Robust microfabricated field-effect sensor for monitoring molecular adsorption in liquids

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We show that a microfabricated field-effect sensor located at the terminus of a freestanding cantilever can detect surface potential changes resulting from the adsorption of charged molecules in an aqueous environment. The charge sensitive region, defined by lightly doped silicon, is embedded within the heavily doped silicon cantilever. Since both the electrical trace and sensitive region are passivated with thermally diffused silicon dioxide, the entire cantilever can be immersed in buffer solutions and cleaned with strong acids without degrading its electrical response. As an example, we demonstrate that the device can reproducibly detect adsorption of positively charged poly-L-lysine (PLL) on silicon dioxide. We also demonstrate that PLL adsorption and *p*H can be measured in discrete solutions by scanning the cantilever through parallel, distinct streams within a microfluidic channel array. © *2001 American Institute of Physics.* [DOI: 10.1063/1.1423776]

Molecular adsorption at the solid–liquid interface plays an important role in various areas of industry and research ranging from water purification systems[1] to coatings for contact lenses and biomedical implants,[2] up to state-of-the-art biosensors such as high-density DNA arrays[3] and protein chips.[4] For many of these areas, it is desirable or even necessary to measure molecular adsorption on a glass or quartz surface in real time without labeling the molecules for detection. This is most commonly accomplished by detecting the change in the refractive index due to molecular adsorption with techniques such as ellipsometry,[5] scanning angle reflectometry (SAR),[6] or optical grating wavemode light spectroscopy (OWLS).[7] However, these techniques are difficult to miniaturize for high throughput screening assays or for *in vivo* applications. One recent approach for detecting unlabeled biomolecules with a microfabricated device is to monitor the mechanical bending of cantilevers due to molecular binding at their surfaces.[8]

We are currently exploring the capabilities of microfabricated potentiometric sensors for electrical monitoring of molecular adsorption. Electrical detection has the advantage of providing direct access to interfacial parameters such as surface potential or surface charge densities. In addition, microelectronic devices are well suited for parallel detection and have the potential to be packaged for low volume analysis with simple readout circuitry.

The scanning probe potentiometer (SPP)[9] is a field-effect device that consists of an electrolyte-insulator-semiconductor (EIS) structure. This capacitive structure achieves a similar surface potential resolution[10] to the ion-sensitive field-effect transistor (ISFET),[11] but requires only one electrical connection to the silicon portion of the device. The extent of the depletion region in the silicon portion of the device, which provides a measurement of the potential at the electrolyte–insulator interface, is typically measured in two ways. The first, demonstrated by the light addressable potentiometric sensor (LAPS),[12] measures the current of photogenerated carriers drifting in the high electric field of the depletion region. The second measures the depletion region capacitively, by applying a small ac voltage to the bias voltage.[13] We employ the latter. The SPP miniaturizes the EIS concept from the millimeter scale to the micrometer scale and integrates it onto an atomic force microscope cantilever. In previous work,[9] we have shown that the SPP can profile the *p*H of nanoliter volumes in a plurality of discrete solutions. These measurements were accomplished by submerging the entire cantilever in microfluidic channels with widths below 100 μm. The cantilever design allows the sensor to be rapidly (<1 s) scanned through many analytes which themselves remain in spatially distinct locations. This enables measurement of reaction dynamics, without complications of volume exchange within a fixed chamber, including adsorptive losses to chamber walls and mechanical disturbances.

The application of field effect sensors to bioanalytical tasks makes evident the need for an EIS sensor that is not only reliable during operation in electrolyte solutions, but also robust to a wide variety of stringent cleaning procedures necessary to run multiple experiments with a single device. Drawing from integrated-circuit design methodology, our previous devices relied on metal electrical traces passivated by plasma enhanced chemical vapor deposition (PECVD) oxide and nitride films along the length of the cantilever. Unfortunately, cleaning with acids can exacerbate defects in imperfect PECVD passivation films, making it difficult to repeat experiments reliably. In this letter, we demonstrate that a SPP with a heavily doped silicon electrical trace passivated with thermally grown silicon dioxide is robust to aggressive cleaning procedures and can reliably monitor the adsorption of poly-L-lysine (PLL). We have verified robustness by measuring similar current–voltage characteristics before and after the entire device is cleaned in 3:1 H_2SO_4:H_2O_2 (piranha). We demonstrate the capability of the SPP to measure molecular adsorption dynamics in a system of microfluidic channels.

Processing begins with double-side-polished silicon-on-

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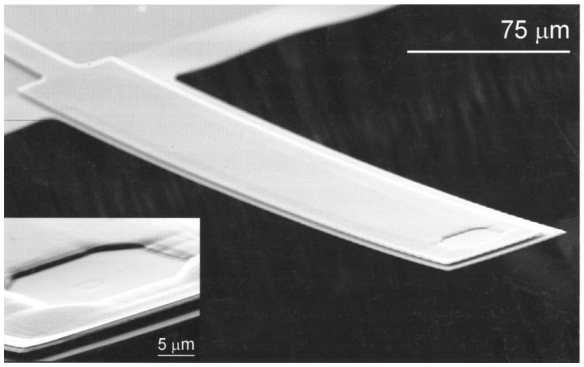

FIG. 1. Scanning electron micrograph of a scanning probe potentiometer. Inset shows detail of sensor region that is 25 μm^2.

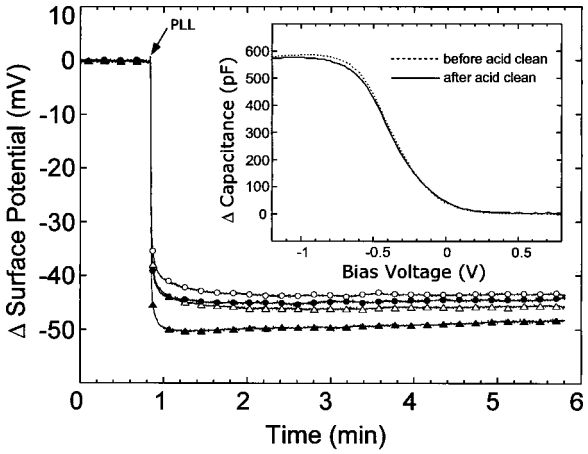

FIG. 2. Change in surface potential vs time during poly-L-lysine adsorption on the silicon dioxide sensor surface. After allowing the system to reach a stable baseline, the PLL solution was injected (arrow) in the fluidic chamber. (△) Sensor A, run 1; (▲) sensor A, run 2. (○) Sensor B, run 1; (●) sensor B, run 2. Inset shows capacitance vs voltage characterization of SPP with $100\times100\ \mu m^2$ sensor region acquired in PBS buffer *p*H 7.5, showing response of the device before and after 60 s clean in piranha solution. Characteristic was measured at 7.9 kHz.

insulator (SOI) substrates. The device layer is initially 2.2 μm thick *p*-type (boron-doped) silicon with 4–6 Ω cm resistivity, and the buried oxide layer is 1.1 μm thick. After masking a small area for the charge sensitive region, the rest of the wafer is implanted with boron to achieve a relatively uniform doping level of $\sim10^{18}$ atoms/cm^3 after the anneal. Electrical traces are defined by patterning the highly doped silicon region. The mask is removed, and a thick (1.1 μm) thermal oxide is grown to passivate the device. The thick oxide is then cleared from the charge sensitive region of the SPP, and replaced with a 100 nm layer of thermally diffused oxide, which forms the final surface of the charge sensitive region. Next, contact cuts are made in the die, and aluminum is deposited and patterned to make contacts. Finally, the cantilevers are released with a deep reactive ion etch.

A scanning electron micrograph of a completed device is shown in Fig. 1. We have fabricated SPPs with active sensing areas ranging from 5 μm on a side to 100 μm on a side and demonstrated similar surface potential resolution for the range of sensors. In a closed fluidic chamber, 50 μV changes are observable in a 1 Hz bandwidth, commensurate with the demonstrated resolution of commercial LAPS devices.[14] The thickness of the passivation oxide and the high doping level of the electrical trace guarantee that the response of the SPP is dominated by the response of the lightly doped active area.

A Ag/AgCl wire is used as the counterelectrode in solution. The device is typically biased such that the lightly doped silicon is depleted to about half of its maximum depletion depth, where the capacitive response to surface potential changes is linear and most sensitive. A 0.1 V ac signal at 1–10 kHz is applied to the bias voltage to generate a charging current. The charging current is amplified with a current amplifier and then its root-mean-square (rms) amplitude is monitored with a lock-in amplifier. Gain and offset are adjusted with a differential amplifier and captured with data acquisition software.

Devices are characterized by measuring differential capacitance versus applied bias voltage ($\Delta C-V$ curve). Figure 2 (inset) shows the $\Delta C-V$ characterization of a device with a $100\times100\ \mu m^2$ sensitive region. The robustness of the passivation layer is demonstrated by the similarity of $\Delta C-V$ characteristics taken before and after cleaning the device for 60 s in piranha.

For biosensing applications, silicon dioxide surfaces are often functionalized with a sensing layer of biomolecules or cells. A standard method for activating glass or silicon dioxide surfaces for further immobilization of biomolecules is to adsorb pure or modified poly-l-lysine.[15] Since PLL is a highly positively charged polypeptide it binds electrostatically to the negatively charged silicon dioxide of the SPP. Here we use the PLL to demonstrate the detection of adsorption of charged molecules with the SPP. The adsorption of the positively charged PLL layer is expected to compensate negative surface charges of silicon dioxide and therefore significantly change the surface potential of the SPP.

The PLL adsorption data in Fig. 2 was taken with devices mounted in a small-volume fluidic chamber. Two small inlet and outlet ports allowed injection of analytes. Data are shown for two trials with each of two devices. For each trial, the sensor surface was first cleaned by piranha solution and then equilibrated in 10 mM HEPES, 5 mM NaCl, *p*H 7.4, for several hours. The sensor signal was calibrated by measuring the response to a 10 mV change in bias potential between the SPP and the Ag/AgCl electrode. First, it was determined that injection of buffer solution did not alter the steady state sensor signal. Next, buffer solution containing 0.1 mg/mL PLL (25 kDa poly-L-lysine·HCl, Sigma, in 10 mM HEPES, 5 mM NaCl, *p*H 7.4) was injected in the fluidic chamber. Figure 2 shows the rapid drop of the surface potential by ~45 mV due to adsorption of PLL to the sensor surface. Within several seconds the surface was saturated and a second injection of the same PLL solution did not further decrease the sensor output. Nor was the sensor response changed by additional injection of buffer solution, indicating that the PLL surface layer is stable and does not desorb significantly in solution. As shown in Fig. 2, these measurements are reproducible to within 5% (±2 mV) among different sensors and even if the sensors were cleaned with a piranha etch between the measurements. In separate experiments, concentrations down to 0.1 μg/mL could be detected. In this case, the time response was slowed down and successive injections of identical concentrations lead to successive drops in surface po-

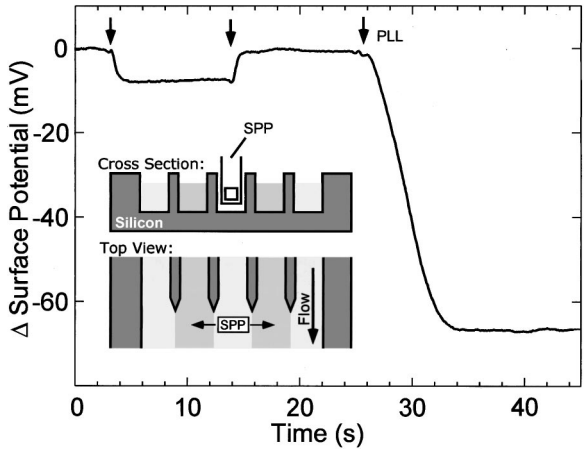

FIG. 3. Change in surface potential vs time during *p*H change, and poly-L-lysine adsorption on sensor in microfluidic channels. (Inset) Schematic views of silicon microfluidic channels. Top side of channels are open, device is partially immersed as shown in cross section. SPP can be scanned among channels in reservoir (shown in top view) without removing it from solution or changing the level to which it is immersed.

tential until the saturation value was reached (data not shown).

With a typical surface charge density of silicon dioxide[16] of 0.8 C/m^2 and a typical surface potential at the electrolyte–insulator interface[17] of approximately −200 mV, we estimated[18] that a surface potential drop of about 50 mV, as we observe with PLL adsorption, corresponds to a change in surface charge density on the order of $10^6 e/\mu m^2$. This number is in good agreement with typical molecular densities of PLL on SiO_2 as determined by radio labeling experiments. Given that the rms noise within each experiment in Fig. 2 was about 150 μV in a 1 Hz bandwidth, we can further estimate the change in surface charge density resolvable with the SPP to be $10^4 e/\mu m^2$ in this bandwidth.

A system of microfluidic channels, shown schematically in Fig. 3 (inset) allow the comparison between the response of the sensor surface to change in *p*H and the response to PLL adsorption shown in Fig. 3. The microfluidic system has the advantage of offering fast switching times between solutions, with a low mechanical settling time. By flowing solutions of different colored dyes through the channels, we have verified that the streams remain parallel and distinct in the reservoir region where we scan the SPP. The device starts in a channel with the baseline buffer, 10 mM HEPES+5 mM NaCl (*p*H 7.62). It is then moved (arrow) to a channel with the same HEPES buffer that has been adjusted with HCL to *p*H 7.12. The *p*H response of this device is about 16 mV/*p*H unit. This sub-Nernstian response indicates that the sensor surface is not fully hydrated, as has often been observed with EIS devices with SiO_2 surfaces.[16,19] At the following arrow, the SPP is introduced into a stream with the baseline buffer (*p*H 7.62). Finally, the device is scanned (arrow) to a stream with 5 μg/mL PLL in the baseline buffer, and a rapid drop in surface potential is seen with adsorption. Switching the device into a stream with the baseline buffer, as well as further introducing the device to the PLL stream do not alter the surface potential after adsorption. The rms noise of the sensor in the microfluidic channels is ~150 μV in a 1 Hz bandwidth.

In conclusion, we have shown that the scanning probe potentiometer can monitor the adsorption of charged molecules. Sensor performance is reliable through multiple experiments and cleaning cycles for more than 90 days, providing robust and reproducible surface charge detection. We anticipate the future use of the SPP to monitor interactions between charged molecules or enzymatic reactions occurring close to the sensor surface. The cantilever design allows independent chemical functionalization of sensors, enabling differential measurements. The direct electrical detection scheme and the miniaturized configuration could lead to the development of parallel sensor arrays for low-volume, high-throughput analysis. The simplicity of the electrical readout shows promise for wireless applications.

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[1] D. Meyers, *Surfaces, Interfaces, and Colloids*, 2nd ed. (Wiley, New York, 1999).
[2] G. L. Kenausis, J. Voros, D. L. Elbert, N. Huang, R. Holfer, L. Ruiz-Taylor, M. Textor, J. A. Hubbell, and N. D. Spencer, J. Phys. Chem. B **104**, 3298 (2000).
[3] E. Southern, K. Mir, and M. Shchepinov, Nature Genetics **21**, 5 (1999).
[4] S. Nock and P. Wagner (unpublished).
[5] H. Elwing, Biomaterials **19**, 397 (1998).
[6] P. Huetz, P. Schaaf, J. Voegel, E. Mann, B. Miras, V. Ball, M. Freund, and J. Cazenave, Proteins Interfaces II **602**, 334 (1995).
[7] J. J. Ramsden, J. Mol. Rec. **10**, 109 (1997).
[8] J. Fritz, M. K. Baller, H. P. Lang, H. Rothuizen, P. Vettiger, E. Meyer, H.-J. Güntherodt, Ch. Gerber, and J. K. Gimzewski, Science **288**, 316 (2000).
[9] S. R. Manalis, E. B. Cooper, P. R. Indermuhle, P. Kernen, P. Wagner, D. G. Hafeman, S. C. Minne, and C. F. Quate, Appl. Phys. Lett. **76**, 1072 (2000).
[10] A. Fanigliulo, P. Accossato, M. Adami, M. Lanzi, S. Martinoia, S. Paddeu, M. T. Parodi, A. Rossi, M. Sartore, M. Grattarola, and C. Nicolini, Sens. Actuators B **32**, 41 (1996).
[11] P. Bergveld, IEEE Trans. Biomed. Eng. **19**, 70 (1970); **19**, 342 (1972).
[12] D. G. Hafeman, J. W. Parce, and H. M. McConnell, Science **240**, 1182 (1988); H. M. McConnell, J. C. Owicki, J. W. Parce, D. L. Miller, G. T. Baxter, H. G. Wada, and S. Pitchford, *ibid.* **257**, 1906 (1992).
[13] L. Bousse and P. Bergveld, J. Electroanal. Chem. **152**, 25 (1983).
[14] L. Bousse, J. C. Owicki, and J. W. Parce, *Chemical Sensor Technology*, edited by S. Yamaguchi (Kodansha, Tokyo, 1992), Vol. 4, p. 145; J. C. Owicki and J. W. Parce, Biosens. Bioelectron. **7**, 255 (1992).
[15] L. A. Ruiz-Taylor, T. L. Martin, F. G. Zaugg, K. Witte, P. Indermuhle, S. Nock, and P. Wagner, PNAS **98**, 852 (2001); B. S. Jacobson and D. Branton, Science **195**, 302 (1977).
[16] L. Bousse, N. F. de Rooij, and P. Bergveld, IEEE Trans. Electron Devices **ED-30**, 1263 (1983).
[17] Y. Dong, S. V. Pappu, and Z. Xu, Anal. Chem. **70**, 4730 (1998).
[18] J. Israelachvili, *Intermolecular and Surface Forces*, 2nd ed. (Academic, New York, 1992)
[19] W. H. Baumann, M. Lehmann, A. Schwinde, R. Ehret, M. Brischwein, and B. Wolf, Sens. Actuators B **55**, 77 (1999).