Electronic Supplementary Information

Label Free Detection of Nucleic Acids by Modulating Nanochannel Surfaces

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Contents

Materials

DNA oligonucleotides were purchased from Integrated DNA Technologies (IDT) or the Stanford Protein and Nucleic Acid Facility with standard desalting purification and dissolved in nuclease free water (Invitrogen) to 1 mM stock solutions. Gibco 1x Phosphate Buffered Saline (PBS, pH 7.4) was purchased from Invitrogen. Tris(hydroxymethyl)aminomethane, hydrochloric acid, disodium ethylendiamine tetraacetate, potassium chloride, and magnesium chloride hexahydrate were purchased from Fisher Scientific. BS3 was purchased from Thermo Scientific. Boric acid was purchased from EMD Millipore. 3-(aminopropyl)trimethoxysilane, 3-(chloropropyl)trimethoxysilane and sodium azide were purchased from Sigma-Aldrich. DNase I was purchased from New England Biolabs. All chemicals were used as received. 100 nm height, 5 mm length, 5 μm width straight nanochannels were purchased from Micronit or fabricated using the outlined materials and procedure below.

All current measurements were performed on a Keithley 2410 1100V Sourcemeter using platinum electrodes. Fluorescence microscopy was performed on an Olympus IX71 inverted fluorescence microscope using an Andor Ixon+ CCD camera and Olympus LUCPlanFL N 20x/0.45 objective and processed using Andor SOLIS software. Bulk conductivity of solutions were determined on an Oakton Con2700 Conductivity Meter using 23 μS/cm, 447 μS/cm, and 12,880 μS/cm calibration standards.

Nanochannel Fabrication Process

Fused silica nanochannels were fabricated by high temperature bonding of two fused silica wafers [100mm Quartz wafers (HOYA)]; one "top" wafer that contained the access holes, and a "bottom" wafer containing the etched channels. To create nanochannels on the bottom wafer, positive photoresist (AZ4110) was spun onto 100mm Quartz wafers (HOYA) using HMDS as an adhesion promoter. Lithography was performed using a contact aligner (SUSS MA-6) and the exposed photoresist was developed using AZ300K. Substrates were etched 89nm deep with CHF_4 using an Inductive Coupled Plasma Etcher (Panasonic E640) and photoresist was subsequently removed with an oxygen/plasma clean.

The top wafer was temporarily bonded to a silicon backing wafer using a resin (CrystalBond), and a thin layer of photoresist (AZ4110) was spun onto the wafer and then developed (AZ300K) in order to act as a protective layer for drilled access holes. Holes were drilled using a diamond tipped bit on a 3-axis CNC drill, and the wafers were then debonded and cleaned thoroughly by soaking in acetone, isopropanol, and rinsing with DI water. Organic residues were removed from both the etched wafer and the drilled wafer using Nano-Strip (CYANTEK). Wafers were then rinsed in DI water, briefly submerged in Buffered HF Improved (Transene), and then rinsed in DI water and dried thoroughly with nitrogen. The drilled wafers were placed in contact with the etched wafers and light pressure was applied to initiate van der Waals bonding. The wafers were then heated to 1050 ^{0}C for 6 hours inside a furnace (Tystar 8300) to create a permanent fusion bond. After optical inspection of the bonds, the chips were then separated using a dicing saw (ADT).

Modeling Channel Conductivity[1]

Comsol Multiphysics was used to model the conductivity in the channel. To avoid the complexity of modelling PBS buffer, KCl was used in the simulations. K^+ and Cl^- have valences of +1 and -1 and mobilities of $7.62 * 10^{-8}$ and $7.91 * 10^{-8}$ [m²/(V s)], respectively. To achieve a bulk conductivity of 16.0 [mS/cm], the equations below were used assuming a bulk concentration of 106.76 [mmol/L]. First, the concentration, ρ, and electrostatic potential, Ψ, profiles were calculated via the Poisson-Boltzmann equation:

$$\epsilon_0 \epsilon_r \nabla^2 \Psi(z) = -e_0 \sum_i z_i \rho_{i\infty} \exp\left(-\frac{e_0 z_i}{k_B T} \Psi(z)\right)$$

where ϵ_0 is the permittivity of free space, ϵ_r is the relative permittivity of water, e_0 is the charge of an electron, z_i is the valence of species i, $\rho_{i,\infty}$ is the bulk concentration of species i, k_B is the Boltzmann constant, and T is the temperature. This assumes a one-dimensional geometry with the potential at each wall equal to the zeta potential, ζ, which was varied. A finely-spaced mesh with increased resolution near the walls was employed to capture the sharp gradients in potential near the wall. We calculate the electromigrative conductivity:

$$\sigma_{mig} = \frac{1}{h} \int_0^h e_0 \sum_i \nu_i |z_i| \rho_{i\infty} \exp\left(-\frac{e_0 z_i}{k_B T} \Psi(z)\right) dz$$

and advective conductivity:

$$\sigma_{adv} = \frac{1}{h\, E_0} \int_0^h e_0 \sum_i z_i \rho_{i\infty} \exp\left(-\frac{e_0 z_i}{k_B T} \Psi(z)\right) \vec{u(z)}\, dz$$

assuming a unidirectional velocity profile of:

$$\vec{u(z)} = -\frac{\epsilon_0 \epsilon_r E_0}{\mu} (\zeta - \Psi(z))$$

where h is the channel height (either 89 or 100 [nm]), ν_i is the mobility of species i, and E_0 is the applied electric field in the channel, equal to 120[kV/m], and μ is the viscosity of water. The velocity profile is derived from the steady Stokes equations with an electric body force:

$$0 = -\nabla p + \mu \nabla^2 \vec{u} + \rho_E E_0$$

where ρ_E is the charge density. Assuming a one-dimensional geometry, zero applied pressure gradient, and no slip at the channel walls, the above velocity profile is achieved.

Finally, the current in the channel is given by taking the conductivity multiplied by the area and electric field:

$$I = I_{adv} + I_{mig} = (\sigma_{adv} + \sigma_{mig}) h\, w\, E_0$$

where w is the channel width of 5 μm.

Channel Modification Procedures

Aminosilanization of Channel Wall

4.8 mL MilliQ 18 MΩ water and 0.2 mL 3-(aminopropyl)trimethoxysilane were mixed. 5 µL of this solution was added to the east reservoir of a dry, unmodified nanochannel and allowed to capillary fill for approximately 15 minutes. Complete channel filling was confirmed by examining the channel under light microscopy. 5 µL was added to the west reservoir then the entire nanochannel chip was immersed in the silane solution and allowed to incubate at room temperature overnight (16 to 20 hours). The chip was then removed from the silane solution, the exterior surface dried, and the nanochannel connected to a Keithley 2410 via platinum electrodes and electrokinetically washed for 15 minutes at +600 V with MilliQ 18 MΩ water. Wells were constantly replenished with 1 – 2 µL volumes in each reservoir, replaced every 2 minutes to avoid evaporation. Surface charge inversion of the nanochannel wall was assayed by applying 2 µL of PBS (1.06 mM KH_2PO_4, 2.97 mM Na_2HPO_4, 155.2 mM NaCl, pH 7.4) to the east reservoir, where a large rise in current (greater than 20 nA in 1 minute) confirmed successful modification. The channel was equilibrated with PBS for 5 minutes at +600 V then washed with TE Buffer (10 mM Tris HCl, 1 mM EDTA, pH 7) for 10 minutes at +600 V. The nanochannel chip was stored immersed in TE buffer at +4°C until subsequent use or modification.

Azidosilanization of Channel Wall

80 µL of 3-(azidopropyl)trimethoxysilane[2] was mixed with 4 mL of isopropyl alcohol. Approximately 2 µL of this solution was added to the east reservoir of a dry, unmodified nanochannel and allowed to capillary fill for approximately 15 minutes. As the isopropanol is prone to evaporation, the east well was replenished with silane solution every 5 minutes. After capillary filling was complete, approximately 2 µL of the silane solution was added to each well and platinum electrodes connected to a Keithley 2410 were attached and a field of +500 V was applied for 1 minute. The entire nanochannel chip was then immersed in the silane solution and allowed to incubate at room temperature for 22 hours. The chip was removed from the silane solution and immersed in isopropyl alcohol for 90 minutes, then the nanochannel connected to a Keithley 2410 via platinum electrodes and electrokinetically washed with MilliQ 18 MΩ water for 20 minutes under an applied field of +500 V. Chips were stored immersed in MilliQ 18 MΩ water at +4 °C until further use or modification.

ssDNA Channel Wall Modification (of Aminosilanized Channels)

An aminosilane modified chip was electrokinetically washed at +600 V with MilliQ 18 MΩ water for 5 minutes. 99 µL PBS, 1 µL of a stock solution of BS3 (freshly prepared, 2 mg per 150 µL MilliQ 18 MΩ water, final concentration 0.23 mM) and 0.5 µL of 1 mM stock 5'-amino modified DNA oligo (final concentration 5 µM) were briefly mixed then electrokinetically loaded into the nanochannel at +600 V for 15 minutes (refilling wells as above). After loading, the channel was placed in a humid chamber and incubated at room temperature for 6 hours, then washed for 15 minutes at +600 V with MilliQ 18 MΩ water followed by 15 minutes at +600 V with TE buffer. The nanochannel chip was stored immersed in TE buffer +4°C until use.

For high concentration modification, 3 µL stock 5'-amino ssDNA (final concentration 100 µM) and 1 µL BS3 stock (2 mg/150 µL) were added to 27 µL PBS, mixed briefly via vortexing and the resulting solution used for electrokinetic loading.

ssDNA Channel Wall Modification (of Azidosilanized Channels)

1 μL of 5'-DBCO modified DNA stock solution (1 mM) was mixed with 99 μL phosphate buffered saline to afford a 10 μM working solution. An azidosilanized chip was removed from storage, the surface cleaned, and attached to a Keithley 2410 via platinum electrodes and a field of +500 V was applied. Both wells of the channel were replaced with 1 μL of the DNA solution to provide electrokinetic loading of DNA into the nanochannel for 15 minutes. The loaded chip was then incubated for 4 hours at 37 °C in a humid chamber. After completion of the modification, the chip was electrokinetically washed for 15 minutes under an applied field of +500 V with TE buffer. Chips were stored in TE buffer at 4 °C until use.

BS3 Modification

The same procedure as above was employed, omitting addition of 5'-amino modified DNA oligo. For increased BS3 modification, 10 μL of BS3 stock solution was added to 90 μL PBS (2.3 mM final concentration).

General DNA Incubation Procedures

ssDNA Solution

0.5 μL of ssDNA (1 mM stock solution) was added to 99.5 μL loading buffer (70 mM tris borate, 10 mM $MgCl_2$, pH 7.55) and the solution briefly mixed by vortexing to provide a 5 μM concentration. Dilution was performed as needed in loading buffer and the ssDNA was stored at -20°C when not in use.

Annealing of dsDNA

To 99 μL loading buffer (70 mM tris borate, 10 mM $MgCl_2$, pH 7.55) was added 0.5 μL each of 1 mM stock solutions of complimentary oligonucleotides (5 μM final concentration). The solution was vortexed to mix then heated at 80°C for 10 minutes then allowed to slowly cool to room temperature over 30 minutes. The resulting dsDNA was then diluted as needed in loading buffer and stored at -20°C when not in use.

Nanochannel incubation

Note: as small volumes are employed in channel reservoirs (1 – 2 μL), replacement of reservoirs every 1 – 2 minutes during all processes but incubation in a humid environment are necessary to avoid evaporation. Such reservoir replacements are implied when buffers are being electrokinetically driven through the nanochannel.

The nanochannel chip was removed from storage buffer and the exterior surface dried, then each reservoir was filled with 1 – 2 μL TE buffer and platinum electrodes connected to a Keithley 2410 were placed in each reservoir. The east reservoir was replaced with PBS and a field of +600 V was applied, with an increase in measured current indicating charge inversion of the nanochannel wall, while no change indicated regular direction of flow. Both reservoirs were then replaced every 1 – 2 minutes with PBS until a stable current (e.g. the measured current was unchanged on consecutive reservoir replacements) was measured at +600 V and recorded to the nearest 0.5 nA (see tables below). The nanochannel was then washed with TE buffer in both reservoirs for 5 minutes at +600 V. ssDNA or dsDNA at the desired concentration (see main text) in loading buffer (70 mM tris borate, 10 mM $MgCl_2$, pH 7.55) was electrokinetically loaded at +600 V for 10 minutes. The nanochannel chip was then allowed to incubate

in a humid chamber at room temperature for 3 hours, then the nanochannel was washed with loading buffer (containing no DNA) for 10 minutes at +600 V, followed by refilling of the east reservoir with PBS. A substantial increase in current (greater than 20 nA within 1 minute) indicated charge inversion while no or small changes indicates regular direction of flow. Both reservoirs were then replaced every 1 – 2 minutes with PBS until a stable current was measured at +600 V and recorded to the nearest 0.5 nA. The west reservoir was then replaced with TE buffer and a substantial decrease in current (greater than 20 nA within 1 minute) indicated regular flow, while no or small change in current indicated charge inversion. The nanochannel was then washed with TE buffer in both reservoirs until the measured current was less than 10 nA and the channel was stored immersed in TE buffer at 4°C for subsequent experiments.

For fluorescence imaging, the chip was mounted on the fluorescence microscope and images were taken when the channel was filled with PBS prior to sample loading, when the channel was filled with sample in loading buffer, and when the sample was filled with PBS after sample washing. Fluorescein filters in the camera provided excitation at 480 nm and emission at 515 – 555 nm.

DNase I Degradation of dsDNA

5 μM dsDNA was annealed in DNase I buffer (10 mM Tris HCl, 2.5 mM $MgCl_2$, 0.5 mM $CaCl_2$, pH 7.6) at 80°C for 10 minutes then allowed to slowly cool to room temperature over 30 minutes. 2 units of DNase I (1 μL of 2,000 units/mL) were added, the mixture was briefly vortexed then incubated at 37°C for 10 minutes followed by heat inactivation at 75°C for 10 minutes. The resulting reaction mixture was then employed in channel incubations as described in the general procedure, with 1x DNase I buffer being used for the ten minute channel wash after incubation.

Determining Charge Leakage through Channel

A 150 mM solution of potassium chloride was prepared and electrokinetically loaded at +600 V through both wells until stable current of 99.2 nA was attained in a 100 nm Micronit channel. The bulk conductivity of the potassium chloride solution was measured to be 17.92 mS/cm which afforded an expected current of 107.5 nA for the given channel dimensions (100 nm depth, 5 μm width, 5 mm length).

Discussion of BS3 Surface

Control experiments were also performed with channels having aminosilane modification followed by reaction with BS3 crosslinker but no 5'-amino ssDNA (providing a surface charge of +20 mV), and observed a considerable 80% increase in measured current and re-inversion to a negatively charged wall (-155 mV) upon incubation with free ssDNA, indicating considerable nonspecific adsorption to the surface. To further understand these effects, amino modified channels were incubated with different amounts of BS3 (0.23 mM or 2.3 mM), generating"low" and "high" BS3 modified surfaces that were subsequently exposed to increasing concentrations of ssDNA or dsDNA. The high concentration BS3 modification created a negatively charged surface that repelled all ssDNA and dsDNA, as represented by only minor changes in measured current after sample exposure, similar to unmodified channels (see Table S3). Similarly, higher BS3 concentrations during surface attachment of 5'-amino ssDNA reduced sensitivity of the surface to detection of a complimentary strand when forming dsDNA at the surface (Table S2), again likely because of increased negative surface charge repelling DNA.The "low" BS3 modification proved to be more sensitive to nucleic acid interactions at the channel wall (Table S11). Although there was an insignificant decrease in measured current using 5 nM ssDNA (from 75 nA to 74 nA), incubation with 50 nM ssDNA created a strong decrease in measured current (from 72.5 nA to 45.5

nA) as well as the ability of the channel to flow in either direction, indicating either a neutral channel surface, or zwitterionic groups on the surface. Increased concentration to 500 nM ssDNA raised the measured current to 86 nA and created a negative surface charge presumably due to considerable DNA adsorption. No further increase in measured current was observed for the nanochannel at 5 μM ssDNA, indicating 500 nM created a saturated surface and repelled subsequent DNA adsorption, particularly as the surface had become negatively charged. With dsDNA, wall interactions (and changes in current) were readily observed at 5 nM, as seen by both charge re-inversion and an 8% decrease in measured current (from 104 nA to 95.5 nA). Exposure of this channel to higher concentrations of dsDNA did not further increase current (Table S3), again consistent with the generated negative surface charge repelling DNA. Furthermore, a new BS3 modified nanochannel was prepared and incubated with 50 nM dsDNA, and considerable adsorption was observed with a 38% increase in measured current (61.5 nA to 85 nA) and reversion to a negatively charged surface, which is a markedly different response than experiments with the same concentration of ssDNA. We hypothesize this is because of the higher charge density of dsDNA generating a higher negative charge on the wall. Again, exposure to even higher dsDNA concentrations only changed measured current slightly, further confirming our data that negatively charged surfaces repels subsequent adsorption.

Rigorous explanations of the effects of BS3 on the surface are complicated by the possible reactions of the crosslinker alone with the aminosilanized surface, making application of our model to this surface difficult. The sulfosuccinimidyl esters may react with the surface in a variety of possible ways, each with different impact on observed surface charge. For example, both sulfosuccinimidyl esters could react with amino groups on the surface, forming two amides and thus creating neutral surface charge groups. Nonspecific ion pairing could also occur, again neutralizing surface charge. Alternatively, only one end may react (forming an amide and neutralizing a single amine charge), in which case a free sulfosuccinimidyl ester remains from the crosslinker. A negative charge results regardless of whether aqueous hydrolysis of the sulfosuccinimidyl ester occurs and the net surface charge is a balance between unreacted positively charged amino groups and negatively charged sulfonate or carboxylate groups available. Alternatively, tris from buffer during a wash could displace unreacted sulfosuccinimidyl esters, functioning to generate a neutral species. Such partial charge passivation may explain the improved detection limits for nucleic acids, although further experiments and modelling are needed.

Table S1: Absolute measured currents and flow directions for various modified channels exposed to 5 μM ssDNA or 5 μM dsDNA.

Channel Wall	Sample	PBS current before sample	PBS current after sample	Change in current	Direction of Flow
Unmodified 1	ssDNA	94.5 nA	96.0 nA	1.6%	Normal
Unmodified 2	ssDNA	88.0 nA	90.0 nA	2.3%	Normal
Unmodified 3	ssDNA	92.0 nA	92.5 nA	0.5%	Normal
ssDNA Modified 1	ssDNA	87.5 nA	148.0 nA	69.1%	Normal
ssDNA Modified 2	ssDNA	80.0 nA	144.0 nA	80.0%	Normal
ssDNA Modified 3	ssDNA	88.0 nA	125.0 nA	42.1%	Normal
Unmodified 1	dsDNA	99.5 nA	102.0 nA	2.5%	Normal
Unmodified 2	dsDNA	88.0 nA	89.0 nA	1.1%	Normal
Unmodified 3	dsDNA	89.0 nA	90.5 nA	1.7%	Normal
Amino	ssDNA	101.0 nA	89.0 nA	-11.9%	Inverted
Amino/adsorbed ssDNA	ssDNA	97.0 nA	97.0 nA	0.0%	Inverted
Amino/adsorbed ssDNA	dsDNA	91.0 nA	108.5 nA	19.2%	Normal
Amino/BS3	ssDNA	84.0 nA	150.0 nA	78.6%	Normal
20 μm chip, ssDNA Modified*	ssDNA	2820 nA	2810 nA	-0.4%	Normal
ssDNA Modified, high concentration	ssDNA	84.5 nA	93.5 nA	10.7%	Normal
ssDNA Modified, random sequence	ssDNA	96.0 nA	137.0 nA	42.7%	Normal
ssDNA Modified, random sequence, high concentration	ssDNA	90.0 nA	127.0 nA	41.1%	Normal

*An applied field of +100 V was used as the larger channel dimensions afforded considerably higher measured currents.

Table S2: Calculated surface charges before and after incubation for various modified channels with 5 μM ssDNA or 5 μM dsDNA.

Channel Wall	Sample	Calculated surface charge before incubation	Calculated surface charge after incubation	Change in calculated surface charge
Unmodified 1	ssDNA	-82.25 mV	-85.0 mV	-2.75 mV
Unmodified 2	ssDNA	-43.0 mV	-45.0 mV	-2.0 mV
Unmodified 3	ssDNA	-70.0 mV	-73.7 mV	-3.7 mV
ssDNA Modified 1	ssDNA	+37.5 mV	-150.0 mV	-187.5 mV
ssDNA Modified 2	ssDNA	+10.0 mV	-150.0 mV	-160.0 mV
ssDNA Modified 3	ssDNA	+38.0 mV	-140.0 mV	-140.0 mV
Unmodified 1	dsDNA	-100.0 mV	-10.0 mV	-6.0 mV
Unmodified 2	dsDNA	-38.0 mV	-39.0 mV	-1.0 mV
Unmodified 3	dsDNA	-39.0 mV	-45.0 mV	-6.0 mV
Amino	ssDNA	+100.0 mV	+38.0 mV	-62.0 mV
Amino/adsorbed ssDNA	ssDNA	+88.0 mV	+88.0 mV	0.0 mV
Amino/adsorbed ssDNA	dsDNA	+71.0 mV	-119.0 mV	-190.0 mV
Amino/BS3	ssDNA	+20.0 mV	-155.0 mV	-175.0 mV
20 μm chip, ssDNA Modified*	ssDNA	N/A**	N/A**	N/A**
ssDNA Modified, high concentration	ssDNA	-21.0 mV	-84.0 mV	-63.0 mV
ssDNA Modified, random sequence	ssDNA	+85.0 mV	-148.0 mV	-233.0 mV
ssDNA Modified, random sequence, high concentration	ssDNA	+42.0 mV	-141.0 mV	-183.0 mV

*An applied field of +100 V was used as the larger channel dimensions afforded considerably higher currents.

**See discussion in main text regarding bulk flow as primary determinant of measured current

Table S3: Absolute measured currents and flow directions for BS3 modified channels exposed to different concentrations of ssDNA or dsDNA. See Figure S5 for graphical interpretation of data.

Entry	Channel Wall	Sample	PBS current before sample	PBS current after sample	Change in Current	Direction of Flow
A	Low BS3, chip 1	5 nM ssDNA	75.0 nA	74.0 nA	-1.3%	Inverted
B	Low BS3, chip 1	50 nM ssDNA	72.5 nA	45.5 nA	-37.2%	Both Directions
C	Low BS3, chip 1	500 nM ssDNA	50.5 nA	86.0 nA	70.3%	Normal
D	Low BS3, chip 1*	5 µM ssDNA	49.0 nA	82.0 nA	67.4%	Normal
E	Low BS3, chip 2	5 nM dsDNA	104.0 nA	95.5 nA	-8.2%	Normal
F	Low BS3, chip 2*	50 nM dsDNA	104.0 nA	94.5 nA	-9.1%	Normal
G	Low BS3, chip 2*	500 nM dsDNA	102.5 nA	94.5 nA	-7.8%	Normal
H	Low BS3, chip 2*	5 uM dsDNA	102 nA	96.5 nA	-5.4%	Normal
I	Low BS3, chip 3*	50 nM dsDNA	61.5 nA	85.0 nA	38.2%	Normal
J	Low BS3, chip 3*	500 nM dsDNA	47.5 nA	84.5 nA	77.9%	Normal
K	Low BS3, chip 3*	5 µM dsDNA	96.0 nA	84.5 nA	-12.0%	Normal
L	High BS3*	5 nM ssDNA	91.0 nA	91.0 nA	0.0%	Normal
M	High BS3*	50 nM ssDNA	92.5 nA	93.0 nA	0.5%	Normal
N	High BS3*	500 nM ssDNA	93.0 nA	95.0 nA	2.2%	Normal
O	High BS3*	5 µM ssDNA	94.5 nA	94.5 nA	0.0%	Normal
P	High BS3*	5 nM dsDNA	94.5 nA	93.5 nA	-1.1%	Normal
Q	High BS3*	50 nM dsDNA	93.0 nA	93.5 nA	0.5%	Normal
R	High BS3*	500 nM dsDNA	94.5 nA	95.5 nA	1.1%	Normal
S	High BS3*	5 µM dsDNA	93.0 nA	95.0 nA	2.2%	Normal

*Indicates channel surface was negatively charged prior to incubation

Table S4: Absolute measured currents and flow directions of amine-modified channels incubated with various concentrations ssDNA. Surface saturation at 500 nM and subsequent low value for 5 μM experiments informed the preparation of new channels to retest 5 μM. See Figure S6 for graphical interpretation of data.

Entry	Channel Wall	Sample	PBS current before sample	PBS current after sample	Change in Current	Direction of Flow
A	Amine 1	5 nM ssDNA	79.5 nA	82.5 nA	3.8%	Inverted
B	Amine 2	5 nM ssDNA	85.5 nA	82.5 nA	-3.5%	Inverted
C	Amine 3	5 nM ssDNA	96.5 nA	93.5 nA	-3.1%	Inverted
D	Amine 1	50 nM ssDNA	73.0 nA	72.5 nA	0.7%	Inverted
E	Amine 2	50 nM ssDNA	79.5 nA	80.5 nA	1.3%	Inverted
F	Amine 3	50 nM ssDNA	88.5 nA	94.5 nA	6.8%	Inverted
G	Amine 1	500 nM ssDNA	71.5 nA	83.5 nA	16.8%	Both
H	Amine 2	500 nM ssDNA	79.0 nA	91.0 nA	15.2%	Both
I	Amine 3	500 nM ssDNA	90.5 nA	92.0 nA	1.7%	Both
J	Amine 1	5 μM ssDNA	61.0 nA	77.5 nA	27.1%	Normal
K	Amine 2	5 μM ssDNA	73.5 nA	81.0 nA	10.2%	Normal
L	Amine 3	5 μM ssDNA	86.0 nA	91.0 nA	5.8%	Normal
M	New Amine 1	5 μM ssDNA	87.5 nA	96.0 nA	9.7%	Both
N	New Amine 2	5 μM ssDNA	83.5 nA	86.5 nA	3.6%	Both
O	New Amine 3	5 μM ssDNA	84.5 nA	90.0 nA	6.5%	Both

Table S5: Calculated surface charges before and after incubation of amino modified channels with various concentrations of ssDNA. See Figure S7 for graphical interpretation of data.

Entry	Channel Wall	Sample	Calculated surface charge before incubation	Calculated surface charge after incubation	Change in calculated surface charge
A	Amine 1	5 nM ssDNA	+84.7 mV	+95.0 mV	+10.3 mV
B	Amine 2	5 nM ssDNA	+106.0 mV	+95.0 mV	-11.0 mV
C	Amine 3	5 nM ssDNA	+127.0 mV	+121.0 mV	-6.0 mV
D	Amine 1	50 nM ssDNA	+49.0 mV	+52.6 mV	+3.6 mV
E	Amine 2	50 nM ssDNA	+84.7 mV	+88.5 mV	+3.8 mV
F	Amine 3	50 nM ssDNA	+111.0 mV	+123.0 mV	+12.0 mV
G	Amine 1	500 nM ssDNA	+31.5 mV	±97.9 mV*	+66.4 mV and -129.4 mV
H	Amine 2	500 nM ssDNA	+82.8 mV	±116.0 mV*	+33.2 mV and -198.8 mV
I	Amine 3	500 nM ssDNA	+115.0 mV	±118.4 mV*	+3.4 mV and -233.4 mV
J	New Amine 1	5 μM ssDNA	+108.5 mV	±125.5 mV*	+17 mV and -234 mV
K	New Amine 2	5 μM ssDNA	+97.0 mV	±106.0 mV*	+9 mV and -203 mV
L	New Amine 3	5 μM ssDNA	+101.0 mV	±114.0 mV*	+13 mV and -215 mV

*Indicates bidirectional flow properties in the channel after incubation of ssDNA

Table S6: Absolute measured currents and flow directions of amino modified channels incubated with various concentrations of dsDNA. Surface saturation of the first set of channels at 500 nM dsDNA and subsequent low value for 5 μM experiments informed the preparation of new set of channels to retest 5 μM. See Figure S8 for graphical interpretation of data.

Entry	Channel Wall	Sample	PBS current before sample	PBS current after sample	Change in Current	Direction of Flow
A	Amine 1	5 nM dsDNA	89.0 nA	87.5 nA	-1.7%	Inverted
B	Amine 2	5 nM dsDNA	84.5 nA	86.0 nA	1.8%	Inverted
C	Amine 3	5 nM dsDNA	89.5 nA	92.0 nA	2.8%	Inverted
D	Amine 1	50 nM dsDNA	84.5 nA	85.0 nA	0.6%	Inverted
E	Amine 2	50 nM dsDNA	82.0 nA	80.5 nA	-1.8%	Inverted
F	Amine 3	50 nM dsDNA	86.5 nA	90.5 nA	4.6%	Inverted
G	Amine 1	500 nM dsDNA	82.0 nA	92.5 nA	12.8%	Normal
H	Amine 2	500 nM dsDNA	80.0 nA	86.0 nA	7.5%	Normal
I	Amine 3	500 nM dsDNA	79.5 nA	90.5 nA	13.8%	Normal
J	Amine 1	5 μM dsDNA	70.0 nA	88.5 nA	26.4%	Normal
K	Amine 2	5 μM dsDNA	63.0 nA	84.5 nA	34.1%	Normal
L	Amine 3	5 μM dsDNA	56.0 nA	86.5 nA	54.5%	Normal
M	New Amine 1	5 μM dsDNA	76.0 nA	79.5 nA	4.6%	Normal
N	New Amine 2	5 μM dsDNA	89.0 nA	93.0 nA	4.5%	Normal
O	New Amine 3	5 μM dsDNA	80.0 nA	88.5 nA	10.6%	Normal

Table S7: Calculated surface charges before and after incubation of amino modified channels with various concentrations of dsDNA. See Figure S9 for graphical interpretation of data.

Entry	Channel Wall	Sample	Calculated surface charge before incubation	Calculated surface charge after incubation	Change in calculated surface charge
A	Amine 1	5 nM dsDNA	+112.0 mV	+109.0 mV	-3.0 mV
B	Amine 2	5 nM dsDNA	+101.0 mV	+105.0 mV	+4.0 mV
C	Amine 3	5 nM dsDNA	+113.0 mV	+118.5 mV	+5.5 mV
D	Amine 1	50 nM dsDNA	+101.0 mV	+102.4 mV	+1.4 mV
E	Amine 2	50 nM dsDNA	+93.6 mV	+88.0 mV	-5.6 mV
F	Amine 3	50 nM dsDNA	+106.0 mV	+115.5 mV	+9.5 mV
G	Amine 1	500 nM dsDNA	+93.5 mV	-119.4 mV	-212.9 mV
H	Amine 2	500 nM dsDNA	+86.5 mV	-105.0 mV	-191.5 mV
I	Amine 3	500 nM dsDNA	+84.9 mV	-115.5 mV	-200.4 mV
J	New Amine 1	5 μM dsDNA	+69.0 mV	-84.9 mV	-153.9 mV
K	New Amine 2	5 μM dsDNA	+112.0 mV	-120.5 mV	-232.5 mV
L	New Amine 3	5 μM dsDNA	+87.0 mV	-111.0 mV	-198.0 mV

Table S8: DNase I degradation of dsDNA prior to sample incubation.

Channel Surface	PBS current before sample	PBS current after sample	Change in Current	Flow
Unmodified 100 nm	91.0 nA	90.5 nA	-0.6%	Normal
Amine 89 nm	83.5 nA	84.5 nA	+1.2%	Inverted
Amine/BS3 89 nm	76 nA	Unstable	N/A	Normal

Table S9: Bulk conductivity measurements of buffers used for experiments.

Buffer	Conductivity (mS/cm)
Phosphate Buffered Saline, pH 7.4	16.01
Loading Buffer (70 mM tris borate, 10 mM $MgCl_2$, pH 7.55)	4.33
Tris EDTA Buffer (10 mM tris HCl, 1 mM EDTA, pH 7.0)	1.24
150 mM potassium chloride	17.92

Table S10: Measured currents and calculated surface charges for azide and azide/ssDNA surface modified nanochannels upon incubation with compliment or mismatch DNA sequences.

Surface	**Sample**	**Current Before Sample**	**Surface Charge Before Sample**	**Current After Sample**	**Surface Charge After Sample**
Azide/ssDNA	Compliment	-94.5 nA	-80.3 mV	-98 nA	-93.6 mV
Azide/ssDNA	Compliment	-82 nA	N/A*	-85.5 nA	N/A*
Azide/ssDNA	Compliment	-95.5 nA	-84.5 mV	-98 nA	-93.6 mV
Azide/ssDNA	Mismatch	-93 nA	-73.2 mV	-94 nA	-78.0 mV
Azide/ssDNA	Mismatch	-94.5 nA	-80.3 mV	-96.5 nA	-88.3 mV
Azide/ssDNA	Mismatch	-94 nA	-78.0 mV	-95.5 nA	-84.5 mV
Azide	Compliment	-80.5 nA	N/A*	-82 nA	N/A*
Azide	Compliment	-89 nA	-45.5 mV	-89.5 nA	-50.2 mV
Azide	Compliment	-85.5 nA	N/A*	-88 nA	-33.7 mV

*****Indicates measured current is lower than the model calculated minimum value (86.9 nA) based upon channel dimension.

Table S11: Changes in properties of aminosilane and subsequent 0.23 mM BS3 modified 100 nm nanochannels with varying concentrations of single- or double stranded DNA.

Sample	**Change in Current**	**Direction of flow***
5 nM ssDNA	-1.3 %	Inverted
50 nM ssDNA	-37 %	Both
0.5 μM ssDNA	70 %	Normal
5 nM dsDNA	-8.2 %	Normal
50 nM dsDNA	38 %	Normal

*Normal indicates a negative wall charge

Figure S1: Modification of nanochannel walls. (A) 3-(aminopropyl)trimethoxysilane, H_2O, Room temperature (B) BS3, 5'-amino DNA, phosphate buffered saline (C) Complimentary DNA, magnesium chloride/tris borate buffer

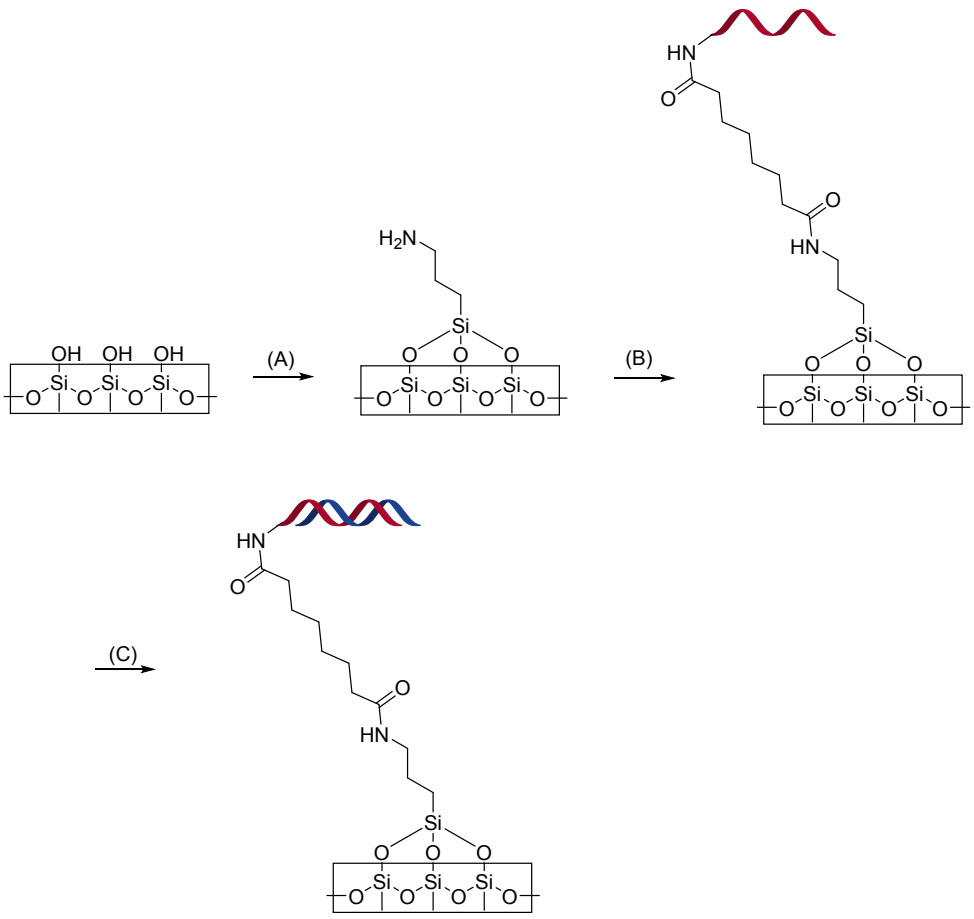
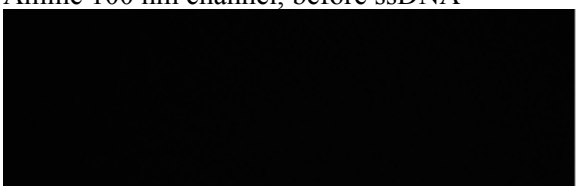


Figure S2: Fluorescence images of variously modified channel before incubation of 5 μM ssDNA (left column) and after incubation/washing of ssDNA (right column).

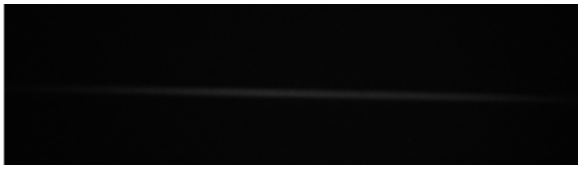
Amine 100 nm channel, before ssDNA



Amine 100 nm channel, adsorbed ssDNA

Random ssDNA 100 nm channel, before ssDNA

Random ssDNA 100 nm channel, adsorbed ssDNA

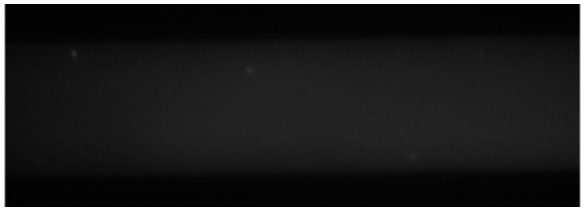


Matching ssDNA 100 nm channel, before ssDNA

Matching ssDNA 100 nm channel, hybridized DNA

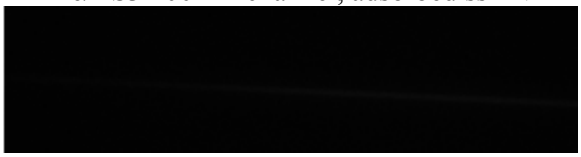
Matching ssDNA 20 μm channel, before ssDNA

Matching ssDNA 20 μm channel, hybridized DNA

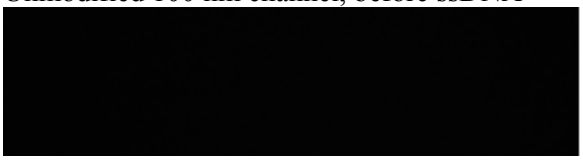


Amine/BS3 100 nm channel, before ssDNA

Amine/BS3 100 nm channel, adsorbed ssDNA



Unmodified 100 nm channel, before ssDNA



Unmodified 100 nm channel, adsorbed ssDNA

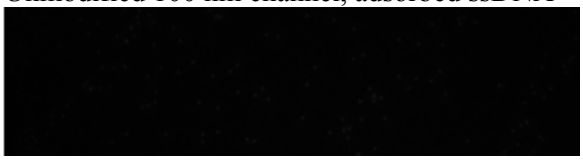
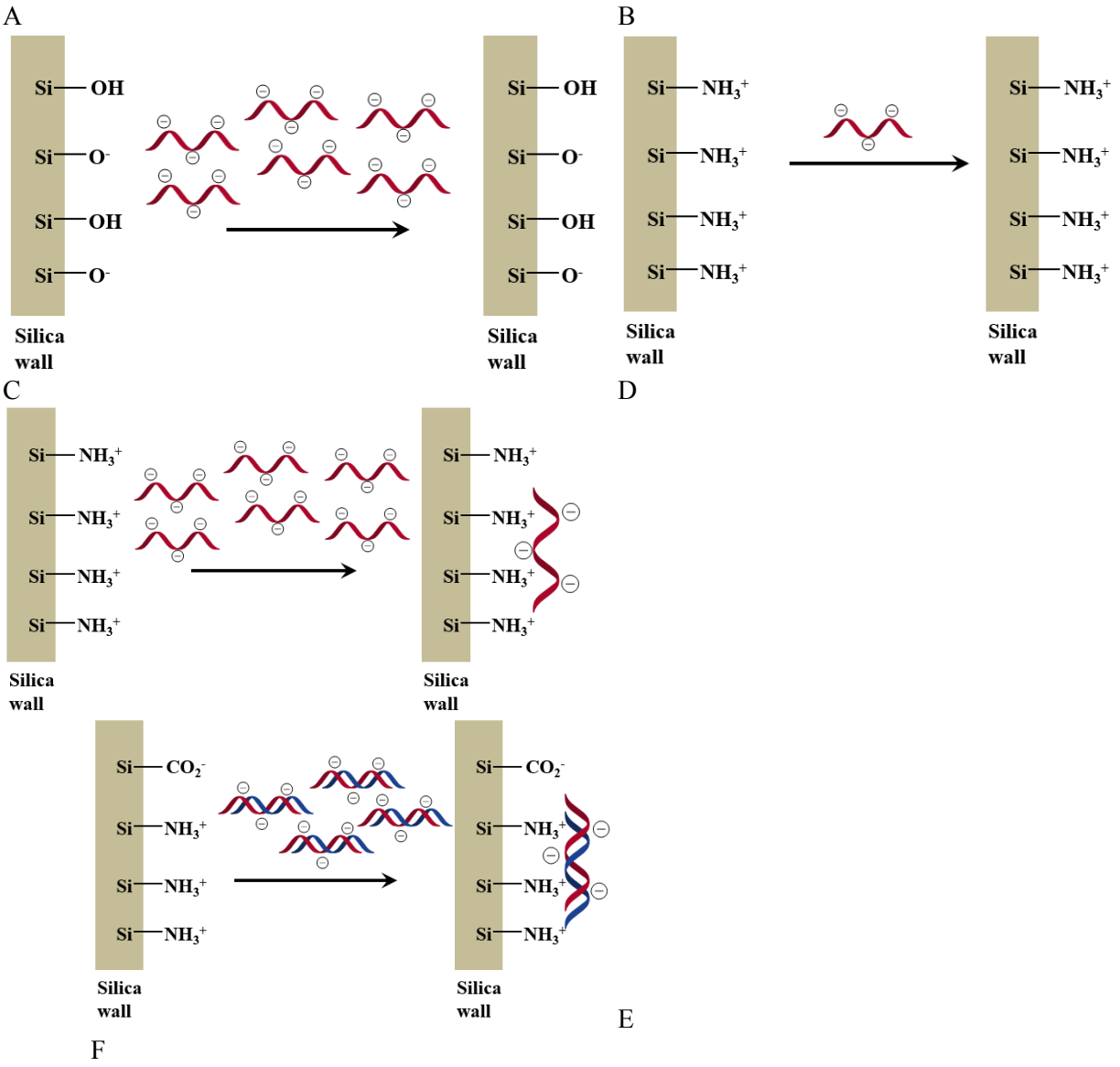


Figure S3: Possible interactions of nucleic acids with nanochannel walls. Displayed are no change in wall properties resulting from (A) a negatively charged surface repelling nucleic acid adsorption or (B) dilute concentrations of nucleic acids not binding. Reducing the measured current without changing sign of surface charge is shown in (C), when ssDNA adsorbs to aminosilanized walls. Reduced measured current accompanied by change in sign of surface charge is shown in (D) when dilute amounts of dsDNA adsorbs to amine/BS3 modified channels. Finally, increase of measured current and inversion of surface charge occurs when (E) sufficient dsDNA adsorbs to amino modified walls or (F) dsDNA is formed at the surface by surface ssDNA modification hybridizing to complementary solution ssDNA. Channels with bidirectional flow are not modeled in this figure as a result of uncertainty in exact channel wall properties.



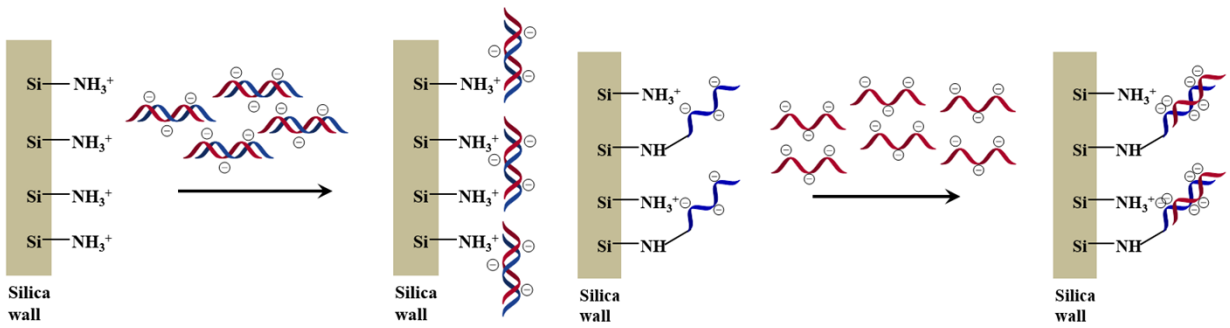


Figure S4: Surface functionalization with 3-(azidopropyl)trimethoxysilane and subsequent copper free click modification with 5'-DBCO modified DNA. (A) 3-(azidopropyl)trimethoxysilane, iPrOH, Room temperature (B) 5'-DBCO DNA, phosphate buffered saline (C) Complimentary DNA, magnesium chloride/tris borate buffer

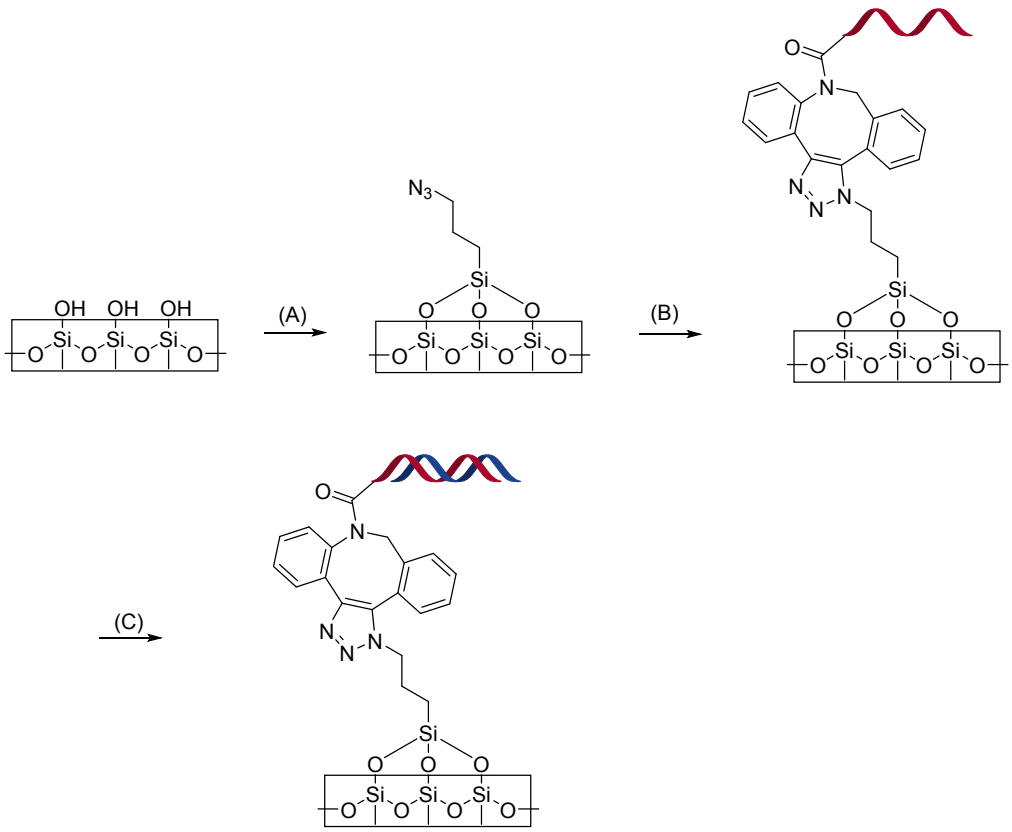


Figure S5: Histogram representing data in Table S3. Alphabetical legend corresponds to entry column in Table S3. Green bars indicate a positive surface after DNA exposure, blue bars indicate bidirectional flow after DNA exposure, and red bars indicate negative surface charge after DNA exposure.

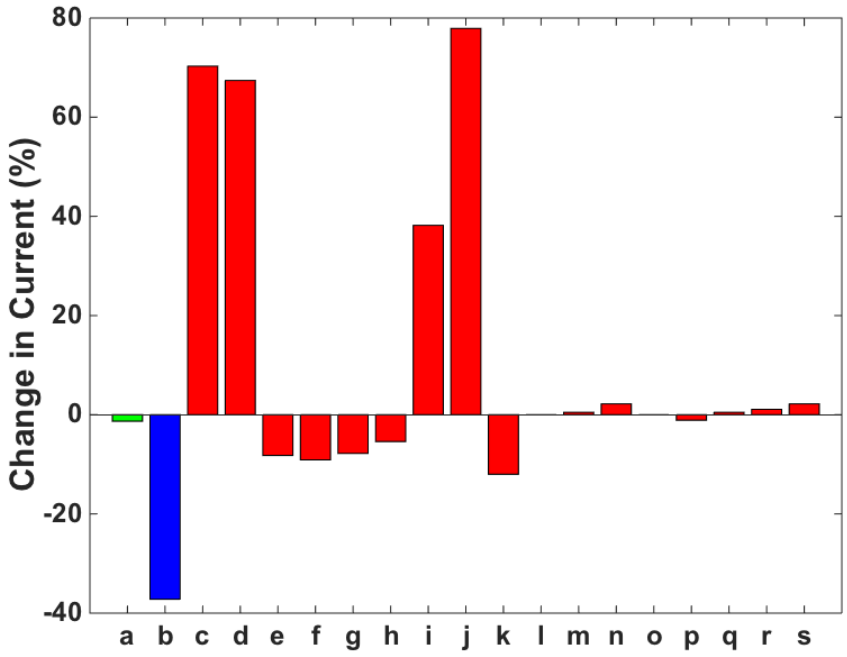


Figure S6: Histogram representing data in Table S4. Alphabetical legend corresponds to entry column in Table S4. Blue bars indicate a positive surface after DNA exposure, green bars indicate bidirectional flow after DNA exposure, and red bars indicate negative surface charge after DNA exposure.

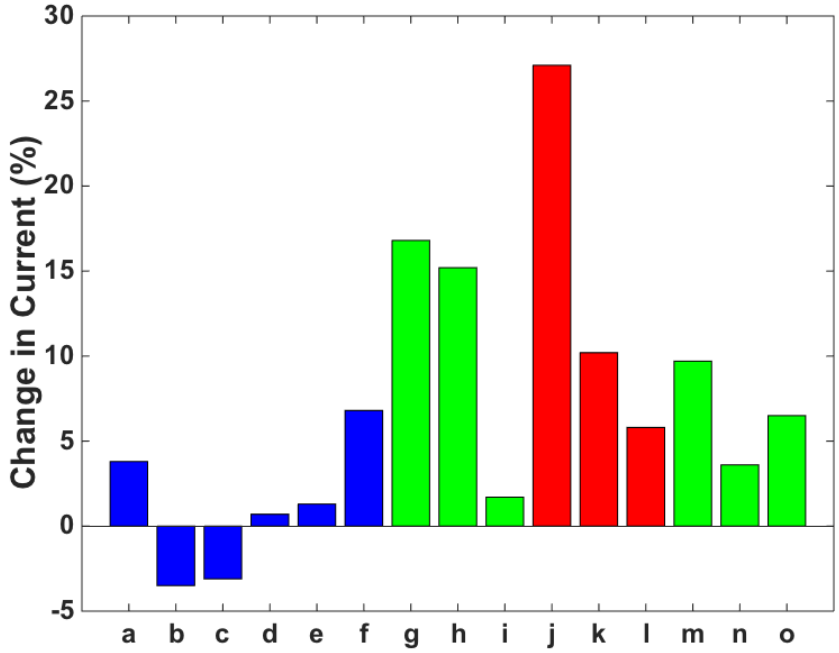


Figure S7: Histogram representing data in Table S5. Alphabetical legend corresponds to entry column in Table S5. Green bars indicate a positive surface and increased measured current after DNA exposure, blue bars indicate bidirectional flow after DNA exposure, and red bars indicate a positive surface charge but decreased measured current after DNA exposure.

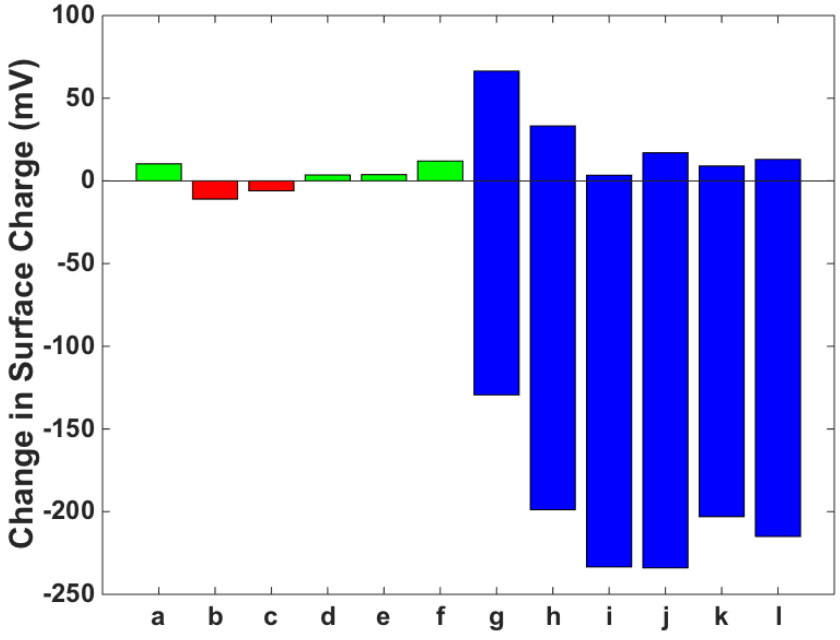


Figure S8: Histogram representing data in Table S6. Alphabetical legend corresponds to entry column in Table S6. Green bars indicate a positive surface after DNA exposure and blue bars indicate negative surface charge after DNA exposure.

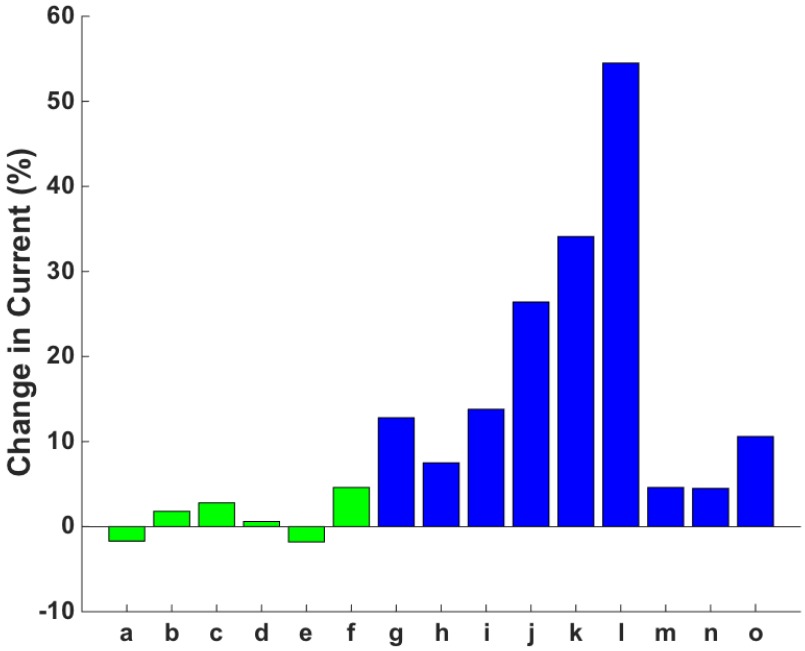
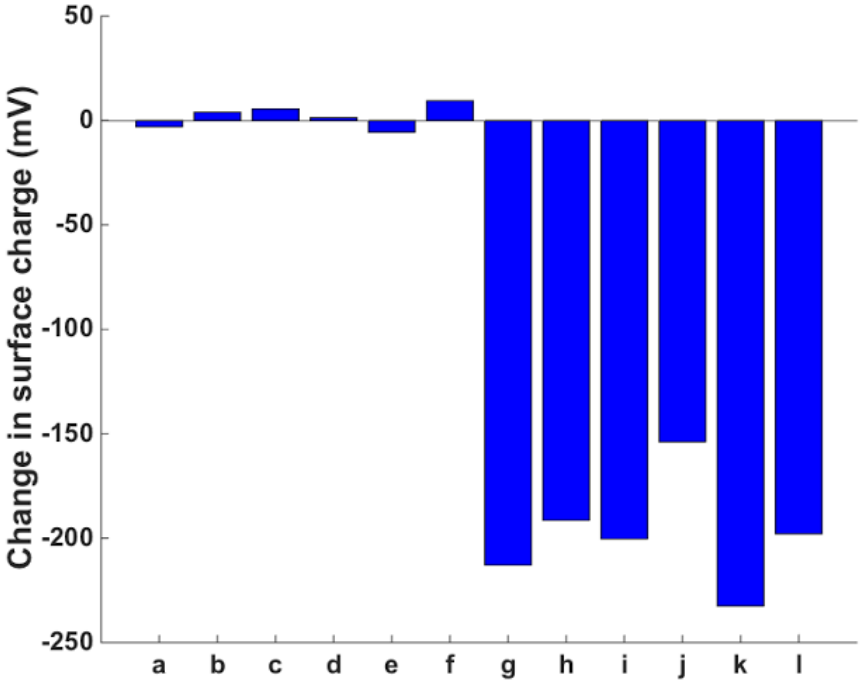


Figure S9: Histogram representing data in Table S7. Alphabetical legend corresponds to entry column in Table S7.



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1. Gillespie, D., & Pennathur, S. (2013). Separation of Ions in Nanofluidic Channels with Combined Pressure- Driven and Electro-Osmotic Flow. *Analytical Chemistry*, *85*, 2991–2998.
2. Azam, M. S., Fenwick, S. L., and Gibbs-Davis, J. M. (2011). Orthogonally Reactive SAMs as a General Platform for Bifunctional Silica Surfaces. *Langmuir*, *27*, 741-750.