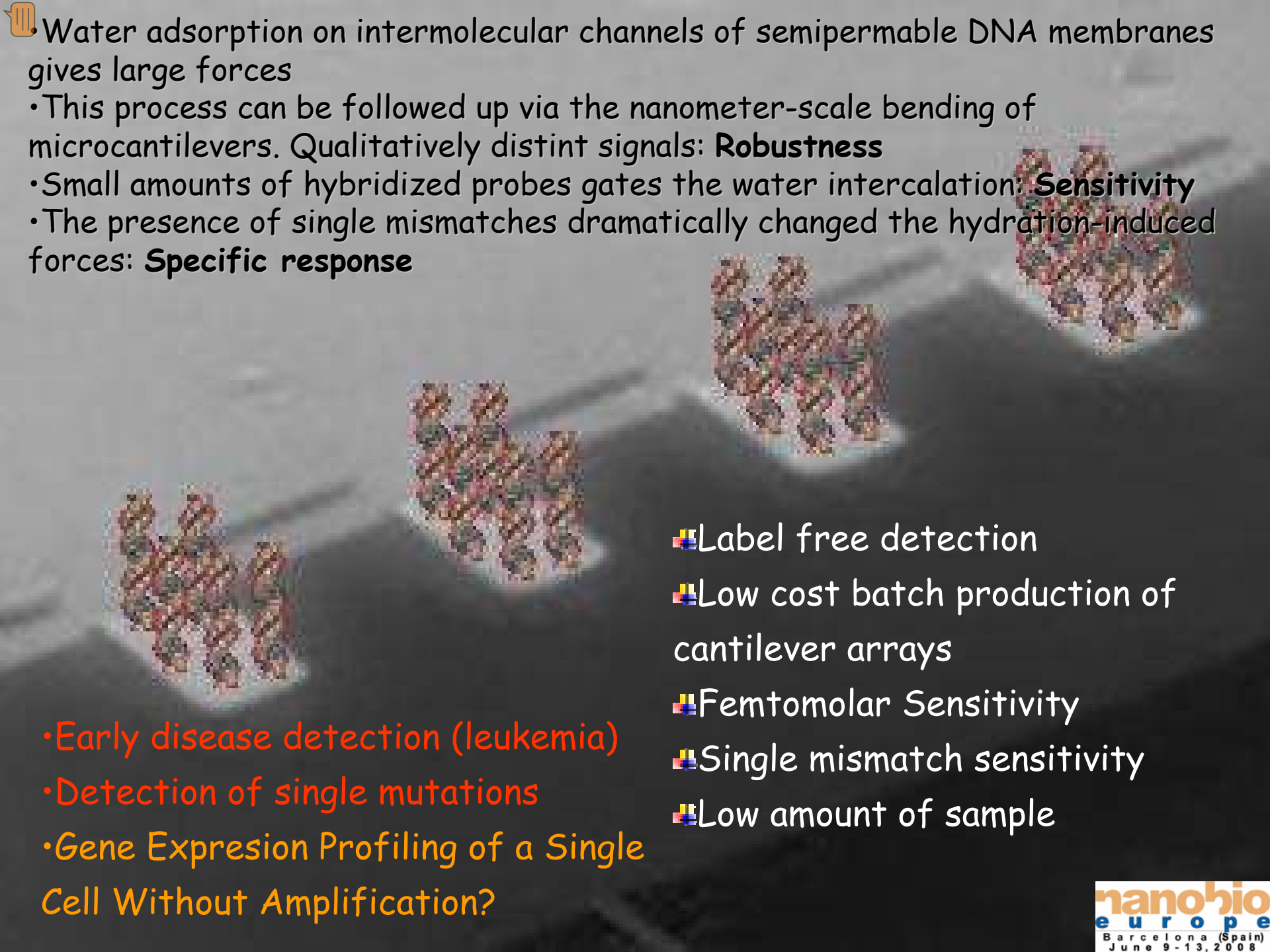


At least 100 times more sensitive than label-dependent DNA arrays

Patent: 1.2077.0423, CSIC

Nature Nanotechnology 3, 301 (2008)

- Water adsorption on intermolecular channels of semipermable DNA membranes gives large forces
- This process can be followed up via the nanometer-scale bending of microcantilevers. Qualitatively distint signals: **Robustness**
- Small amounts of hybridized probes gates the water intercalation: **Sensitivity**
- The presence of single mismatches dramatically changed the hydration-induced forces: **Specific response**



- Label free detection
- Low cost batch production of cantilever arrays
- Femtomolar Sensitivity
- Single mismatch sensitivity
- Low amount of sample

- Early disease detection (leukemia)
- Detection of single mutations
- Gene Expresion Profiling of a Single Cell Without Amplification?

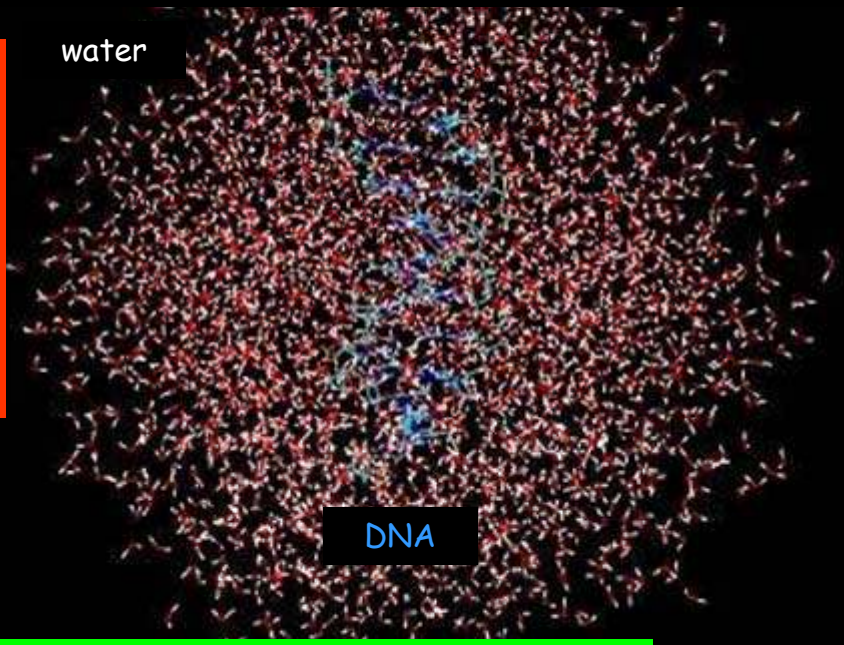
Innovating in mechanics for your *Bio* applications...

CSIC – Bionanomechanics Lab

spin-off company

Technology protected by: EP04381000, 08/March/2004, CSIC, EP05380157.7, 15/July/2005, CSIC and 1.2077.0423, Europe (pending)

- Label free detection
- Robust response
- Fast and simple detection with cantilever arrays
- Femtomolar Sensitivity
- Single mismatch sensitivity
- Low amount of sample
- Scalable technology



Candidate for next generation of DNA arrays?

Innovating in mechanics for your *Bio* applications...

CSIC – Bionanomechanics Lab

spin-off company

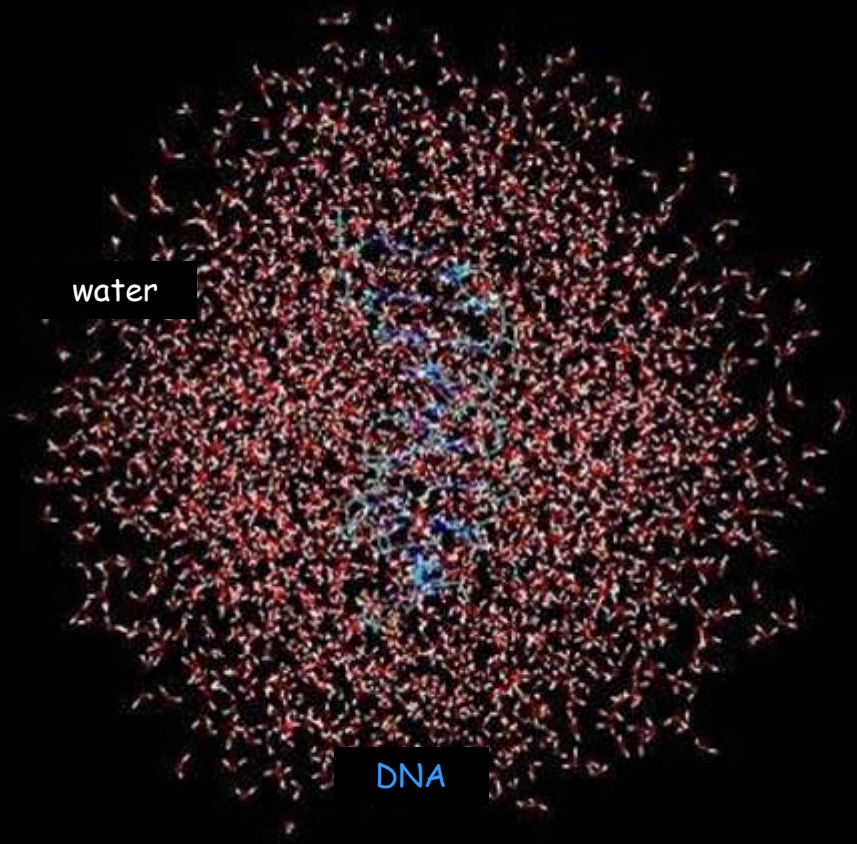
Technology protected by: EP04381000, 08/March/2004, CSIC, EP05380157.7, 15/July/2005, CSIC and 1.2077.0423, Europe (pending)

We are currently seeking candidates for the following positions:

Research and business director

Project engineers

MecWins is committed to developing new biosensing technologies addressing the needs of the Biotechnology companies for fast, easy and highly sensitive genetic analysis. The goal of Mecwins is providing cutting edge tools for genetic analysis for disease prognosis and diagnosis and pharmacogenomics. Mecwins applies the latest advancements of Nanotechnology in the Sensors field through close collaboration with key research groups in Nanomechanical sensing. The company has strong intellectual property rights of highly innovative nanotechnologies.
We are searching for a candidate with extensive scientific experience together with expertise in project management and with a strong interest in business development.



Acknowledgements

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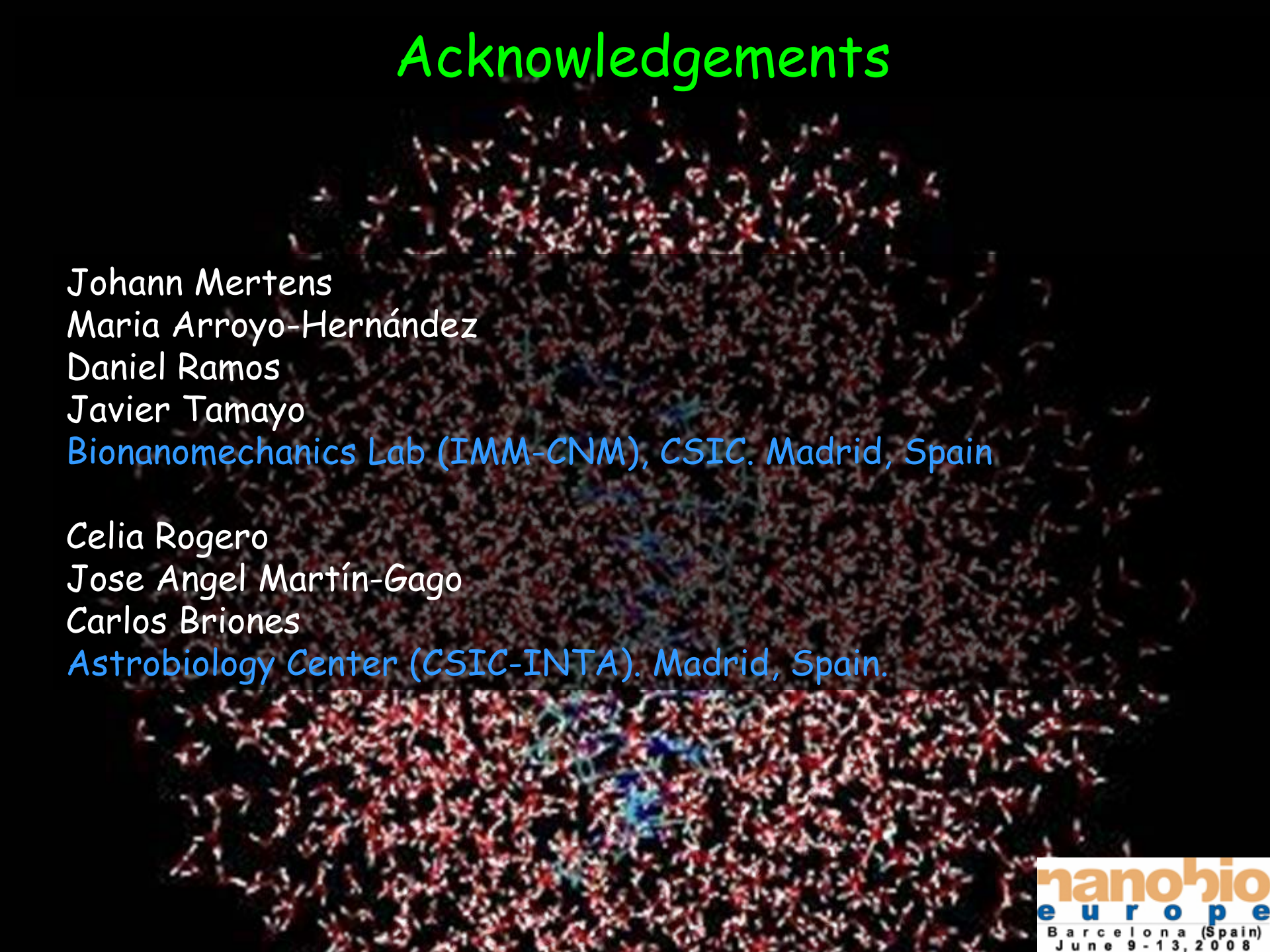

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