Time Resolved Analysis of Molecular Interactions Using Nanomechanical Cantilever Sensors

J. Koeser (1, 2), P. Shahgaldian (1), M. Bammerlin (2), F. M. Battiston (2), U. Pieles (1)
(1) University of Applied Sciences Northwestern Switzerland (Basel/Muttenz, CH); (2) Concentris GmbH (Basel, CH);
koeser@concentris.ch

Abstract. Cantilever sensors have created a widespread interest in recent years due to their unique nanomechanical signal generation mechanism. Their applications range from sensing of small molecules, chemicals and biomolecules to on-line monitoring of surface-associated phenomena, such as molecular reorganization and formation of self assembled monolayers (SAMs). Cantilever sensors allow real-time monitoring, which is the basis for the kinetic description of interactions at the sensor surface. In this paper, we present examples of cantilever sensor measurements in continuous liquid flow using a commercially available instrument (Cantisens® Research, Concentris GmbH, Switzerland) and demonstrate successful approaches for
a) the description of molecular interaction kinetics,
b) the improvement of calibration curves of cantilever biosensors and
c) the study of SAM formation, protein immobilization and surface-related conformational changes.

1. Introduction
Cantilever sensors are mechanical sensors made from silicon, which monitor surface associated phenomena. Due to their small dimensions (length 500 µm, width 100 µm, thickness 0.5 -10 µm), they are extremely sensitive towards changes of mechanical properties like changes of cantilever mass and surface stress. Such property changes can be measured by using two parallel cantilever read-out modes [1]:
a. Dynamic Mode
In the dynamic mode changes in the resonance frequency of the cantilever is measured. These resonance frequency shifts can be attributed to a mass change on the surface of the cantilever.

b. Static Mode
In the static mode the bending of the cantilever due to a change of surface stress on one side of the cantilever is measured.

According to the direction of the resulting force, two types of surface stress can be distinguished: compressive and tensile surface stress (scheme 1).
Scheme 1: The two types of surface stress occurring in static mode cantilever sensing. The schematic drawing shows a cross-section through a cantilever sensor (black) with a functional layer on the top side (grey). Compressive stress leads to a downward bending of the cantilever whereas upward bending is induced by tensile stress.

Surface stress changes can originate from (i) the formation of a surface layer on one side of the cantilever, (ii) changes within surface layers and (iii) the interaction of a functional coating with an analyte. Thus cantilevers detect surface layer associated processes as well as chemical and biological interactions.

Due to their mechanical read-out cantilever sensors monitor surface stress changes in real-time which allows their use for the characterization of dynamic processes. In the following chapters we demonstrate the suitability of a commercially available cantilever sensor platform, Cantisens® Research, for real-time measurements under continuous buffer flow and present examples for the application of such measurements.

2. Liquid flow through the cantilever sensor measurement chamber
To have homogeneous liquid flow through a measurement chamber is a pre-requisite for reproducible (bio-)chemical sensor response and for kinetic measurements which are important for the determination of chemical and biological affinities.

The Cantisens® Research platform uses a 5 µl measurement chamber, which harbors arrays of 8 cantilevers arranged to be exposed to homogenous liquid flow. Figure 1a is a screenshot of this arrangement. The buffer flow and cantilever numeration is from the left to the right.

To test the homogeneity of the liquid flow through the cantilever measurement chamber a simple test was performed, which uses the interaction between glutathione and calcium ions.

Four cantilevers of a cantilever array were functionalized with glutathione (cantilevers 2, 4, 6, 8), the other four served as references. When calcium ions are injected in the measurement chamber the glutathione-functionalized cantilevers bind calcium and generate a signal. Figure 1b shows the time-resolved reaction of the glutathione-functionalized cantilevers to the injection of a calcium solution at a constant flow of 0.42 µl/sec. A sequential signal of cantilevers 2, 4, 6 and 8 is generated. The points in time when the individual functionalized cantilevers deflect are pitched by approximately 2-4 seconds which corresponds to the time it takes to pump the calcium ion sample from one to the other functionalized cantilever. The sample injection is followed by a wash with EDTA which rapidly removes the cantilever-bound calcium ions. Again, the liquid flow reaches the cantilevers sequentially as can be seen by the sequential signal decrease. This experiment demonstrates an accurate and homogenous sample flow through the measurement chamber.
3. Monitoring of binding kinetics and calibration of cantilever-sensors

The hybridization of nucleic acids immobilized on one side of a cantilever sensor with free single stranded nucleic acids creates a surface stress and as a result a cantilever bending which can be used for biosensing and quantification of the analyzed nucleic acids [2]. To further investigate the hybridization dependent cantilever bending and the kinetics of the reaction we functionalized gold-coated cantilevers with thiol-terminated oligonucleotides and monitored the deflection of these cantilevers upon addition of complementary DNA.

Figure 2 summarizes the results of a series of injections of varying concentrations of complementary DNA. The injection of increasing concentrations of ssDNA resulted in increased cantilever bending as can be seen from figure 2a. Furthermore the deflection signal increases faster when higher concentrations of complementary DNA are injected. For each set of experiments the same cantilevers were used. The cantilevers were regenerated after each experiment by washing them with a 30% urea solution, which completely removes hybridized complementary DNA, and were reused again.

Figure 2b shows a calibration curve where the maximal cantilever bending upon hybridization and the speed of the signal generation (slope of the curves in figure 1a) as a function of the injected ssDNA concentration are plotted. It can be seen that whereas the maximal deflection reaches saturation, the slope of the signal generation increases steadily. Thus a combination of the two parameters greatly enlarges the dynamic range of detection with cantilever sensors.
Figure 2: Quantification of the DNA-hybridization dependent cantilever signals
a) Differential deflection of oligonucleotide-functionalized cantilevers upon injection of 0.1-2 µM solutions of complementary DNA. Note that the plotted cantilever deflections are inverted for better visual understanding. As an example for the calculation of the values presented in figure 2b the dashed orange line represents the speed of a signal generation upon injection of 0.1 µM complementary DNA. The blue double arrow marks the maximal cantilever deflection of the same measurement. b) Graph showing the results from a series of complementary DNA injection experiments. The concentration dependent maximal cantilever deflections (orange, squares) and the speed of the signal generation (blue, diamonds) at different DNA concentrations are plotted.

4. SAM formation and molecular reorganization of proteins
The formation of self assembling monolayers (SAMs) is of great interest for surface sciences especially in the growing field of (bio)-chemical modification of nanotechnological devices. One of the most investigated SAMs are those formed by the binding of alkanethiols on gold where the thiol group binds covalently to the gold layer [3]. Cantilever sensors monitor the stress created during the process of SAM-formation in real-time [4]. Figure 3a shows the bending of a gold-coated cantilever due to surface stress change upon injection of mercaptohexanol solution. Considerable compressive stress is generated resulting in a downward bending of several hundred nm.

Such time-resolved measurements can also be used to investigate the behavior of immobilized biomolecules. As an example (figure 3b) two different thiolated proteins are immobilized on the gold side of the cantilever, which leads to a complex successive generation of tensile and compressive stress. The different behavior between the two proteins can be attributed to different conformational reorganizations within the immobilized protein layers. The results gained from these experiments provide valuable information for e.g. the optimization of protein immobilization protocols and for biosensor design [5].
Figure 3: Monitoring of SAM formation and conformational changes in protein layers
a) Surface stress generated during the formation of a mercaptohexanol SAM on the gold layer on the
top of the cantilever sensor. The fast process of thiol-binding to the gold is followed by a slower slight
decrease in the surface stress. b) Monitoring of protein immobilization followed by a molecular
rearrangement process. The different behaviour of a large, approximately 90kDa, importin-β (blue
line) and a smaller protein construct representing two neighboring Ig-domains of E-cadherin
(approximately 12 kDa, orange line) are shown. Bars below the time axis mark the duration of the
sample injection.

5. Outlook
Cantilever sensors monitor surface associated processes and molecular interactions. Our results show
that, due to their mechanical sensing mechanism, they can follow reactions in real-time, which allows
their application for a growing area of research spanning the whole range from basic research in
surface sciences to the development of sensors for chemical and biological detection.

6. Acknowledgments
The authors gratefully acknowledge financial support from the Commission for Technology and
Innovation (CTI), and the European Commission within the 6th Framework Programme (FP6).

7. Appendix
All measurements were performed in static mode on a Cantisens® Research cantilever sensor platform
(Concentris GmbH, Basel, Switzerland) at a flow rate of 0.42 µl/sec. Cantilever arrays (CLA-500-010-
08, Concentris GmbH) were coated with a 3 nm adhesion layer of titanium followed by a 20 nm thick
gold layer. Thiol modified oligonucleotides (one 22-mer and one 25-mer) for cantilever sensor
functionalization and complementary oligonucleotides for hybridization were ordered from
Microsynth (Balgach, Switzerland). Measurements were performed in Tris-buffer with varying
concentrations of NaCl. Tris-EDTA buffer contained 0.5 mM EDTA. Recombinant proteins were
kindly provided by K. Schwarz-Herion (β-importin) and Dr. D. Haeussinger (cystein terminated Ig-
domains of E-cadherin). Data analysis and presentation was done with the Cantisens® Data Viewer.
All cantilever deflections presented are differential deflections of functionalized cantilevers minus
reference cantilevers.

References
[1] Carrascosa LG, Moreno M, Alvarez M, Lechuga LM, 2006 TrAC, 25 (3) 196-206
[2] Fritz J, Baller M K, Lang H P, Rothuizen H, Vettiger P, Meyer E, Guntherodt H J, Gerber C, Gimzewski J K, 2000 Science 288, 316-318.
McKendry R A, Zhang J, Arntz Y, Strunz T, Hegner M, Lang H P, Baller M K, Certa U, Meyer E, Güntherodt H-J, Gerber Ch. 2002 Proc. Natl. Acad. Sci. U.S.A. 99, 9783-9788.

[3] Love JC, Etsroff LA, Kriebel JK, Nuzzo RG, Whitesides GM, 2005, Chem Rev 105, 1103-1169

[4] Berger R, Delamarche E, Lang HP, Gerber C, Gimzewski JK, Meyer E, Guentherodt HJ, 1997, Science 267, 2021-2024.

[5] Zhang Y, Venkatachalan SP, Xu H, Xu X, Joshi P, Ji H-F, Schulte M, 2004 Biosensors and Bioelectronics 19(11), 1473-1478