Nanomechanical Cantilever Bio-Sensors
for Time-Resolved Detection of DNA and Surface Layer Formation

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ABSTRACT

Nanomechanical cantilevers are small and extremely sensitive force and mass detectors. By coating (functionalizing) their surface with specific receptor molecules, cantilevers can be converted into highly sensitive and selective chemical sensors or label-free biosensors. Biosensor measurements with cantilever sensors in liquids have so far mostly been carried out in "stop and go" mode, i.e. the functionalized cantilever is exposed to the analyte liquid and the resulting sensor response is monitored. Here, we will present an instrumental concept, which allows measuring at constant liquid flow rates. Cantilever sensor arrays with eight cantilevers are inserted into a small liquid cell (volume approx. 5 µl) and a homogeneous liquid flow across the cantilevers is maintained using a dedicated, programmable liquid handling system. In this setup, the analyte concentration in the vicinity of the cantilever sensor is kept constant and interaction dynamics as well as the dynamics of surface processes can be analyzed.

Keywords: cantilever sensors, biosensors, molecular interactions, surface processes, scientific instruments

1 INTRODUCTION

Typically, nanomechanical cantilevers are micro fabricated silicon beams with a length of a few hundred µm and a thickness between 500 nm and a few µm. Due to their small size and low spring constant (most commonly in the order of a few 10 mN/m), cantilevers are highly sensitive force and mass detectors. To transform a cantilever into a chemical or bio sensor, its surface can be functionalized with a variety of chemical coatings, which are able to selectively bind or adsorb molecules from the surrounding. Examples for functionalization layers in the field of biosensors include single stranded DNA molecules [1, 2, 3], which are used to detect specific DNA sequences through hybridization, or antibodies used to detect specific antigens [4, 5]. Among other applications described in the literature are e.g. the detection of heavy metal ions [6], the investigation of small molecules such as glucose [7], or the analysis of interaction of small ligands with receptor molecules immobilized on the cantilever surface [8].

These biosensor measurements are typically done in liquids in the so-called static mode, where the deflection (bending) of a cantilever caused by surface stress is measured. Surface stress can be caused by a number of factors such as steric repulsion of the molecules binding to the functionalization layer, electrostatic forces or conformational changes within the functionalization layer. Recently, the dynamic operation mode has successfully been used for cantilever sensing [9]. Here the changes in the resonance frequency (or higher harmonics) of a cantilever sensor are monitored in order to gain information on mass load changes on the cantilever. Combining static and dynamic mode read out, complementary information about the processes taking place at the cantilever surface are obtained.

Since cantilever sensors transform chemical processes occurring at their surfaces directly into a mechanical response, all these measurements are label-free and allow observing molecular interactions or surface-related phenomena in real-time.

2 INSTRUMENTAL SETUP

Measurements in liquids are often done in "stop an go mode", where the measurement cell containing the cantilever sensors is flushed with the analyte liquid and the response of the cantilevers in the motionless liquid is observed. In this setup, interaction processes will be diffusion controlled and the analyte concentration will vary throughout the measurement cell and change over time due to molecules binding to the cantilever surface. In contrast, measuring in a constant liquid flow keeps the concentration constant throughout the measurement. Benefits of this method are e.g. a significantly expanded concentration range (dynamic range) of biosensor measurements, as illustrated below, or the possibility to observe dynamic interaction processes at well-defined analyte concentrations.

The liquid handling system of the Cantisens Research instrument illustrated in fig. 1 has been designed to support controlled liquid flow rates of 2.5µl/min and above. At the heart of the system is a measurement cell with a liquid volume of only 5µl. The syringe pump employed for the liquid transportation is located behind the measurement cell in

order to avoid contamination of the flow path. Used analyte liquid is disposed of into a waste container connected directly to the pump. The inlet of the measurement cell is connected to a valve, which is able to switch between the analyte liquid contained inside a so-called sample loop and buffer flow from a buffer container. The sample loop allows injecting a well-defined amount of sample and can be chosen according to the needs of a specific analysis. All components are mounted and connected in a way to eliminate mechanical coupling to the cantilever sensors and allow an uninterrupted, homogeneous liquid flow across the cantilevers. A typical experiment starts with buffer flow at a user-defined flow-rate, before the analyte in the sample loop is switched into the flow path and transported through the measurement cell. In order to achieve highest thermal stability, the measurement cell as well as the liquid flow path in front of the measurement cell are both temperature controlled with a stability well below 0.05ºC. This ensures that the liquid is entering the measurement cell already at the desired temperature.

Figure 2: Cantilever array holder used with the Cantisens Research instrument. The holder allows fast sensor handling and adjustment towards the optical read-out.

An optical beam deflection system is used to accurately read-out cantilever movement with nanometer precision. An integrated CCD camera in combination with the intelligent adjustment mechanism of the cantilever array holder allows aligning the cantilevers the optical read-out system within seconds. Using cantilever arrays instead of just one cantilever enables multiplexed measurements, i.e. different cantilevers can be functionalized differently in order to interact with different analyte substances. Moreover, one or more cantilevers within the array can be used as references to increase the reliability of measurements – e.g. unspecific interactions or sample contaminations are detected immediately. When employing reference cantilevers, the differential signal (i.e. the difference between the signals of the functionalized and the reference cantilever) can be monitored directly.

Last, but not least, the instrument incorporates vibrational damping means, which isolate the cantilever sensors from any harmful mechanical interferences and account for the excellent mechanical stability of the system. The instrument operates without the need for any external vibrational damping system.

3 EXPERIMENTAL

3.1 General

The experiments described below have been done using cantilever arrays with eight silicon cantilevers (Cantisens Arrays type CLA500-010-08). The dimensions of each cantilever amount to a length of 500µm, a width of 100µm and a nominal thickness of 1µm. This results in a calculated spring constant of 2.6×10^{-4} N/m and a minimal detectable surfaces stress in static operation mode in the order of 10^{-4} N/m.

Prior to functionalization, the cantilever arrays were coated with a 20nm gold layer on top of a 3nm titanium adhesion layer. Homogeneity of the cantilevers was assessed by subjecting the gold coated cantilever arrays to various heat pulses and quantifying the deflection of each cantilever (resulting as a consequence of the different ther-
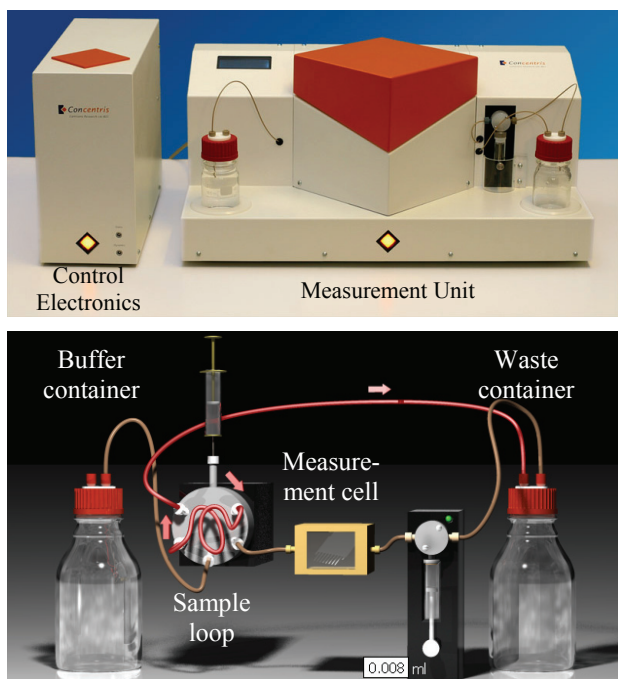

Figure 1: The Cantisens Research instrument. Top: Photograph of the instrument with measurement unit (right) and control electronics (left). Bottom: Schematic setup of the Cantisens Research instrument illustrating the liquid flow path. The sample loop allows to inject a defined analyte volume, which is then pumped through the 5µl measurement cell at a controlled flow rate (the picture is a screen shot taken from the Cantisens Research control software).

Cantilever arrays with eight cantilevers are mounted in the measurement cell by means of a cantilever array holder. This holder has been designed to allow fast an easy handling and exchange of cantilever arrays.

mal expansion coefficients of silicon and gold) as a function of temperature.

3.2 DNA Functionalization

To monitor hybridization of complementary DNA strands, thiol-terminated oligonucleotides were immobilized on the gold-coated side of the cantilevers using the Cantisens Functionalization Unit FU-401. Here, four cantilevers within an array are immersed into glass capillaries filled with 200 mM NaCl buffer containing the SH-terminated DNA sequences. According to [10], these functionalization conditions result in a density of approx. 10^{14} oligonucleotides/cm^2. Two different DNA sequences were chosen and applied to four cantilevers each: Nl4-3 (5' thiol-GTTACAATAGGAAAAATAGGAA-3') and Sf162 (5' thiol-CATACAACAGGAAGAATAATAGGAG-3').

3.3 Surface Layer Formation

For the observation of the dynamics of surface layer formation, reference cantilevers were passivated with a dense layer of mercaptohexanol using the glass capillary technnique. The levers for the investigation of formation dynamics was left unmodified prior to the measurement.

4 RESULTS AND DISCUSSION

4.1 DNA Hybridization Measurement

When cantilevers functionalized with single stranded DNA are brought into contact with matching, free nucleic acids, hybridization will occur and generate surface stress. The surface stress in turn results in a bending of the cantilever, which is used to quantify the concentration of the matching free DNA in the analyte liquid. All data shown in figures 3 to 5 are differential deflections, i.e. the difference between the deflections of a cantilever functionalized with the Nl4-3 sequence and a reference cantilever functionalized with the Sf162 sequence.
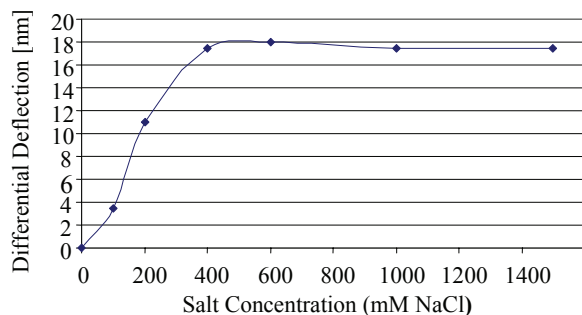
Figure 3: Steady-state differential cantilever deflections as a function of NaCl concentration.

Figure 3 shows the response of a cantilever functionalized with the Nl4-3 sequence upon hybridization with the complementary nucleotide Nl4-3' in a 1 µM concentration and at a liquid flow rate of 0.42 µl/s. The equilibrium or steady-state differential deflection was measured for different concentrations of NaCl. Initially the hybridization response increases with salt concentration, until a stable plateau is reached at a concentration of approximately 400 nM NaCl. For higher salt concentrations, no change in the steady state deflection is observed up to the highest concentration level used in this study (1.5 M NaCl). This behavior is attributed to the fact that the DNA melting temperature is low at low concentrations of NaCl leading to a lesser number of hybridized DNA molecules on the cantilever. With increasing NaCl concentration, the melting temperature of DNA increases logarithmically resulting in a saturation of the cantilever at concentrations above 400 nM NaCl, i.e. all immobilized nucleotides have hybridized.
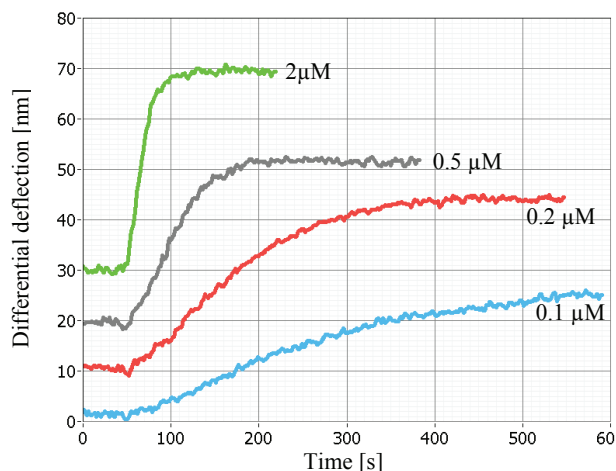
Figure 4: Differential cantilever deflection upon hybridization of DNA molecules as a function of time for four different concentrations of DNA.

Differential cantilever deflections as a function of time are shown in figure 4 for four different oligonucleotide concentrations. The measurements were done with 1M NaCl at a liquid flow rate of 0.42 µl/s. Most notably, while the steady-state deflection increases with increasing DNA concentration, it saturates at around 0.5 µM DNA. This again is due to the fact that at this concentration all oligonucleotides immobilized on the cantilever have hybridized. However, taking into account the interaction dynamics by analyzing the steepest slope of the deflection curves expands the dynamic range of the concentration measurement by more than one order of magnitude. Note that for this kind of analysis, not only a reliably measurement of the time-dependence of the differential deflection is necessary, but also a homogeneous liquid flow across the cantilever sensors must be maintained in order to keep the DNA concentration in the measurement cell constant.
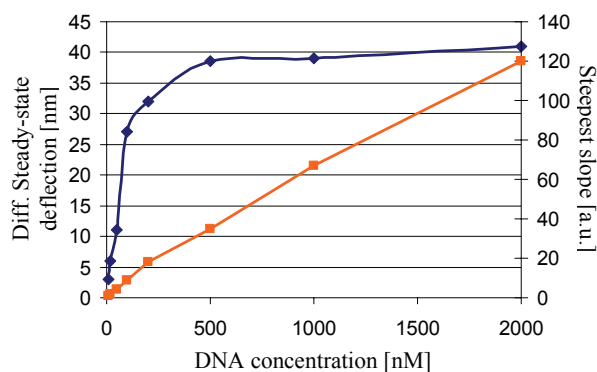
Figure 5: Cantilever deflection (blue) and steepest slope (orange) of the time-dependence of the cantilever deflections plotted as a function of DNA concentration.

4.2 Dynamics of Surface Layer Formation

Self-assembled monolayers (SAMs) are of great interest for surface science in general and the (bio-) chemical modification of biosensor surfaces or nanotechnological devices in particular. Analyzing the deflection of cantilevers during the formation of SAMs helps to gain valuable information into the layer formation process.

Figure 6 shows the cantilever response to a layer of proteins forming on a gold-coated cantilever array. The data shows the differential deflection between the cantilever, where the protein layer forms on, and a passivated reference lever. Formation of a SAM (e.g. from alkanethiols or thiolate oligonucleotides) is expected to result in compressive stress, which bends the cantilever downwards, i.e. away from the surface containing the SAM. Here, a more complex process is observed: After initial build-up of compressive surface stress at point 1, the surface stress reverses its sign and turns into tensile stress at point 2. This effect suggests a conformational change of the proteins. At point 3, the flow of proteins is stopped resulting in a relaxation releasing the surface stress. This indicates a less stable surface arrangement than would be expected for a simple SAM.

5 SUMMARY AND OUTLOOK

Measuring cantilever signals in real-time and under continuous liquid flow allows studying a variety of aspects of time-dependent phenomena and interaction kinetics. Depending on the experimental setup, formation of surface layers, conformational changes, phase transitions or the dependence of surface processes and structures on the chemical environment or temperature can be studied. Using time-resolved data can also serve to extend the dynamic range of cantilever sensor measurements. In addition, combining deflection measurements with complementary data from resonance frequency analysis has recently become possible and holds the promise of providing additional insight into the dynamics of surface-related processes.
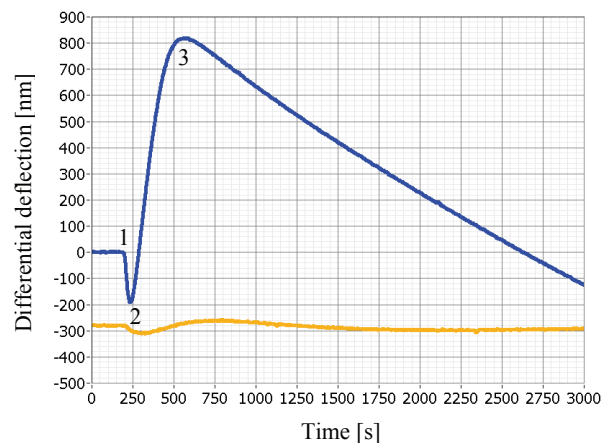

Figure 6: Formation of protein layers on a gold coated cantilever. The complex behavior suggests a conformational change of the proteins.

REFERENCES

[1] J. Fritz, M.K. Baller, H.P. Lang, H. Rothuizen, P. Vettiger, E. Meyer, H.-J. Güntherodt, Ch. Gerber and J.K. Gimzewski, Science 288, 316-318, 2000.

[2] J. C. Stachowiak, M. Yue, K. Castelino, A. Chakraborty, A. Majumdar, Langmuir 22, 263-268, 2006.

[3] J. Zhang, H. P. Lang, F. Huber, A. Bietsch, W. Grange, U. Certa, R. Mckendry, H.-J. Güntherodt, M. Hegner and Ch. Gerber, Nature Nanotechnology 1, 214 – 220, 2006.

[4] G. Wu, R.H. Datar, K.M. Hansen, T. Thundat, R.J. Cote, A. Majumdar, Nature Biotechnology 19, 856, 2001.

[5] N. Backmann, C. Zahnd, F. Huber, A. Bietsch, A. Plückthun, H.P. Lang, H.-J. Güntherodt, M. Hegner, Ch. Gerber, PNAS 102, 14587–14592, 2005.

[6] S. Cherian, R.K. Gupta, B.C. Mullin, T. Thundat, Biosensors and Bioelectronics 19, 411-416, 2003.

[7] J. Pei, F. Tian, T. Thundat, Anal. Chem. 76, 292-297, 2004.

[8] Y. Zhang, S.P. Venkatachalan, H. Xu, X. Xu, P. Joshi, H.-F. Ji, M. Schulte, Biosensors and Bioelectronics 19, 1473–1478, 2004.

[9] T. Braun, N. Backmann, M. Vögtli, A. Bietsch., A. Engel, H.P. Lang, Ch. Gerber and M. Hegner, Biophysical Journal 90, 2970-2977, 2006.

[10] K. Castelino, B. Kannan, A. Majumdar, Langmuir 21, 1956-1961, 2005.