Observation of an anomalous mass effect in microcantilever-based biosensing caused by adsorbed DNA

Seonghwan Kim,[1] Dechang Yi,[1] Ali Passian,[1,2] and Thomas Thundat[1,2,3,a)]
[1]*Biosciences Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37831, USA*
[2]*Department of Physics, University of Tennessee, Knoxville, Tennessee 37996, USA*
[3]*Department of Mechanical, Aerospace and Biomedical Engineering, University of Tennessee, Knoxville, Tennessee 37996, USA*

(Received 28 January 2010; accepted 23 March 2010; published online 15 April 2010)

Quantifying adsorbed mass using resonance frequency variation in a microcantilever is an established technique. However, when applied to adsorbed mass determination in liquids, the resonance frequency variations represent several contributions. While the discrepancy between the apparent and real adsorbed mass is negligible for measurements in air, it can be significant in liquids. Here we present an anomalous effect of adsorbed DNA on the resonance frequency of microcantilevers which cannot be explained using current models. Our findings suggest that the measured frequency shifts may be explained on the basis of a hydrodynamic interaction caused by the adsorbed molecules on the cantilever. © *2010 American Institute of Physics*.
[doi:10.1063/1.3399234]

Measurement of adsorbed mass using resonating devices offers one of the most sensitive techniques, approaching single-molecule detection.[1,2] Recently, cantilever-based mass determination using molecular adsorption-induced changes in the resonance frequency has been attracting much attention.[3] Extremely high sensitivity for the determination of adsorbed mass has been demonstrated for cantilever sensors, where for example attogram level mass resolution has been frequently reported.[4,5] However, when the sensor operates in a fluid, the observed resonance frequency shift is overshadowed by fluid coupling with the vibrating cantilever. Here we refer the determined adsorbed mass including that caused by the participating fluid as added apparent mass (AAM).[6] The AAM depends on the material properties of the fluid such as density and viscosity, as well as the dynamic attributes of the oscillator such as the resonance frequencies in the fluid medium. In gases at ordinary pressures, the effect of the participating fluid is often very small. Based on our preliminary experiments, the variations in the resonance frequency of a cantilever in vacuum and air are less than 0.9%. On the contrary, the resonance frequency of a microcantilever is much decreased when the cantilever is operated in liquid. Although it has been reported that the resonance frequency of a cantilever in liquid is much lower than its value in air, few direct experiments have been reported on the delineation of the effect of surrounding liquid on the *in situ* measurement of adsorbed mass.[7–11] While a number of works have been emerging on the effect of fluid on the dynamics of the cantilever, little attention has been paid to the consequences of adsorbate-induced effects for biosensing measurements.

In this paper, we report on the anomalous contribution of a monolayer of single stranded DNA (ssDNA) on the resonance frequency measurements in liquid and discuss the implications of the technique in experimental determination of adsorbed mass using cantilevers operating in liquid. This investigation was motivated by the observation that during real-time adsorption of ssDNA on a cantilever immersed in water (under a flow of 2mL/h), the determined amount of ssDNA based on the resonance frequency shifts was an order of magnitude larger than that of the amount of ssDNA based on the number of adsoprtion sites available on the cantilever surface. Adsorption of a monolayer of ssDNA with less than 10 nm thickness is not expected to drastically affect the resonance frequencies due to flexural rigidity change. Thus, this observation shows that a monolayer of ssDNA adsorbed on the cantilever can significantly affect the resonance frequencies. The fluid-solid interface, in the absence of molecular adsorption, imposes both a roughness and a material dependence on the dynamics of the system. For an ideal surface, approximate models can account for the observed dynamics, at least qualitatively.[12] Under the process of ssDNA adsorption, the interface is modified and as such one may consider this modification to effectively yield a new set of boundary conditions. Therefore, we conjecture the observed frequency change to be due to a local hydrodynamic mechanism in the adsorbed layer.

Adsorption of ssDNA has been well investigated by cantilever sensor platforms.[13–15] As summarized in Table I, we used commercially available silicon microcantilevers. Three different types of tipless silicon microcantilevers were selected for experiments (see Table I for their physical layouts,[16] material properties, and fundamental resonance frequencies in air at 20 °C). The cantilevers were cleaned with acetone, isopropyl alcohol, methanol, and ethanol for 10 min in a sequential manner. Using electron beam evaporation, all cantilevers were coated with 20 nm of gold on both sides with 5 nm of chromium as adhesion layer. For each cantilever, the reference spring constant k was calibrated from the method described by Sader *et al.*[17] The selected microcantilevers were mounted on a liquid-compatible cantilever holder of an atomic force microscope (MFP-3D-BIO™, Asylum Research Inc., Santa Barbara, CA). In order to maintain a uniform liquid temperature, a closed fluid cell was used with the BioHeater™ System (Asylum

[a)]Author to whom correspondence should be addressed. Electronic mail: thundattg@ornl.gov.

 © 2010 American Institute of Physics

Downloaded 15 Apr 2010 to 128.219.49.8. Redistribution subject to AIP license or copyright; see http://apl.aip.org/apl/copyright.jsp

TABLE I. Physical dimensions and properties of three different tested tipless microcantilevers (MikroMasch Inc., Tallinn, Estonia) for a temperature of $T_o = 20$ °C.

	Dimensions					Physical properties		
Cantilever	L (μm)	b (μm)	h (μm)	L/b	Material	ρ (kg m^{-3})	k (N m^{-1})	f_{air} (kHz)
NSC12-A	104	35	2	2.97	Si	2 320	10.71	235.5
			0.005		Cr	7 140		
			0.02		Au	19 300		
NSC12-B	83	35	2	2.37	Si	2 320	20.52	365.0
			0.005		Cr	7 140		
			0.02		Au	19 300		
NSC12-C	124	35	2	3.54	Si	2 320	6.48	167.7
			0.005		Cr	7 140		
			0.02		Au	19 300		

Research Inc.) that provides temperature uniformity within ±0.1 °C. The thermal noise spectra, the fundamental resonance frequencies, and the quality factors of 30 microcantilevers from the same batch were measured in the experiments.

The adsorption experiments were carried out using 20mer ssDNA oligomers modified with thiol chains at the 5′ end [5′-HS-$(CH_2)_6$-ACTTCAACTTCAACTTCAAC-3′] (Oligos Etc., Wilsonville, OR). Thiolated oligomers were supplied in lyophilized form and were dissolved in deionized water containing 10 mM dithiothreitol (DTT) to cleave the disulfide functionality. Prior to exposure of thiolated ssDNA to fresh gold surfaces, the DTT was removed by running the ssDNA solution over a NAP™-5 desalting column (GE Healthcare, Piscataway, NJ). The concentration of the thiolated ssDNA was typically 10 μM. The immobilization was achieved by incubating the cantilevers in a thiolated ssDNA solution for 1, 16, 40, and 65 h to control the amount of adsorbed ssDNA or grafting density of ssDNA on the cantilever. After each incubation time step, the cantilevers were thoroughly washed with deionized water to remove physisorbed ssDNA and frequency measurements were performed both in air and deionized water, respectively.

Recent studies have shown that the resonance frequencies of a microcantilever in air can also be affected by adsorption-induced surface stress,[18–20] flexural rigidity change due to adsorbate,[21] and position of added mass.[22] Furthermore, it is known that the frequency response of a microcantilever in liquid is significantly influenced by hydrodynamic loading.[12] Previously, we reported that the immobilization of the thiolated ssDNA molecules resulted in a surface stress of approximately 20 mN/m.[15] Considering the nominal thickness of the cantilevers used in the present work, we expect negligible resonance frequency shifts from ssDNA adsorption-induced surface stress and flexural rigidity change.[19,21,23]

Figure 1 shows the measured resonance frequency shifts from the reference frequency in air and the determined AAM as functions of incubation time. Assuming evenly distributed adsorbates on the cantilever surfaces, the AAM of ssDNA molecules in air, δm_{air}, can be calculated from the simple approximate equation, as follows:[10]

$$\delta m_{air} = -2m_c \frac{\Delta f_{exp}}{f_0}, \tag{1}$$

where, m_c is the cantilever mass, Δf_{exp} is the measured resonance frequency shift, and f_0 is the initial resonance frequency of a microcantilever in air.

Figure 2 shows the measured resonance frequency shifts from the reference frequency in deionized water as functions

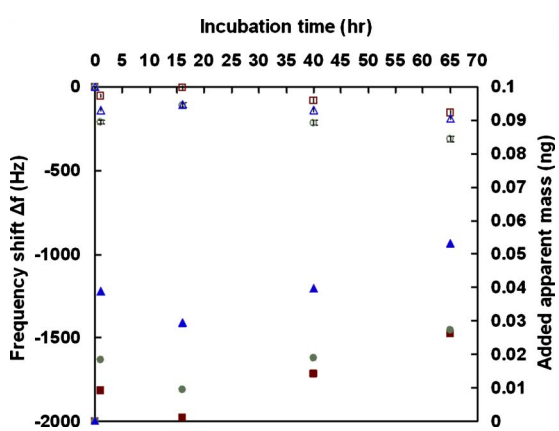

FIG. 1. (Color online) Measured fundamental resonance frequency shifts and the determined AAM in air as a function of incubation time for the three tested cantilevers. Each open symbol represents the averaged frequency shifts from 10 measurements, with the error bar corresponding to the standard deviation and each solid symbol represents the determined AAM in air: NSC12-A (red squares), NSC12-B (green circles), and NSC12-C (blue triangles).

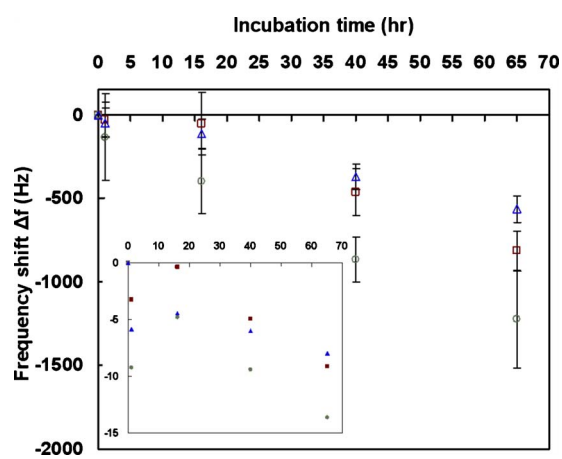

FIG. 2. (Color online) Measured fundamental resonance frequency shifts and the predicted resonance frequency shifts based on Sader's viscous model in deionized water as a function of incubation time for the three tested cantilevers. Each open symbol represents the averaged frequency shifts from 10 measurements, with the error bar corresponding to the standard deviation and each solid symbol in the inset figure represents the predicted resonance frequency shifts: NSC12-A (red squares), NSC12-B (green circles), and NSC12-C (blue triangles).

Downloaded 15 Apr 2010 to 128.219.49.8. Redistribution subject to AIP license or copyright; see http://apl.aip.org/apl/copyright.jsp

of incubation time and the predicted resonance frequency shifts based on Sader's viscous model.[12] Before calculating theoretical resonance frequency shifts, we note that currently no model exists for the dynamic response of an adsorbate-modified microcantilever in a viscous fluid. When assuming a homogeneously smooth thin elastic film of ssDNA of mass δm_{air} is formed on the cantilever surfaces and this film does not affect the flexural rigidity of the microcantilever, Sader's viscous model can be employed to analytically predict the frequency response of a microcantilever in a viscous fluid. Since our experiments were carried out in air and liquid at temperature T, to obtain the corresponding vacuum resonance frequency $\omega_{\mathrm{vac},1}$ of a cantilever of mass m_c, we introduced an empirical mass correction factor for the vacuum resonance frequency to obtain the following:

$$\omega_{\mathrm{vac},1}(T) = \omega_{\mathrm{vac}}(T)\sqrt{\frac{m_c}{m_c + \delta m_{\mathrm{air}}}}, \tag{2}$$

where the vacuum frequency ω_{vac} is calculated from the modified SHO model by evaluating the hydrodynamic function Γ at the measured fundamental resonance frequency in air ω_{air}.[12]

$$\omega_{\mathrm{vac}}(T) = \omega_{\mathrm{air}}(T)\sqrt{1 + \frac{\pi\rho b^2}{4\mu_c}\Gamma_r[\omega_{\mathrm{air}}(T)]}, \tag{3}$$

where, ρ is a fluid density, b is a width of the cantilever, μ_c is the mass per unit length of the cantilever. Following Eq. (3) in Green and Sader,[24] we calculated the resonance frequency shifts Δf_{theo} in deionized water. Large discrepancies up to two orders of magnitude between the predictions and measurements were identified in this case.

Although previous studies explained this discrepancy on the basis of an increase in the hydrophilicity of a cantilever surface due to biomolecular interactions[10,11] that affect hydrodynamic loading and thus change the resonance frequency of the cantilever, we believe that such hydrophilic effects appear insufficient to explain the two orders of magnitude variations in resonance frequency shifts. To investigate the origin of the anomalous resonance frequency shifts measured in liquid, we studied the normalized resonance frequency shifts, $\Delta f_{\mathrm{exp}}/f$, in air and deionized water for all cantilevers as a function of incubation time.[25] Measurements in air imply that most thiolated ssDNA molecules adsorb on the cantilever surfaces in the early stages of incubation and the amount of adsorption slightly increases for long incubation time. However, frequency shifts measured in deionized water dramatically decrease for long incubation time. This observation is in line with a recent study where for long incubation time, adsorbed thiolated ssDNA molecules were reported to change configuration from an initial lying down on the surface to a standing-up, exposing sites for further immobilization.[26] Therefore, we primarily attribute our observations to such molecular rearrangements and configuration changes which we believe induce additional inertial and dissipative hydrodynamic loading on the adsorbate-modified microcantilever.

In conclusion, we have shown that a monolayer of adsorbed ssDNA on a cantilever operating under solution significantly decreases the resonance frequency due to increased hydrodynamic effect. We postulate that such variations are primarily due to molecular rearrangements and configuration changes in the adsorbed thiolated ssDNA causing additional hydrodynamic loading in addition to the DNA-induced increased interfacial hydrophilicity. An increase in the surface roughness due to the nanoscale distribution of the ssDNA causes a larger fluid coupling and the amount of the fluid participating in the coupling causes an additional inertial and dissipative hydrodynamic loading. Therefore, current *in situ* measurement of adsorbed biomolecular mass in liquids can only provide qualitative information or an evidence of adsorption rather than a quantitative measure of the adsorbed mass. The process is further complicated since the adsorption-induced surface roughness and condition can be different for different biomolecules.

This research at ORNL's CNMS was sponsored by the Scientific User Facilities Division, OBES, U.S. DOE. ORNL is managed by UT-Battelle, LLC for the U.S. DOE under Contract No. DE-AC05-00OR22725.

[1] K. L. Ekinci and M. L. Roukes, Rev. Sci. Instrum. **76**, 061101 (2005).
[2] Y. T. Yang, C. Callegari, X. L. Feng, K. L. Ekinci, and M. L. Roukes, Nano Lett. **6**, 583 (2006).
[3] P. S. Waggoner and H. G. Craighead, Lab Chip **7**, 1238 (2007).
[4] M. Li, H. X. Tang, and M. L. Roukes, Nat. Nanotechnol. **2**, 114 (2007).
[5] J. Verd, A. Uranga, G. Abadal, J. Teva, F. Torres, F. Perez-Murano, J. Fraxedas, J. Esteve, and N. Barniol, Appl. Phys. Lett. **91**, 013501 (2007).
[6] We note that the term AAM has appeared in the literature [C. A. Van Eysden and J. E. Sader, J. Appl. Phys. **100**, 114916 (2006)] to describe the fluid inertial loading. Here, we use this term to describe the real adsorbed mass plus the inertial and dissipative loading due to the hydrodynamic interaction between biological molecules on cantilever and fluid.
[7] T. Braun, V. Barwich, M. K. Ghatkesar, A. H. Bredekamp, Ch. Gerber, M. Hegner, and H. P. Lang, Phys. Rev. E **72**, 031907 (2005).
[8] A. P. Davila, J. Jang, A. K. Gupta, T. Walter, A. Aronson, and R. Bashir, Biosens. Bioelectron. **22**, 3028 (2007).
[9] K. Rijal and R. Mutharasan, Anal. Chem. **79**, 7392 (2007).
[10] T. Kwon, K. Eom, J. H. Park, D. S. Yoon, T. S. Kim, and H. L. Lee, Appl. Phys. Lett. **90**, 223903 (2007).
[11] T. Kwon, K. Eom, J. Park, D. S. Yoon, H. L. Lee, and T. S. Kim, Appl. Phys. Lett. **93**, 173901 (2008).
[12] J. E. Sader, J. Appl. Phys. **84**, 64 (1998).
[13] K. M. Hansen, H. Ji, G. Wu, R. Datar, R. Cote, A. Majumdar, and T. Thundat, Anal. Chem. **73**, 1567 (2001).
[14] M. Su, S. Li, and V. P. Dravid, Appl. Phys. Lett. **82**, 3562 (2003).
[15] S. Jeon, N. Jung, and T. Thundat, Sens. Actuators B **122**, 365 (2007).
[16] Using scanning electron microscopy, we measured the length L and the width b of the cantilevers, while data on the thickness h was provided by the manufacturers (with 15% variations from the nominal value).
[17] J. E. Sader, I. Larson, P. Mulvaney, and L. R. White, Rev. Sci. Instrum. **66**, 3789 (1995).
[18] G. Y. Chen, T. Thundat, E. A. Wachter, and R. J. Warmack, J. Appl. Phys. **77**, 3618 (1995).
[19] P. Lu, H. P. Lee, C. Lu, and S. J. O'Shea, Phys. Rev. B **72**, 085405 (2005).
[20] M. J. Lachut and J. E. Sader, Phys. Rev. Lett. **99**, 206102 (2007).
[21] J. Tamayo, D. Ramos, J. Mertens, and M. Calleja, Appl. Phys. Lett. **89**, 224104 (2006).
[22] D. Lee, S. Kim, N. Jung, T. Thundat, and S. Jeon, J. Appl. Phys. **106**, 024310 (2009).
[23] D. Ramos, M. Arroyo-Hernández, E. Gil-Santos, H. D. Tong, C. V. Rijin, M. Calleja, and J. Tamayo, Anal. Chem. **81**, 2274 (2009).
[24] C. P. Green and J. E. Sader, J. Appl. Phys. **98**, 114913 (2005).
[25] See supplementary material at http://dx.doi.org/10.1063/1.3399234 for supporting data.
[26] J. Mertens, C. Rogero, M. Calleja, D. Ramos, J. A. Martin-Gago, C. Briones, and J. Tamayo, Nat. Nanotechnol. **3**, 301 (2008).

Downloaded 15 Apr 2010 to 128.219.49.8. Redistribution subject to AIP license or copyright; see http://apl.aip.org/apl/copyright.jsp