In article number 1602601, Michel Godin and co-workers demonstrate that on-chip microvalves enable the regulation of electrical and fluidic access to an array of nanopore sensors integrated within microfluidic networks. Using a single pair of electrodes, multiple independent nanopores are fabricated in situ by a controlled breakdown process and are used to interrogate biomolecular samples compartmentalized within a single device equipped with microvalves.
Nanopore sensors most commonly rely on an ionic current measurement to detect and characterize the conformation and structure of biological molecules such as DNA, protein, or molecular complexes. Intensive research efforts over the past two decades have explored their use in a variety of applications including DNA sequencing, single molecule force and mass spectroscopy, protein–aptamer interactions, and drug discovery. Solid-state nanopores have drawn attention for their tunable size, environmental tolerance, and propensity for integration in lab-on-a-chip technologies.

A solid-state nanopore device typically consists of a single nanoscale channel in a thin insulating membrane that separates two electrolyte solutions. Ionic current is induced across the pore by applying an electric potential difference across the membrane. The passage of molecules through the nanopore causes a disruption in the ionic conductance, which can be used to determine the structure and identity of the translocated molecule. Typically, a nanopore experiment requires the translocation of many individual biomolecules to reliably extract information with statistical significance. Therefore, arrays of nanopores have compelling advantages in terms of rapid and high-throughput data acquisition. In addition, nanopore sensors are typically situated between macroscale fluidic reservoirs. This configuration limits the ability of a nanopore device to perform on-demand sample preparation, manipulation, and processing.

Sample manipulations within microfluidic architectures are recognized to enhance experimental outcomes through automation and miniaturization of analytical sensors. Many researchers are adapting microfluidic technologies to perform a variety of diagnostic tests with built-in and low-cost analysis capabilities due to their spatial and temporal control. They can confine, preprocess, purify, mix, sort, and fractionate a small volume (micro to nanoliters) of fluidic sample in their microscale architectures.

Some techniques and strategies have been reported to integrate solid-state nanopores in microfluidics. Nanopores used in these studies were milled in a thin insulating membrane using a high energy electron or ion beam or derived from quartz capillaries with a laser puller prior to integration. Beam-based techniques of nanopore fabrication are complex, expensive, and ill-suited for integration within microfluidic networks since nanopores must be fabricated prior to integration, i.e. milled ex situ. For instance, the fragility of large-area membranes, handling risks, wetting issues, and inaccurate alignment of multiple channels on prefabricated nanopores are among the commonly reported challenges. On the other hand, while glass nanopores fabricated by laser pulling reduce cost and offer good performance, reproducibly achieving sub-20 nm diameter pores in this manner is generally difficult, and scaling to large arrays of glass pipettes can be challenging.

We recently reported a microfluidic device in which an array of low-noise solid-state nanopores was fabricated in situ by controlled breakdown (CBD). Due to its simplicity, the CBD method can easily accommodate nanopore fabrication in solid-state membranes enclosed in a range of fluidic configurations. Importantly, we found that controlling the electrical field symmetry during the nanopore fabrication process and biomolecular sensing improved the sensor’s detection efficiency. We demonstrated that a symmetric electric field can be ensured by including microfluidic vias on the membrane at the site of nanopore formation and/or by using equally biased electrodes within the same microchannel, one located upstream of the membrane and another downstream. However, the device previously presented required that each microfluidic channel be controlled independently with its own fluidic and electrical access. Indeed, the large number of fluidic and electronic connections (two fluidic tubes and two electrodes per channel) crowded the device and complicated the surrounding setup.

In an effort to mitigate this issue, this work highlights the use of on-chip pneumatic microvalves, which also provides...
new opportunities for fluidic manipulation before and during sensing.\textsuperscript{31,32} Typically, pneumatic valves are formed by stacking two microfluidic channels formed in separate layers using an elastomeric material. When a high pressure is applied to one channel, the other one will be compressed, restricting flow. This technology has been developed as a practical way to obtain microfluidic large-scale integration\textsuperscript{33} in the design and fabrication of biosensors.\textsuperscript{34–36}

Herein, we demonstrate a microfluidic device that includes multiple microvalves and an array of five independently addressable solid-state nanopores on a single membrane. This feature allows for precise manipulation of fluidic and electrical access to various regions of the embedded silicon nitride (SiN) membrane from a common inlet with a single pair of electrodes. Importantly, we show that the pressurized (actuated) microvalves provide electrical resistances that are high enough (>50 GΩ) to isolate desired regions of the SiN membrane for the serial fabrication and independent use of nanopore sensor in each flow channel. We introduce a looped flow channel design to ensure a symmetric electric field profile at the pore location on the insulating membrane.

In addition, we discuss chemical compatibility when nanopores are integrated within polydimethylsiloxane (PDMS) microchannels and report improved performance when the PDMS components constituting the microchannel layers of the device are treated with organic solvents prior to embedding the solid-state chip. This treatment removes uncross-linked contaminants from the bulk polymer.\textsuperscript{37}

Finally, we present the ability to store samples on chip prior to nanopore analysis using microvalves. One of the more intriguing features of this design is the ability to use the microvalves in a partially pressurized configuration to restrict fluid flow while allowing for electrical access to the nanopores for biomolecular sensing.

The microfluidic device integrating a solid-state membrane supported by a silicon chip is pictured in Figure 1. Five connected flow channels equipped with on-chip microvalves allow for the fabrication of individually addressable nanopores by CBD\textsuperscript{29,30} and subsequent enlargement with high electric fields.\textsuperscript{38,39} Figure 1a is a top view of the device showing the microvalves (red) and flow channels (blue) made from multilayered PDMS pieces.\textsuperscript{40} Only a small (=1000 µm\(^2\)) region of the membrane inside each flow channel is exposed to electrolyte through a microfluidic via (Figure 1b). As previously reported,\textsuperscript{29} these microvias limit the exposed area of the membrane to the aqueous solutions, thus localizing the formation of the nanopores by confining the electric field. This also results in a significant reduction of high-frequency noise in the ionic current signal through reduction of chip capacitance.\textsuperscript{41} The fluid is flushed in and out of the device through a common inlet and outlet. The etched side of the silicon chip sits atop a wide microfluidic channel containing an electrode that is common to all nanopores on the device (shown as purple in Figure 1). On the other side of the membrane, five connected flow channels (shown in blue) equipped with on-chip microvalves (shown in red) allow for the fabrication of individually addressable nanopores by CBD. A routing microvalve (green in Figure 1a) is also included next to the common inlet and pressurized during fluid introduction to ensure that flow channels are filled uniformly. Valves pressurized to 30 psi act as electrical resistors, providing more than 50 GΩ of electrical resistance in each flow channel that can effectively isolate different regions of the SiN membrane during serial nanopore fabrication and biomolecular sensing. Measurements validating the microvalve functionality are described in Section S7 of the Supporting Information.

During the fabrication of each nanopore, electrical access is restricted in the other four flow channels by pressurizing the corresponding microvalve pairs (Figure 1c). The pair of microvalves addressing the targeted flow channel remains unpressurized (Figure 1d). A cross-sectional view of a typical device containing a pair of microvalves, a flow channel, and a microvia atop the membrane is presented in Figure S3 of the Supporting Information.

While elastomeric microvalves are extensively used in microfluidic devices, we found that controlling the hydrophobicity of the flow channel wall was particularly important to ensure a high electrical resistance seal. High hydrophobicity prevents electrolyte solution (and thus parasitic ionic current) from remaining in the channel cross section when a flow channel is compressed by its valves. However, wetting the insulating membrane prior to nanopore fabrication is important and this can be challenging when the hydrophobicity of the device is reduced. In most cases, it was adequate to flush the hydrophobic flow channels with ethanol prior to the introduction of an aqueous solution. This flushing procedure increases the wettability of the flow channels and the SiN membrane due to the low surface tension of ethanol. Although plasma-treating\textsuperscript{42} the assembled device prior to nanopore fabrication could still be a useful method to facilitate wetting of the flow channels and the membrane surface, we found that all of the valves in the device needed to be

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**Figure 1.** a) Micrograph of the integrated SiN chip in a microfluidic network containing the flow channels (blue), microvalves (red), routing valve (green), and a common bottom channel (purple). b) Five flow channels, each containing a 50 × 20 µm\(^2\) microvia, cross over a 500 × 500 µm\(^2\) SiN membrane. c) Pressurized (actuated) mode: during nanopore fabrication or biomolecular sensing in the desired flow channel, all other valve pairs are pressurized to 30 psi to obtain high electrical resistance seal. d) Unpressurized (unactuated) mode: valve pairs corresponding to the target flow channel are charged to atmospheric pressure to allow for electrical and fluidic access to the nanopore. See the Experimental Section for more details.
pressurized during plasma treatment in order to maintain the hydrophobicity of the flow channels at the valve locations. This process is described in Section S5 of the Supporting Information.

The high electrical resistance seal provided by the on-chip microvalves enables the fabrication of individual nanopores by CBD, while the other areas of the same SiN membrane that are exposed to the electrolyte in other flow channels are electrically isolated and remain intact. We also confirmed that existing nanopores remain unaffected when isolated by the microvalves even when high electric fields are applied in adjacent flow channels. Each solid-state nanopore is formed by applying 14–18 V across the 20 nm thick SiN membrane, thus producing a high electric field (0.7–0.9 V nm⁻¹). A typical nanopore fabrication curve is shown in Figure S6 of the Supporting Information. Nanopore fabrication typically takes a few minutes⁴⁴ and the resulting nanopores are assessed and electrically validated immediately prior to performing molecular sensing experiments.

Figure 2a shows current to voltage (I–V) measurements of five nanopores fabricated sequentially in a single device. The diameter d of each nanopore was extracted from its conductance, G, which was measured by recording the ionic current over a range of applied potentials.⁴³ Equation (1) describes the relationship between nanopore diameter and conductance, where σ is the conductivity of the electrolyte and \( L_{\text{eff}} \) is the effective thickness of the nanopore assuming a cylindrical geometry,

\[
G = \frac{\sigma}{\pi d^2} \left( \frac{4L_{\text{eff}}}{\pi d^2} + \frac{1}{d} \right)^{-1}
\]

(1)

Generally, the effective thickness of the nanopore is smaller than the nominal thickness of the SiN membrane due to deviations from the assumed cylindrical shape of the nanopore (60%–85% of its nominal thickness).⁴⁴ Interestingly, nanopores formed at different locations within a single membrane can show different effective thicknesses, possibly due to varying SiN thickness across the membrane window or most likely as a result of slight deviations of the actual nanopore shape from the assumed perfect cylindrical geometry. Note that we have found that this effect is more pronounced on membranes with thicknesses that are ≥20 nm than on <10 nm membranes.

Double-stranded DNA (dsDNA) translocation experiments were conducted to assess the suitability of these integrated nanopores for biomolecular sensing applications. Figure 2b shows typical ionic current traces through five nanopores in the same device, each having different diameters. Here we present an example of a device in which the nanopore in the first flow channel exhibited high 1/f noise and was unsuitable for biomolecular sensing, while the remaining nanopores formed in the same membrane but in the other flow channels were fully functional and exhibited a clean, stable conductance prior to the addition of biomolecular samples. This highlights one of the advantages of working with arrays of nanopores that are individually addressable using on-chip microvalves, namely increasing the experimental yield of functional pores by enabling the analysis of either the same injected sample with multiple nanopores or multiple samples independently within a single device.

In this example, the biomolecular samples consisting of 100, 250, 5k and 10k base pair (bp) dsDNA fragments were independently introduced to the flow channels and the resulting translocation characteristics were consistent with results obtained using standard nanopore setups (Section S14 of the Supporting Information).⁴³

In PDMS-based microfluidic devices, incomplete curing of the two-part elastomer can result in unpolymerized monomers leaching out into the fluidic microchannels. In some instances, we have observed a degradation in the performance of a device over time (days) which we have linked to this leaching process. This can manifest itself as an increase in 1/f noise, frequent device clogging due to a gradual change in nanopore surface properties or a reduction in the nanopore capture rate. However, these issues were mitigated by immersing the PDMS components in a series of organic solvents prior to assembly,⁴⁷ which effectively removed unreacted low-molecular weight monomers from the PDMS

![Figure 2. a) Current to voltage (I–V) curves used to infer each nanopore diameter. b) Traces of the ionic current through five independently fabricated nanopores in a single device. The first nanopore (black) exhibited high noise and was unsuitable for biomolecular sensing experiments. The remaining four showed the translocation of various lengths of dsDNA under an applied potential difference of 200 mV. All measurements were done in 2 M KCl buffered at pH 8.0 and ionic current traces were sampled at 250 kHz, low-pass filtered at 100 kHz using a four-pole Bessel filter, and multiplied by −1 for visualization.](image-url)
bulk phase (see the Experimental Section). This procedure ensures that the devices and their integrated nanopores perform reliably even when stored for up to 10 days prior to nanopore fabrication or being used in molecular sensing experiments.

The device configuration allowed for experiments to be conducted in various modes of operation depending on whether the sample was introduced in one of the independent flow channels or the common microchannel located on the opposite side of the membrane. Samples introduced to the common channel could be analyzed by several different nanopores sequentially. This might be useful if the performance of a nanopore degrades during an experiment, as other nanopores can be used to continue acquiring translocation data to obtain a statistically significant number of single molecule events. This is particularly advantageous when a precious, low-volume sample is undergoing analysis. Experiments can also be conducted using the independent channels under constant flow conditions with the ability to exchange solutions and dilute samples at any time to be analyzed either by the same nanopore or by others in the array.

An interesting extension is the ability to use on-chip valves to store different samples in the vicinity of a nanopore until they are ready to be analyzed. In Figure 2b, sensing was performed as each flow channel was sequentially filled with the desired sample type while other flow channels were fluidically isolated by their corresponding valves, as described in the Experimental Section. After >1000 translocation events were recorded, the sample was then trapped in the flow channel by pressurizing the appropriate microvalve pair while the device was flushed with fresh solution and a new sample was loaded to a neighboring flow channel. After all samples were loaded, a specific sample could then be analyzed by opening the appropriate pair of valves. While we successfully conducted experiments under these conditions, great care was required in this operational mode to equalize pressures to minimize unintended flow through the channel. Any pressure differential across a channel, even one induced by a difference in height of the vials that accommodate the inlet and outlet tubing, would rapidly remove a plug of solution containing sample from the vicinity of the nanopore. This small but unintended flow could result in a drastic decrease in sample concentration and nanopore capture rate (Section S8 and Figure S8 of the Supporting Information).

We can address this issue by using the microvalves in a partially pressurized configuration. As shown in Figure S10 of the Supporting Information, it is possible to reduce the cross-sectional area without complete collapse of the flow channel using a moderate valve pressure. This constriction increases flow resistance, minimizing the removal of sample from the vicinity of the nanopore while still allowing electrical access for biomolecular sensing. In the case of a small sample volume (nanoliters) trapped in a flow channel between valves while the remainder of the channel is filled with pure electrolyte solution, the reduced cross-sectional area of the constricted channel also minimizes diffusion of biomolecules away from the sensing region of the nanopore. In order to facilitate the partial collapse of flow channels, a device with modified flow channel dimensions (wider flow channels under narrower valves) was used (Figure S2, Supporting Information). A schematic cross section of this operational mode is shown in Figure 3a, where 5 kb dsDNA was first introduced into a flow channel containing a nanopore. Figure 3b(i) shows an ionic current trace containing biomolecular translocation events while the valves remained unpressurized. As pressure is applied to the microvalves, the flow channel is partially collapsed on either side of the nanopore, trapping the sample in the vicinity of the pore. As shown in Figure 3b(ii), applying moderate pressure to the microvalves initiates a reduction in the ionic current measured through the system due to the increased electrical resistance imparted by the partially collapsed flow channel. However, the desired electric field strength at the nanopore was then re-established by increasing the applied electric potential difference (Figure 3b(iii)) until the ionic current through the nanopore was equal to its initial value when the valves were unpressurized.

In order to show that nanopore sensing is possible while the microvalves are partially actuated and test the limits of channel constriction while maintaining molecular sensing ability, a proof-of-concept experiment was performed in which dsDNA was introduced into an independent flow channel while the corresponding microvalve pair was pressurized. To determine the limits of channel compression

![Figure 3. a) Schematic cross section of a device showing a flow channel (light blue) and microvalves (dark red). By pressurizing the microvalves to a moderate pressure $P_v$, the active PDMS membrane is deflected downward to reduce fluid flow and sample loss through the flow channel (dashed lines). b) Ionic current traces showing the translocation of 5 kbp dsDNA in 2 mM KCl pH 8.5 through a 7.8 nm pore in three different conditions: (i) an applied bias $V_b = 200$ mV and pressure $P_v = 0$ psi, (ii) $V_b = 200$ mV and $P_v = 20 \pm 1$ psi, and (iii) $V_b = 500$ mV and $P_v = 20 \pm 1$ psi. Traces of individual linear (unfolded) translocation events before and after the valves are pressurized are shown in the insets. Dashed red lines show fits to the events using Equation (S7) of the Supporting Information to extract rise times and blockage levels of translocation events. Ionic current measurements were acquired at 250 kHz and low-pass filtered at 100 kHz using a four-pole Bessel filter.](image)
while maintaining the ability to sense biomolecular translocations, the experiment presented in Figure 3 reflects the maximum valve pressure that could be applied before completely preventing electrical measurements of the nanopore sensor (20 ± 1 psi for this device). In this configuration, the flow channel is almost completely collapsed, with a cross sectional area reduced from 12 500 µm² to <50 µm² at the location of the microvalves. Cross-sectional profile of the flow channel during microvalves actuation is presented in Section S9 of the Supporting Information. While this constriction had negligible effect on the equivalent capacitance of the device in this regime (20 ± 5 pF), the total electrical resistance of the flow channel was increased by almost three orders of magnitude, from \( R_{ch} = 0.075 \pm 0.005 \) MΩ to \( R_{ch} = 45 \pm 5 \) MΩ, and the ionic current was reduced accordingly. Even after increasing the applied voltage from 200 to 500 mV in order to re-establish the ionic current level through the channel, this added resistance results in a smaller ionic current blockade upon translocation, as shown in Figure 3b. The relative change in the current blockage in the pressurized and partially pressurized valve configurations is given by

\[
\frac{\Delta' I}{\Delta I} = \frac{R_{DNA}}{R_{ch} + R_{DNA}}
\]

(2)

where \( \Delta' I \) and \( \Delta I \) are the ionic current changes caused by DNA translocation while the valves are, respectively, partially pressurized and open. \( R_{ch} \) is the resistance of the channels when the valves are partially pressurized, and \( R_{DNA} \) is the nanopore resistance during the passage of a DNA molecule. In this example, the ratio of blockage levels for unfolded dsDNA in the pressurized and unpressurized valve regimes is 0.41, in agreement with what is expected for the electrical resistance values measured (Section S10 of the Supporting Information).

The increase in electrical resistance of the flow channels when the valves are partially pressurized can also affect the shape of the ionic current trace corresponding to the translocation of dsDNA molecules by significantly slowing the response of the ionic current to step changes in the pore resistance. The current response is dominated by the slower shape of the ionic current trace corresponding to the translocation of dsDNA molecules by significantly slowing the response of the ionic current to step changes in the pore resistance. The current response is dominated by the slower response of the ionic current to step changes in the pore resistance. This distortion effect is present in configurations of extreme flow channel compression, such as that shown in Figure 3, we note that it is possible to apply a lower pressure to the microvalves (<10 psi) such that flow channel constriction does not contribute significant electrical resistance while still restricting flow (Figure S10, Supporting Information). However, the minimal signal attenuation inherent in such configurations comes at the expense of a decreased ability to confine sample in the vicinity of a nanopore for sensing.

Here, we have utilized PDMS-based pneumatic microvalves to manipulate electrical and fluidic access to solid-state nanopore arrays fabricated by controlled breakdown within microfluidic architectures. While unpolymerized monomers from the PDMS components of a device can interfere with nanopore sensing, their extraction using a series of organic solvents improves device performance. This is providing a biocompatible environment for rapidly generating prototype lab-on-a-chip devices. By fully collapsing flow channels using pressurized microvalves, it is possible to obtain a high electrical resistance seal (>50 GΩ) for the serial on-demand fabrication of nanopores in different regions of a single embedded silicon nitride membrane. This integrated sensing platform allows for biomolecular analysis while offering microfluidic sample processing capabilities, all while efficiently minimizing the required number of electrodes and fluidic tubing to increase scalability. To improve nanopore fabrication and molecular sensing, a symmetric electric field is provided within a looped flow channel and using a single electrode pair. In addition, microvalves allow for the introduction and sequestration of multiple samples to be analyzed by different nanopores within a single device. Furthermore, partially pressurizing microvalves can effectively segment a biomolecular sample in the vicinity of a nanopore while allowing electrical access for sensing. While the flow channel cross section can be reduced to sub-50 µm² to minimize parasitic flow and sample loss, this can impart an electrical resistance comparable to the integrated nanopore, limiting the bandwidth of the sensor. However, varying microvalve pressure can precisely control cross section collapse to provide <1 MΩ of electrical resistance while still segregating fluidic samples. Such sample control can ultimately be used to effectively purify, sort, and mix biomolecular samples for on-chip analysis using large-scale arrays of nanopores.

**Experimental Section**

**Microfabrication:** Low-pressure 500 × 500 µm², 20 nm thick silicon nitride membranes supported on 100 µm thick, 3 mm frame size silicon substrate (SiMPore Inc., SN100-A20Q05) were embedded between PDMS pieces (Sylgard 184 kit, Dow Corning). Each PDMS piece was replicated from a master mould fabricated by multilayered soft lithography with SU-8 2050 (MicroChem)
and AZ 50X (Electronic Materials) photoresists. Full microfabrication protocols and methods are provided in Sections S2 and S3 of the Supporting Information.

Device Specifications and Assembly: Figure 1a is a top view of an assembled device in which aqueous food coloring (Club house) was used to feature flow channels and valves. A three-layer PDMS piece (valves, flow channels, and the microvia layer) was aligned and bonded to the membrane side of the silicon chip using an oxygen plasma system. The etched side was placed on a hand-punched hole in the middle of a single channel (purple) to allow electrical and fluidic access to the etched side of the chip. This common bottom channel is 400 µm wide and 100 µm high.

The flow channel layer (blue) consisted of five connected microfluidic channels, which narrowed over the membrane. The valve layer (red) located above the flow channels contained five pairs of independent channels, which intersected perpendicularly with flow channels of similar width. In order to avoid undesired collapsing of a fluidic channel, valve channels narrowed over such intersections (passive crossovers). A routing valve (green) was discharged to atmosphere during nanopore fabrication and biomolecular sensing, resulting in a symmetric electric field across the membrane in each flow channel.

A thin (5–7 µm) deflectable PDMS membrane separates the flow channel layer from the valves. The thickness of this membrane is dictated by the thickness of the PDMS elastomer covering the flow channels. Therefore, uncured PDMS was spun onto the flow channels mould whereas the elastomer was directly cast on the valves mould. Consequently, the valve layer was several millimeters thick to securely accommodate the tubing and electrodes. Detailed device specifications and assembly are presented in Sections S1 and S4 of the Supporting Information. Figure S13 (Supporting Information) is an overview of a typical integrated nanopore-microfluidic device with five connected flow channels while all the microfluidic channels are filled with food coloring dyes.

Solvent Extraction of PDMS Pieces: Significant improvement in the yield of nanopores functional for biomolecular sensing was observed when residual uncross-linked PDMS monomers were extracted from the PDMS device components. For this purpose, the PDMS pieces were immersed sequentially in three different organic solvents prior to mounting the silicon chip. The PDMS pieces were first soaked in 20% v/v hexane in ethanol for 1 hour, followed by ethyl acetate (1 day), then acetone (2 days). The components were then dried at 70 °C for 2 days in an oven. This extraction resulted in an ~0.4%–1.0% reduction in overall weight of the PDMS pieces.

Electrical and Fluidic Setup: The location of the electrodes was chosen to limit the electrical resistance of the flow channels approaching each nanopore to ~100 kΩ in 1 m solution of potassium chloride, KCl (~1% of the total electrical resistance of a 10 nm nanopore). Fluid was introduced to the flow channels and microvalves via polyether ether ketone (PEEK) tubes (IDEX Health & Science), which were connected to vials pressurized using high-precision pressure regulators. To avoid introducing air bubbles in the flow channels, the valves were filled and pressurized with deionized water. Prior to nanopore fabrication, a resistance seal of ~100 GΩ was measured between the different flow channels while the valves were pressurized up to 30 psi.

Nanopore Fabrication: Individual nanopores were fabricated within a few minutes by CBD in 1 m KCl buffered to pH 8.3 (σ = 11.0 ± 0.1 S m⁻¹). As previously described, this was achieved by applying high electric fields across the SiN membrane (using a potential difference of 14–18 V) through each of the integrated flow channels using a custom-built current amplifier circuit. A typical nanopore fabrication by CBD is shown in Section S6 of the Supporting Information. Nanopores were conditioned and enlarged as required by the cyclic application of moderate electric field pulses. Afterward, nanopore conductance was measured in the same electrolyte solution that was used for biomolecular sensing experiments. Ionic current power spectral density plots of the presented nanopores are provided in Section S11 of the Supporting Information. This protocol was used to produce 15 microfluidic devices, each containing three to five stable low-noise nanopores. Pores showing excess 1/f noise (defined as >10 pA²/Hz at 1 Hz under an applied potential of 200 mV) were not used in sensing experiments.

Sample Preparation and Sensing Methods: Double-stranded DNA fragments (NoLimits, Thermo Scientific) of 100, 250, 5k and 10k bp in length were diluted in 2 m KCl (σ = 20.0 ± 0.1 S m⁻¹) buffered with 10 × 10⁻⁵ m HEPES (an organic chemical buffering agent) to pH 8.0 to a final concentration of 750 ng µL⁻¹. To avoid mixing of different DNA lengths between experiments or between flow channels within a device, the common inlet channel was flushed with aqueous salt solution prior to the introduction of each new sample. For this purpose, all valves were pressurized while the routing valve was unpressurized. The target dsDNA sample was sent through the next flow channel and sensed with the appropriate nanopore. All sensing experiments were performed until ~1000 translocation events were acquired for each nanopore.

Data Acquisition and Analysis: All experiments were performed inside a Faraday cage to minimize environmental electrical noise pickup. Custom-written LabVIEW programs interfaced with a USB-6351 DAQ card (National Instruments) were used for nanopore fabrication and sensing. Nanopore fabrication was monitored at 10 Hz, while data acquisition for biomolecular sensing experiments was performed at 250 kHz and low-pass filtered at 100 kHz with a four-pole Bessel filter using an Axopatch 200B (Molecular Devices). Translocation data were analyzed using a custom implementation of the CUSUM algorithm, as well as the adept2State module of MOSAIC v1.3. Figures were plotted in Originlab. Nanopore and microchannel capacitance measurements were performed using a handheld capacitance meter (Keysight U1701B) connected to electrodes embedded in the appropriate microchannels under the indicated operational modes.

Supporting Information
Supporting Information is available from the Wiley Online Library or from the author.

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for Innovation (CFI). The authors would also like to thank Sebastian Hadjiantoniou for his help in analyzing the fluorescence images.

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