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Analyzing Gene Expression Using Combined Nanomechanical Cantilever Sensors

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Abstract. In diseases such as cancer or during viral infections gene expression is greatly altered. These changes in gene activity can be analysed at different levels of cellular activity, like transcription activation, transcription and translation. Currently, no simple method is available to detect all these biochemical signals simultaneously and rapidly. Micromechanical cantilever sensor array technology is applied, because it has the advantage that sample preconditioning like labelling and amplification is not required. Furthermore, DNA, RNA, protein or combinations thereof could be detected in parallel on a single cantilever array. With such a device, diagnosis and therefore treatment of diseases can be improved. Here we present successive detection of DNA hybridization and antigen using the same micromechanical cantilever sensor array.

1. Introduction

Cells adapt in different ways to changes in their environment by varying expression patterns or expression levels of proteins. In recent years new ways of regulation of gene expression have been discovered especially at the level of translation and RNA degradation [1]. Special RNAs, micro RNA (miRNA) and small interfering RNA (siRNA), expressed by a normal cell, a cancer or virus infected cell, form double stranded RNA (dsRNA) with messenger RNA (mRNA) as a result they either enhance mRNA degradation or interfere with the translation machinery and thus directly with protein synthesis. These events either cause the destruction of a particular mRNA or the presence of mRNA which is not translated. Furthermore miRNA and siRNA are part of the large group of non coding RNAs (ncRNA), which make up 62% of the whole mammalian genome [2]. Due to the effects of miRNAs or siRNAs on mRNA, there is no direct correlation between mRNA levels and the subsequent amount of expressed protein. These findings indicate that regulation of gene expression is more complex than previously thought. With current methods it is not easily possible to analyze transcription (mRNA synthesis) and translation (protein synthesis) in parallel.

We propose to use micromechanical cantilever array sensors to investigate these complex biochemical signals. It was already shown that DNA hybridization [3] and protein binding [4, 5] can be analyzed with cantilevers, albeit on separate arrays. Micromechanical cantilever arrays offer a method to investigate levels of transcription and translation in parallel. The label- and amplification-free nature of the method enables monitoring transcription and translation without creating artifacts, giving a more accurate picture of gene expression either in a healthy or a diseased state.

2. Material and Methods

2.1. Reagents. Oligonucleotides 5'-functionalized by a hexyl spacer with a thiol group were obtained from Microsynth GmbH (Balgach, Switzerland). They had the following sequences SH-BioB2: 5'-TGC TGT TTG AAG-3'; SH-unsp12: 5'-ACA CAC ACA CAC-3'. The oligonucleotide complementary to BioB2 (BioB2C) was synthesized without the hexyl spacer and the thiol group and

had the following sequence: 5'-CTT CAA ACA GCA-3'. The thiolated single-chain fragments (scFv) C11L34cys, G9cys, and the antigen to C11L34cys m3.16-GCN4 used in this study are described elsewhere [4].

2.2. Cantilever Preparation. A microfabricated silicon cantilever array, with a cantilever pitch of 250 µm, lengths of 500 µm, thickness of 500 nm and a spring constants of 0.0025 N/m were provided by the Micro- and Nanomechanics group at the IBM Zurich Research Laboratory. The cantilevers were prepared as described in detail elsewhere [3, 6]. Briefly, 4 microcapillaries (OD 250 µm; ID 150 µm; from Garner Glass, Claremont, CA) were either filled with thiol modified oligonucleotide in solution or scFv solution in an alternating pattern, thereby functionalizing the cantilevers either with a sensing layer or a reference layer (figure 1A).

2.3. Instrument. The functionalized cantilever array is inserted into a liquid chamber (volume: 50 µl) and mounted at an angle of 11° with respect to the incident laser beam (time-multiplexed vertical-cavity surface-emitting laser; wavelength 760 nm, Avalon Photonics, Zurich, Switzerland). The laser beam is redirected by a mirror to a PSD (position-sensitive detector, SiTek, Partille, Sweden). Data are acquired using a multifunctional data-acquisition board (National Instruments, Austin, TX) driven by LabView software. The software also controls the liquid-handling system of the setup, the syringe pump (GENIE, Kent Scientific Corp, Torrington, CT), and a ten-position valve system (Rheodyne, Rohnert Park, CA). The entire setup (figure 1B) is placed inside a temperature-controlled box (Intertronic; Interdiscount, Switzerland), which is temperature-equilibrated through a fuzzy logic controller by the LabView software at ±0.02 °C accuracy.

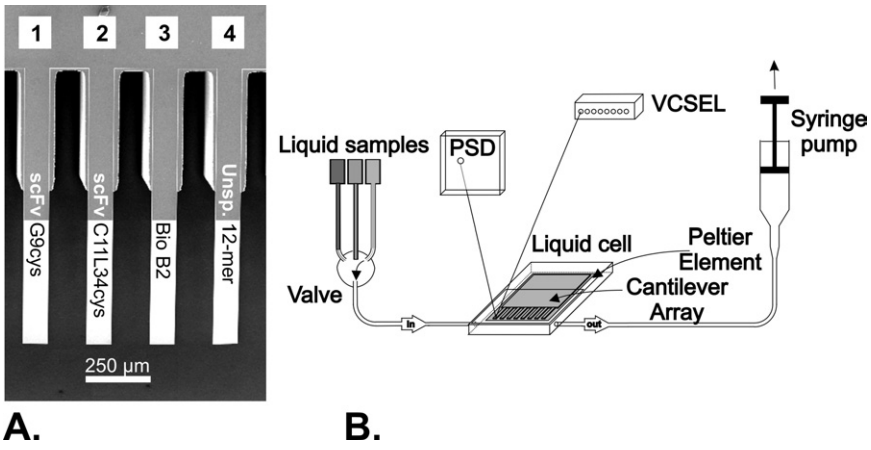

Figure 1A. Cantilever array showing 4 cantilevers functionalized with cystein modified single-chain antibody fragments, scFv G9cys used as reference, scFv C11L34cys probe, thiolated oligonucleotide BioB2 probe, and Unsp. 12-mer thiolated oligonucleotide used as reference. **B.** Device setup for measuring cantilever responses upon biomolecular interaction. A cantilever array is placed inside a liquid filled cell, which is on top of a peltier element. A laser beam is deflected at the end of a cantilever and its position is detected with a position sensitive detector (PSD) to measure cantilever bending. Target solutions or buffer are delivered by a syringe pump. Different analytes or buffers can be selected with a many fold valve. The whole setup is put in a temperature controlled box.

2.4. Binding Experiment. Binding experiments were conducted at 23°C; for this purpose the environmental box was equilibrated for two hours. Then the cantilever array was first kept in PBST (100mM NaP_i pH 7, 150 mM NaCl, 0.05% Tween). After a stable baseline has been reached 200 µl 500 nM m3.16-GCN4 in PBST was injected. Antigen binding was allowed to proceed for 12 min. before cantilevers were rinsed with 200 µl PBST. Development of the signal was followed for an additional 10 min. before the cantilever array was washed and equilibrated 3 times with 200 µl 7xSSC

(100 mM NaCitrate pH 7, 1 M NaCl). After a stable baseline has been reached 200 µl BioB2C in 7xSSC was injected and development of the signal was observed for 12 min., when the array was washed with additional 200 µl 7xSSC.

2.5. Data analysis. Prior to the binding experiment, bimetallic response and mechanical properties of the cantilevers were assessed by a 30 s/1 V thermal cycle, resulting in a 0.5°C pulse, using a Peltier element directly below the chamber. The cantilever with the highest deflection response was used to normalize the response of the others provided the responses did not differ by more than 10% of magnitude. Normalized data from reference cantilevers were subtracted from the data of the sensing cantilevers to obtain a differential signal. A baseline correction, needed because the drift behavior of different cantilevers varies slightly, was applied, using a linear fit of data in the time interval between the start of the experiment and the injection of biological material. The slope of the linear fit then was subtracted from all differential signals.

3. Results

For the purpose of developing a biosensor capable of analyzing transcription and translation at the same time individual cantilevers are modified with model oligonucleotides BioB2 and unsp12 for the detection of hybridization and model single-chain antibody fragments scFvC11L24cys and G9cys for the detection of antigen binding (figure 1A). Binding of antigens and DNA hybridization usually require different buffer conditions. While buffer conditions, like high salt concentrations, favorable for DNA complement binding could be deleterious to the antibodies structure or antigen binding, we first investigated antigen antibody interaction (figure 2). After a 5 minute delay, we injected 200 µl 500 nM of the antigen m3.16-GCN4 at a rate of 40 µl/min. A signal of 5 nm developed which increased to 10 nm, this showed that the antibodies on the cantilever surface could not be saturated at this concentration in this timeframe. Then the cantilevers were washed with 200 µl PBST. The signal came almost back to the baseline before application of the DNA complement, indicating partial unbinding. It is possible that some of the antigen will bind nonspecifically to the gold surface and can not be removed by washing with PBST alone.

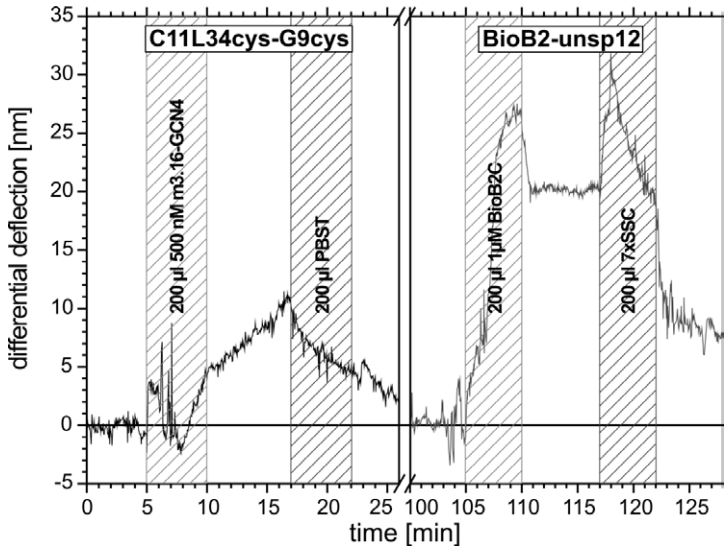

Figure 2. Differential signals upon sequential injections of 500 nM antigen m3.16-GCN4 at 5 min. and 1 µM complement oligonucleotide BioB2C at 105 min. 200 µl of analyte are injected at a flow rate of 40 µl/min. A deflection of 5 to 10 nm is recorded for the antigen binding and a signal of 20 nm is measured for the hybridization of the DNA complement. Cantilevers are washed after 17 min. with 200 µl PBST and at 117 min. with 200 µl 7xSSC.

The cantilever array was now prepared for DNA binding by extensively washing it with 7xSSC until the baseline was stable. Then 200 µl 1µM BioB2C was injected at a rate of 40 µl/min. A signal of 20 nm was observed (figure 2) which did not change during the course of the experiment indicating saturation of the cantilever, confirming previous findings at the same concentration [3]. Washing of the cantilever array with 200 µl 7xSSC at a rate of 40 µl/min resulted in partial dehybridization of BioB2C with a remaining signal of 7 nm. Using more stringent conditions like urea treatment or heating during washing could remove more of the complement.

4. Discussion and Outlook

With our experiments we were able to show that protein and DNA detection on the same array is possible. The next experiments will investigate the conditions for measuring hybridization and antigen binding at the same time, showing the feasibility for a combined DNA/protein sensor. These promising experiments will allow us to explore the effects of RNA interference on RNA and protein synthesis on a single platform without labeling and amplification.

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