NANOMECHANICAL DEVICES: LABEL- FREE ULTRASENSITIVE BIOSENSORS

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In the last years, a large variety of ultrasensitive nanomechanical sensors have been developed and used as biological sensors. The results demonstrate that rapid detection of biomolecules with high sensitivity and specificity without need of sample pre-treatment and labeling with fluorescent dyes is attainable[1]. This technology has the potential to revolutionize the fields of molecular biology and preventive medicine. Here, we present results in several of the battle fronts of nanomechanical biosensors faced by our group in collaboration with several multidisciplinary scientific and industrial partners. We split the results into the dynamic and static modes used for nanomechanical sensing. In the dynamic mode, we have found that the added mass is not the sole responsible for the changes in the resonant frequency. Actually, the mechanical properties of adsorbates not only play a relevant role in the dynamic response, they can be taken advantage off in order to obtain more specific biosensors. In the static mode, we have found a new detection method to track DNA hybridization that is based on hydration induced tension in nucleic acid films. These results are under exploitation by the CSIC spin-off company Mecwins S.L.

Dynamic Nanomechanical Biosensors

In order to develop nanomechanical devices for ultrasensitive pathogen detection, we have measured the effect of the bacteria adsorption on the resonant frequency of microcantilevers as a function of the adsorption position and vibration mode. The resonant frequencies were measured from the Brownian fluctuations of the cantilever tip. Our results indicate that the resonance shift is not solely depending on the adsorbed mass and adsorbate location. Our results indicate that there exist three mechanisms that can produce a significant resonant frequency shift: **the stiffness**, the surface stress gradient and the mass [3,4].. The combination of high vibration modes and the confinement of the adsorption to defined regions of the cantilever allow detection of single bacterial cells by only measuring the Brownian fluctuations, i.e., without any use of external energy. The results of this study have been applied for a new design of arrays of nanomechanical resonators, with a volume about 10^4 times smaller for ultrasensitive detection of nucleic acids. The fabricated arrays have alternate nanomechanical resonators with differently sensitized regions to obtain a double signature of the target based either on its mass or the stiffness of the molecule. We have been able to detect DNA hybridization at the level of few femtograms in air and without any external excitation, which implies one of the highest sensitivities obtained in these conditions [5].

Static Nanomechanical Biosensors

The change in the structural and dynamic properties of water at nanoscale is crucial in a wide variety of phenomena, from the stability of a sandcastle to the structure and function of

nucleic acids and proteins. Advances in nanotechnology, in particular those based in micro- and nanomechanical sensors, can potentially be used to analyze the role played by water molecules in macromolecular interactions. Here we show that adsorption of water on a highly-packed self-assembled monolayer (SAM) of single stranded (ss) DNA has an extraordinary effect on the intermolecular interactions. We have followed the process by measuring the nano-scale bending of a silicon microcantilever, on which the ssDNA monolayer is attached, under controlled relative humidity. More importantly, the hydration-induced tension pattern undergoes dramatic changes when complementary and single mismatched DNA hybridizes with the ssDNA monolayer. Based on these new phenomena we have developed a novel nucleic acid biosensor with two key features: its optimal specificity (one mutation or single-nucleotide polymorphism, SNP), as well as its outstanding sensitivity (in the sub-picomolar range, at least 100 times more sensitive than the label-dependent DNA microarrays) [2].

Figures:

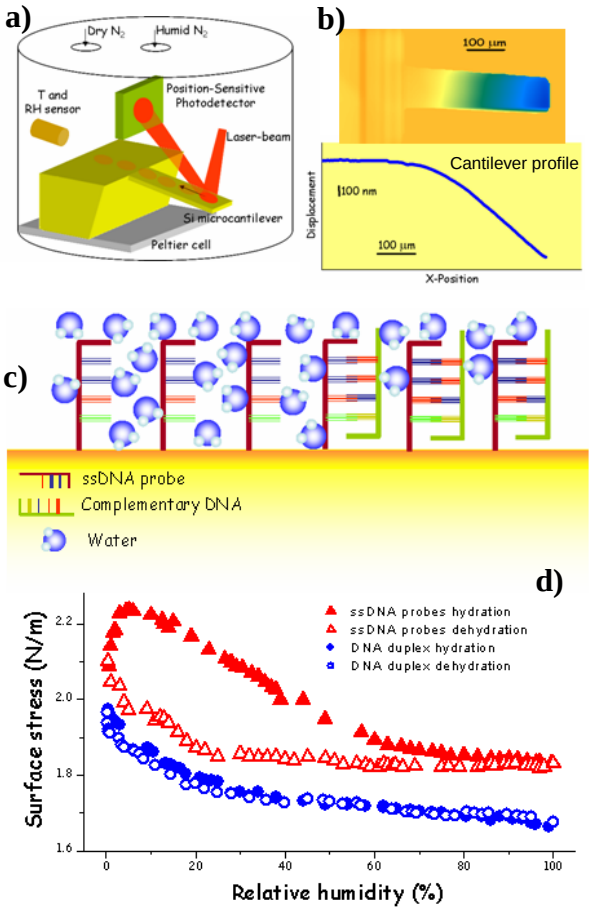

Fig. 1. a) Schematic depiction of the experimental set-up. The cantilever is placed in a humidity-controlled chamber. The relative humidity (RH) was controlled by adjusting the ratio between dry and water saturated nitrogen. **b)** The cantilever profile was obtained by scanning a laser beam over the cantilever and measuring the reflected beam deflection by a position-sensitive photodetector (patent pending). A three dimensional image of the cantilever obtained by this technique is also shown. **c)** A cartoon of the ssDNA oligonucleotides on the gold-coated side of the cantilever is shown. **d)** Surface stress measured under hydration and dehydration cycles for the cantilever sensitised with the ssDNA probes and for the same cantilever after hybridization upon exposure to a solution containing the complementary ssDNA target. We obtain a qualitatively distinct signal when hybridization occurs on the cantilever surface.

References:

[1] J. Tamayo, Nature Nanotechnology **2** (2007) 342.

[2] Mertens, J., Rogero, C., Calleja, M., Ramos, D., Martín-Gago, J.A., Briones, C. & Tamayo, J., Nature Nanotechnology **3** (2008) 301.

[3] Ramos, D., Tamayo, J., Mertens, J., Calleja, M. & Zaballos, A., J. Appl. Phys. **100** (2006) 106105.

[4] Tamayo, J., Ramos, D., Mertens, J. & Calleja, M., Appl. Phys. Lett. **89** (2006) 224104. Also in News&Views Nature Nanotechnology **2** (2007) 18.

[5] Ramos, D., Arroyo-Hernández, M., Tong, H.D., Calleja, M., Tamayo, J., submitted