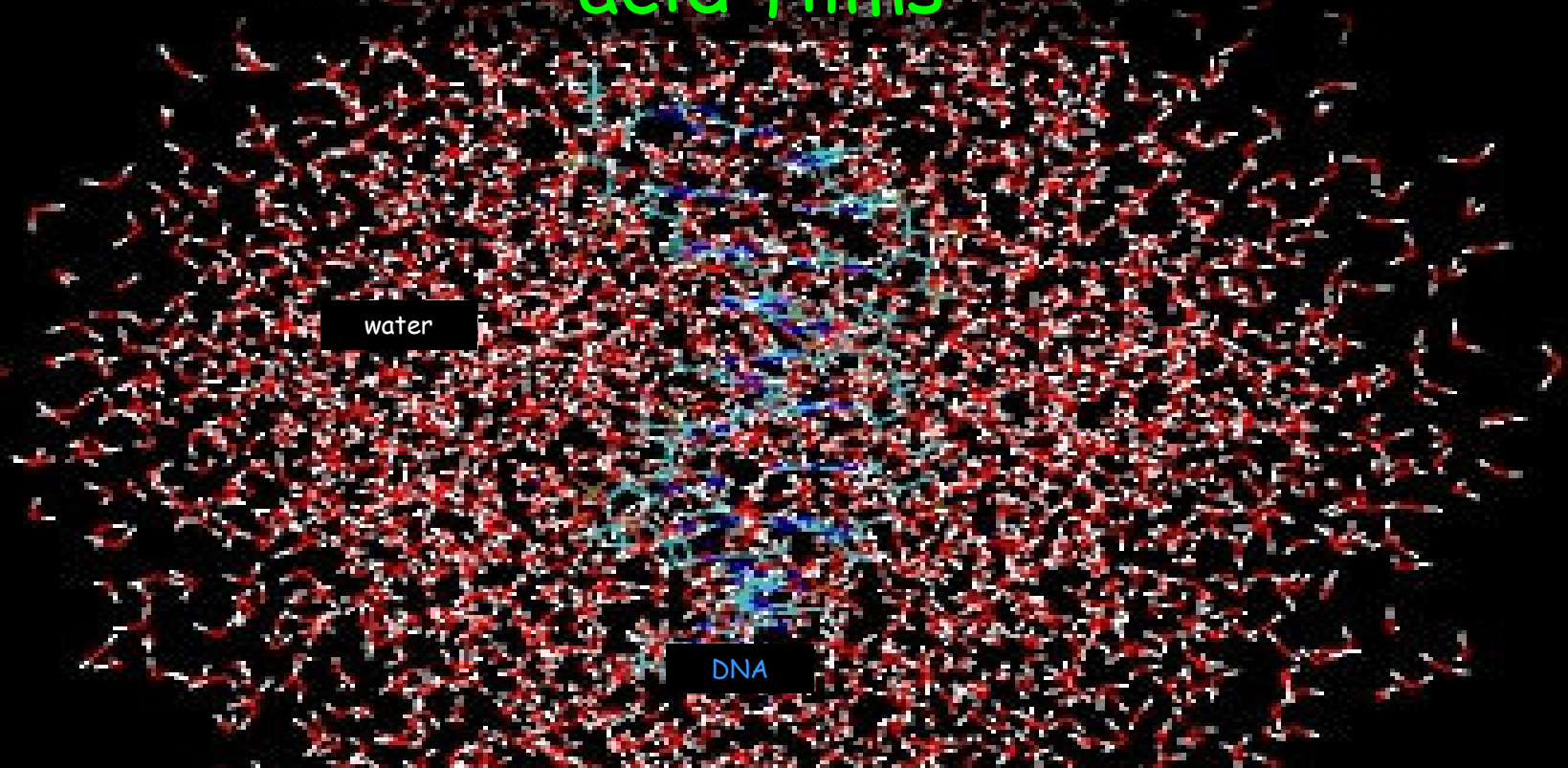


Label-free detection of DNA hybridization based on hydration induced tension in nucleic acid films



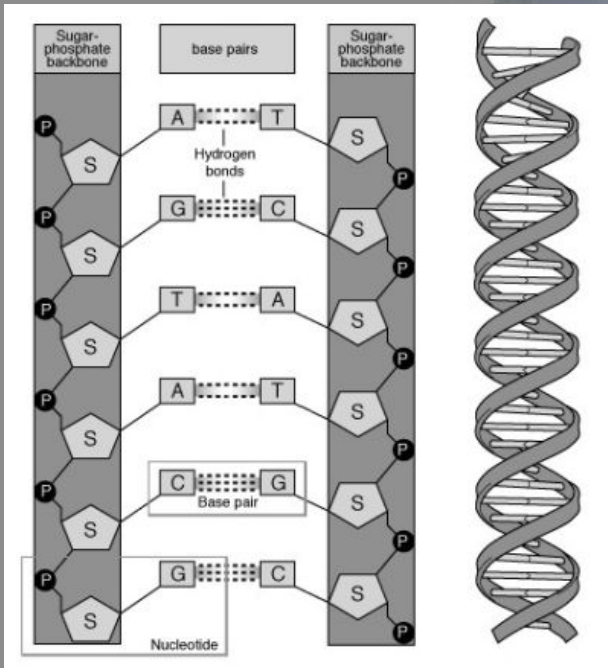
Johann Mertens, Maria Arroyo-Hernández, Montserrat Calleja, Daniel Ramos, and Javier Tamayo

Bionanomechanics Lab (IMM-CNM), CSIC. Madrid, Spain

Celia Rogero, Jose Angel Martín-Gago, Carlos Briones

Astrobiology Center (CSIC-INTA). Madrid, Spain.

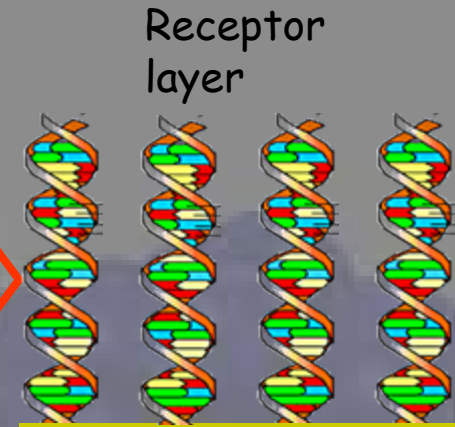
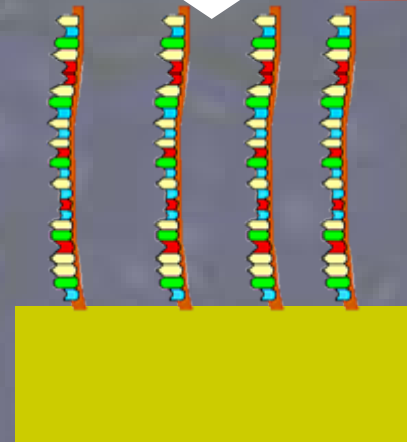
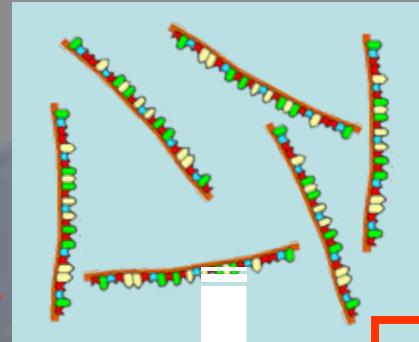
DNA structure



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

- DNA nanotechnology
- Sensors

DNA sensor

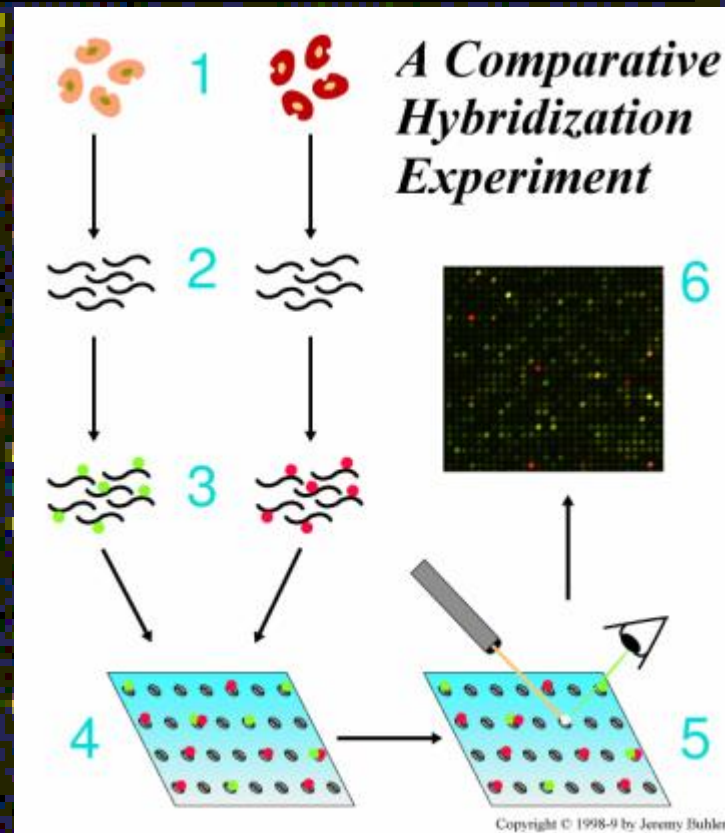
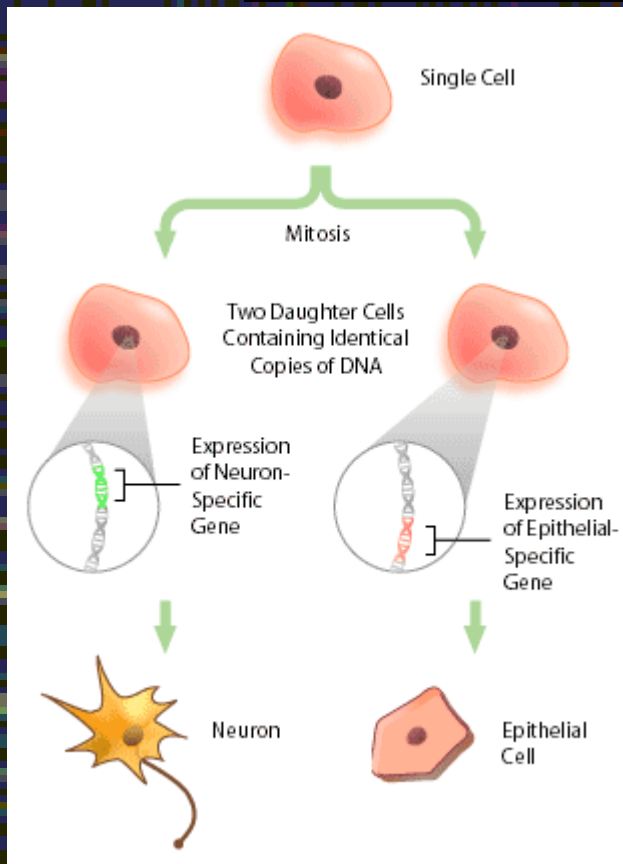


Transduction

- 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 \Rightarrow low genetic purity, change of the sample stoichiometry

MOLECULAR RECOGNITION DETECTION BY SURFACE STRESS BIOSENSORS

CANTILEVER PREPARATION

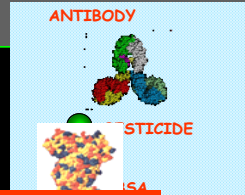
20-50 nm Gold

~100 μm

<1 μm

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

25 nM Antibody



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

HORMONE/ANTIBODY RECOGNITION

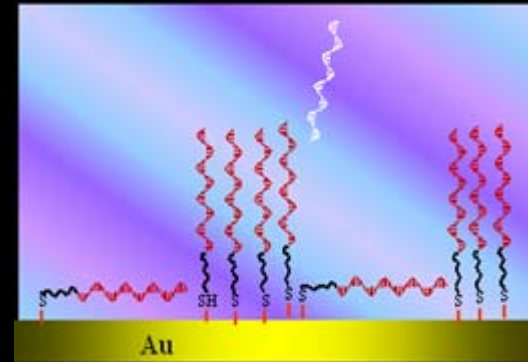
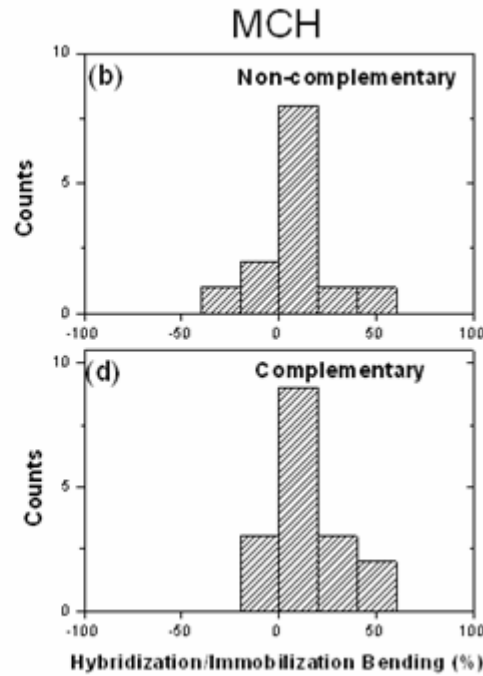
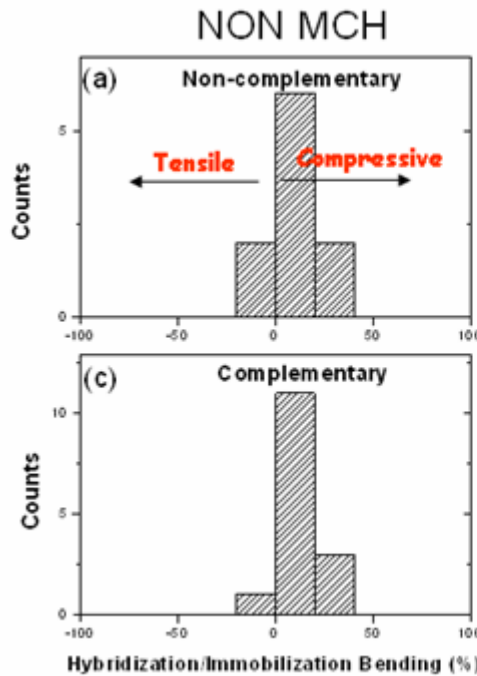
Polymer cantilever

Candidate for next generation of DNA arrays?

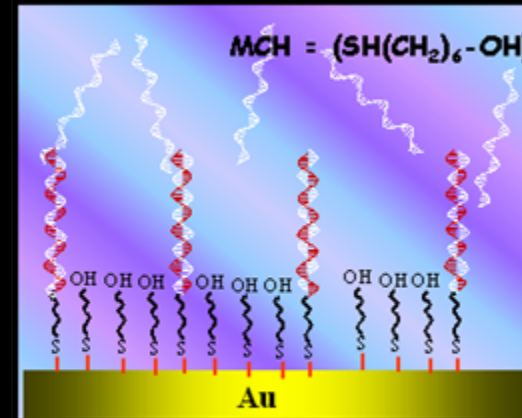
10 minutes

100 μm

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 fluorescense and SPR experiments give significant hybridization signals



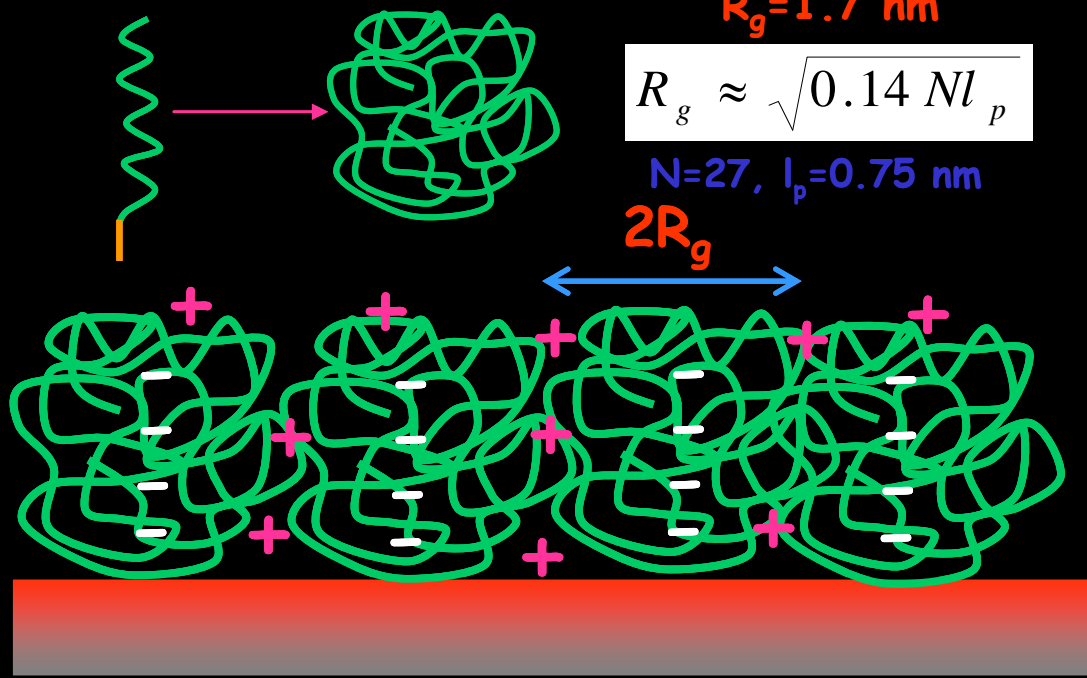
■ DNA hybridization produces a surface stress below the detection sensitivity for a single microcantilever ≈ 5 mN/m. It is necessary the use of differential signals with a reference cantilever (remove temperature, turbulences, ion fluctuations). See Hegner, McKendry and Gerber work

■ This is in contrast with the surface stress generated by the antigen/antibody recognition $\approx 100-200$ 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



$$R_g \approx \sqrt{0.14 N l_p}$$

$R_g = 1.7 \text{ nm}$
 $N = 27, l_p = 0.75 \text{ nm}$

Electrostatic repulsion

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

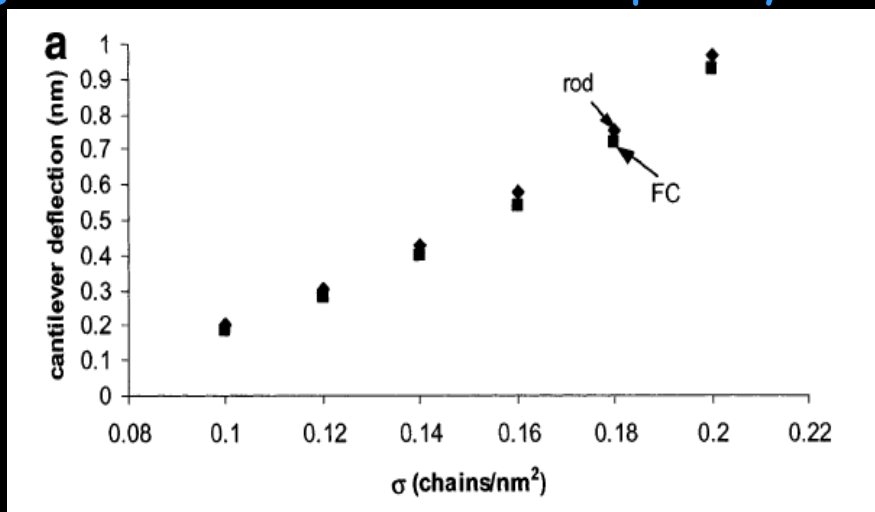
$\lambda_D \approx 0.3 \text{ nm}$

Hydration force

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

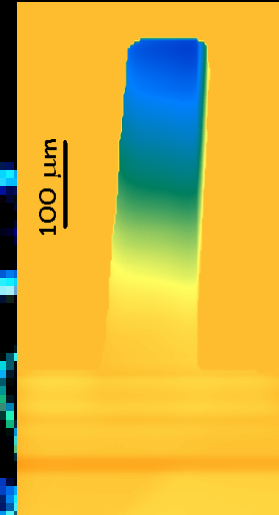
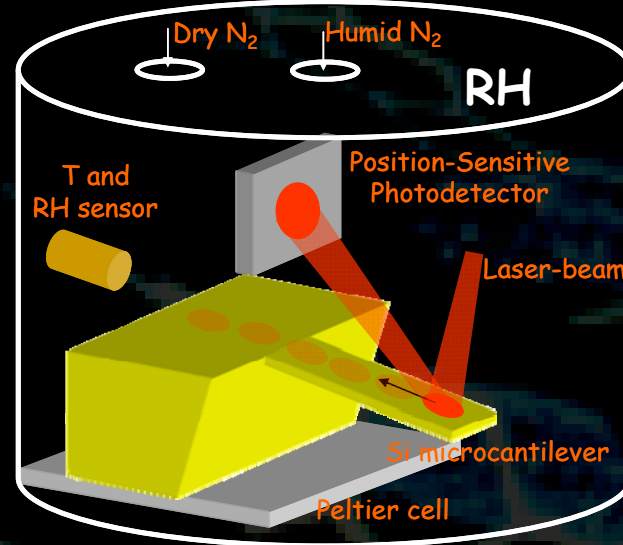
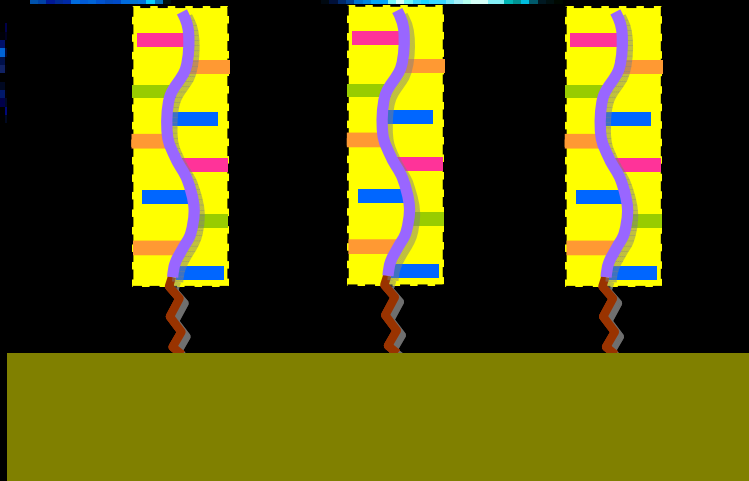
$\lambda_H \approx 0.3 \text{ nm}$

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

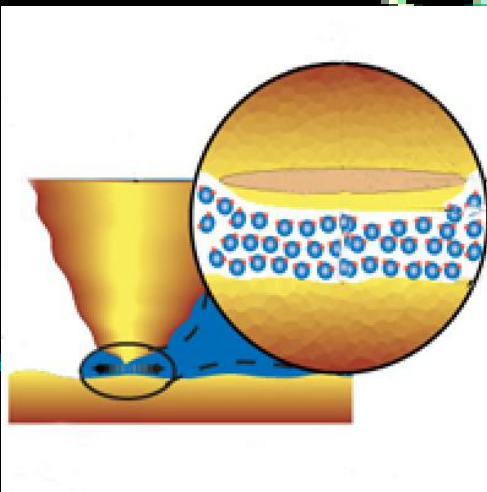


Hagan, Majumdar and Chakraborty, J. Phys. Chem. B, 106, 10163 (2002)

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



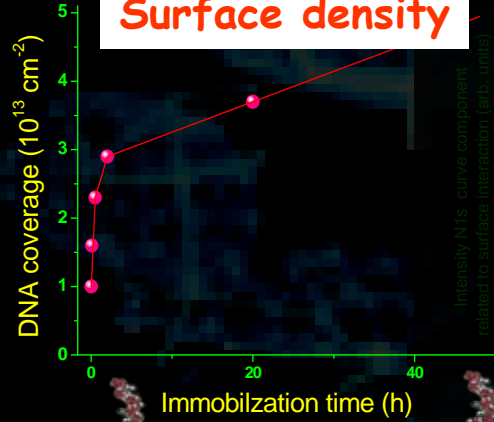
Exploit the high forces generated when water is confined in subnanometer channels: Disruption of the hydrogen bond network



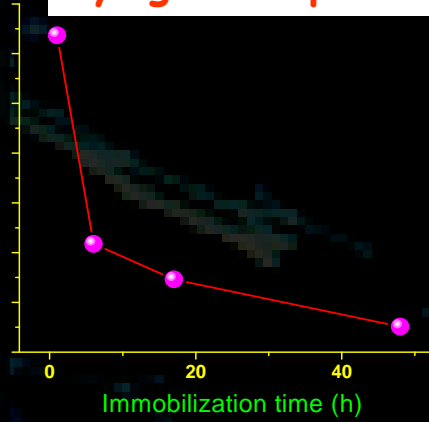
- Water confined in pores with diameters between 1 and 3 nm: water is in a liquid phase, short range order and a strong decrease of the solidification temperature by several tens of degrees.
- When the pore diameter is below 1 nm, the water is in an intermediate state between liquid water and crystalline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

XPS analysis

Surface density

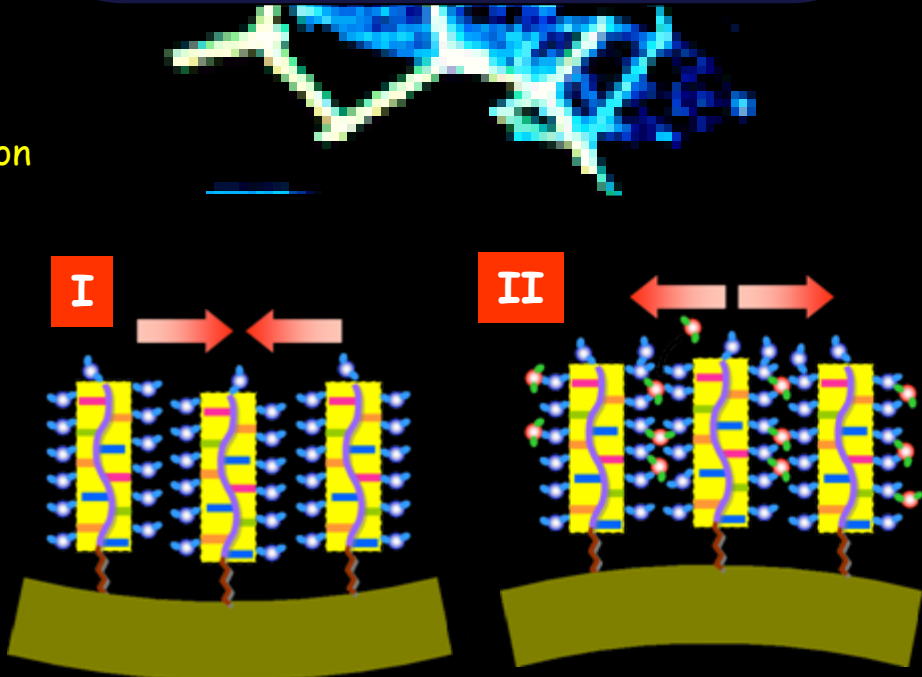
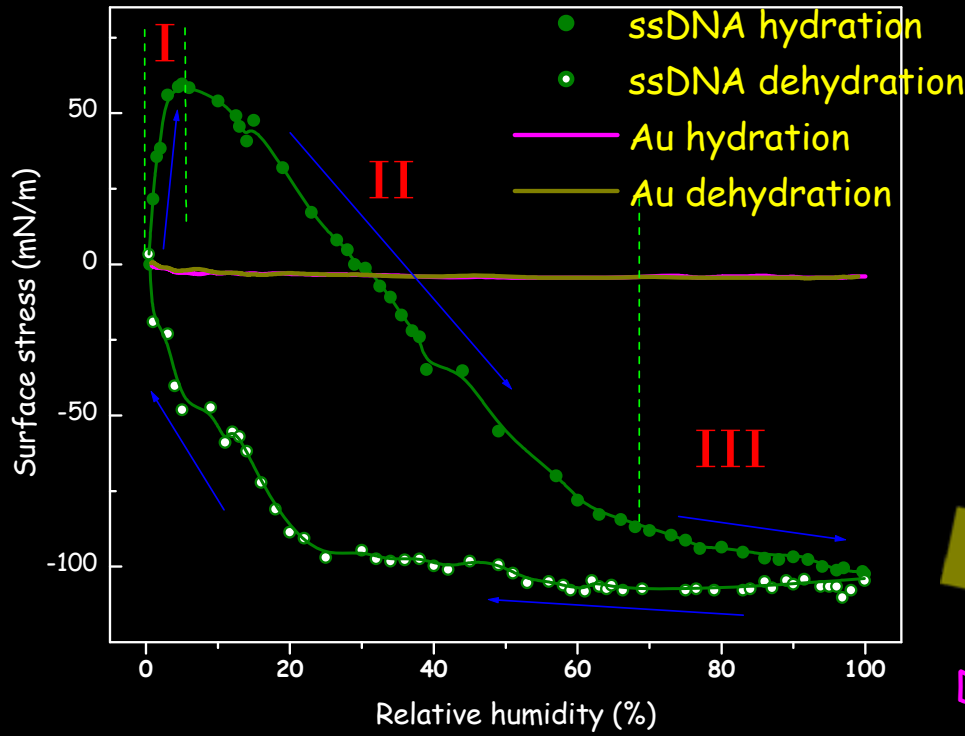
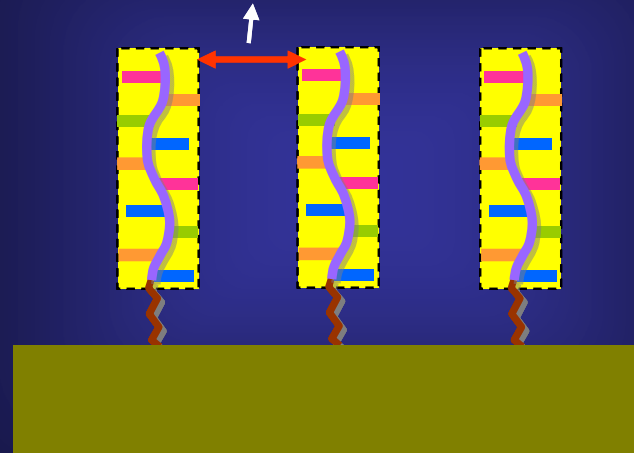


Lying down phase



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

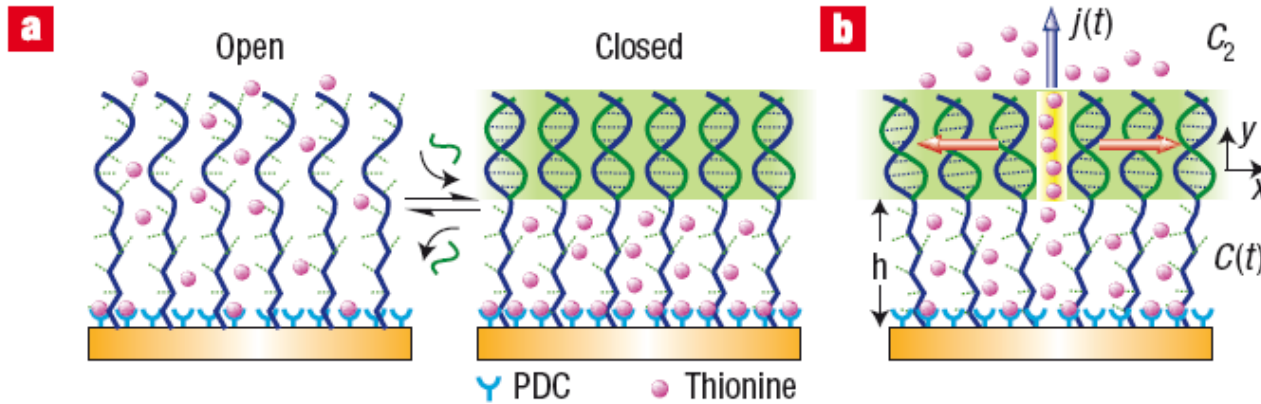
0.5-0.8 nm \approx 2-3 water molecules



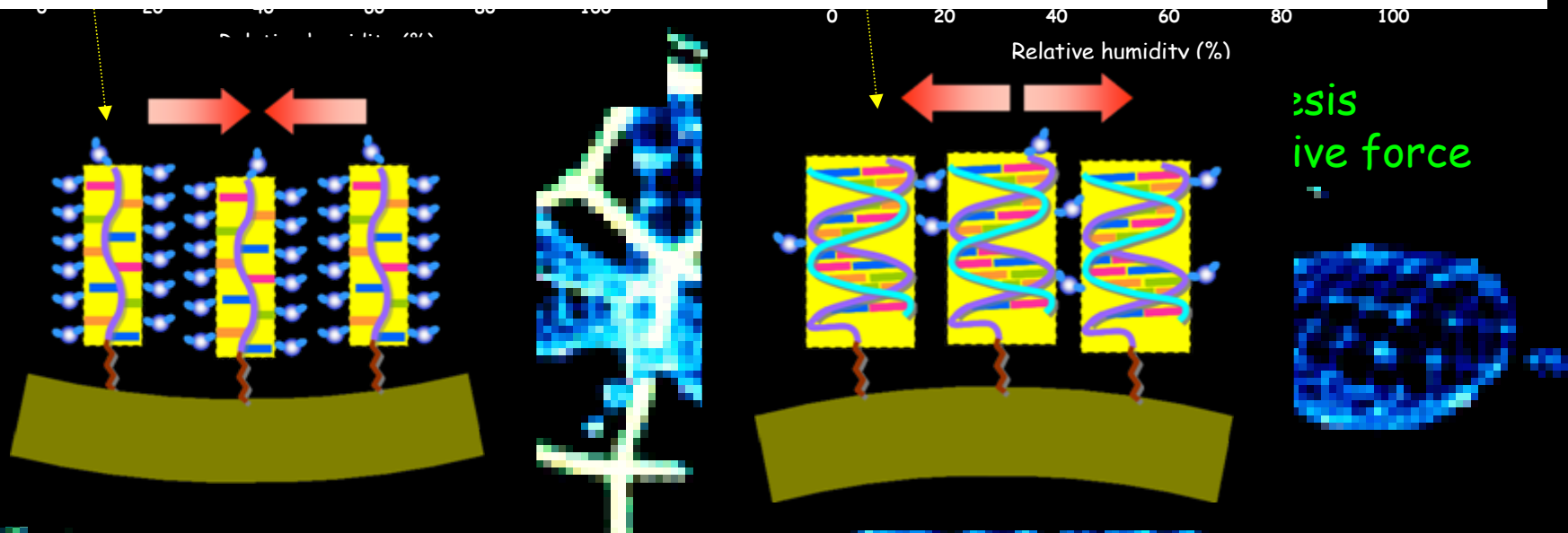
Dipole/Dipole forces

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?

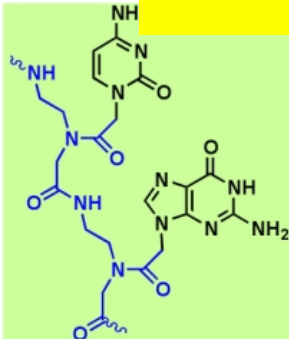
Electrostatic phenomena play a fundamental role in the DNA structure

DNA has an 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.

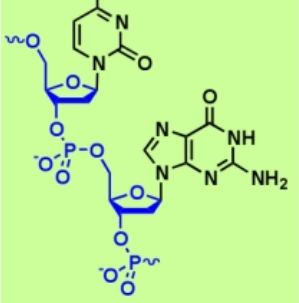
PNA v

PNA is a charged by a pep

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



PNA Peptide Nucleic Acid



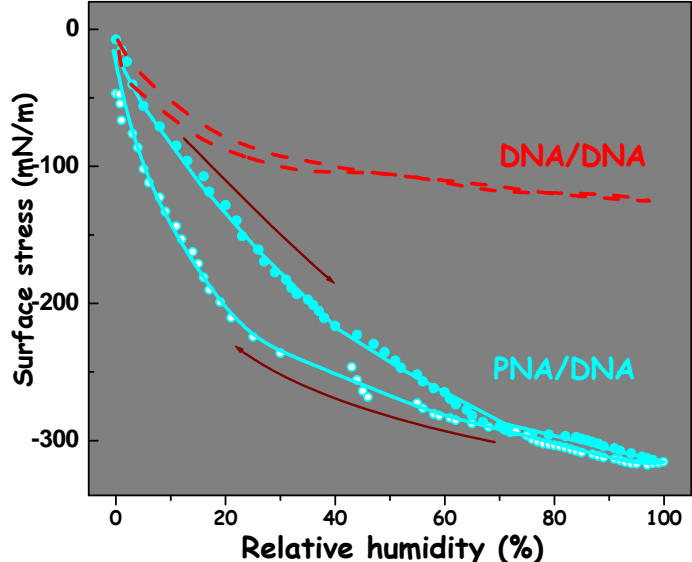
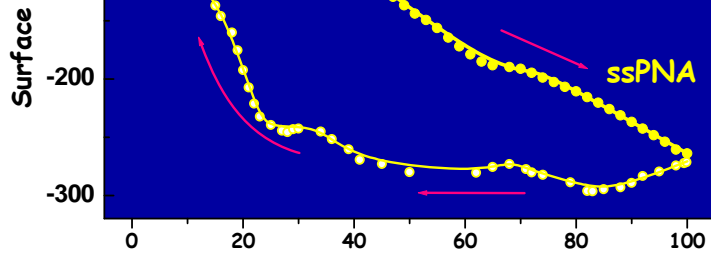
DNA Deoxyribo Nucleic Acid

- no nucleic acid \Rightarrow no degradation by nucleases
- no peptide \Rightarrow no degradation by proteases
- forms extremely stable complexes with DNA and RNA
- high sequence selectivity

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

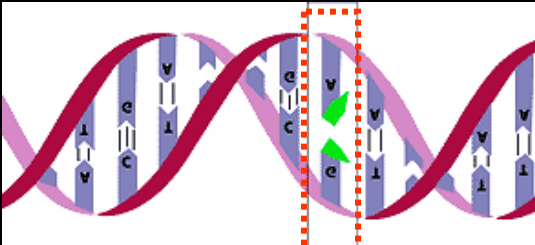
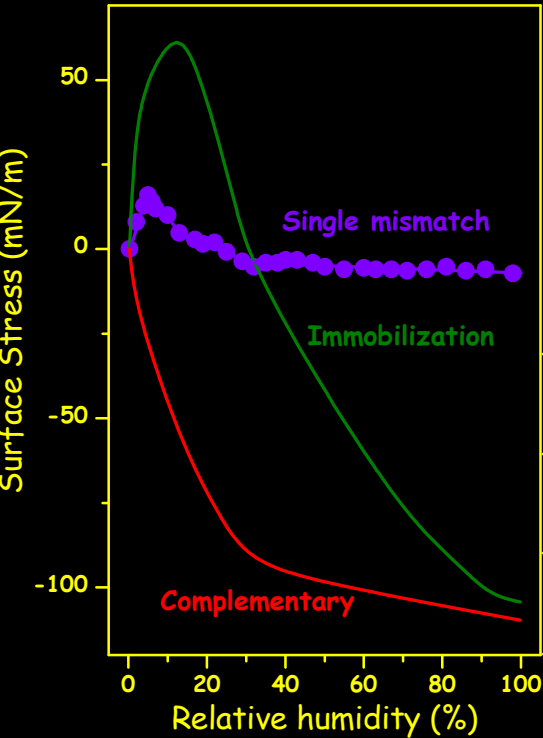
Nielsen, Egholm, Berg, Buchardt, Science 1991, 254, 1497

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



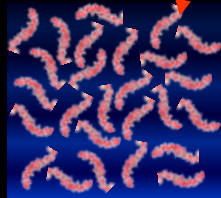
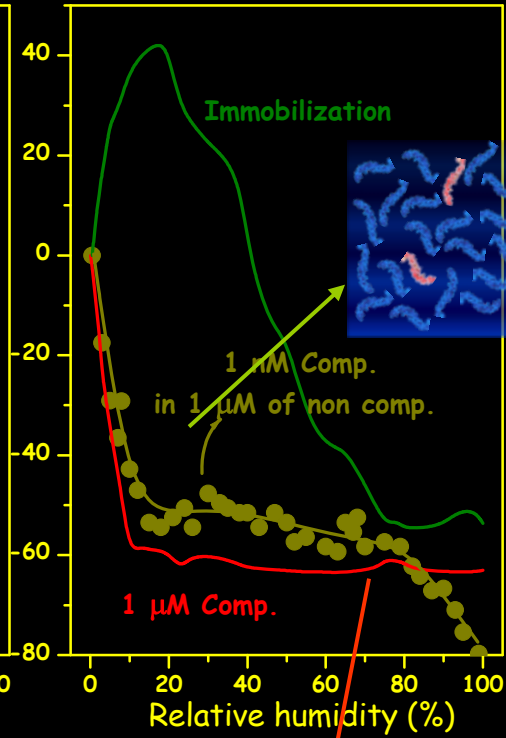
Towards DNA biochips

HIGH SPECIFICITY



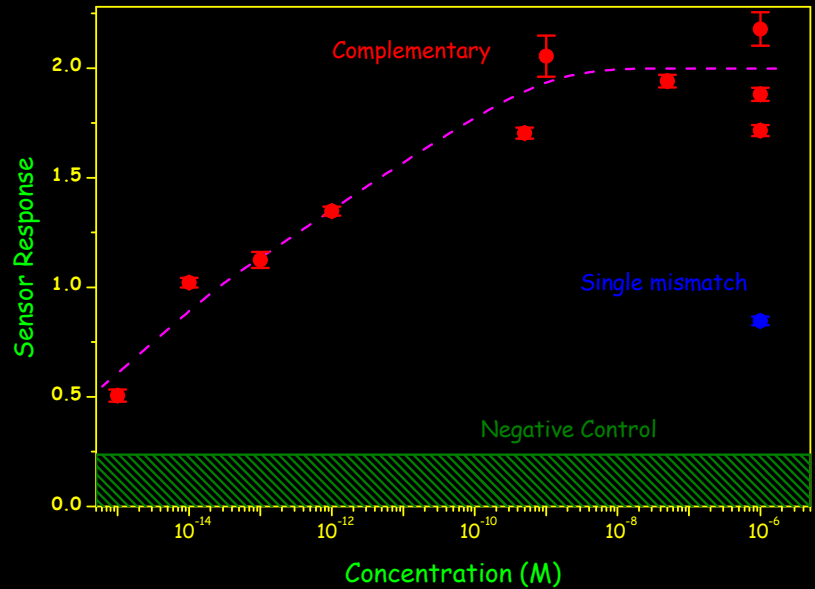
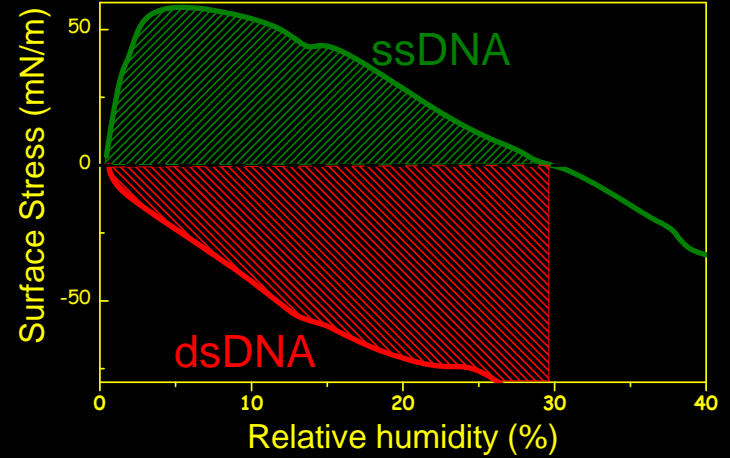
Sensitivity to a single mismatch

HIGH SENSITIVITY



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

ROBUSTNESS

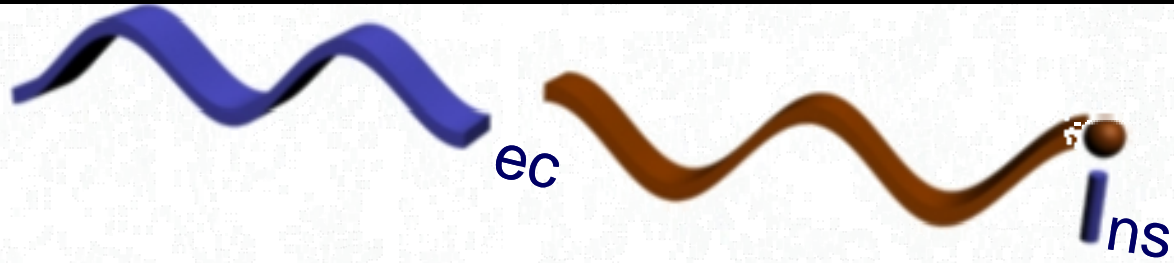


At least 100 times more sensitive than label-dependent DNA arrays

- Water adsorption on intermolecular channels of semipermeable DNA membranes gives large forces
- This process can be followed up via the nanometer-scale bending of microcantilevers. Qualitatively distinct 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**

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

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



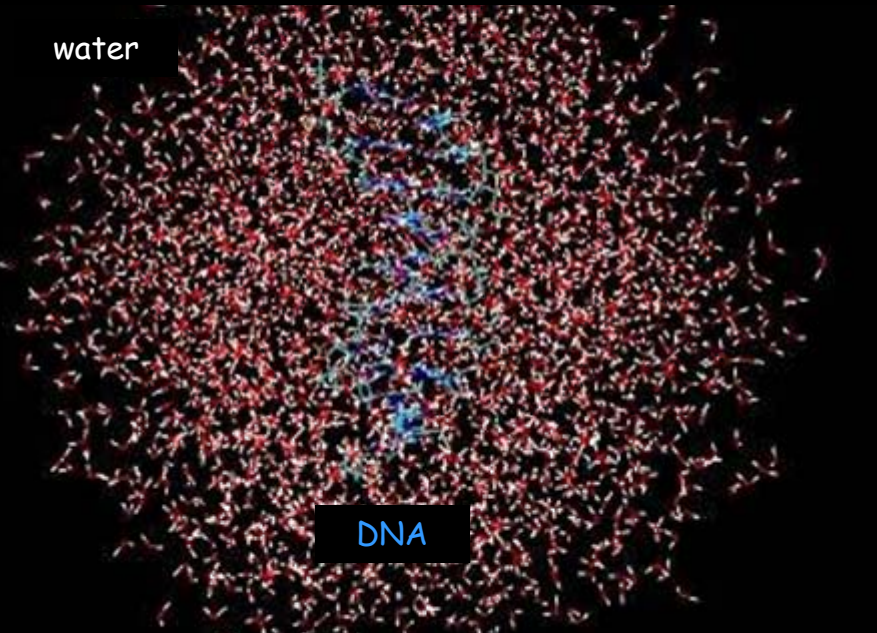
Innovating in mechanics for your *Bio* applications...

CSIC - Bionanomechanics Lab
spin-off company

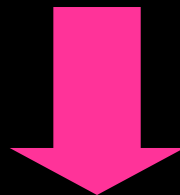
Technology protected by: EP04381000, 08/March/2004,
CSIC, EP05380157.7, 15/July/2005, CSIC and
1.2077.0423, Europe (pending)

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

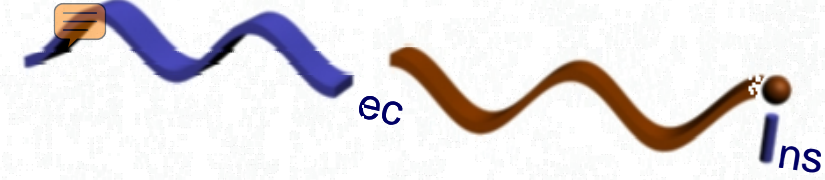
water



DNA



Candidate for next generation of DNA arrays?



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

CSIC - Bionanomechanics Lab
spin-off company

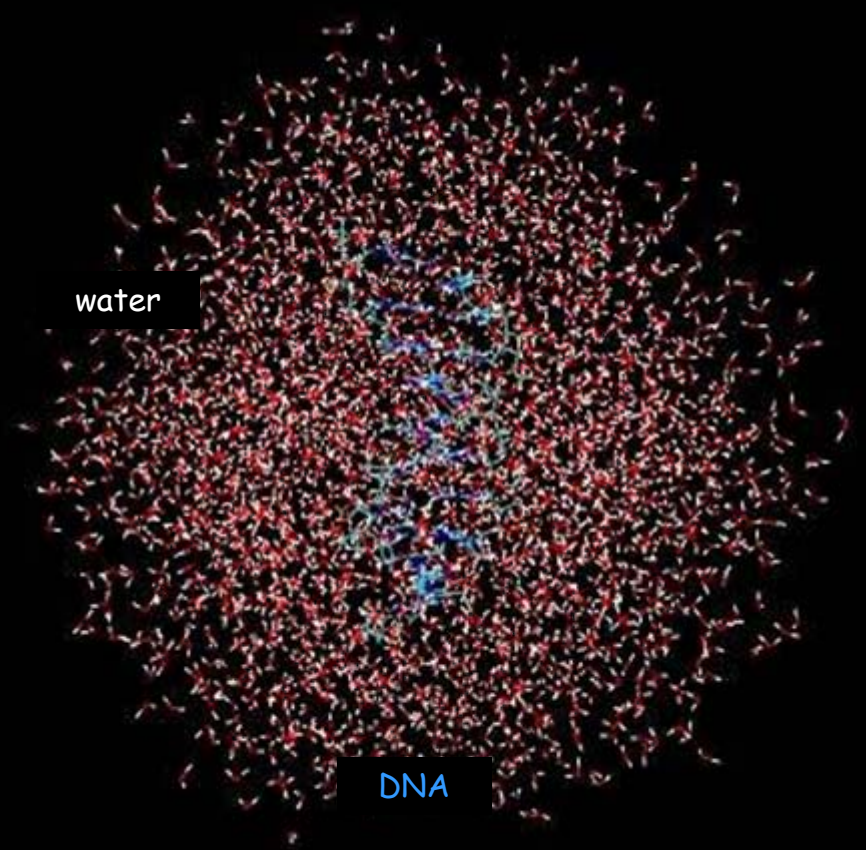
Technology protected by: EP04381000, 08/March/2004,
CSIC, EP05380157.7, 15/July/2005, CSIC and
1.2077.0423, Europe (pending)

*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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