Nanofluidic channels and nanopores having dimensions comparable to the size of biological macromolecules such as proteins and DNA have attracted attention in applications such as single molecule detection, analysis, separation, and control of biomolecules. Biological nanopores such as α-hemolysin offer single molecule sensitivity but are labile and difficult to handle. On the other hand, inorganic channels are robust and offer better control over channel geometry and are more amenable to integration into functional systems. Nanochannels fabricated in a controlled fashion have enabled the exploration of charge-related effects such as concentration enhancement and depletion of small molecules such as aminosilane or biotin mainly changes surface charge, affecting conductance only in the low concentration regime. However, streptavidin not only modifies surface charge but also occludes part of the channel, resulting in observable conductance changes in both regimes. Our observations reflect the interplay between the competing effects of charge and size of streptavidin on nanochannel conductance.
any functionalization of nanochannel surfaces with different surface groups and biomolecules can be expected to change surface charge and the nanochannel conductance. A signature of this regime is that conductance becomes independent of bulk ionic concentration as well as the channel height. In the high concentration regime, $\frac{\alpha}{e}h \ll n$ and conductance becomes largely independent of surface charge.\(^{14,16}\) In this regime, conductance depends on channel height and increases linearly with ionic concentration

$$G = 2n\varepsilon h \left(\frac{\mu_+ + \mu_-}l\right)w$$  \hspace{1cm} (2)

If the size of the biomolecules in the channel is comparable to the channel size, the resulting change in channel geometry ($h$) would result in a change in nanochannel conductance. Hence, in both regimes, measurement of electrical conductance of nanochannels offers means of probing biological reactions and modifications on surfaces, as illustrated in Figure 1. Electrokinetic measurements in microslits have been used for the characterization of surfaces and measurement of protein adsorption.\(^{20,21}\) However, electrokinetic characterization is cumbersome, involving the application of pressure and the measurement of flow rates. In nanochannels, surface effects dominate and we can expect biological modifications and reactions to be detected directly by simply measuring the conductance.

To fabricate the nanochannel devices, a 30 nm thick layer of polysilicon was deposited on a fused silica wafer using low-pressure chemical vapor deposition (LPCVD) process and subsequently patterned, forming sacrificial material that defined the nanochannels. Polysilicon film thickness was measured using a Nanospec 3000 film analysis system (Nanometrics) as well as with an Alpha-Step IQ surface profiler (KLA-Tencor) after patterning the thin film. A 2 \(\mu\)m thick low-temperature oxide was then deposited in a LPCVD process, annealed, patterned, and etched down to access the nanochannel ends. Microchannels with access holes were fabricated on another fused silica wafer. The nanochannel and microchannel components were then bonded together using a transfer bonding technique with poly(dimethylsiloxane) (PDMS) (Sylgard 184, Dow Corning) as adhesive.\(^{22}\) A number of staggered nanochannels were used in the fabrication process such that only one set of nanochannels bridged the microchannels. After bonding, nanochannels were formed by etching the sacrificial polysilicon with xenon difluoride gas at 3 Torr for 1.5 h (Figure 2). Once the channels were formed, the entire device was treated with oxygen plasma at 300 W for 10 min in a plasma etcher (Technics).

Plasma-treated surfaces of the channels were immersed in a 2% solution of (3-aminopropyl)trimethoxysilane (APTM) (Gelest Inc.) in ethanol for 1 h at room temperature, followed by a 5-min ethanol rinse. The devices were then immersed in a 0.1 M phosphate-buffered saline (PBS, pH 7.2, Invitrogen; 10 mM PBS is an aqueous solution of 1.55 M NaCl, 0.015 M KH2PO4, and 0.027 M Na2HPO4). Biotinylation of the surface was done by treating the aminosilane-coated surface with a 10 mM solution of Sulfo-NHS-SS-Biotin (sulfosuccinimidyl 2-(biotinamido)-ethyl-1,3-dithiopropionate) (Pierce Biosciences) in 0.1 M PBS for 1 h at room temperature. The succinimide moiety reacts readily with the primary amine group of the APTM resulting in cross-linking biotin to the surface. The NHS-SS-biotin cross-linker was used because of its long spacer arm, which reduces steric constraints leading to better binding efficiency of avidins. Residual amine groups, if any, were then passivated by treating the surface with a 10 mM solution of N-hydroxysuccinimide (NHS) (Sigma Aldrich) in 0.1 M PBS for 1 h at room temperature.

Following each step of surface reactions, electrical conductance of the nanochannels was measured at a range of

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**Figure 1.** Effect of biomolecules on nanochannel conductance. At high ionic concentrations, nanochannel conductance is governed by channel geometry, while at low ionic concentrations, conductance is governed by surface charge (red curve). Immobilization of biomolecules results in a decrease in conductance at high ionic concentrations due to biomolecule size and an increase in conductance at low concentrations due to biomolecule charge (green curve).

**Figure 2.** Nanochannel device. Microchannels interface with a single nanochannel device out of a set of staggered devices and enable conductance measurements while providing for good control over electrolyte concentration. Each device consists of 10 120 \(\mu\)m long, 3.5 \(\mu\)m wide, and 30 nm thick silica nanochannels. (Inset a, before etching polysilicon. Inset b, bonded device, showing two microchannels and inlet-outlet ports.)
buffer concentrations using a Keithley 6430 sub-femtoamp source meter controlled through a GPIB interface by a real-
time control and analysis MATLAB program. Ag/AgCl
electrodes were used to make electrical contact with solutions
through access holes at the ends of microchannels. While
solutions were changed, conductance measurements and
rinses were carried out alternately to ensure complete rinsing.

Nanochannel conductance for a range of concentrations
from 0.001× to 10× PBS after each step of surface
modification is shown in Figure 3A. This device was first
treated with APTMS, followed by NHS-SS-biotin and then
NHS. At higher buffer concentrations, conductance varied
linearly with concentration. Approximating 10
-150
μM NaCl with an equivalent conductivity of 10
-15
S/mol 23
(or μ+ + μ− = 10.4 × 10−8 m2/(V s)) and device geometry
of 10 parallel 120 μm × 3.5 μm × 30 nm channels (first
two dimensions estimated from micrographs), eq 2 gives an
expected conductance of 1.35 × 10−7 S, which is in
reasonable agreement with the measured nanochannel con-
ductance under the same conditions. The conductance was
repeatable from device to device, confirming the integrity
of nanochannels and the microchannel interface. However,
at low buffer concentrations, nanochannel conductance
deviated significantly from linearity and was seen to level
off for the APTMS treated nanochannels. At pH 7.2, the
amino groups may be expected to be positively charged.
Subsequent treatment with NHS increased conductance slightly. No appreciable change in conductance was observed at high ionic
concentrations indicating absence of steric blocking. (B) Detailed plot of conductance at the highest and lowest buffer concentrations. The
error bars (1σ) correspond to five measurements at each point. PBS buffer was used for the measurements (1× PBS corresponds to an ionic
centrations, presumably due to reaction of the amino
group with the NHS group resulting in a moiety with no
charge. In this case, surface charge was lowered to such an
extent that and ωleh is comparable to n and eqs 1 and 2 are
not valid. However, since conductance decreases monotonically with bulk concentration, eq 1 puts an upper bound of
about 1 mC/m2 on the surface charge. Further treatment with
NHS did not result in a large change in conductance. To
clearly illustrate charge-governed and geometry-governed
regimes, conductance values at 10−3× and 10× PBS (∼150
μM and 1.5 M NaCl) for the three surfaces are shown in
Figure 3B. It is seen that functionalization of nanochannel
surfaces with small molecules resulted in a large change in
surface charge, detected at low buffer concentrations, while
conductance values at high buffer concentration remained
unchanged, indicating no change in nanochannel geometry.

To study the effect of biological binding reactions on
nanochannel surfaces, 1 mg/mL Alexa Fluor 488 labeled
streptavidin (Molecular Probes, Eugene, OR) in 0.1× PBS
was introduced into the above test device for 10 h at room
temperature, followed by rinsing in buffer. To ensure that
any observed changes were not due to effects external to
nanochannels such as blocking of nanochannel inlets, another
nonbiotinylated control device was prepared by treatment
with APTMS followed by NHS and was similarly treated
with streptavidin. Use of fluorescently labeled streptavidin
enabled electrical measurement as well as direct optical
confirmation of the presence or absence of streptavidin on
nanochannel surfaces. Conductance measurements (Figure
4) revealed large changes in conductance of the biotinylated
nanochannels (test device) at both low and high ion
concentrations, but little change in conductance of the
nonbiotinylated nanochannels (control device). Further, this
is corroborated by fluorescence images obtained with a Nikon
TE2000-U inverted epifluorescence microscope using an
ORCA-ER (Hamamatsu Photonics Gmbh) camera, which
clearly showed immobilization of streptavidin in the test
device but not in the control device (Figure 4, insets).

Conductance measurements in charge-governed and ge-
ometry-governed regimes (Figure 5) reveal that immobiliza-
tion of streptavidin in the nanochannels resulted in changing
not only the surface charge but also the device geometry.
At 10× PBS, conductance of the biotinylated nanochannels
dropped by about 15% when streptavidin was introduced,
indicating an effective reduction in channel size from about 30 to 25 nm or an immobilized layer effectively 2.5 nm thick on the surface. This change is consistent with the globular size of streptavidin (5–6 nm) and the change in size of colloids on protein binding reported in other studies. At low ion concentrations, conductance of the biotinylated nanochannels showed an increase, which implies an increase in the surface charge due to immobilization of streptavidin. Streptavidin with a mildly acidic pI of 5 is reported to have about two electron charges at pH 7.2 (ref 26), which qualitatively explains the increase in conductance. The conductance of the nonbiotinylated nanochannels remained relatively unchanged, indicating that the changes observed in the test device can be attributed to the streptavidin binding reaction.

Our experiments clearly demonstrate that biological binding events modulate surface charge and change nanochannel geometry. Moreover, conductance values were highly repeatable even after rinsing with different buffer concentrations. At the lowest buffer concentration, APTMS-treated surfaces showed the largest variability in conductance. This variability may be due to the presence of bivalent phosphate counterions in PBS, since multivalent ions are known to adsorb and sometimes even reverse charge on highly charged surfaces. In contrast, NHS and NHS-SS-biotin treated surfaces were extremely stable after rinsing with different buffer concentrations; conductance varied by less than 10^{-11} S in some cases. Assuming that change in conductance is roughly equivalent to a change in ionic concentration of \( \Delta \sigma / e \), it corresponds to variations in surface charge of approximately 0.1 mC/m^2 or one electron charge per 400 Å area. This observation suggests that electrochemically stable nanochannel surfaces could be used as highly sensitive probes for measuring changes in surface charge. On the other hand, at high ionic concentrations, we observed a variation of about 1% in nanochannel conductance. This could be due to slight variations in concentration and temperature since the viscosity of water changes by 2% per 1 °C change in temperature in the 20–30 °C range, resulting in changes in ionic mobilities and conductance. Since the room temperature remained at 23 ± 0.5 °C during the course of the experiment, these variations are not expected to materially affect the results. Another aspect of streptavidin immobilized on nanochannel surfaces is the deviation of conductance at low concentrations from that of nanochannels with a constant surface charge. This behavior could arise from a number of effects including charge regulation of streptavidin due to changes in pH, discreteness of charge, adsorption of ions, nonplanar geometry due to streptavidin, etc. Moreover, interpretation of electrokinetic characteristics of adsorbed proteins in terms of a Debye layer at a plane surface was shown to be inadequate. Further investigations are required to study and explain this behavior.

Our experiments indicate that biomolecule charge and volume have opposite effects on nanochannel conductance: biomolecule charge increases the number of conducting ions
in the nanochannel whereas volume exclusion of ions decreases the number of conducting ions. This argument is valid when the nanochannel has a comparatively low surface charge to begin with, as in the present case. Consider a biomolecule with charge $q$ and volume $V$ present in a solution with ionic concentration $n$ in the nanochannel. The number of charges on the biomolecule is $q/e$, where $e$ is the charge of an electron. The number of conducting ions introduced due to biomolecule charge is expected to be of the order of $q/e$, while the number of excluded ions is of the order of $nV$. These contributions are analogous to eqs 1 and 2, the first contribution depending only on charge and the second one varying with geometry and ionic concentration. The biomolecule charge effect dominates at lower ionic concentration, but as the ionic concentration increases, the number of ions displaced due to the biomolecule volume increases. This exactly offsets the effect of biomolecule charge at a certain concentration, i.e., when $n \sim q/eV$. At higher concentrations, the volume exclusion effect dominates. For streptavidin, using $V = 5.4 \times 5.8 \times 4.8 \text{nm}^3$ (ref 24) and $q/e = 2$ (ref 26), we get $q/eV = 22 \text{mM}$, which is in agreement with the data in Figure 4A. Interestingly, we observed an analogous transition in the conductance of DNA translocation through nanotubes though the transition occurred at a much higher ionic concentration.

In conclusion, our experiments demonstrate that the ionic conductance of nanochannels reflects an interplay between the competing effects of biomolecule charge and size. Nanochannel conductance could be used to sense surface charge and the presence of biomolecules immobilized on nanochannel surfaces in both surface-charge-governed and geometry-governed regimes. Our devices provide for an integrated nanofluidic platform with a robust electronic probing scheme that is amenable to scaling and multiplexing. This technique could be developed for charge-sensitive biosensing, having the potential of label-free detection of binding of small molecules and kinase activity, which are hard to detect conventionally.

Acknowledgment. We acknowledge the support of Basic Energy Sciences, Department of Energy; the IMAT program of the National Cancer Institute; and the National Science Foundation. R.K. would like to acknowledge Min Yue and Deyu Li for input on device fabrication. We also thank Richard Cote, Ram Datar, Hirofumi Daiguji, and Andrew Szeri for their collaboration. We acknowledge the UC Berkeley Microfabrication Laboratory for the use of their facilities.

References