 MCB, vol.1, no.3, pp.211-220, 2004

Cantilever Arrays for Multiplexed Mechanical Analysis of Biomolecular Reactions

Min Yue[1], Jeanne C. Stachowiak[1,2], and Arunava Majumdar[1,3]

Abstract: Microchips ;ontaining arrays of cantilever beams have been used to mechanically detect and quantitatively analyze multiple reactions of DNA hybridization and antigen-antibody binding simultaneously. The reaction-induced deflection of a cantilever beam reflects the interplay between strain energy increase of the beam and the free energy reduction of a reaction, providing an ideal tool for investigating the connection between mechanics and chemistry of biomolecular reactions. Since free energy reduction is common for all reactions, the cantilever array forms a universal platform for label-free detection of various specific biomolecular reactions. A few such reactions and their implications in biology and biotechnology are discussed.

keyword: Cantilevers, array, DNA hybridization, protein interaction, multiplexing.

1 Introduction

Biomolecules such as nucleic acids and proteins are three dimensional structures that interact with each other through highly specific reactions. Such reactions lead to various kinds of cellular functions: (i) formation of new complexes that provide mechanical strength to a cell; (ii) catalytic reactions that lead to energy conversion as well as molecular synthesis and breakdown; (iii) signal transduction and amplification; etc. The reaction specificity arises from a combination of three-dimensional structure and chemical forces that produce the largest reduction in free energy. The interaction forces are produced either through hydrogen bonding, electrostatic interactions, van der Waals interactions, or a combination of all of them. In many cases, the mechanical compliance of a molecule in conjunction with the interaction forces plays a critical role in the free energy of the reaction. In other words, the free energy landscape of biomolecular reactions reflects a rich interplay between mechanics, chemistry and geometry.

Given this nature of biomolecular reactions, an approach to detect such interactions that capitalizes on this interplay is intellectually appealing. Furthermore, given the large number of such reactions that occur in a cell, a high-throughput way of doing so is technologically attractive as well. Here we report progress on the development and assessment of cantilever arrays as a universal platform for high-throughput mechanical detection of biomolecular interactions.

High-throughput genomic analysis can now be performed using DNA microarrays. Over the last decade, their development and wide use have encouraged many researchers to develop protein microarrays for high-throughput protein analysis. Great progress has been made despite the challenges accompanying studying and handling proteins. Proteins have been arrayed in a high-density format on modified glass slides [Haab *et al.* (2001), Macbeath and Schreiber (2000), Zhu *et al.* (2001)], in 3-D matrixes [Guschin *et al.* (1997); Afanassiev *et al.* (2000)], and in nanowells made of poly[dimethyl-siloxane] (PDMS) [Zhu *et al*. (2000)]. The binding events are usually detected by attaching labels or enzymes to analytes directly or to secondary probes. The labels include widely used fluorophores [Haab *et al.* (2001), Macbeath and Schreiber (2000)], radioisotopes [Zhu *et al*. (2000)] or quantum dots [Sutherland (2002)]. Enzymes are used in chemiluminescence detection of microarrays [Wiese *et al.* (2001), Angenendt *et al.* (2003)], in which enzymes catalyze certain reactions to emit light. The presence of labels or enzymes directly indicates the occurrence of the binding events. Such high-throughput assays generally use very little amount of sample ($\sim$ nanoliters). However, labeling itself is a time-consuming and costly process, besides

[1] Department of Mechanical Engineering, University of California, Berkeley, CA 94720, USA
[2] Presently at Sandia National Laboratory, Livermore, CA 94551, USA
[3] Materials Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA. Corresponding author: majumdar@me.berkeley.edu

raising safety concerns of radioisotopes and issues related to the volatility of fluorophores. More problematic is the fact that proteins are very labile and their activities can be readily affected by labeling. Different results were obtained from the same antibody-antigen binding when the antibody or the antigen was labeled respectively [Haab *et al.* (2001)]. Surface plasmon resonance (SPR) has caught significant attention as a label-free technique for protein interaction analysis [McDonnell (2001)]. A great advantage of SPR lies in its ability to yield both kinetic and equilibrium information of protein interactions, in contrast to the only end-point report from the label-based technologies. Since light does not penetrate the sample, measurements can also be made on turbid or opaque samples with no interference from light absorption or scattering. However, multiplexed SPR is still in its infancy due to the sophisticated microfluidics and optics involved. Furthermore, it is still quite a challenge for using SPR to analyze target molecules with a molecular weight less than 1000 Dalton.

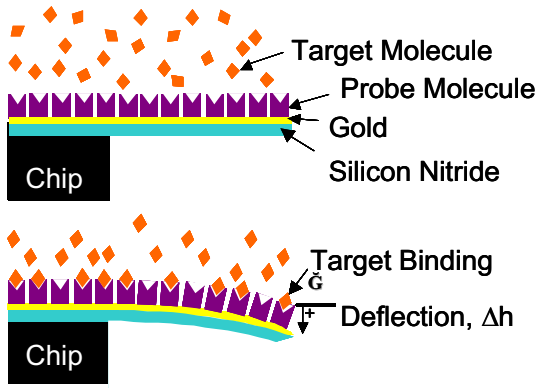

Figure 1 : Specific biomolecular interactions between target and probe molecules alter intermolecular interactions within a self-assembled monolayer on one side of a cantilever beam. This can produce a sufficiently large surface stress to bend cantilever beam and generate motion.

In recent years, researchers have reported a new type of mechanical sensor for chemicals and biological molecules [Thundat and Majumdar (2003)]. In particular, it was discovered that when chemical or biological reactions occur on one side of a microcantilever beam, they produced a bending moment that deflected the cantilever (see Figure 1). The motion of the cantilevers can be detected either electronically or optically. Berger *et al.* (1997) demonstrated the detection of various alkanethiol molecules and hexylamine with gold-coated cantilevers, while Thundat *et al.* (1997) demonstrated adsorption of gas molecules and antigen-antibody binding [Raiteri *et al.*, 1999]. The quantitative measurement of DNA hybridization, including single base pair mismatch detection, was reported by Fritz *et al.* (2000), Wu *et al.* (2001a) and Hansen et al. (2001). It was later shown by Wu *et al.* (2001b) that prostate specific antigen (PSA), a serum marker for prostate cancer, can be detected at clinically relevant concentration and conditions. Recently, Savran *et al.* (2004) showed the detection of Taq DNA polymerase using aptamer-coated cantilevers.

What is the science underlying reaction-induced cantilever bending? When occurring on a cantilever, the reaction-induced free energy reduction on one cantilever surface is balanced by the strain energy increase due to bending, such that at equilibrium the free energy of the whole system reaches the minimum [Hagan *et al.* (2002)]. In other words, the penalty of increasing the strain energy must be compensated by a larger reduction in free energy due to the reaction, reflecting the interplay between mechanics and chemistry. Hence, the cantilever bending can be construed as a measure of free energy reduction due to the chemical reaction on one surface. What is worth noting is that because free energy reduction is common for all reactions, the cantilever-based sensing is a universal platform for studying all reactions. Furthermore, the technique does not require attachment of labels to the biomolecules. Changes of free energy density in biomolecular reactions were reported to be in the range of 1 to 50 mJ/m^2 [Berger *et al.* (1997), Fritz *et al.* (2000), Wu *et al.* (2001a, 2001b), Hansen *et al.* (2001), Marie *et al.* (2002)], or as high as 900 mJ/m^2[Marie *et al.* (2002)].

While all previous work has focused on studying individual reactions using single or at most 8 cantilevers, a truly high-throughput technique is missing. Our previous work demonstrated 2-D microcantilever array containing 9 cantilevers to study the drift and physical characteristics of the device and the system [Yue *et al.* (2004)]. In this work, we report the development of a cantilever array chip that allows simultaneous monitoring of about 500 cantilevers. This enables various types of statistical analysis to produce redundancy and mitigate false positive/negative signals. Multiplexed DNA hybridization

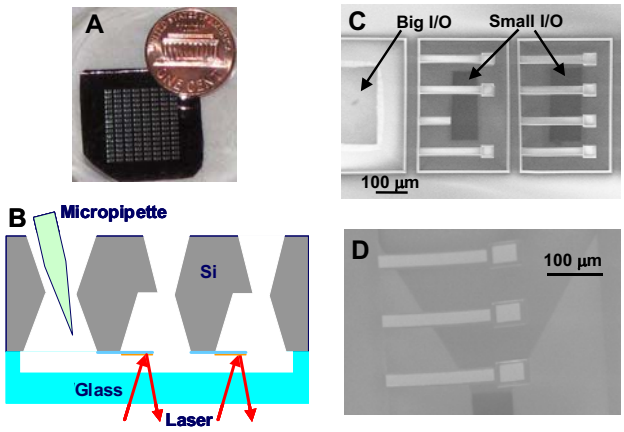

Figure 2 : (A) The cantilever array chip containing a 2-D array of reaction wells, each well containing multiple cantilevers. The array is roughly the size of a penny; (B) A schematic diagram of the sideview of a single reaction well containing fluidic inlet and outlet in the silicon chip, the cantilevers, and the transparent glass cover for the laser beam to be used for measuring cantilever deflection; (C) Electron micrograph of a single reaction well showing 7 cantilever beams, a big inlet/outlet (I/O) port and two small I/O ports; (D) A close-up picture of three cantilever beams that contain a gold film on the top surface and a flat paddle at the end of each cantilever for reflecting the laser beam.

and protein interaction assays are demonstrated using the new 2-D microcantilever arrays.

2 Experimental System

2.1 Cantilever Array Chip

Figure 2 shows schematic diagrams and optical and electron micrographs of the cantilever array chip. The silicon nitride cantilevers were 200-400 μm long, 0.5 μm thick and 40 μm wide. They were covered a thin film of gold on one side, which were used for attaching biomolecules using Au-thiol binding. The cantilevers had square paddles at the end, which were used as laser reflectors for optically measuring cantilever motion (Yue et al., 2004). As opposed to the compliant arm of the cantilever, each paddle was made rigid and flat by increasing the moment of inertia using ridges along the four sides.

To effectively combat the inaccuracies arising from sensor drift and fabrication variations between sensors, the design included multiple cantilevers per reaction well, each of which received the same analytes at all stages of an experiment. The response from all the sensors in a given reaction well could then be used to obtain a more statistically relevant response for each well. Each such reaction well contained a large fluidic inlet (called big I/O) and two small fluidic outlets (called small I/O). The small I/O was designed to prevent vapor bubbles to be trapped, such that when a fluid sample is injected into the big I/O the gas was ejected through the small I/O.

The fabrication process for the cantilever array chip utilizes conventional microelectromechanical systems (MEMS) fabrication including bulk and surface micromachining, which are described in detail by Yue *et al.* (2004). The yield (percent of cantilevers on each chip surviving the fabrication process) achieved from this fabrication process ranged from 95-98%.

In order to use the cantilever array chip as a multiplexed sensor array, each reaction well must be physically separated from the neighboring wells. This is achieved using a pyrex substrate that is patterned and etched to produced the reaction well, and bonded to the silicon chip. This is accomplished using an adhesive stamping technique [Satyanarayana *et al.* (2004)]. First, UV curing adhesive is spin coated onto a glass slide. The etched pyrex cover was carefully brought into contact with the adhesive coated slide and then removed in a "stamping" process. During this process, a layer of adhesive was transferred to the pyrex cover. Next, the silicon chip and the pyrex chip were aligned and bonded, and then checked under microscope to assess the degree of bonding. If some wells were not separated, a small amount of mechanical force was applied manually to spread the contact front into the region of the connected wells. If the pyrex cap and silicon chip were both smooth and free of dust at the time of bonding, separation in excess of 90% of the wells on the chip would occur. Figure 3 depicts examples of separated and connected wells after Pyrex capping.

2.2 Optomechanical readout

In order to simultaneously image the entire cantilever array chip of about 20 mm^2 area, it was necessary to construct a whole field illumination system that would detect cantilever motion by ray optics. This system consists of a low power helium neon laser (15mW, 1mm beam diameter, 632.8nm, CoherentInc.), a neutral den-

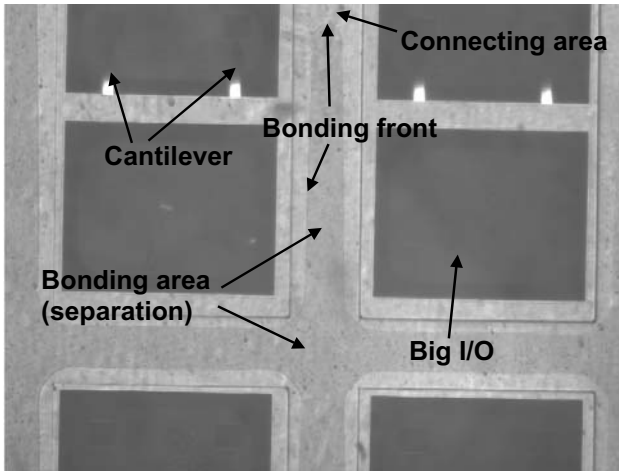

Figure 3 : Capping of microfluidic well using etched glass cover. Top two wells are connected, as indicated by bonding front. They are separated from the bottom two wells.

sity filter, a 20X beam expander (expands beam to about 20mm), a simple flat mirror, a 50/50 beam splitter, a temperature controlled chip holder, a simple plano-convex lens (BK7 glass, focal length of 100mm, diameter of 80mm, Melles Griot Inc.), and a CCD camera (Apogee Inc. 9E, 3072x2048 pixels, 14bit, 27.6x18.4mm) (Figure 4A). The laser beam was reflected by the cantilevers and the rest of the chip in various directions. However, the reflections from the flat paddles at the end of the cantilevers formed collimated beams in a particular direction, which could then be separated from spurious reflections and directed towards a CCD for imaging. Figure 4B shows a CCD image of an entire cantilever array chip, where each spot corresponds to the reflection from the paddle of an individual cantilever. Any motion of a cantilever would lead to corresponding motion of the CCD spot, which can be estimated using ray optics. Following acquisition by the CCD camera, images were transferred to a Matlab script, which tracks each cantilever paddle image "spot", by calculating the intensity centroid of each spot.

As depicted in Figure 5, the bending of a cantilever results in a change in angle of the light deflected from its paddle. This light then reflects from the beam splitter and passes through the lens at a different angle than that from an undeflected reference cantilever. At the image plane, the images from the two cantilevers appear at the same location. However, as the CCD camera is moved further

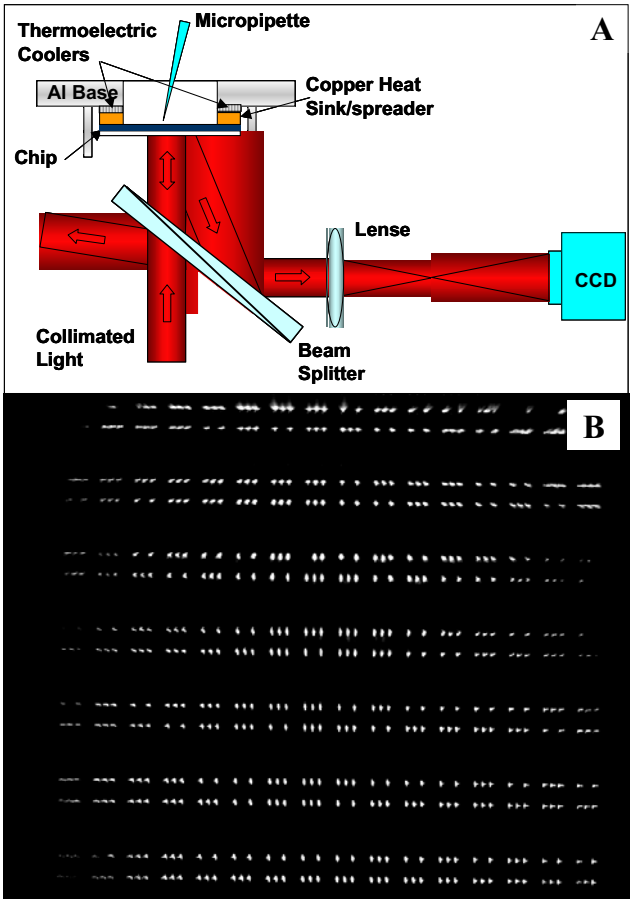

Figure 4 : (A) Components of the optical system; (B) A CCD snap shot of about 500 spots, each spot corresponding to the reflection of the laser beam from the paddle of a cantilever.

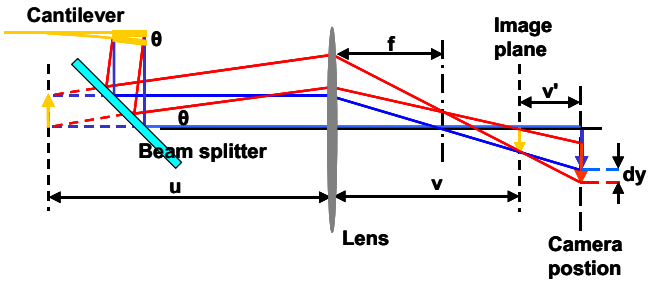

Figure 5 : Ray optics principles underlying optical detection of cantilever motion.

from the lens, away from the image plane, the relative positions of the two images diverge (dy). It is this divergence, which constitutes the measured sensor response to surface stress. Applying the lens equation of geometrical optics,

$$\frac{1}{f} = \frac{1}{u} + \frac{1}{v} \tag{1}$$

and conventional principles of triangle geometry, the motion dy can be derived as

$$dy = v' \left(\frac{u}{f} - 1 \right) \tan\theta \approx v' \left(\frac{u}{f} - 1 \right) \theta \tag{2}$$

where f is the focal length of the lens, u is the distance between the object and lens, and v' is the distance between the image and the lens.

Clearly the sensor signal is proportional to the distance between the camera and the image plane. Curvature of the paddles and the imperfection of the light beam collimation cause the spots to blur at the position. If the spots were allowed to blur excessively, increased noise in the sensor signal was observed. The optimum position of the camera away from the image plane was achieved when the signal to noise ratio was a maximum, as determined by a trial and error process for each device.

2.3 Calibration

While the goal of our research is to perform multiplexed biomolecular analysis using cantilever arrays, thermomechanical actuation of these bimorph cantilevers provides a means to study them and calibrate bioreaction-induced actuation. Derived from Stoney's formula, a cantilever's theoretical deflection (at the center of the paddle), $S_\sigma$sgaused by surface stress change $\Delta\sigma$, can be calculated as [Yue *et al.* (2004)],

$$S_\sigma = 3 \left(\frac{n+1}{K} \right) \left(\frac{L_b^2}{d_{SiN}} \right) \left(1 + \frac{L_p}{L_b} \right) \cdot \Delta\sigma \tag{3}$$

where

$$K = 4 + 6n + 4n^2 + \phi n^3 + \frac{1}{\phi n}; \quad n = \frac{d_{Au}}{d_{SiN}}; \quad \phi = \frac{E_{Au}}{E_{SiN}} \tag{4}$$

andd_{SiN} is the SiN_x film thickness, d_{Au} is the gold film thickness, L_b and L_p are the length of the cantilever beam and the paddle, E_{SiN} and E_{Au} are the Young's moduli for silicon nitride and gold, respectively. Because the gold layer is much thinner than the nitride layer, the surface stress change $\Delta\sigma_T$ caused by temperature change ΔT can be described as

$$\Delta\sigma_T = (\alpha_{Au} - \alpha_{SiN}) \cdot \Delta T \cdot d_{Au} \cdot E_{Au} \tag{5}$$

where α_{SiN} *and* α_{Au} are the thermal expansion coefficients for silicon nitride and gold, respectively. A 200-μm long, 40-μm wide and 0.5-μm thick cantilever, with a 100×100 μm^2 paddle, coated with 25 nm Au on one side of the cantilever, has a thermomechanical sensitivity of about 210 nm/K [Yue *et al.* (2004)]. Because a cantilever's deflection strongly depends on geometry, the surface stress change, which is directly related to biomolecular bindings on the cantilever surface, was chosen to be the quantitative measurement for the bindings. The thermomechanical response of the cantilevers was used to calibrate the signal caused by biomolecular bindings. All the cantilevers used in this work had a thermal response of $\sim$ 25 J/m^2-K. In each experiment, the thermal response for all the cantilevers was first simultaneously measured. This value for each cantilever was later used to normalize the cantilever bending induced by biomolecular reactions. The normalized signal was finally converted to the absolute surface stress change using Stoney's formula.

3 Experiments

3.1 Materials

The thiolated 20mer single-stranded DNA (ssDNA) was modified at its 5' end with the thiol-modifier, HS-$(CH_2)_6$, to serve as probe molecules for DNA hybridization assay (IDT, IA). The 20mer non-complementary and complementary ssDNA was also purchased from IDT and was used for target molecules. Phosphate buffer (pH 7.0, 100mM) was the buffer used for DNA hybridization assay. The hepatitis virus DNA was 70mer long and was received from Frank Chen (Life Sciences Division, LBL). To attach the probe 70mer ssDNA to the Au surface of the cantilever, first a Sulfo-NHS-SS-Biotin was immobilized on the gold surface, and then incubated with NeutrAvidin^{TM} Biotin-Binding Protein (Pierce, IL). The probe 70mer ssDNA was biotinylated, which was used to bind to the NeutrAvidin^{TM} on the surface.

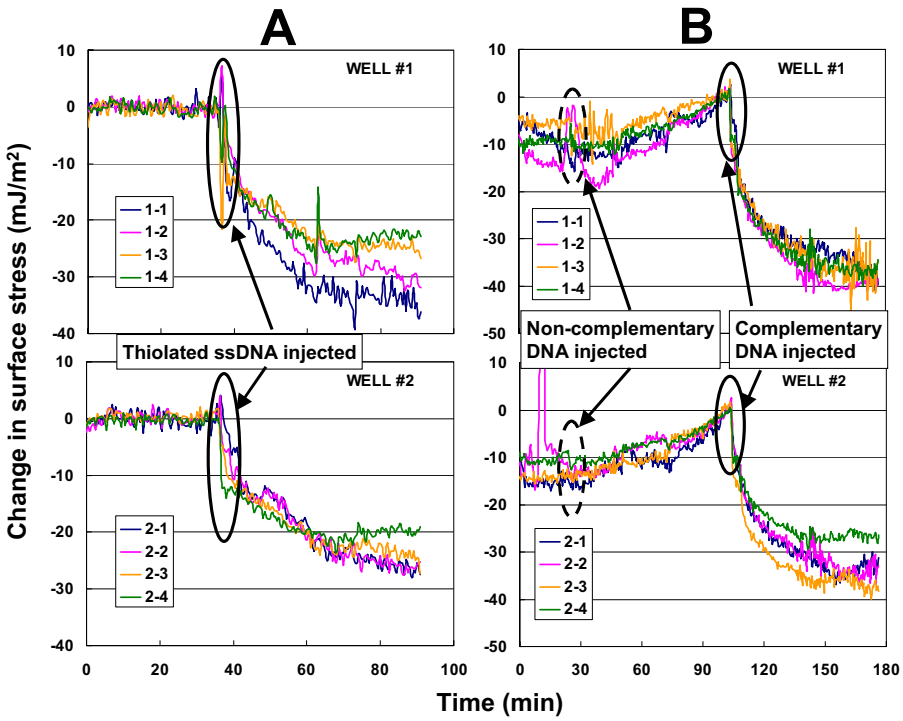

Figure 6 : Deflections of eight cantilevers plotted as a function of time for: (A) DNA immobilization in wells 1 and 2, each well containing four cantilevers; (B) DNA hybridization in the two wells. Dashed circles represent the injection of non-complementary DNA. Solid circles represent the injection of complementary DNA.

Highly pure human free prostate specific antigen (fPSA) and its specific mouse anti-human antibody (MAH-PSA) were chosen for demonstration of multiplexed antibody-antigen binding assay (Fitzgerald, MA). 3,3' -Dithiobis[sulfo-succinimidylpropionate] (DTSSP) was the cross-linker used to immobilize the antibody to the gold surface of the cantilevers (Pierce, IL). Citrate buffer (5mM, pH 5.0) was used for DTSSP immobilization. Phosphate buffer saline (PBS, pH 7.5) was used in the rest of protein experiments. 2-[Methoxy(polyethylenoxy)propyl]trimethoxysilane (PEG-silane) was immobilized on the nitride surface of the cantilevers to block the non-specific absorption (Gelest, PA). D-Salt excellulose desalting columns were used to purify the DNA and protein samples (Pierce, IL).

3.2 Methods and results

A chip was cleaned in acetone and isopropanol sequentially and was gently rinsed with deionized (DI) water before an experiment. The chip was then equilibrated in either phosphate or citrate buffer for DNA hybridization or antibody-antigen binding assay.

3.2.1 DNA hybridization assay

The DNA samples arrived in a freeze-dried form and were first hydrated in phosphate buffer. Dithiothreitol (DTT) was added into the thiolated ssDNA solution for 15-minute resuspension to cleave the di-sulphide bond. The mixture was then passed through excellulose columns to remove DTT. The DNA was dilute to the desired concentration of 5μM in phosphate buffer thereafter. A PDMS-covered chip was used in this assay. The thiolated-ssDNA solution was injected to multiple wells. Each well contained 3-5 cantilevers equilibrated already in phosphate buffer immediately after the majority of the phosphate buffer in the wells was aspirated out by a micropipette. The motion of the cantilevers in multiple wells was monitored simultaneously. The binding of the thiolated-DNA to the gold surface through the nearly covalent Au-S bond, which is also called immobilization, resulted in a surface stress change of approximately 25$\pm$5 mJ/m^2 (Figure 6A). The cantilevers in multiple wells were washed several times and re-equilibrated in phosphate buffer after 2-hour in-

cubation in thiolated-ssDNA solution. The 8μM non-complementary DNA was first injected into the wells in which the cantilevers were functionalized with the thiolated-ssDNA. Only marginal deflection was observed for the non-specific binding (Figure 6B). The 5μM complementary DNA was injected to these wells after an hour or so. The specific binding between the DNA strands caused significant deflection of all the cantilevers, corresponding to the surface stress change of 35$\pm$5 mJ/m^2. These experiments clearly demonstrated that ssDNA immobilization and DNA hybridization on the gold surface induced significant cantilever deflection while the deflection from non-specific binding was almost negligible. As evident in Fig. 6, both reaction steps produced repeatable deflections from the four cantilevers within the same well. Furthermore, the cantilevers in different wells also showed the same degree of deflections, indicating the well-to-well consistency.

Figure 7 summarizes the quantitative experimental results obtained for DNA hybridization, with DNA of different length and for different target concentrations. Each point represents the average value of the hybridization signals obtained from multiple cantilevers and the error bar is the standard deviation of the signals. The number in the parenthesis next to each point is the number of the cantilevers from which the signals were obtained. It is very clear that the hybridization at lower target DNA concentration caused smaller deflection of the cantilevers, which indicates the equilibrium of the DNA hybridization reaction depended on the DNA concentrations. Figure 7 also shows that the hybridization between longer DNA single-strands resulted in larger deflection of the cantilevers, which suggests the total free energy reduction in longer DNA's hybridization is more than that of the shorter ones.

These experiments clearly demonstrate the capability of the multiplexed cantilever chip to quantitatively detect biomolecular interactions. The platform is allowing us to rapidly search the parameter space of DNA hybridization, and thus help us understand the origin of nanomechanical forces that lead to cantilever deflection, as well as the dependence of such deflection on the identity and concentration of the target molecules.

3.2.2 Protein interaction assay

MAH-PSA and fPSA were run through DSalt columns to remove sodium azide from the stocking solution. The ni-

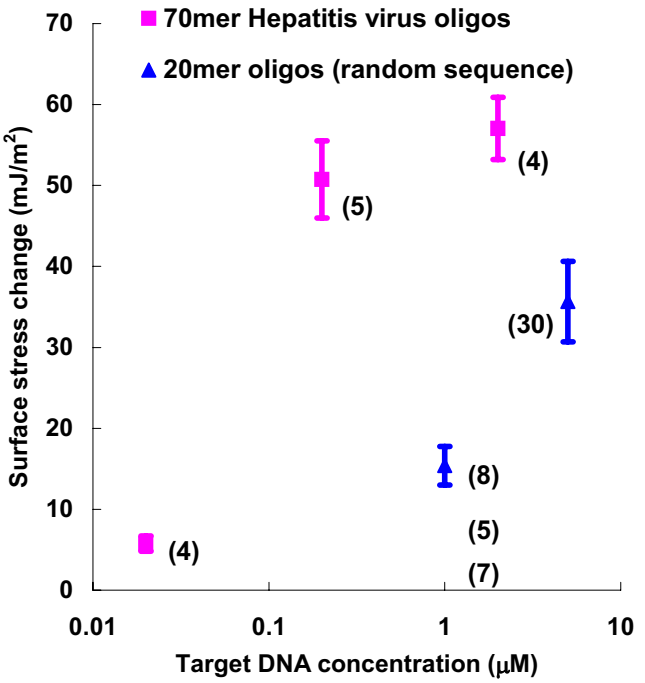

Figure 7 : Summary of quantitative cantilever response to DNA hybridization plotted as a function of target DNA concentration. The numbers in the parenthesis denotes the number of reaction used for statistical analysis.

tride surface of the chip was first coated with PEG-silane which were attached to the nitride surface using silane chemistry, and were used to block non-specific absorption of proteins [Papra *et al*. (2001)]. DTSSP of 2mM in citrate buffer was injected to the multiple wells of the chip previously equilibrated in citrate buffer. Cantilevers were kept in the solution for 2 hours to allow a self-assembly monolayer of DTSSP formed on the gold surface of the cantilevers. The chip was then washed three times with citrate buffer and three times with PBS. Immediately after the wash, 100 μg/ml MAH-PSA in PBS was injected to the wells. After incubating in the antibody solution for 5 hours to maximize the cross-reaction between DTSSP and the antibody, the chip was washed again with PBS and was equilibrated in PBS before the analyte was injected. The cantilevers functionalized with MAH-PSA deflected negligibly after the injection of human serum albumin (HSA), which only non-specifically binds the antibody (Figure 8A). Upon the injection of 20-μg/ml fPSA to the other two wells, specific binding between the antibody and the antigen resulted in the surface

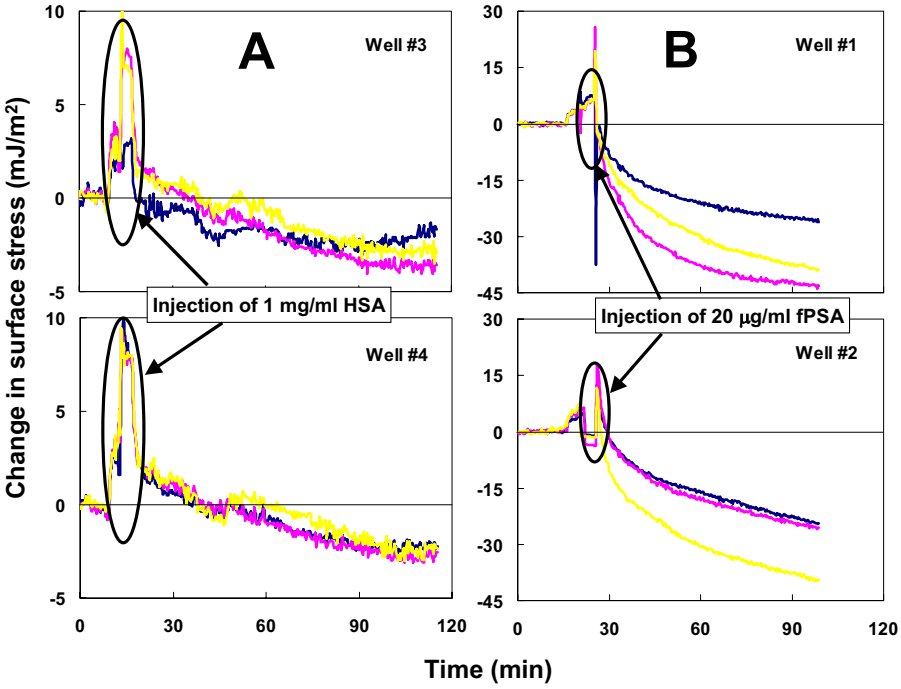

Figure 8 : Deflections of eight cantilevers plotted as a function of time for: (A) DNA immobilization in wells 1 and 2, each well containing four cantilevers; (B) DNA hybridization in the two wells. Dashed circles represent the injection of non-complementary DNA. Solid circles represent the injection of complementary DNA.

stress change of 30 ± 10 mJ/m^2 (Figure 8B). These experiments clearly demonstrated multiplexed protein interaction assay using the microcantilever array.

3.3 Discussion

The variation in the deflection of cantilevers could be due to several reasons. From one fluid well to another, there might be differences in local environment conditions, like salt concentration and sample concentration, which are known to be critical to the reaction. This is very likely because all the aspiration and injection operations were done manually. On the other hand, there is intrinsic non-uniformity in every fabrication step. The non-uniformity of the chip itself could easily cause the different conditions in different wells, as well as the variations in cantilevers' surface condition and mechanical properties. Finally, in the case of antigen-antibody binding, the antibodies were randomly oriented on the cantilever surface. The surface chemistry for immobilization has to be optimized in protein binding assay so that the antibody could be ideally oriented for best specific binding. Efforts are underway to make the cantilever array system a practical tool for rapid mechanical analysis of biomolecular reactions.

Although only simultaneous 8-10 reactions are reported here, more than 20 reactions can be conducted concurrently. By integrating an automated fluid injection system with current array, it would be possible to conduct 100 binding reactions simultaneously.

4 Summary and Outlook

In this paper, we report the development of a cantilever array chip for high-throughput mechanical analysis of biomolecular reactions. Because the response of a cantilever sensor is based on the free energy reduction of biomolecular reactions, this approach forms a universal platform for studying all biomolecular reactions in a label-free manner. Here we demonstrate this by reporting multiplexed DNA hybridization and antigen-antibody binding assays.

The universality of the cantilever biosensor and its mechanical interface with biomolecules are unique among many biosensors. This provokes the question: Besides DNA hybridization and antigen-antibody binding, what else can this be used for? It is clear that other specific

binding reactions, such as DNA-protein, RNA-protein, and protein-protein binding, can be studied. What is more intriguing though are enzymatic reactions that are involved in signaling, such as phosphorylation by kinases, dephosphorylation by phosphatases, metabolism by proteases, etc. There are several questions that remain unanswered. Would the addition or removal of phosphate groups on a substrate attached to the cantilever produce deflections? Would this result from the ionic effects, or from the fact that the substrate protein may undergo conformational changes? Could this be used for studying small molecule inhibitors? If so, can the cantilever array play any role in drug discovery and development? Another fascinating question is whether proteins involved in energy conversion can be studied. These include motors such as DNA and RNA polymerase, ATPase, kinesin, myosin etc. Clearly these molecules symbolize the connection between mechanical motion and chemistry. Can this be exploited by cantilever sensors for detecting minute quantities of specific biomolecules or for microscopic actuation? Finally, there is reason to believe that mechanical interactions of cells can also be studied using cantilever sensors. This opens up issues regarding cell-cell and cell-molecule interactions, which have implications on various biological functions.

We believe that we are at a very early stage of development and understanding with regards to cantilever biosensors. The development of the cantilever array chip reported and demonstrated here is likely to expedite the exploration of various aspects of the mechanics and chemistry of biomolecules.

Acknowledgement: We are thankful for the support from the Innovative Molecular Analysis Technologies (IMAT) program of the National Cancer Institute (NIH), the DARPA SIMBIOSYS program, the Basic Energy Sciences, Department of Energy, and the National Science Foundation. We would like to thank Michael Hagan and Arup Chakraborty of Chemical Engineering Department at University of California, Berkeley; Ram Datar and Richard Cote of Pathology Department at University of Southern California; Karolyn Hansen, Jerry Hu and Thomas Thundat of Oak Ridge National Lab; Frank Chen, Lawrence Berkeley National Laboratory. Their suggestions were very helpful. We would also like to thank the Microfabrication Laboratory at University of California, Berkeley for providing all the facilities of the microfabrication.

References

Afanassiev, V.; Hanemann, V.; Wolfl, S. (2000): Preparation of DNA and protein micro arrays on glass slides coated with an agarose film. *Nucleic Acids Research*, vol. 28, E66.

Angenendt, P., Glökler, J., Konthur, Z.; Lehrach, H.; Cahill, D. J. (2003): 3D protein microarrays: performing multiplex immunoassays on a single chip. *Anal. Chem.*, vol. 75, pp. 4368-4372.

Berger, R.; Delamarche, E.; Lang, H.; Gerber, C.; Gimzewski, J. K.; Meyer, E.; Güntherodt, H. J. (1997): Surface stress in the self-assembly of alkanethiols on gold. *Science*, vol. 276, pp. 2021-2024.

Duffy, D. C.; McDonald, J. C.; Schueller, O. J.; Whitesides, G. M. (1998): Rapid prototyping of microfluidic systems in poly(dimethylsiloxane). *Anal. Chem.*, vol. 70, pp. 4974-4984.

Fritz, J.; Baller, M. K.; Lang, H. P.; Rothuizen, H.; Vettiger, P.; Meyer, E.; Güntherodt, H. J.; Gerber, C.; Gimzewski, J. K. (2000): Translating biomolecular recognition into nanomechanics. *Science*, vol. 288, pp. 316-318.

Guschin, D.; Yershov, G.; Zaslavsky, A.; Gemmell, A.; Shick, V.; Proudnikov, D.; Arenkov, P.; Mirzabekov, A. (1997): Manual manufacturing of oligonucleotide, DNA, and protein microchips. *Analytical Biochemistry*, vol. 250, pp. 203-211.

Haab, B. B.; Dunham, M. J.; Brown, P. O. (2001): Protein microarrays for highly parallel detection and quantitation of specific proteins and antibodies in complex solutions. *Genome Biology*, vol. 2, pp. R4.1-4.13.

Hagan, M. F.; Majumdar, A.; Chakraborty, A. K. (2002): Nanomechanical forces generated by surface grafted DNA. *J. Phys. Chem. B*, vol. 106, pp. 10163-10173.

Hansen, K.; Ji, H.; Wu, G.; Datar, R.; Cote, R.; Majumdar, A. (2001): Cantilever-based optical deflection assay for discrimination of DNA single-nucleotide mismatches. *Analytical Chemistry*, vol. 73, pp. 1567-1571.

MacBeath, G.; Schreiber, S. L. (2000): Printing proteins as microarrays for high-throughput function determination. *Science*, vol. 289, pp. 1760-1763.

Marie, R.; Jensenius, H.; Thaysen, J.; Christensen,

C. B.; Boisen, A. (2002): Adsorption kinetics and mechanical properties of thiol-modified DNA-oligos on gold investigated by microcantilever sensors", *Ultramicroscopy*, vol. 91, pp. 29-36.

McDonnell, J. M. (2001): Surface plasmon resonance: towards an understanding of the mechanisms of biological molecular recognition. *Curr. Opin. Chem. Biol.*, vol. 5, pp. 572-577.

Papra, A.; Gadegaard, N.; Larsen, N. B. (2001): Characterization of ultrathin poly(ethylene glycol) monolayers on silicon substrates. *Langmuir*, vol. 17, pp. 1457-1460.

Raiteri, R.; Nelles, G.; Butt, H-J.; Knoll, W.; Skladal, P. (1999): Sensing biological substances based on the bending of microfabricated cantilevers. *Sensors Actuators B,* vol. 61: pp. 213–217.

Savran, C. A.; Knudsen, S. M.; Ellington, A. D.; Manalis, S. R. (2004): Micromechanical detection of proteins using aptamer-based receptor molecules. *Anal. Chem.*, vol. 76, pp. 3194-3198.

Satyanarayana, S.; Karnik, R.; Majumdar, A. (2004): Stamp and Stick Room Temperature Bonding Technique for Microdevices. *J. MEMS*, in press.

Sutherland, A. J. (2002): Quantum dots as luminescent probes in biological systems. *Curr. Opin. Solid State & Materials Sci.*, vol. 6, pp. 365-370.

Thundat, T.; Oden, P. I.; Warmack, R. J. (1997): Microcantilever sensors. *Microscale Thermophys. Eng.,* vol. 1, pp. 185–199.

Thundat, T.; Majumdar, A. (2003): Microcantilevers for Physical, Chemical, and Biological Sensing. In *Sensors and Sensing in Biology and Engineering*, eds. F. G. Barth, J.A.C. Humphrey, T.W. Seecomb, Springer-Verlag, New York.

Tyers, M.; Mann, M. (2003): From genomics to proteomics. *Nature*, vol. 422, pp. 193-197.

Wiese, R.; Belosludtsev, Y.; Powdrill T.; Thompson, P.; Hogan, M. (2001): Simultaneous multianalyte ELISA performed on a microarray platform. *Clinical Chemistry*, vol.47, pp. 1451-1457.

Wilkins, M. R.; Pasquali, C.; Appel, R. D.; Ou, K.; Golaz, O.; Sanchez, J. C.; Yan, J. X.; Gooley, A. A.; Hughes, G.; HumpherySmith, I.; Williams, K. L.; Hochstrasser, D. F. (1996): From proteins to proteomes: Large scale protein identification by two-dimensional electrophoresis and amino acid analysis. *Biotechnology*, vol. 14, pp. 61-65.

Wu, G.; Ji, H.; Hansen, K.; Thundat, T.; Datar, R.; Cote, R.; Hagan, M. F.; Chakraborty, A. K.; Majumdar, A. (2001a): Origin of nanomechanical cantilever motion generated from biomolecular interactions. *Proceedings of National Academy of Science*, vol. 98, pp. 1560-1564.

Wu, G.; Datar, R.; Hansen, K.; Thundat, T.; Cote, R.; Majumdar, A. (2001b): Bioassay of prostate specific antigen (PSA) using microcantilevers. *Nature Biotechnology*, vol. 19, pp. 856-860.

Zhu, H.; Bilgin, M.; Bangham, R.; Hall, D.; Casamayor, A.; Bertone, P.; Lan, N.; Jansen, R.; Bidlingmaier, S.; Houfek, T.; Mitchell; T., Miller, P.; Dean, R. A.; Gerstein, M.; Snyder, M. (2001): Global analysis of protein activities using proteome chips. *Science*, vol. 293, pp. 2101-2105.

Zhu, H.; Klemic, J. F.; Chang, S.; Bertone, P.; Casamayor, A.; Klemic, K. G.; Smith, D.; Gerstein, M.; Reed, M. A.; Snyder, M. (2000): Analysis of yeast protein kinase using protein chips. *Nature Genetics*, vol. 26, pp. 283-289.