Nanomechanical Detection of DNA Melting on Microcantilever Surfaces

Sibani Lisa Biswal,† Digvijay Raorane,† Alison Chaiken,‡ Henryk Birecki,‡ and Arun Majumdar*,†

Department of Mechanical Engineering, UC Berkeley, Berkeley, California 94720, and HP Labs, Palo Alto, California 94306

We observe surface stress changes in response to thermal dehybridization, or melting, of double-stranded DNA (dsDNA) oligonucleotides that are grafted on one side of a microcantilever beam. Changes in surface stress occur when one complementary DNA strand melts and diffuses away from the other, resulting in alterations of the electrostatic, counterionic, and hydration interaction forces between the remaining neighboring surface-grafted DNA molecules. We have been able to distinguish changes in the melting temperature of dsDNA as a function of salt concentration and oligomer length. This technique also highlights differences between surface immobilized and solution DNA melting dynamics, which allows us to better understand the stability of DNA on surfaces. The transduction of phase transitions into a mechanical signal is ubiquitous for DNA, making cantilever-based detection a widely useful and complementary alternative to calorimetric and fluorescence measurements.

Configuration and conformational changes in biomolecules, including phase transitions, play a critical role in many biological processes. Given that they are driven by free energy reductions that usually involve enthalpy changes, they are often detected by calorimetric measurements.[1] Differential scanning calorimetry has shown to effectively measure the melting temperature (T_m) and enthalpy of homogeneous and heterogeneous DNA strands.[2] Despite the fact that enthalpy microarrays[3] have been developed for multiplexed calorimetry, thermal insulation of a calorimeter from its surroundings becomes increasingly difficult for small samples where the surface-to-volume ratio increases.

Calorimetry is also used as a label-free technique to study specific biomolecular binding reactions and has been widely employed in applications such as drug discovery.[4,5] The detection of specific biomolecular interactions in complex mixtures is also very important for disease diagnosis, genomics, and proteomics research. Most current approaches that use ligand−receptor binding, such as DNA microarrays and enzyme-linked immunosorbent assays, rely on the labeling of samples with a fluorescent or radioactive tag.[6,7] For small molecules, the tag could significantly change the chemistry or structure of the molecule itself, thereby motivating label-free approaches. Label-free methods include the surface plasmon resonance[8] and mass-based detection using a quartz crystal microbalance.[9,10] Most recently, microcantilever sensors have been shown to detect surface stress changes upon specific ligand−receptor binding, and thereby forming a new way for label-free detection.[11−16] What has remained elusive is whether such sensors can be used to study phase transitions or conformational changes such as those observed by calorimetry. In this paper, we use surface-grafted DNA as a model system to demonstrate that it is indeed possible.

The increasing number of applications for surface-grafted DNA has spurred a surge of activity aimed at increasing the understanding of the properties of DNA as a thin film. Of particular interest is the analysis of the melting temperature of DNA bound to surfaces. In this configuration, DNA has physical properties different from those of the bulk material due to interfacial interactions and effects of molecular confinement.[17−22] Many groups have used surface-bound DNA analysis to detect single-

* Corresponding author. E-mail: majumdar@me.berkeley.edu.
† UC Berkeley.
‡ HP Labs.

(1) Cooper, A.; Johnson, C. M.; Lakey, J. H.; Nollmann, M. *Biophys. Chem.* **2001**, *93*, 215−230.
(2) Riccelli, P. V.; Hall, T. S.; Pancoska, P.; Mandell, K. E.; Benight, A. S. *J. Am. Chem. Soc.* **2003**, *125*, 141−150.
(3) Torres, F. E.; Kuhnt, P.; De Bruyker, D.; Bell, A. G.; Wolkin, M. V.; Peeters, E.; Williamson, J. R.; Anderson, G. B.; Schmitz, G. P.; Recht, M. I.; Schweizer, S.; Scott, L. G.; Ho, J. H.; Elrod, S. A.; Schultz, P. G.; Lerner, R. A.; Bruce, R. H. *Proc. Natl. Acad. Sci. U. S. A.* **2004**, *101*, 9517−9522.
(4) Cooper, M. A. *Anal. Bioanal. Chem.* **2003**, *377*, 834−842.
(5) Perozzo, R.; Folkers, G.; Scapozza, L. *J. Recept. Signal Transduction* **2004**, *24*, 1−52.
(6) Mansfield, E. S.; Worley, J. M.; McKenzie, S. E.; Surrey, S.; Rappaport, E.; Fortina, P. *Mol. Cell. Probes* **1995**, *9*, 145−156.
(7) Stimpson, D. I.; Hoijer, J. V.; Hsieh, W. T.; Jou, C.; Gordon, J.; Theriault, T.; Gamble, R.; Baldeschwieler, J. D. *Proc. Natl. Acad. Sci. U. S. A.* **1995**, *92*, 6379−6383.
(8) Homola, J.; Yee, S. S.; Gauglitz, G. *Sens. Actuators, B* **1999**, *54*, 3−15.
(9) Babacan, S.; Pivarnik, P.; Letcher, S.; Rand, A. G. *Biosens. Bioelectron.* **2000**, *15*, 615−621.
(10) Bailey, L. E.; Kambhampati, D.; Kanazawa, K. K.; Knoll, W.; Frank, C. W. *Langmuir* **2002**, *18*, 479−489.
(11) Hansen, K. M.; Ji, H. F.; Wu, G. H.; Datar, R.; Cote, R.; Majumdar, A.; Thundat, T. *Anal. Chem.* **2001**, *73*, 1567−1571.
(12) Wu, G. H.; Ji, H. F.; Hansen, K.; Thundat, T.; Datar, R.; Cote, R.; Hagan, M. F.; Chakraborty, A. K.; Majumdar, A. *Proc. Natl. Acad. Sci. U. S. A.* **2001**, *98*, 1560−1564.
(13) Yue, M.; Lin, H.; Dedrick, D. E.; Satyanarayana, S.; Majumdar, A.; Bedekar, A. S.; Jenkins, J. W.; Sundaram, S. *J. Microelectromech. Syst.* **2004**, *13*, 290−299.
(14) Tian, F.; Hansen, K. M.; Ferrell, T. L.; Thundat, T. *Anal. Chem.* **2005**, *77*, 1601−1606.
(15) Thundat, T.; Majumdar, A. In *Sensors and Sensing in Biology and Engineering*; Springer-Verlag: New York, 2003.
(16) Marie, R.; Jensenius, H.; Thaysen, J.; Christensen, C. B.; Boisen, A. *Ultramicroscopy* **2002**, *91*, 29.
(17) Peterlinz, K. A.; Georgiadis, R. M.; Herne, T. M.; Tarlov, M. J. *J. Am. Chem. Soc.* **1997**, *119*, 3401−3402.
(18) Peterson, A. W.; Heaton, R. J.; Georgiadis, R. *J. Am. Chem. Soc.* **2000**, *122*, 7837−7838.

10.1021/ac052171y CCC: $33.50
Published on Web 00/00/0000

nucleotide polymorphisms (SNPs) for genomic information.[23,24] Dynamic allele-specific hybridization is a novel SNP detecting method based on allele-specific hybridization followed by dynamic heating and simultaneous monitoring of the denaturation profile using fluorescently labeled probes or intercalating dyes.[25] The melting temperature of the sequence containing a SNP is lower than fully hybridized strands. One recent paper has reported combining molecular beacons with microfluidics to study the thermal dehybridization of surface-bound DNA.[26] Other studies using gold nanoparticles have shown sharper and higher melting transitions when using a nanoparticle−DNA aggregate or a nanoparticle/complementary strand/surface-bound DNA complex compared to conventional fluorophore probe studies.[27,28]

We propose using microcantilever sensors to detect DNA melting by heating the microcantilevers while monitoring the cantilever deflection. When biomolecules are immobilized onto one surface of the cantilever, changes in the surface stress on that side of the cantilever induce bending. Stoney's formula relates the change in surface stress to the change in cantilever deflection:[29]

$$\Delta h = \frac{3\Delta\gamma(1-\nu_1)L^2}{E_1 t_1^{\,2}} \qquad (1)$$

where $\Delta\gamma$ is the change in surface stress, ν_1 is Poisson's ratio for the thick layer, E_1 is Young's modulus for the thick layer, t_1 is the thickness of the thick layer, L is the length of the cantilever, and Δh is the deflection of the cantilever. The advantage of microcantilevers is that they allow detection of phase transitions in the steady state without the use of a labeling probes or costly reagents. We use this concept of surface stress change to monitor the melting of surface immobilized DNA. As the temperature of the cantilever is raised, there is a conformational change of the DNA molecules as it undergoes a transition from double- to single-stranded, resulting in a change in the surface stress of the layer. The cantilever either shrinks due to tensile stress or expands due to compressive stress. Berger et al. has demonstrated a similar detection of phase transitions using alkanethiols.[30] In this paper, we have utilized the thermally induced bending phenomenon to study phase transitions in DNA films on microcantilevers for varying DNA lengths and solution ionic strengths.

(19) Peterson, A. W.; Heaton, R. J.; Georgiadis, R. M. *Nucleic Acids Res.* **2001**, *29*, 5163−5168.
(20) Peterson, A. W.; Wolf, L. K.; Georgiadis, R. M. *J. Am. Chem. Soc.* **2002**, *124*, 14601−14607.
(21) Petrovykh, D. Y.; Kimura-Suda, H.; Whitman, L. J.; Tarlov, M. J. *J. Am. Chem. Soc.* **2003**, *125*, 5219−5226.
(22) Meunier-Prest, R.; Raveau, S.; Finot, E.; Legay, G.; Cherkaoui-Malki, M.; Latruffe, N. *Nucleic Acids Res.* **2003**, *31*.
(23) Mao, H. B.; Holden, M. A.; You, M.; Cremer, P. S. *Anal. Chem.* **2002**, *74*, 5071−5075.
(24) Mukhopadhyay, R.; Lorentzen, M.; Kjems, J.; Besenbacher, F. *Langmuir* **2005**, *21*, 8400−8408.
(25) Prince, J. A.; Feuk, L.; Howell, W. M.; Jobs, M.; Emahazion, T.; Blennow, K.; Brookes, A. J. *Genome Res.* **2001**, *11*, 152−162.
(26) Dodge, A.; Turcatti, G.; Lawrence, I.; de Rooij, N. F.; Verpoorte, E. *Anal. Chem.* **2004**, *76*, 1778−1787.
(27) Jin, R. C.; Wu, G. S.; Li, Z.; Mirkin, C. A.; Schatz, G. C. *J. Am. Chem. Soc.* **2003**, *125*, 1643−1654.
(28) Taton, T. A.; Mirkin, C. A.; Letsinger, R. L. *Science* **2000**, *289*, 1757−1760.
(29) Stoney, G. G. *Proc. R. Soc. London, Ser. A* **1909**, *82*, 172−175.
(30) Berger, R.; Gerber, C.; Gimzewski, J. K.; Meyer, E.; Guntherodt, H. J. *Appl. Phys. Lett.* **1996**, *69*, 40−42.

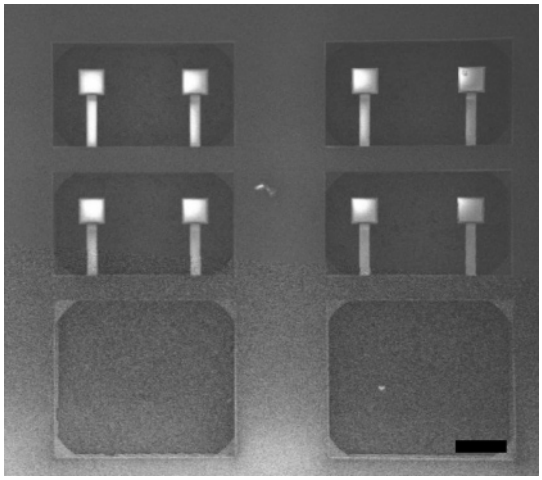

Figure 1. SEM picture of microcantilver array chip used in melting experiments. Scale bar, 200 μm.

Denaturation of DNA is induced by using heat or chemical treatments to separate the double helix into two single strands. This process is often thought to be cooperative so that the AT-rich regions melt first, destabilizing the neighboring helical structure. The separated strands can either rehybridize or undergo a coil-to-globule transition in solution.[31] The melting temperature, T_m, of bulk DNA in solution is well determined by spectroscopy, and many empirical formulas have been developed to account for both the ionic strength and the mole fraction of G−C base pairs.[32] However, the environment of oligomers can differ between a solid interface and the bulk solution due to difference between ionic strengths. We use the cantilevers to highlight the differences between T_m in solution and on a solid support.

MATERIALS AND METHODS

Microcantilever Array. Preparation of the microcantilever array has been described previously.[13] A low-pressure chemical vapor deposition of low-stress silicon nitride and thermal evaporation of chromium and gold layers is performed on a silicon wafer. Each cantilever sensor consists of a released silicon nitride beam that is 500 nm thick, 40 μm wide, and 200 μm long. A rigid paddle structure is fabricated at the end of the beam to provide a flat surface that assists with the optical detection of the beam's reflection. On one side of the beam, there is a 5-nm chromium layer, which is used to facilitate the adhesion of a 25-nm-thick gold layer. The gold layer provides a reflective surface as well as an interface for attaching functional probe molecules. A Pyrex wafer containing wet-etched microfluidic chambers is bonded to the chip using a UV curing adhesive (Norland 121). The 2-cm^2 cantilever chip shown in Figure 1 is made up of a 15 × 6 array of individual fluid chambers, each of which contains four cantilevers. We average the responses of the four cantilevers to increase the accuracy of the measurements.

To image the cantilever array, a helium−neon laser (632.8 nm, 15 mW) is expanded to illuminate the entire chip as it sits in a temperature-controlled holder. All reflections pass through a beam splitter, which directs half the reflected intensity from the

(31) Marky, L. A.; Kallenbach, N. R.; McDonough, K. A.; Seeman, N. C.; Breslauer, K. J. *Biopolymers* **1987**, *26*, 1621−1634.
(32) Blake, R. D.; Delcourt, S. G. *Nucleic Acids Res.* **1998**, *26*, 3323−3332.

Table 1. DNA Sequences Used and Corresponding Calculated Using OligoAnalyzer 3.0 in SciTools (http://www.idtdna.com/)

name	sequence	$T_{m,calculated}$ (°C) 25 mM Na^+	at 50 mM Na^+	at 100 mM Na^+
ls15	5′-thiol-CTACTAAATACAAAT-3′	24.7	30.6	35.8
comp15	5′-ATTTGTATTTAGTAG-3′			
ls20	5′-thiol-TTTTTTTTATTCAATTTATT-3′	30.2	36.7	42.1
comp20	5′- AATAAATTGAATAAAAAAAA-3′			
ls25	5′thiol-TTTTTTTTATTCATTTTACTTTTTT-3′	37.1	43.8	49.9
comp25	5′-AAAAAAGTAAAATGAATAAAAAAA A-3′			
ss20	5′thiol- GTGGTAGATGAAGGTGAGAG −3′	46.1	51.9	
dcomp20	5′- CTCTCACCTTCATCTACCAC −3′			

cantilever array onto a CCD camera chip (Apogee Inc., 3072 × 2048 pixels, 16 bit). The frame capture rate is 1 frame every 10 s. Once aligned with the optical system, individual cantilever reflections appear as spots in the CCD image. A Matlab particle tracking program tracks each cantilever paddle image by calculating the intensity centroid of the spot.

The mismatch between the thermal expansion of the silicon nitride and the gold causes the well-known thermal biomorph effect. For each experiment, the thermomechanical response of each cantilever is simultaneously measured in units of CCD pixels/°C. This value is later used to normalize the cantilever bending induced by biomolecular reactions. A white-light interferometer (Veeco) is then used to measure the thermomechanical response of the cantilevers (in nm/°C). As described previously,[23] this interferometry provides the calibration (pixel/nm) between motion of the laser spot on the CCD image and the absolute cantilever deflection in nanometers. The deflection of the cantilevers is then used to calculate the surface stress caused by biomolecular reactions using Stoney's formula. The previously measured thermomechanical sensitivity of the cantilevers is 208 nm/K with a standard deviation of 14 nm/K.[13] In terms of surface stress, a 1 K change in temperature corresponds to a stress change of 24.5 mJ/m^2.

Before an experiment, a chip is cleaned in 2-propanol, rinsed with deionized water, and equilibrated in phosphate buffer for at least 15 min. Then the chip is mounted in the chip holder with a temperature controller (model LCP-3215, Wavelength Electronics). The temperature controller has a control band of ∼10 mK. The temperature of the chip holder is monitored using a thermocouple temperature recorder (VWR). The cantilever deflection due to a temperature change of 1 °C is recorded to establish the thermomechanical sensitivity of each cantilever. Using this measurement, the movement of the spot reflection in response to a surface reaction can be converted into a surface stress change.

Oligonucleotide Immobilization. To prevent nonspecific binding to the silicon nitride side of the cantilever, a PEG-silane coating is prepared. A solution of 100 μL of 2-methoxy(polyethylenoxy)propyltrimethoxysilane (Gelest Inc.) with 40 μL of HCl in 50 mL of toluene is sonicated for 15 min. The chip is incubated in the solution overnight.

Four different capture singl-stranded (ss) DNA probes and their complementary (Integrated DNA Technologies (IDT), Coralville, IL) strands were used for the measurements. As shown in Table 1, the sequences differ in length and melting temperatures. The ssDNA has a thiol group attached to the 5′-end through a $(CH_2)_6$ linker for covalent attachment to the gold surface of the cantilever. DNA disulfide groups were reduced with 0.1 M dithiothreitol (DTT), and the excess DTT was removed using a NAP-10 column. The oligonucleotide attachment on the cantilever was done by injecting each 1.5-μL well with 1.0 μL of 1 μM thiolated ssDNA in 50 mM phosphate-buffered saline (PBS; pH 7.0). The chip was held at room temperature during the adsorption experiments. The cantilever response was monitored for 1 h after exposure to the ssDNA solution to confirm immobilization. The chip was then left to incubate overnight with the solution.

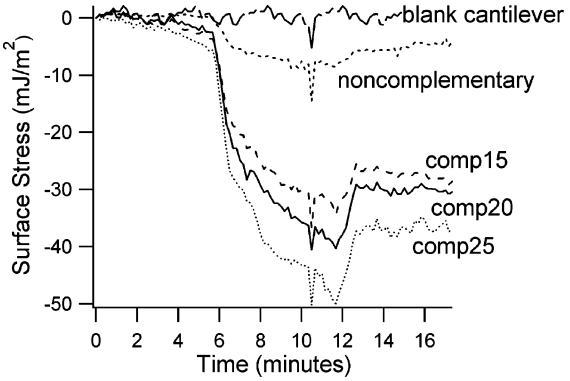

Figure 2. Hybridization results for three sets of DNA. We see that there is no surface stress change on the blank cantilever, a minimal amount when noncomplementrary DNA is added, and a surface stress change in the range of 30−40 mJ/m^2 when complementary target DNA is added.

Hybridization/Dehybridization Experiments. After immobilization, each well is rinsed with a 50 mM PBS buffer several times to remove unattached oligonucleotides. The wells were then injected with the complementary sequence dissolved in a 50 mM PBS buffer. As controls, plain buffer and a solution with a noncomplementary sequence were injected into different wells. Again, the chip was held at room temperature during the hybridization experiments. The cantilever response was monitored for ∼1 h to ensure successful hybridization had occurred. After this time, the wells are washed with PBS buffer at the desired ionic strength and covered with a poly(dimethylsiloxane) piece to prevent evaporation of the solution from the wells.

Melting curves are acquired by slowly ramping up the temperature to anywhere between 25 and 50 °C at a rate of ∼1 °C/min and simultaneously measuring the temperature using the

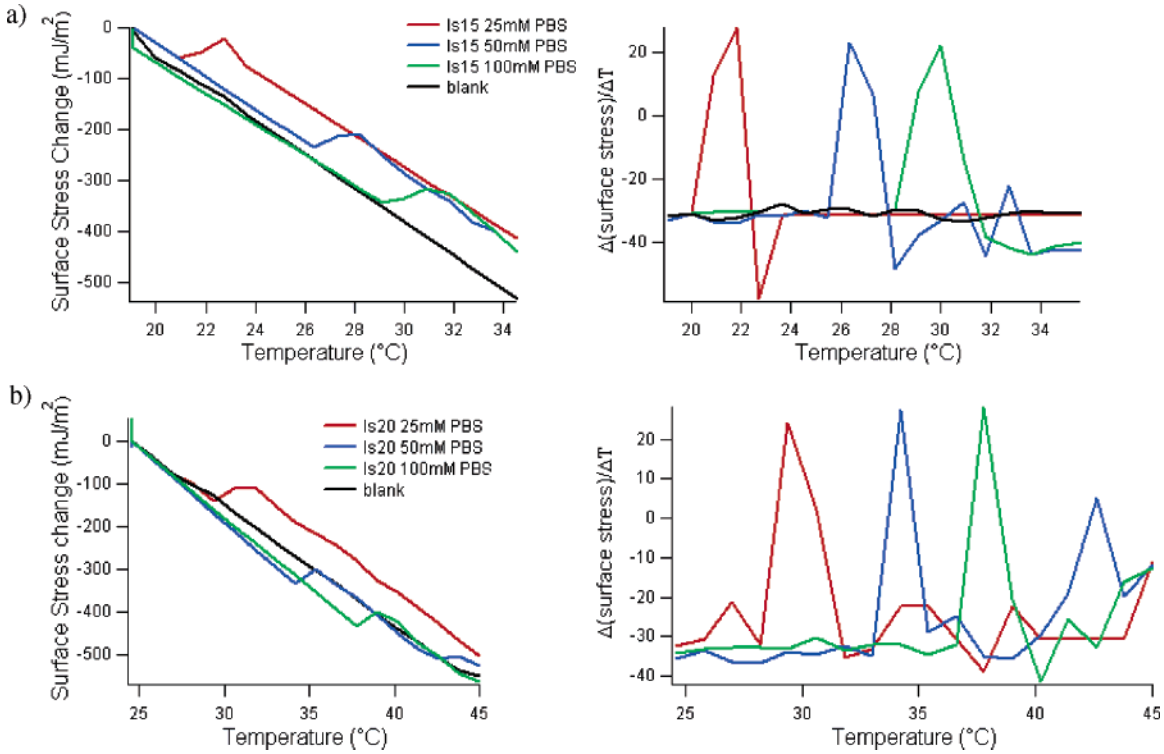

Figure 3. Surface stress vs temperature at various buffer salt concentrations and the derivative of the surface stress with respect to temperature are shown for (a) Is15 and (b) Is20. The melting temperature can be determined by the position of the peaks.

thermocouple recorder. The temperature range is limited to ~20 °C in our optical system. Once the temperature change is greater than 20 °C, the spots become difficult to track because they fade as they go out of focus. For this reason, we align the optics to accommodate the desired temperature range. The total run time is ~20 min.

Repeated measurements were performed on the same immobilized oligonucleoetides. The wells were rinsed with PBS buffer to remove the dehybridized DNA probes leaving the ssDNA strands on the surface.

RESULTS AND DISCUSSION

Immobilization/Hybridization Experiments. As previously shown, cantilever deflection results from an increase in surface stress due to hybridization of DNA strands to complementary DNA strands on the surface.[13] Cantilever deflection as a function of time for the hybridization of target ssDNA to surface immobilized probes is shown in Figure 2. The injection of the sample occurs at $t = 6$ min. The matching sequence hybridizes and produces a change in surface stress of 30−40 mJ/m^2, which in turn leads to bending of the cantilever. The system is sensitive to external disturbances as seen by the spike at $t = 10$ min, but stabilizes afterward. The small decrease in the signal at $t = 12$ min is associated with desorption of loosely bound oligonucleotides. Hybridization between the immobilized probe and the matching target causes the cantilever to bend away from the gold surface. To confirm that the deflection signals are caused by DNA hybridization, 5 μM noncomplementary ssDNA is also injected into a different well for comparison. As shown in Figure 2, injection of noncomplementary ssDNA produces only a minimal deflection produced after this injection, most likely due to nonspecific interactions of the oligonucleotides with the functionalized microcantilever surfaces. The blank cantilevers in wells containing only buffer do not bend when the target sequence is injected. Therefore, those cantilevers serve as reference sensors.

Previously, it was observed that probe immobilization and hybridization on microcantilevers depends on a variety of factors such as probe length, probe surface density, and ionic strength of solution.[33,34] The complex parameter dependence is due to the interplay of forces, including short-range chemical interactions of covalent gold/thiol attachment and long-range electrostatic repulsion and hydration forces between DNA strands. When considering ionic strengths, at low ionic strengths, fewer probes adsorb because of the larger electrostatic repulsion between probe strands. In high ionic strength solutions, the electrostatic repulsions between probe molecules are effectively screened, and higher probe coverage can be reached limited only by hydration interactions. The changes in film thickness due to hybridization also affect the surface stress on the microcantilevers. When single strands are immobilized, the strands are in a more coiled and compact conformation. Upon introduction and subsequent hybridization of complementary target DNA strands, the double-stranded DNA takes on a rodlike conformation, which results in an increase in the DNA film thickness.[35] We observe this increase in film thickness as an increase in surface stress on the microcantilevers surface. In our experiments, we kept both the immobilization and hybridization conditions fixed at 50 mM buffer solution. Previous studies[36] have shown that these conditions

(33) Hagan, M. F.; Majumdar, A.; Chakraborty, A. K. *J. Phys. Chem. B* **2002**, *106*, 10163−10173.
(34) Hagan, M. F.; Chakraborty, A. K. *J. Chem. Phys.* **2004**, *120*, 4958−4968.
(35) Steel, A. B.; Levicky, R. L.; Herne, T. M.; Tarlov, M. J. *Biophys. J.* **2000**, *79*, 975−981.
(36) Castelino, K.; Kannan, B.; Majumdar, A. *Langmuir* **2005**, *21*, 1956−1961.

result in a grafting density of probe immobilization of 0.03−0.05 probes/nm^2.

Melting Experiments. We use the microcantilevers to measure the melting temperature, T_m, for each oligonucleotide in three different ionic strength solutions: 25, 50, and 100 mM. In the presence of buffer solution, the cantilever deflects away from the gold surface with increasing temperature due to the thermal biomorph effect. This effect is also seen with cantilevers bound with DNA. However, there are discontinuities in the melting profile at varying locations depending on the buffer salt concentration. We associate these discontinuities with phase transitions in the surface-bound DNA films. As shown in Figure 3, the deflection data for a blank cantilever, which has been normalized by its thermomechanical response and converted to surface stress versus temperature, display a characteristic linear response to temperature change. Also shown in Figure 3, cantilevers with DNA display sharp transitions in the surface stress data that deviate from a linear response. By plotting the derivative of the surface stess with respect to temperature ($d\delta/dT$), we find the temperature at which the cantilever response deviates from linearity, which we attribute to DNA melting. The abrupt change in the cantilever deflection is characteristic of a two-state melting transition.[37] The melting temperature of untethered complexes is calculated using the following thermodynamic relationship: $T_m = \Delta H°/(\Delta S° + R \ln C_t)$, where the changes in standard enthalpy (ΔH) and entropy (ΔS) associated with duplex formation are calculated using nearest-neighbor thermodynamic parameters, R is the gas constant, and C_t is the concentration of the oligonucleotide.[38,39] These thermodynamic parameters and T_m are calculated using OligoAnalyzer 3.0 in SciTools (http://www.idtdna.com/). The calculated melting temperatures are expected to be accurate within a range of 2 °C.

Figure 4a summarizes the results for different oligonucleotides at varying salt concentrations. As expected, increasing salt concentration results in an increase in the melting temperature. A similar trend is also observed for increasing oligonucleotide length due to a well-known increase in the stability of the duplexes at higher salt concentrations and greater oligonucleotide lengths.[40−42] If we compare the two 20-mer oligonucleotides, represented by the diamond and circles in Figure 4a, we find that ss20, which is the more GC-rich sequence, melts at a higher temperature than ls20, which is more AT-rich DNA sequence. This is due to the fact that GC base pairs have three hydrogen bonds, while AT base pairs have only two.[39]

In general, we observe that surface-bound complexes melt at lower temperatures than their bulk melting temperatures as shown in Figure 4b, in which the difference between $T_{m,calculated}$ and $T_{m,experimental}$ has been plotted as a function of salt concentration for each of the oligonucleotides. We believe that this reduction in melting temperature of surface-bound DNA results from

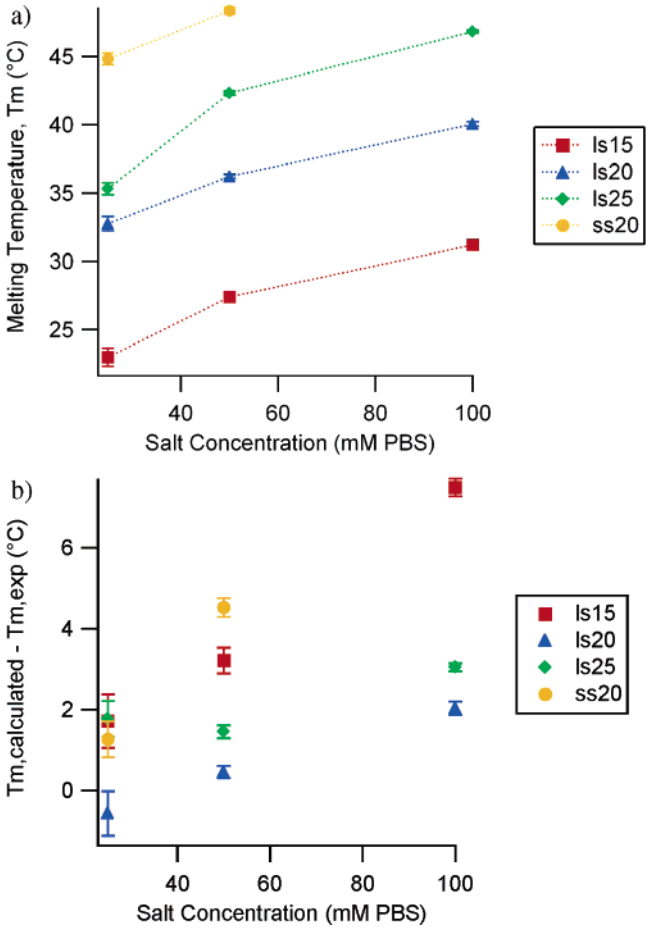

Figure 4. Summary of cantilever melting experiments given by salt concentration. (a) Average experimental melting temperature for each oligonucleotide at 25, 50, and 100 mM ionic strength solutions. (b) Difference between the calculated T_m and the experimental T_m for each oligonucleotide at 25, 50, and 100 mM ionic strength solutions. Error bars show standard deviation from 16 cantilever experiments.

duplexes being less stable on a surface compared to solution because of the difference between DNA dynamics in a bulk environment as compared to dynamics of DNA near an interface. Another interesting observation seen in Figure 4b is that the difference between $T_{m,calculated}$ and $T_{m,experimental}$ becomes more pronounced at higher ionic strengths, which has also been previously reported by Peterlinz et al.[17] We suspect that the enhancement of this destabilization on a surface is likely caused by two effects: the interactions of the strands with the surface and the interactions between neighboring strands. When considering the effect of the surface, the main difference is that access to the immobilized oligomer for hybridization is sterically restricted due to its attachment to the surface. Confining DNA to the surface involves both energetic and entropic effects, with the balance between the two trends controlled by the stiffness of the chain and the length scale of confinement.[40] In the bulk solution, interactions between neighboring strands are oftentimes negligible and the denaturation events are considered independent of each other. However, on a surface, there are interactions between neighboring oligonucleotide strands that must be taken into account. To consider the effects of neighboring strands, we must consider the packing density of probes on the cantilever. For our experiments, the surface coverage is ~0.03 probes/nm^2. So the

(37) Klump, H. H. In *Studies in Modern Thermodynamics 8: Biochemical Thermodynamics,* 2 ed.; Jones, M. N., Ed.; Elsevier: Amsterdam, 1988; pp 100−144.
(38) Sugimoto, N.; Nakano, S.; Yoneyama, M.; Honda, K. *Nucleic Acids Res.* **1996**, *24*, 4501−4505.
(39) Owczarzy, R.; Vallone, P. M.; Gallo, F. J.; Paner, T. M.; Lane, M. J.; Benight, A. S. *Biopolymers* **1997**, *44*, 217−239.
(40) Tikhomirova, A.; Taulier, N.; Chalikian, T. V. *J. Am. Chem. Soc.* **2004**, *126*, 16387−16394.
(41) Rouzina, I.; Bloomfield, V. A. *Biophys. J.* **1999**, *77*, 3242−3251.
(42) Bond, J. P.; Anderson, C. F.; Record, M. T. *Biophys. J.* **1994**, *67*, 825−836.

area occupied by each strand is ~33 nm^2, with an effective radius of 3.3 nm. Podgornik et al.[43,44] showed that arrays of DNA have strong repulsions between neighbors as the intermolecular distance is reduced below 3.2 nm. It is apparent that the thermodynamic stability of the DNA complex is dependent on nearest-neighbor interactions from ssDNA and nearby dsDNA. We are currently investigating the interactions between neighboring strands by probing the effects of surface coverage. When the temperature is reduced back to room temperature, we do not observe the rehybridization signal on the cantilever although we have performed successful melting scans after a 24-h wait for rehybridization. We believe that the lack of rehybridization is due the diffusion of the melted oligonucleotides into the bulk solution.

CONCLUSION

We demonstrate that changes in surface stress in a microcantilever beam can be used to detect melting of surface-grafted DNA. Melting causes ones of the strands to dehybridize and diffuse away from the cantilever surface, leaving the surface with only single-stranded DNA, thereby modifying the force interaction between neighboring strands. Melting temperature decreases with chain length and salt concentration and is lower for surface-grafted DNA than for DNA in solution.

The purpose of using DNA in this project was to demonstrate the concept of utilizing the cantilever surface stresses for studying phase transitions. We feel, however, that the method will be applicable to the study of phase transitions in other biomolecules as well and more generally for study molecular configurational and conformational changes in any surface-grafted biomolecular systems. Such a label-free approach could be potentially very valuable in a variety of applications such as quantifying enzymatic activity and measuring the kinetics of ligand−receptor binding.

(43) Strey, H. H.; Parsegian, V. A.; Podgornik, R. *Phys. Rev, Lett.* **1997**, *78*, 895−898.
(44) Strey, H. H.; Podgornik, R.; Rau, D. C.; Parsegian, V. A. *Curr. Opin. Struct. Biol.* **1998**, *8*, 309−313.

ACKNOWLEDGMENT

This work is supported by the Center for Information Technology Research in the Interest of Society (CITRIS) through a grant provided by HP Labs.

Received for review December 8, 2005. Accepted May 15, 2006.

AC052171Y