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Nanomechanics – The Link to Biology and Chemistry

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Abstract: Biological and chemical processes can be transduced into nanomechanical motion *via* change of surface stress on a cantilever. By coating the surface of each cantilever of a micro-fabricated array of silicon cantilevers with a different polymer, a versatile vapor sensor is obtained that is able to discriminate between various solvent vapors using principal-component analysis techniques. In liquids such sensors allow rapid quantitative and qualitative detection of non-labeled biomolecules. Differential measurements of cantilever deflection (with respect to an unspecific reference cantilever) allow the detection of sequence-specific DNA hybridization. Single-stranded thiolated DNA 12-mer sequences, anchored onto the surface of the gold-coated cantilevers of the array, provide a biosensor for the detection of their complementary strands in buffer solution. The influence of the target-molecule concentration on the cantilever deflection is studied, and a value for the thermodynamic surface-solution equilibrium constant is derived from measurements on a cantilever.

Keywords: Biosensor · Cantilever array · DNA hybridization · Molecular recognition · Thermodynamic surface-solution equilibrium constant

1. Introduction

The technique of scanning force microscopy (SFM) [1] takes advantage of the flexibility of thin cantilever beams, which allow interatomic forces to be measured in the piconewton range. Such cantilevers can not only be used to probe the surface profile of a sample but also as freestanding sensors. Adsorption of molecules on the surface of a cantilever will generate surface stress, resulting in a bending of the cantilever, provided the adsorption preferentially occurs on one surface of the cantilever. Such adsorption is favored if one surface (*e.g.* the upper surface) of a cantilever is coated with a thin layer of a material that shows affinity to molecules in the environment. The continuous bending of the cantilever as a function of molecular coverage with molecules is termed operation in the 'static mode', see Fig. 1. The surface stress change is calculated according to Stoney's formula [2]:

$$\Delta\sigma = Et^2 / [4R(1 - \nu)] \quad (1)$$

where E is Young's modulus ($E_{Si} = 1.7 \times 10^{11}$ N/m^2 for silicon), t the thickness of the cantilever, ν the Poisson's ratio ($\nu_{Si} = 0.25$), and R the bending radius of the cantilever. Because the bending of the cantilever strongly depends on the interaction of the cantilever surface with the adsorbing molecules, it is rather difficult to obtain reliable information on the number of molecules adsorbed. However, mass changes can be determined accurately by operation in so-called 'dynamic mode', where the cantilever is actuated at its resonance frequency. If additional mass is adsorbed on the cantilever surface, the resonance frequency will be shifted to a lower frequency, and the mass change on a rectangular cantilever can be determined [3] according to

$$\Delta m = (k/4\pi) \times (f_1^{-2} - f_0^{-2}) \quad (2)$$

where f_0 is the resonance frequency before the mass change occurs, and f_1 the resonance frequency after the mass change. The spring constant k of the cantilever is calculated using

$$k = Et^3w / 4l^3 \quad (3)$$

where l, w, and t denote the length, width, and thickness of the cantilever, respectively.

If the cantilever is coated with additional metal layers, thermal effects will influence cantilever bending. Operation in 'heat mode' produces cantilever bending due to differing thermal expansion coefficients of the sensor layer material and the cantilever material [4]:

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$$\Delta z = 1.25 \times (\alpha_1 - \alpha_2) \times (t_1 + t_2) / t_2^2 \, \kappa \times l^3 \quad P / (\alpha_1 t_1 + \alpha_2 t_2) w \qquad (4)$$

where α_1, α_2 are the thermal expansion coefficients of the materials involved, t_1, t_2 the material thicknesses, P is the total power generated on the cantilever, and κ a geometry parameter of the cantilever device.

These three measurement modes render cantilever sensors versatile tools for experiments in nanoscience.

However, the bending response of a single cantilever often is not very meaningful because various undesired effects, such as thermal drift and unspecific reactions taking place on the cantilever surface, influence the cantilever bending. To avoid such effects, we have introduced measurements with reference cantilevers [5], *i.e.* cantilever sensors that do not react with the target analyte molecules. Thus a small sensor response can be extracted from large cantilever deflections by calculating the difference in responses of sensor and reference cantilevers.

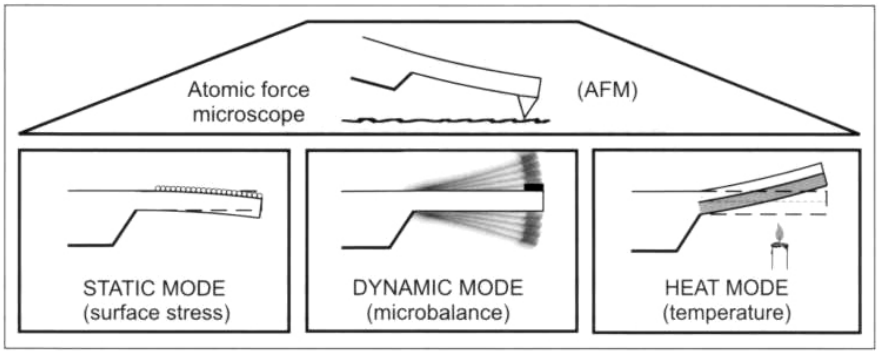

Fig. 1. Schematic of an SFM cantilever and basic operation modes for cantilever sensors derived from it.

2. Experimental

2.1. *Cantilever Arrays*

Silicon cantilever sensor arrays were microfabricated using a combined dry and wet etching fabrication technique developed in the Micro-/Nanomechanics Department at IBM's Zürich Research Laboratory. One chip comprises eight cantilevers with a length of 500 µm, a width of 100 µm, and a thickness of 1 µm, arranged at a pitch of 250 µm. The resonance frequencies of the cantilevers vary by 0.5% only, demonstrating the high reproducibility and precision of cantilever fabrication. A scanning electron microscopy image of a cantilever sensor array chip is shown in the inset of Fig. 2.

The upper surface of these cantilevers is usually coated with 2 nm of titanium and 20 nm of gold to provide a reflective surface and an interface for attaching functional groups of probe molecules, *e.g.* for anchoring molecules with a thiol group to the gold surface of the cantilever. Such small amounts of metal coating are believed not to contribute significantly to the bending produced by surface stress changes, because the temperature is kept constant. We have demonstrated many examples of molecular adsorption on cantilevers, such as the adsorption of alkyl thiols on gold [6], the pH-dependent response of carboxy-terminated alkyl thiols [7], and biomolecular recognition [8][9]. Fig. 2 schematically shows how an array of eight silicon cantilevers functionalized with single-stranded oligonucleotide probes can be used for detection of the complementary oligonucleotide target strands in a biochemical buffer environment. Only the matching sequence will hybridize and produce a change in surface stress, which will in turn lead to a bending of the cantilever. The non-matching probe sequences will not hybridize with the injected target sequence, and therefore those cantilevers serve as reference sensors, because they will not bend. By evaluating the difference in responses of a probe cantilever with the matching sequence and a probe cantilever with a non-matching sequence, the hybridization response can be extracted from the data, even if thermal drift effects, unspecific adsorption of molecules on the cantilever, or turbulence from injection of the liquids into the sample chamber affect individual cantilever responses [8][9].

2.2. *Measurement Chamber*

Fig. 3 shows the schematic setup of the experiments in (a) gaseous environment and (b) liquid (biochemical) environment. The cantilever sensor array is located in an analysis chamber with a volume of 40–300 µl, which has inlet and outlet ports for gases or liquids. The cantilever deflection is determined *via* an array of eight vertical-cavity surface-emitting lasers (VCSELs) at a linear pitch of 250 µm that emit at a wavelength of 760 nm into a cone of 5 to 10 degrees. A time-multiplexing procedure switches the lasers on and off sequentially. The light of each VCSEL is collimated and focused to the apex of the corresponding cantilever. The light is then reflected off the gold-coated surface of the cantilever and

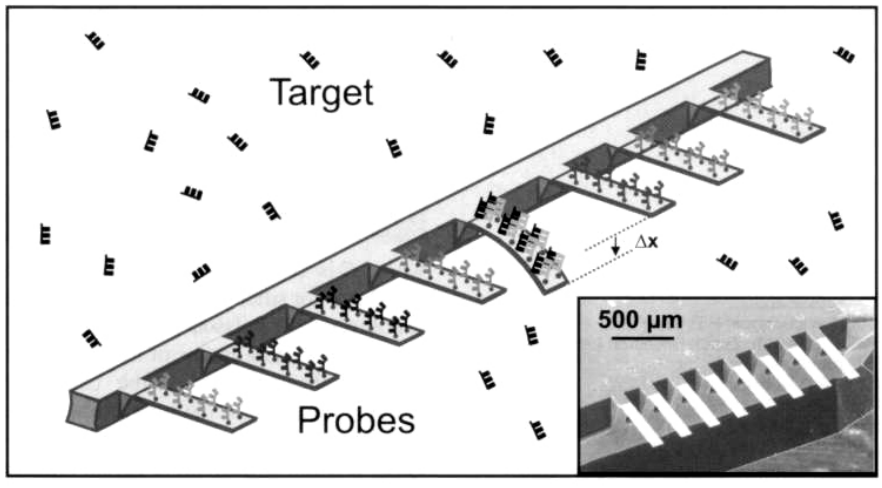

Fig. 2. Schematic drawing of a cantilever array functionalized with single-stranded oligonucleotides and scanning electron microscopy image of a microfabricated cantilever sensor array (inset).

hits the surface of a position-sensitive detector (PSD), a photo potentiometer device with two electrodes that allow the position of an incident light beam to be determined with micrometer precision. The photocurrents are transformed into voltages and amplified in a preamplifier. The deflection signal is converted to digital format and stored together with a time stamp on a personal computer (PC), which also controls the multiplexing of the VCSELs and switching of the valves and mass flow controllers used for setting the composition ratio of the analyte mixture.

The setup for the measurement of liquids consists of a poly-etheretherketone (PEEK) liquid cell, which contains the cantilever array and is sealed by a Viton O-ring and a glass plate. The VCSELs and the PSD are mounted on a metal frame around the liquid cell. After preprocessing of the position of the deflected light beam in a current-to-voltage converter and amplifier stage, the signal is digitized in an analog-to-digital converter and stored on a PC. The liquid cell is equipped with inlet and outlet ports for liquids. The biochemical liquid samples are stored in individual glass containers in a thermally equilibrated water bath. A 10-position valve allows the inlet to the liquid chamber to be connected to each one of the liquid sample containers separately. The liquids are pulled through the liquid chamber by means of a syringe pump connected to the outlet of the chamber. A Peltier element is situated very close to the lumen of the chamber to allow temperature regulation within the chamber.

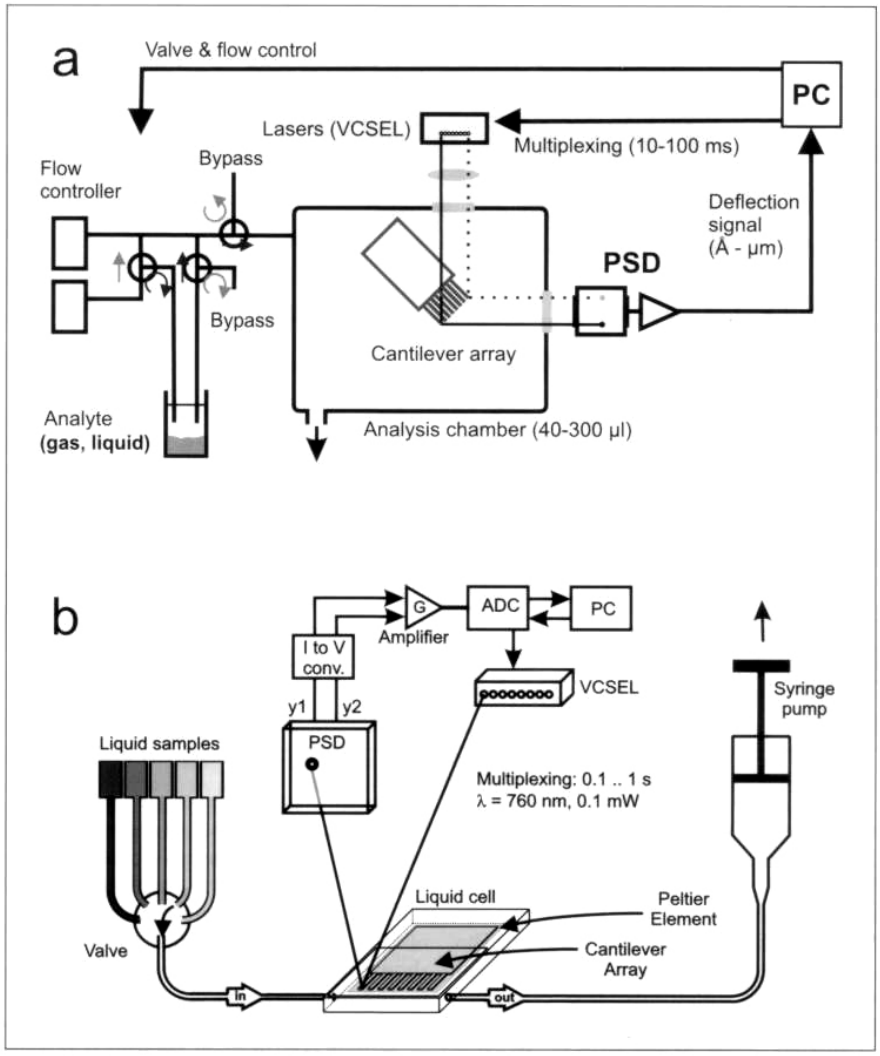

Fig. 3. Schematic views of the experimental setup for measurements in (a) gaseous and (b) liquid environment.

2.3. *Cantilever Coatings*

Various commercial polymers were dissolved in solvents (5 mg/ml). Each solution was sprayed onto one of the cantilevers of the array [10] to obtain a chemical multisensor. Vapor detection proceeds *via* the following mechanism: target analyte molecules, *e.g.* from solvent vapors, diffuse into the polymer layer. This diffusion process results in a swelling of the polymer. The swelling causes a bending of the cantilevers in a way that is specific for the interaction between solvent vapor and polymer in terms of cantilever deflection and time evolution. The resulting bending pattern is then evaluated using principal-component analysis (PCA) techniques.

The self-assembly of thiolated DNA oligonucleotide layers for hybridization experiments in liquids was performed in parallel and under identical conditions by using microcapillaries filled with a 40 µM solution of thiolated probe DNA in triethyl ammonium acetate buffer for 20 min, rinsed, and dried in nitrogen [9].

3. Results and Discussion

3.1. *Measurements of Chemical Vapors*

The deflection traces from eight polymer-coated cantilevers upon injection of ethanol during 10 s (starting at $t = 0$ s) are shown in Fig. 4. After injection the bending signal increases, indicating that the polymer swells and bends the cantilever while the solvent molecules diffuse into the polymer layer. Signal magnitudes at five points in time (dashed lines) are extracted from the data set. These five points per cantilever sufficiently characterize the analyte desorption process during which the analyte molecules diffuse out of the polymer layer again, because the chamber is purged with dry nitrogen. Consequently, the five data points per cantilever are normalized to, for example, the highest magnitude of the bending response. This procedure yields a set of $8 \times 5 = 40$ normalized cantilever magnitudes, representing a so-called 'fingerprint' of the analyte. The data are evaluated using PCA techniques, which extract the most dominant deviations in responses for various analytes. The largest differences in signal amplitudes of the fingerprint patterns are plotted in a two-dimensional graph, whereby an individual measurement (*i.e.* a set of 40 normalized cantilever magnitudes) represents a single point in the PCA space. The axes refer to projections of the multidimensional datasets into two dimensions (principal components). This procedure is targeted at maximum distinction performance between analytes, *i.e.* several measurements of the same analyte should yield a cluster in principal-component space, whereas measurements of differing analytes should produce well-separated clusters of measurements.

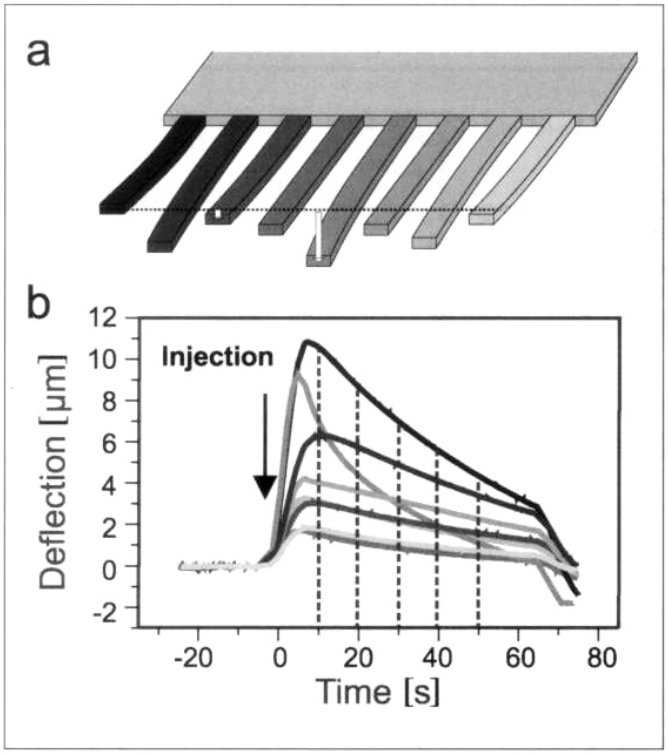

Fig. 4. (a) Schematic of a cantilever sensor array used as an electronic nose. (b) Extraction of a fingerprint from cantilever deflection responses (data by M.K. Baller).

Samples of various, widely used polar solvents such as water, methanol, ethanol, 2-propanol, 1-butanol, and 2-butanol have been used to demonstrate the separation selectivity of the cantilever sensor setup. Vials were filled with 100 µl of solvent. The saturated vapor in the headspace of the vial was extracted with a mass-flow controller that regulated a stream of dry nitrogen gas of 20 ml/min, which was mixed with a dry nitrogen gas stream of 20 ml/min. This mixture was then fed into the analysis chamber. The PCA evaluation of the cantilever sensor response curves is shown in Fig. 5. Clear clustering is observed for the solvents tested, demonstrating successful recognition as well as the selectivity of the method.

3.2. *Measurements in Biochemical Environment*

The detection of specific biomolecular interactions nowadays is very important for disease diagnosis, genome research, and drug discovery. However most current approaches, such as DNA microarrays and ELISAs (enzyme-linked immuno-sorbent assays), rely on the labeling of samples with a fluorescent or radioactive tag. The cantilever sensor technique might turn out to be an alternative to these techniques, because it does not require labeling of molecules [9]. Further label-free methods include surface plasmon resonance (SPR) [11][12] and the quartz crystal microbalance (QCM) [13], which rely on mass detection. The cantilever sensor technique can detect biospecific interactions between a receptor immobilized on one side of a cantilever and a ligand in solution by tracking the cantilever bending. The general applicability of this label-free detection method has been shown for DNA hybridization, the detection of single-base mismatches, protein antibody–antigen recognition of IgG by immobilized protein A [8], and the detection of prostate-specific antigens [14]. Here, we show measurements on hybridization of label-free 12-mer oligonucleotides. The method requires only 100 µl of sample and provides rapid *in situ* analysis within minutes, even in a 80-fold excess background of non-specific complementary oligonucleotides [9]. The signal transduction process is repeatable when denaturation or unbinding agents are used, enabling cyclic operation. In biochemical analysis of life structures, it will be important to detect a certain sequence within longer sections of single-stranded DNA. To demonstrate this capability, we used synthetic oligonucleotides whose structure and properties can be systematically changed. We studied the influence of 5′ and/or 3′ overhanging extensions on the nanomechanical signal with 24-mer targets [9]. Here we focus on the deflection dependence on the concentration of an oligonucleotide that contains a section complementary to the surface-bound probe BioB2 (3'-GAAGTTTGTC GT-C_6-SH-5') and a non-specific poly-adenine tail: 5′-(A_{12})-BioB2C (5'-AAAAAA AAAAAACTTCAAACAGCA-3'). Bio B is a biotin synthetase gene sequence (EMBL accession no. J04423).

Hybridization between the immobilized BioB2 probe and the perfectly matched BioB2C target on the one hand and the 5′-(A_{12})-BioB2C in the other hand produced a compressive surface stress. The magnitude of the differential signal was smaller for the complement with the 5' overhanging extension, see Fig. 6. An injection of 500 nM BioB2C gave a differential signal of 10 nm (the data were corrected for 3 nm/h thermal drift), whereas 5′-(A_{12})-BioB2C gave 8 nm.

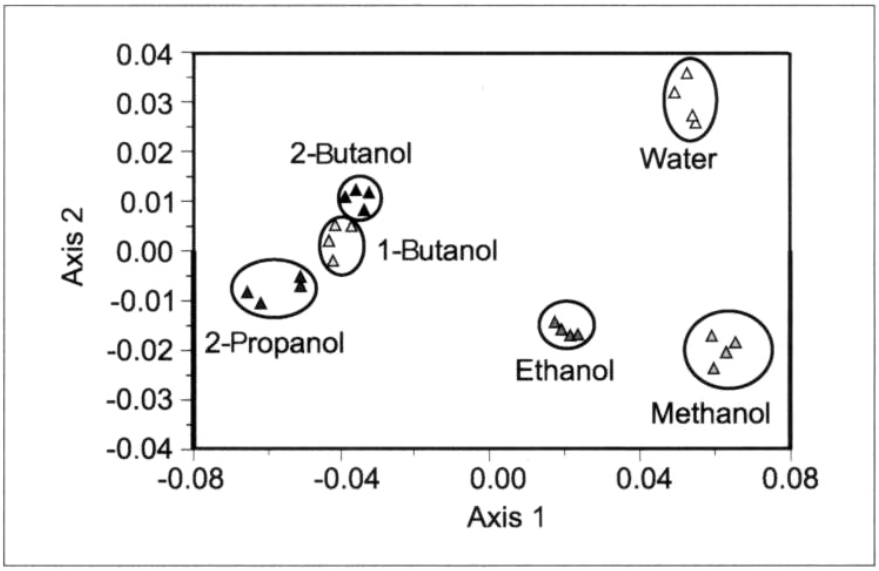

Fig. 5. Principal-component analysis plot of solvent fingerprint patterns (data by M.K. Baller).

Lowering the concentration to 250 nM or 125 nM resulted in cantilever deflections of 6 nm or 3.5 nm, respectively. Under the assumption that the target-probe events are independent and unaffected by surface coverage on the cantilever and that the cantilever bending signal is proportional to the surface coverage, the Langmuir isotherm states:

$$\textit{differential cantilever bending} = a \times c \,/\, (K^{-1} + c) \qquad (5)$$

where c is the concentration of target molecules in solution, K the thermodynamic surface-solution equilibrium constant, and a the proportionality constant. Modeling to the Langmuir isotherm yields K^{-1} (5'-(A_{12})-BioB2C) = 222×10^{-9} M, a = 12.3 nm, and ΔG (5'-(A_{12})-BioB2C) = 38 kJ/mol in 1 M NaCl buffer. As expected [15], the hybridization efficiency of a target with a 5' overhang extension is significantly lower than for the perfectly matching complementary oligonucleotide (K^{-1} (Bio B2C) = 41 nM in 750 mM NaCl buffer) [9]. The results reflect that the steric hindrance of the overhanging extensions of the target DNA decreases the transduction efficiency of hybridization into nanomechanical motion more strongly than for the BioB2C target.

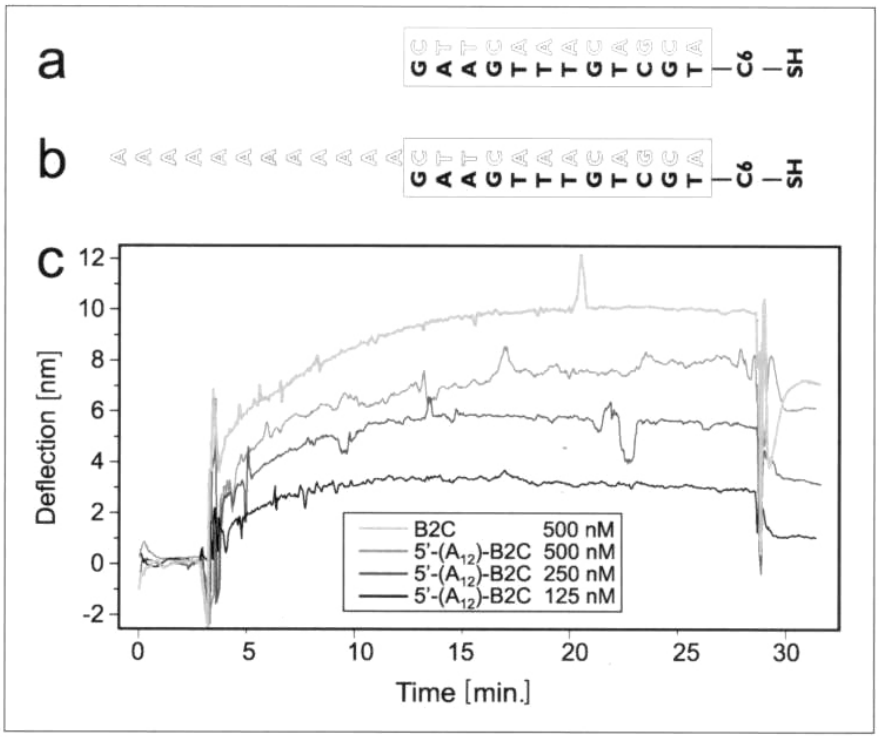

Fig. 6. (a) Single-stranded oligonucleotide (Bio B2) with C6 spacer and thiol end group and matching complementary strand. (b) Bio B2 sequence and complementary strand with an overhanging poly-adenine 5' tail. (c) Hybridization observed with a Bio B2-functionalized cantilever sensor array. The cantilever responses on exposure to a 500 nM solution of the matching complementary sequence and to various concentrations of the complementary sequence with poly-adenine tail are shown (data by R. McKendry).

4. Conclusions

We have demonstrated that a micromechanical array of cantilevers can be used as a selective chemical multisensor for the recognition of chemical vapors, using a previously created fingerprint of the analyte and principal-component analysis techniques. The main advantage of the technique is the capability to use reference sensors for differential measurements that compensate for superimposed disturbances, such as thermal drifts. In biochemical environment, we have shown DNA hybridization of 12-mer oligonucleotides and the effects of modifying the complement with a poly-adenine tail. From the concentration dependence it is possible to extract the thermodynamic surface-solution equilibrium constant K. The hybridization process can be monitored reproducibly and repeatably by breaking the hydrogen bonds between base pairs with denaturation agents. Parallelization of the cantilever sensor array technique beyond eight cantilevers is feasible, because the technology is compatible with silicon microfabrication and is suited for *in situ* operation, opening perspectives for a label-free multi-bio-sensor device suitable for biochemical investigations and medical diagnostics.

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