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Review—Track-Etched Nanoporous Polymer Membranes as Sensors: A Review

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The need to fabricate a nanoporous sensor that can be utilized for the resistive-pulse sensing of particles without labeling them has generated extensive research and led to various methods for nanopore fabrication on several materials. Since the first development of track-etching method and its use on polymer membranes, there has been an ongoing interest in their applications. In this review, we look at the background on tracking technology, chemical etching of these tracks for the fabrication of nanopores with varying geometries and we discuss their applications as electrochemical sensors for biomolecules (i.e. DNA and protein), nanoparticles and others. The main emphasis is on resistive-pulse sensing using single nanopores fabricated by track-etching on polymer membranes. We also discuss sensing based on the specific current – potential (I–V) behavior of asymmetric nanopores as the sensing element. © 2020 The Author(s). Published on behalf of The Electrochemical Society by IOP Publishing Limited. This is an open access article distributed under the terms of the Creative Commons Attribution 4.0 License (CC BY, <http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted reuse of the work in any medium, provided the original work is properly cited. [DOI: 10.1149/1945-7111/ab67a7]



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Nanopore based sensors are very interesting for researchers around the world for their possible applications in medicine, biotechnology and genetics. These sensors work on the well-established Coulter-Counter principle and the method is called resistive-pulse sensing (RPS) (also known as Stochastic Sensing). Coulter-Counter principle is used for measuring the concentration and size of relatively large particles. In a Coulter-Counter, a fixed pore of micrometer scale on an insulating membrane is placed between two reservoirs filled with electrolyte solutions and the particles are driven electrophoretically to the electrodes with opposite charges.¹ As the particle passes through the pore (translocates), there is a momentary drop in the ionic current creating a resistive pulse (a signal) which can then be used for the identification or the counting of particles. RPS mainly focuses on the detection of molecules without the need for labeling as well as separation of particles by their sizes, shapes, surface charge densities etc. RPS is of importance since it emerges as an alternative method among various other electrochemical sensing techniques^{2–5} for the detection of biomolecules such as proteins and DNA.

The first miniaturization of resistive-pulse sensors was established by Kasianowicz et al. in 1996, when he and his colleagues used a biological ion channel, α -Hemolysin, for the molecular detection of single stranded DNA.⁶ Ever since then, the simplicity of the idea, ability to detect particles at a molecular level, and the sensitivity of the measurement have caused a considerable attraction to this technique. In addition to biological nanopores^{7,8} that have fixed sizes and lack of stability due to their lipid bilayers, a variety of synthetic nanopores were also fabricated with the advances in material science and nanofabrication techniques and used as resistive-pulse sensors. The main advantages of synthetic nanopores are that they are stable under high temperature and potentials, they are chemically robust, they can be tailored in size and shape depending on the application, and pore surface can be easily functionalized for a more specific and sensitive analysis.^{9,10} Synthetic nanopores have so far been used as sensors for a variety of analytes such as nanoparticles,¹¹ metal ions,^{12,13} antibiotics,¹⁴ viruses¹⁵ and proteins^{16,17} as well as the most widely implemented DNA sensing.^{18–21} Diversity of possible implementations such as templates for nonwires,^{22–24} selective transport of analytes,²⁵ and sensor applications²⁶ make synthetic nanopores an attractive research area for a considerable number of groups worldwide.

Various routes and methods have been explored so far for the fabrication of synthetic nanopores including ion beam sculpting,^{27,28}

electron beam sculpting,²⁹ thermal annealing,³⁰ micromolding,³¹ dielectric breakdown,³² laser assisted pulling of capillaries³³ and track-etching³⁴ in versatile materials like silicon nitride,³⁵ aluminium oxide,³⁶ boron nitride,³⁷ graphene,³⁸ polymers,^{39–41} quartz⁴² and silicon oxide.⁴³ Track-etching process is especially of interest for us since it allows the fabrication of different nanopore geometries by simply adjusting experimental conditions. Furthermore, track-etching stands out among other techniques because it is a relatively economical and simple option that does not require high-cost equipment or trained personnel.

Selectivity of nanopore sensors are mainly based on size and shape of both the nanopore and the analyte. Especially if a narrow pore size distribution is ensured and the opening of the pore is finely tuned, size selective separation of desired molecules can be attained.⁴⁴ Moreover, with some polymer membranes (i.e. poly(ethyleneterephthalate)- PET) the etching process leaves fixed negative charges (caused by carboxyl groups) on the nanopore walls and such membranes are selective for positively charged analytes over negative ones.⁴⁵ Additionally, track-etched membranes are suitable for surface functionalization which could increase the selectivity towards certain molecules and enhances the detection efficiency.

There are already very informative reviews on the nanopore fabrication technologies,^{46–49} but we will only concentrate on the track-etching process of polymer membranes. We will be focusing on the sensing applications of track-etched single nanopores fabricated on polymer membranes. While the emphasis will be on the recent work and progress in track-etched sensors, we will also provide a detailed background on the tracking process, etching methods and their impact on pore geometry.

Track Technology and Applications

Since the first use of nuclear tracks for the production of porous membranes,⁵⁰ ion track technology has been extensively explored especially for separation purposes. In the last couple of decades, with the developments of ion accelerators and the advances in the material science, the primary focus has been sensing applications.

The first step of fabricating pores on membranes is the production of latent tracks which involves the irradiation of the membrane with high-energy, heavy ions. There are two common methods of producing tracks in the membranes: (1) The irradiation with fragments from the fission of heavy nuclei and (2) the use of ion beams from accelerators.⁵¹ The latter method has become widely popular after the establishment of commercial heavy-ion accelerator facilities such as the UNILAC linear accelerator of GSI (Helmholtzzentrum für Schwerionenforschung, Darmstadt, Germany), cyclotrons at GANIL (Grand Accélérateur National

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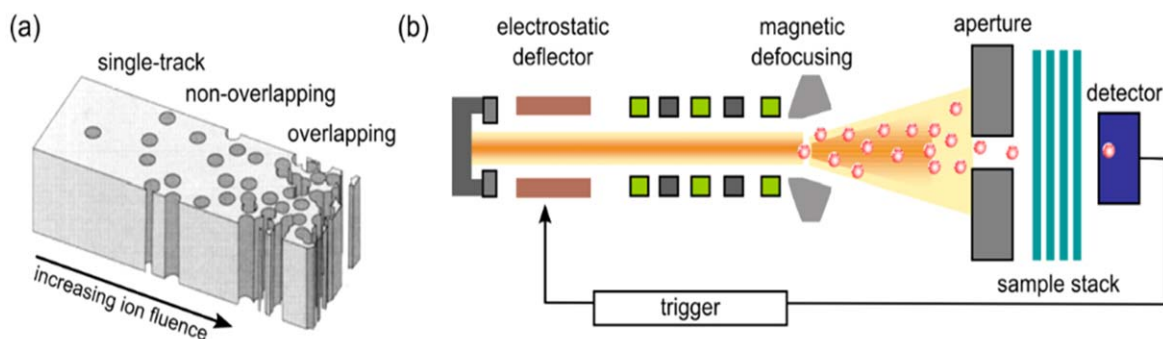


Figure 1. (a) Track-etched membrane illustrating the porosity regime available by means of ion-track technology: single channel, non-overlapping channels, and overlapping channels. (b) Schematic representation of single-ion irradiation setup. (Reproduced with permission from Ref. 52 under Creative Commons Attribution License).

d'Ions Lourds, Caen, France), JINR (Joint Institute for Nuclear Research, Dubna, Russia), and others in China and USA.⁵²

Irradiation of materials with heavy ions produces irreversible damages (tracks) that are a few nanometers in diameter along their trajectories in the membrane. These tracks are more prone to etching than the rest of the material and can turn into nanopores with the appropriate etching methods. Irradiation at various ion densities depending on the application, even down to 1 ion/membrane can be applied.

UNILAC accelerator of GSI, irradiation with heavy ions (i.e. gold or uranium) with energies up to 11.4 MeV can be achieved. This high energy allows multiple polymer membranes to be irradiated simultaneously in stacks.⁵³ By adjusting the ion beam and monitoring the flux, it is possible to irradiate the membrane with multiple ions (i.e. 10^6 – 10^{12} ions/cm²) or a single ion (Fig. 1a). This is succeeded by defocusing the ion beam and using a metal mask with an aperture with a shutter system which shuts down the ion beam as a single ion passage is detected with a detector placed directly behind the sample.⁵⁴ The schematic representation of the single ion irradiation of polymer membranes is given in Fig. 1b. Possibility to alter the ion density allows for the fabrication of single nanopores that are mostly applied for the determination and sensing at a molecular level, or multiple nanopores which can be implemented in templating studies or selective transport and separation of molecules.

Most widely used commercially available polymeric materials for tracking and their subsequent etching are poly(ethyleneterephthalate) (PET), poly(carbonate) (PC), and poly(imide) (PI).⁵¹ Poly(vinylidene fluoride) (PVDF), which is a fluorinated polymer, is also applied in track-etching studies because of its chemical, mechanical and ferro-electric properties.⁵⁵ However, the hydrophobic nature of

PVDF and the need for a chemical treatment of the pore after the etching process is a disadvantage.⁵⁶ PET is mostly preferred because of its stability in various mediums and its mechanical robustness as well as simple and fast etching procedure.⁵⁷ Furthermore, the chemical etching process breaks the ester bonds of polymer chains forming negatively charged carboxylate moieties on the pore walls.⁵⁸ This negative charge also enables easy surface functionalization with desired groups. PC is the oldest polymer material that has been used for the fabrication of track membranes. Although the sensitivity is higher than PET, PC is not as hydrophilic as PET and not stable in some organic solvents.⁵⁷ Similar to that of PET, the surface of PC also has carboxylic groups as functional groups after chemical etching over a wide pH range.⁵⁹ PI, on the other hand, is thermally and mechanically durable but the etching procedure requires high temperatures at which etching chemicals are unstable.⁵⁹ Depending on the desired application and required features, it is possible to decide on the most appropriate polymer material. Chemical structures of the most preferred polymer materials for track-etching are given in Fig. 2.

Etching of the Tracks

The irradiation of polymer membranes with swift heavy ions creates tracks in the material that are essentially damaged parts in the membrane caused by the passing high-energy ions. Second step of the nanopore fabrication process is the chemical etching of these tracks in electrochemical cells. The etching of the sensitive tracks (track-etching rate, v_t) is much faster than the etching of the bulk material (bulk-etching rate, v_b), meaning the nanopores can be created without significantly altering the thickness of the membrane. It was shown that exposing the tracked membranes to UV light prior

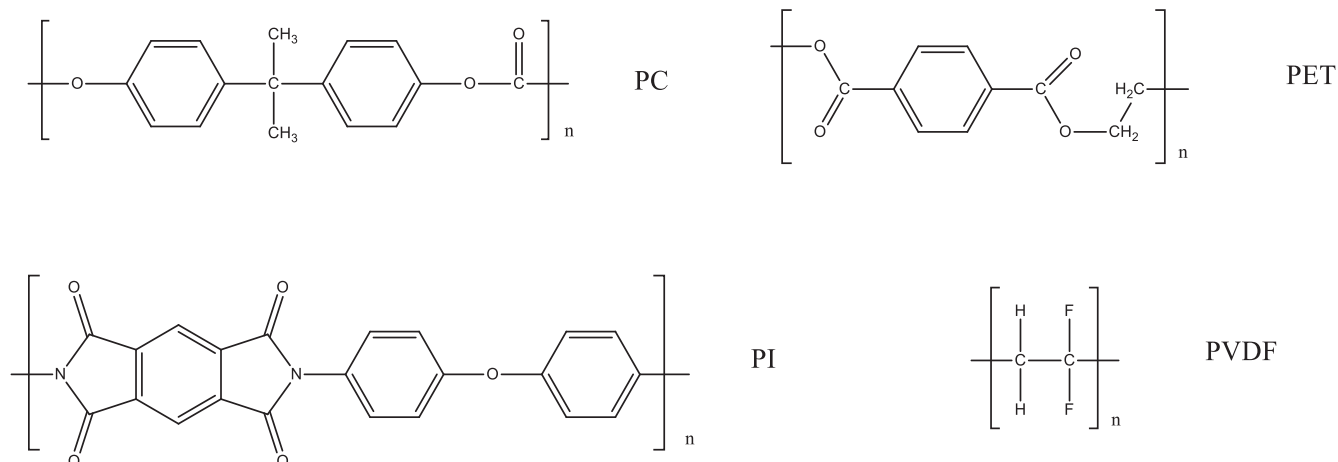


Figure 2. Chemical structures of the most widely preferred polymers for track-etching.

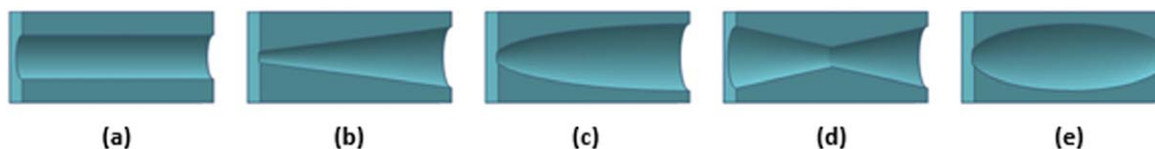


Figure 3. Some nanopore geometries: (a) cylindrical, (b) conical, (c) bullet-like, (d) hourglass (double-conical), (e) cigar-shaped.

to chemical etching, increases the track etching rate by sensitizing the tracks and allows a more homogeneous size distribution of the nanopores.⁶⁰ A concentrated sodium hydroxide (NaOH) solution is generally used as the etchant for PC and PET while PI is usually etched with sodium hypochlorite (NaOCl). PVDF, on the other hand is etched with potassium hydroxide (KOH) at high temperatures.

It is possible to obtain nanopores with various geometries by simply making changes in the etching process (or conditions), which is of importance since the ion transport properties of nanopores are directly affected by the pore geometry.⁶¹ Widely explored nanopore geometries include cylindrical^{62–64} and conical⁶⁵ nanopores that can be fabricated with symmetrical and asymmetrical chemical etching, respectively. Other possible geometries include bullet-like,⁶⁶ cigar shaped,⁶⁷ and hourglass (double conical) shaped⁶⁸ nanopores (nanochannels) (Fig. 3). However, in this review we will be mostly focusing on conical single nanopores and their sensing applications. This special interest in conical nanopores stems from their diode-like, nonlinear current-voltage behavior which can be exploited for sensing purposes.⁶⁹ The preferential direction of ion transport in asymmetrical synthetic nanopores have been thoroughly investigated for promising applications.^{42,70} This phenomenon is called ion current rectification (ICR) and quantified by the ratio of ionic currents at the same potentials with the same value but opposite charges (i.e. $| -1V/+1V |$). Furthermore, the resistance of the conical nanopore is much lower than a cylindrical pore with the same diameter.⁵³ Another interesting feature of conical nanopores is that a “capture (sensing) zone” is formed near the nanopore tip which is the small opening of the conical shape.⁷¹

The preparation of conical nanopores on polymer membranes requires asymmetrical etching conditions. With this purpose an electrochemical cell is used with two compartments divided by the track polymer membrane. During the chemical etching, a constant transmembrane voltage (i.e. 1 V) is applied to the cell via Pt electrodes and the electrical current is monitored using a picoammeter. Schematic representation of the electrochemical cell set-up is given in Fig. 4a. Breakthrough moment, where a meaningful increase in the current is observed, marks the complete etching of the membrane. For the fabrication of conical nanopores in PC and PET at room temperature, one compartment of the electrochemical cell which faces the UV treated side of the membrane is filled with 9 M NaOH, namely the etching solution. The other compartment is filled with the stopping solution which comprises of 1 M formic acid (HCOOH) and 1 M potassium chloride (KCl). The stopping solution

neutralizes the etching solution which prevents the further enlargement of the pore on one side, leading to a narrow cone with one small opening (tip) and one large opening (base). When the observed current reaches a predetermined value, the electrochemical etching can be stopped by replacing the etching solution with the stopping solution. The membrane is washed with deionized water by flushing the chemical cell, in order to remove any possible residues on its surface. By adjusting the etching time and monitoring the current values, nanopores with desired pore diameters can be obtained. The addition of methanol⁶⁵ or ethanol⁷² to the etching solution is known to increase the cone angle of the nanopore, which can be defined as the tip to base diameter ratio of the nanopore, by increasing the wettability of the polymer surface.

Asymmetric chemical etching of PI is also performed in a similar electrochemical cell with Pt electrodes immersed in the compartments on each side of the membrane. A potential is applied across the cell and the current is monitored to determine the breakthrough moment. Unlike PET and PC, chemical etching of the tracks in PI membrane is performed with NaOCl with an active chlorine content of 13% as the etching solution at 50 °C.⁷³ Potassium iodide (KI) is used as the stopping solution so that when the ClO^- ions penetrate the membrane, the iodide ions (I^-) reduce ClO^- to Cl^- ions and stop the etching process.⁷⁴

PVDF films are usually etched in an alkali medium (KOH, 10 M) coupled with KMnO_4 (0.25 M) solution at 65 °C for a predetermined time period which yields cylindrical nanopores. The etched membranes are then washed with potassium metabisulfite ($\text{K}_2\text{S}_2\text{O}_5$) (15 wt%) and deionized water.⁷⁵ This is an example of oxidative etching where oxidation mineralizes the fluorinated organic groups into the aqueous environment.⁵⁹

Characterization of the pores.—The characterization of the obtained track-etched nanopores is routinely performed with a combination of electron microscopy imaging and electrochemical measurements. The base diameter (d_{base}) is the diameter of the large opening of the conical pore and SEM imaging is sufficient for the characterization. It is known that, under same etching conditions, the single nanopore would have the same etching rates and d_{base} as multipore membrane. So, d_{base} can be determined by directly evaluating the SEM images and averaging the diameter values (see Fig. 5a).

Tip diameters, however, can be as small as 2 nanometers and conductivity measurements are needed for their calculations.⁵³

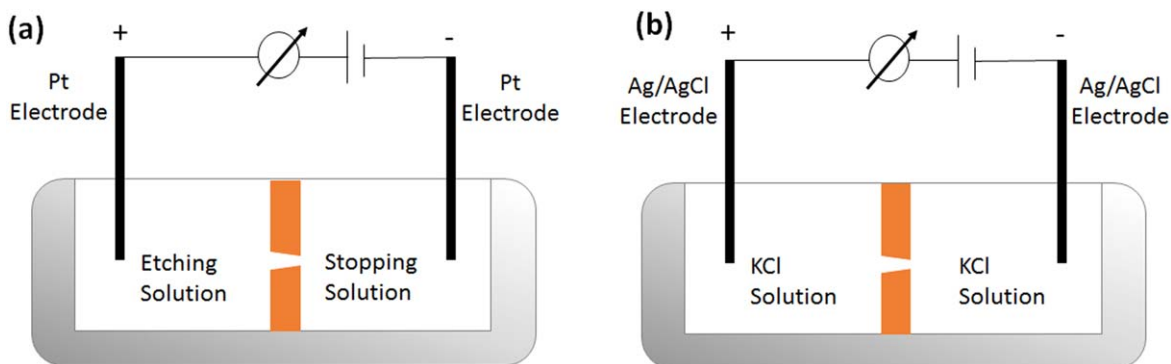


Figure 4. Schematic representations of (a) asymmetrical etching of a single conical nanopore in an electrochemical cell, (b) conductivity measurement in an electrochemical cell.

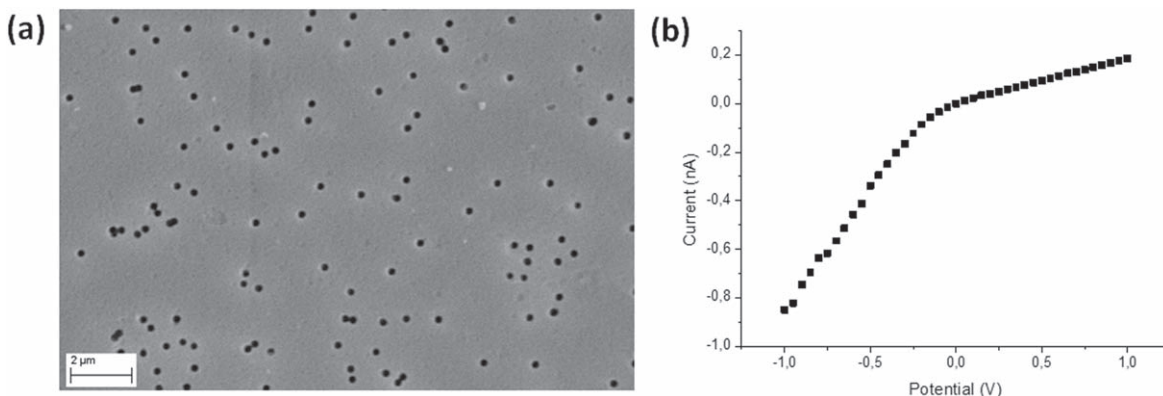


Figure 5. (a) SEM image of a multiporous (10^8 nanopores/ cm^2) PET membrane etched for 120 min (d_{base} : 232 nm) (b) I-V curve of a single conical nanopore PET membrane in 0.1 M KCl (d_{base} : 476 nm, d_{tip} : 3 nm).

Schematic representation of the conductivity measurement set-up is given in Fig. 4b. Briefly, both sides of the electrochemical cell is filled with an electrolyte solution such as KCl with a known concentration (i.e., 1 M) in PBS buffer at pH 7.²¹ Ag/AgCl electrodes are then immersed in the cells and a potential sweep is performed by stepping the voltage between +1 and -1 V.

The dependence of the conical nanopore resistance (R) to the solution conductivity (ρ), membrane thickness (L), and tip and base diameters (d_{tip} , d_{base}) is given in Eq. 1. If the other parameters are known, R can be calculated from the slope of the linear range of the I-V curve and d_{tip} can be found from Eq. 1. An I-V curve obtained with a conical nanopore is given in Fig. 5b.

$$R = \frac{4\rho L}{\pi d_{\text{tip}} d_{\text{base}}} \quad [1]$$

Resistive-pulse measurements.—The current pulses are measured using Ag/AgCl electrodes in the same electrochemical setup filled with the electrolyte solution prepared in buffer (ex. KCl in PBS buffer) as can be seen in Fig. 4b. Firstly, background current measurements are recorded at certain potentials which yields a steady current (Fig. 6a). Afterwards, the analyte is added to the half-cell in contact with the tip side of the nanopore then a

transmembrane potential is applied and the current pulses are recorded. The signal is digitized via a digitizer.

The current pulses are usually characterized by their frequency per unit of time (f) as well as pulse amplitude (Δi) and duration (τ). The current pulse amplitude (Δi) is defined as the difference between the values of lowest and initial currents; whereas current pulse duration (τ) is defined as the time difference between the first drop of current and recovery to the initial value (Fig. 6b).

Resistive-Pulse Sensing Applications

In this section we will give the resistive-pulse sensing applications of single track-etched nanopore membranes under four main subsections based on the frequency of studies: DNA sensing, protein sensing, nanoparticle sensing and sensing of other analytes.

Resistive-pulse sensing of DNA.—Resistive-pulse sensing of DNA gained specific importance due the prospect of sequencing DNA strands with nanopores.⁷⁶ The physicochemical aspects of DNA translocation and understanding dynamics of passage at single-molecule level were discussed. For DNA sensing studies, mostly PC and PET nanopore membranes were preferred. Harell and co-workers used PC single-nanopore membranes to detect two large DNAs which were a single-stranded phage DNA (7250 bases) and a

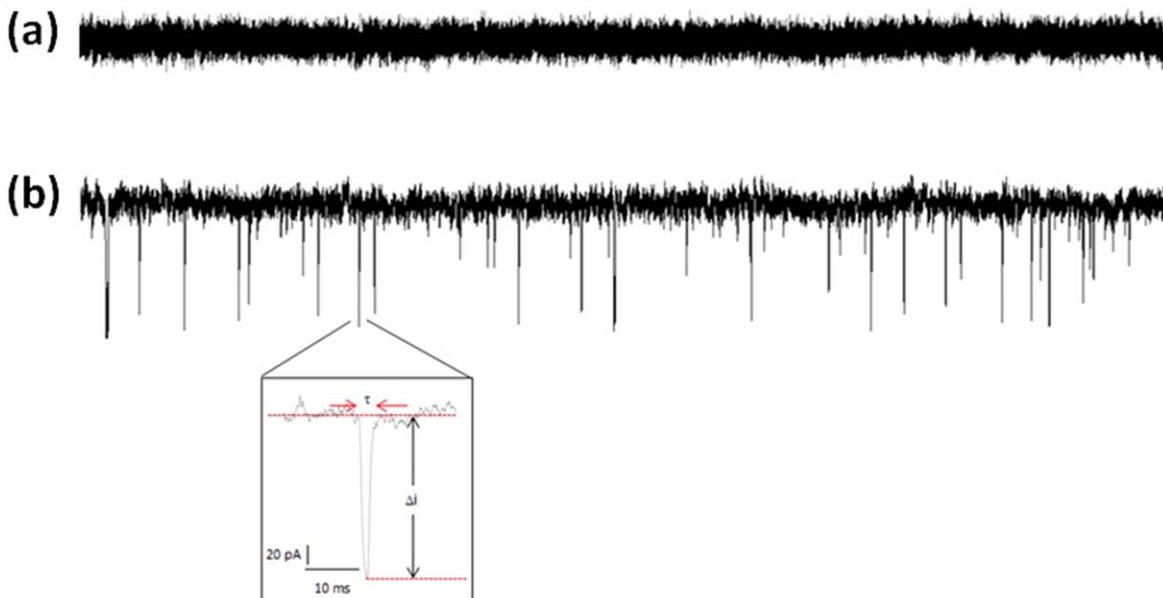


Figure 6. Current-time traces at 1000 mV using PC nanopore (Tip, base diameters: 27 nm, 410 nm) (a) Buffer only, (b) with 10 nM 50 bp DNA, (b, inset) current pulse amplitude, Δi and duration, τ of a signal (Reproduced with permission from Ref. 21).

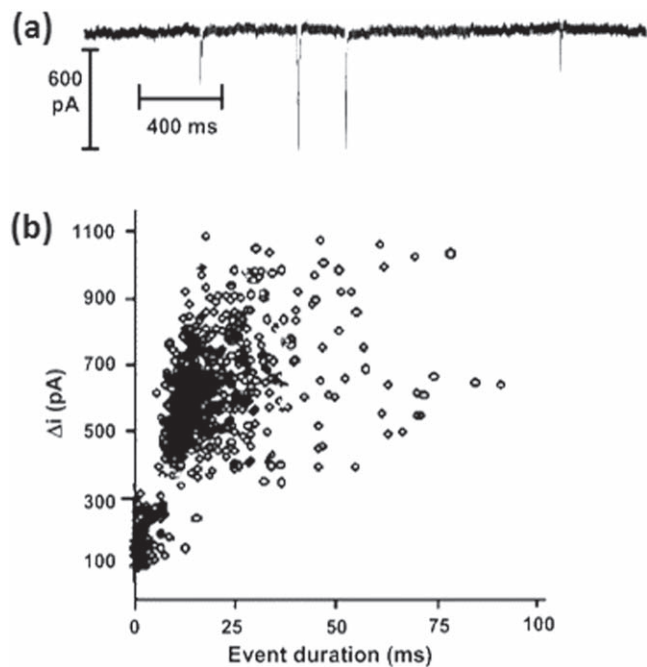


Figure 7. (a) Current-block events for a mixture of ds-DNA and ss-DNA (10 nM each). (b) Scatter plot of current pulse magnitude (Δi) vs. the duration of that pulse for [ss-DNA] = [ds-DNA] = 10 nM. Applied transmembrane potential = 900 mV. Reprinted (adapted) with permission from Ref. 77 (C. C. Harrell, Y. Choi, L. P. Horne, L. A. Baker, Z. S. Siwy, and C. R. Martin, *Langmuir*, 22 (25), 10837–10843). Copyright (2006) American Chemical Society.)

double-stranded plasmid DNA (6600 base pairs).⁷⁷ Conically shaped nanopore had dimensions of 40 nm of tip and 1.5 μm base diameter.

Electrophoretically driven phage DNA showed linear dependency with concentration and applied transmembrane potential. They also gave the inverse relationship between the potential and duration of current-pulse events in Eq. 2, where η is solution viscosity, r is the radius of the ion, l_d is the detection zone's length, z is the charge of the DNA, e is the electronic charge, and E is the electric field.

$$\tau = 6\pi\eta r l_d |z| e E \quad [2]$$

The current-block events obtained with plasmid DNA with small pulse magnitudes and short durations were attributed to the “bumping of the ds-DNA against the tip” rather than entering the tip or completely translocating through the pore. The authors showed that the differences in pulse magnitudes and durations of the current-pulse events made distinguishing ss-DNA and ds-DNA possible (Fig. 7).

Similarly, Kecici and co-workers showed the detection of short DNA strands (i.e., 50 bp) with PC single nanopore membrane and the linear dependency of current-pulse frequency to concentration and potential.²¹ They also compared the translocation events from nanopores with two different tip diameters, 18 nm and 27 nm, and showed that the current-pulse amplitude as a function of tip diameter could be used as an important parameter for the detection and discrimination of molecules (Fig. 8b). They found an inverse correlation with the current pulse duration (τ) and the applied potential, which is in agreement with the previous work.⁷⁷

The negative surface charge (due to carboxyl groups) of PET nanopores can be a disadvantage for the PET nanopores, especially if the analyte also bears an overall negative surface charge causing electrostatic repulsion. This charge could be decreased by surface modification. Kecici and co-workers used ethanolamine to diminish this effect and linked ethanolamine to the carboxylate groups on the nanopore wall through amide bond formation using EDC chemistry.⁷⁸ The modification and change in surface charge was confirmed with XPS and I-V curves (i.e., current rectification). The surface modified PET nanopore was characterized and also used to differentiate the short DNAs such as 50 bp and 100 bp based on their current-pulse amplitudes which were found to be 50 ± 12 pA and 115 ± 32 pA for 50 and 100 bp DNA, respectively.

In another publication, Kaya and co-workers showed the effect of pore geometry on resistive-pulse signal and electric field at the tip for PET nanopores.⁶⁵ With this purpose they altered the cone angle of the nanopore with a 20 nm tip by adding methanol to the etching solution and performed the resistive-pulse sensing of 50-bp DNA. Also, they calculated the electric field strength at the tip region of the nanopore to further understand the effect of cone angle on the translocation phenomena. They found that lower tip-to-base ratio

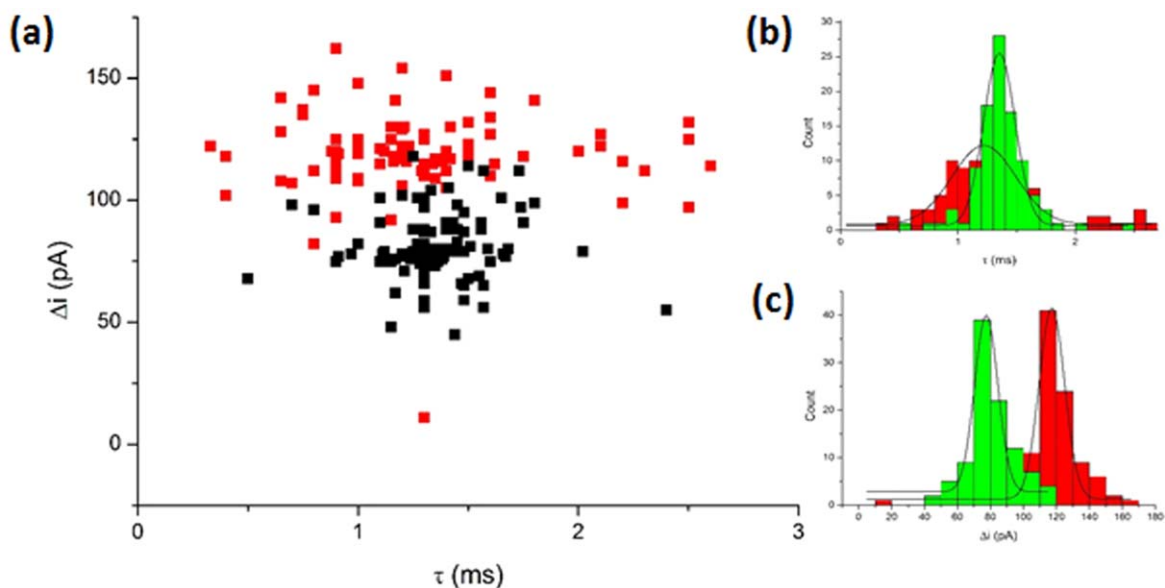


Figure 8. (a) Scatter plot of current-pulse magnitude (Δi) vs. current-pulse duration (τ) for 50-bp (10 nM) for 18 nm tip (red) and 27 nm tip (black). (b) Histogram of τ of 18 nm tip (red) and 27 nm tip (green). (c) Histogram of Δi of 18 nm tip (red) and 27 nm tip (green). Potential = 1000 mV. (Reproduced with permission from Ref. 21).

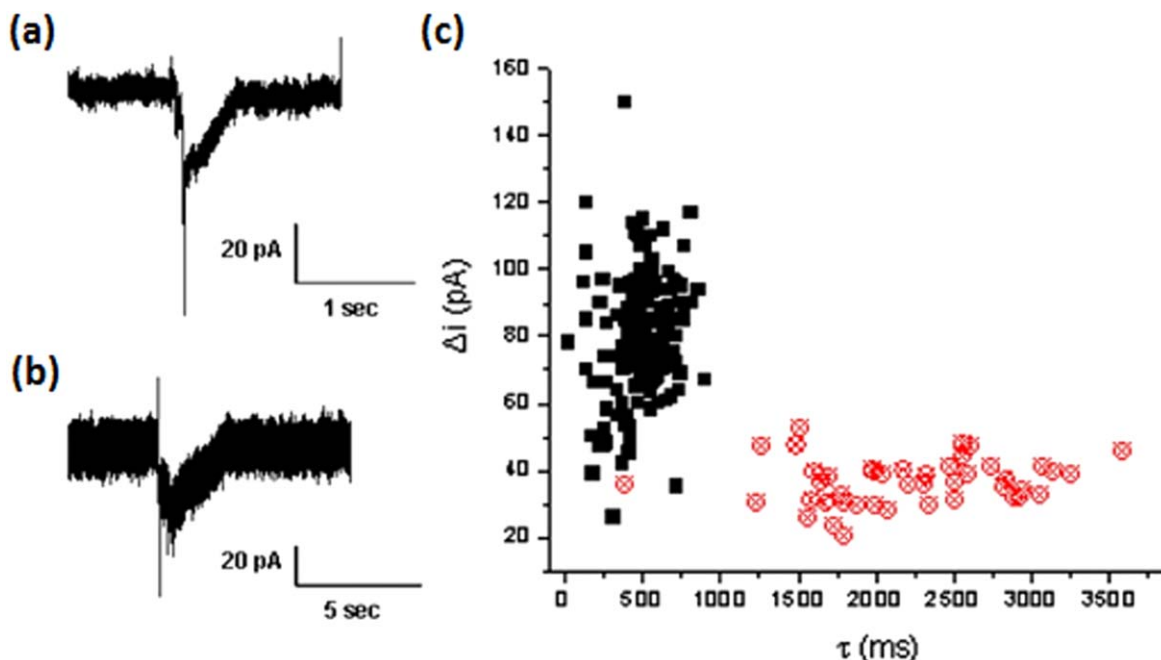


Figure 9. Expanded views of typical current pulses associated with tip to-base translocation of (a) BSA (100 nM) and (b) BSA/anti-BSA-Fab ([BSA] = 100 nM, [anti-BSA-Fab] = 270 nM). (c) Scatter plot of Δi vs τ for 100 nM BSA only (black) and 100 nM BSA plus 270 nM anti-BSA-Fab (red). Applied transmembrane potential: 1000 mV. Tip diameter: 17 nm. Reprinted (adapted) with permission from Ref. 80 (L. T. Sexton, L. P. Horne, S. A. Sherrill, G. W. Bishop, L. A. Baker, and C. R. Martin, *J Am Chem Soc*, **129** (43), 13144–13152). Copyright (2007) American Chemical Society.

have a contribution on electric field strength and extends the capture zone which has an enhancing effect for sensing purposes.

Rarely preferred PI nanopore membranes were used by Mara and co-workers and detected 284 bp and 4.1 kb ds DNA fragments.⁴⁰ They differentiated between these two fragments based on their strand properties (i.e. length) using a conical nanopore with d_{base} of 2 μm and d_{tip} of 4 nm. They were able to distinguish the DNA fragments based on their pulse durations of 0.37 ms and 1.07 ms, corresponding to the translocations of the 286 and 974 bp fragments, respectively. They calculated the ratio of the pulse durations for the 286 and 974 bp fragments as 2.9. They then correlated this value with the ratio of the two fragments' lengths which was 3.41, establishing a linear connection between DNA fragment length and current pulse duration.

Another short DNA detection study was performed with atomic layer deposited PET nanopores by Thangaraj et al.⁷⁹ The nanopore surface was passivated with the coating of Al_2O_3 and also its diameter was controlled by the deposition. Atomic layer deposition also enabled modification of the surface charge since the isoelectric point of Al_2O_3 is approximately 5. Obtained current-pulses showed that DNA samples could indeed be detected with the newly prepared nanopore. Single and double stranded DNAs (10mer and 40mer) were translocated and their durations (dwell time) were found to be 0.85 ms (ss-DNA), 1.07 ms (10mer) and 0.65 ms (40mer), respectively. However these long dwell times were found to be accompanied with weak signal-to-noise ratio, namely 4.16 and 2.3 for ss-DNA and ds-DNA respectively.

Sensing of proteins.—Another class of analytes that were detected with single-nanopore track-etched membranes are protein molecules. Not only the translocations but also possible interactions between the nanopore and the protein were shown by different groups. Sexton and co-workers showed the adsorption model and solution dynamics of proteins.⁸⁰ With this purpose they prepared a conical gold nanotube in a PET membrane with the track-etch method and functionalized it with poly(ethylene glycol) (PEG) to eliminate surface charge. This sensor element was deployed for a selective resistive-pulse sensing of Bovine Serum Albumin (BSA)

molecule using the protein/antibody interaction between BSA and Fab fragment from a BSA-binding antibody. The authors found that the pulse durations were proportional to the size of the BSA/anti-BSA-Fab complex. Interestingly, the larger BSA/anti-BSA-Fab complex showed smaller current amplitude values compared to free BSA molecule (Fig. 9c). This unexpected result was explained by an increase in the overall charge as the BSA/anti-BSA-Fab complex translocates the nanopore tip and brings additional charge carriers, compensating the decrease in the current caused by volume exclusion.

Similar setup and nanopore were also used to understand adsorption—desorption model of proteins by the same group.⁸¹ They studied the resistive-pulse sensing of different proteins with varying size and charge values in order to explain the cause of the signals with long pulse durations. They proposed in their model that the protein analyte goes through a series of adsorption—desorption processes with the nanotube wall during translocation. It was also shown that the pulse durations (τ) could be used for discrimination of proteins based on molecular size rather than pulse amplitudes (Δi). τ values obtained with a $d_{\text{tip}} = 17$ nm pore were 520 ± 110 ms, 1060 ± 230 ms, 1920 ± 870 ms for the studied proteins BSA, phosphorylase B and β -galactosidase. τ values increased with increasing size of the proteins but was found to be independent of nanopore diameter.

Wang and co-workers gave another example on protein sensing using the single track-etched conical nanopore.⁸² They demonstrated the effect of atomic layer deposition (ALD) of Al_2O_3 onto PET nanopore membrane for the detection of BSA molecules and showed high-protein capture rate with excellent signal-to-noise ratios. They found an unusual trend in the current pulses, some of which consisted of biphasic shapes with both an upward rise and a consecutive downward peak. They analyzed each biphasic pulse in five segments given in Fig. 10. This special behavior was explained by the change in the ionic distribution during the translocation of BSA through the ALD PET nanopore, which is caused by the adsorption – desorption events on the nanopore wall.

Selective protein transport based on chirality and their current-pulses was shown by Zhang and co-workers in a recent paper.⁸³

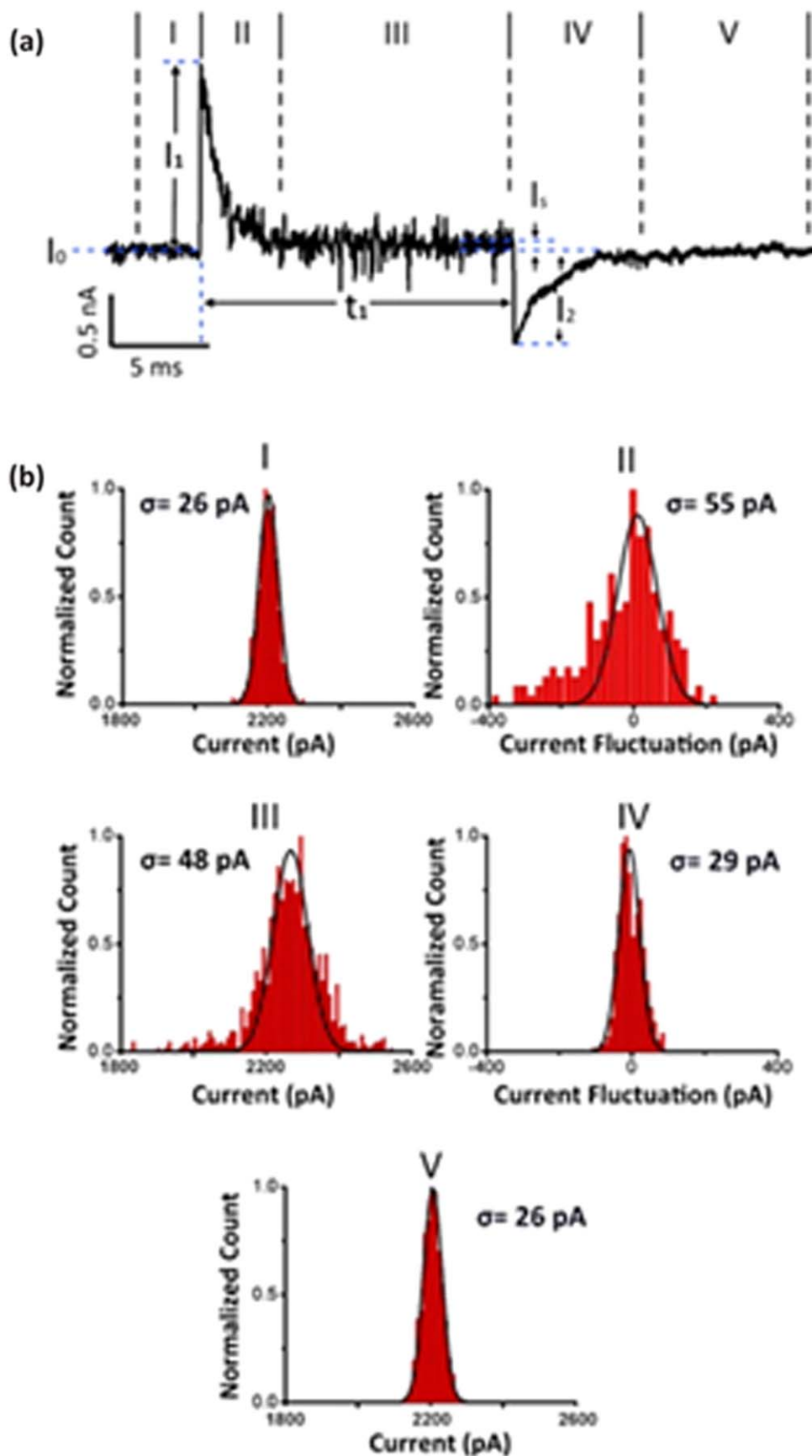


Figure 10. (a) Biphasic wave shape. The waveshape was divided into five segments, labeled as I–V. (b) Histogram analysis of the current fluctuation in each segment. (Reprinted (adapted) with permission from Ref. 82 (C. Wang, Q. Fu, X. Wang, D. Kong, Q. Sheng, Y. Wang, Q. Chen, and J. Xue, *Analytical chemistry*, 87 (16), 8227–8233). Copyright (2015) American Chemical Society.)

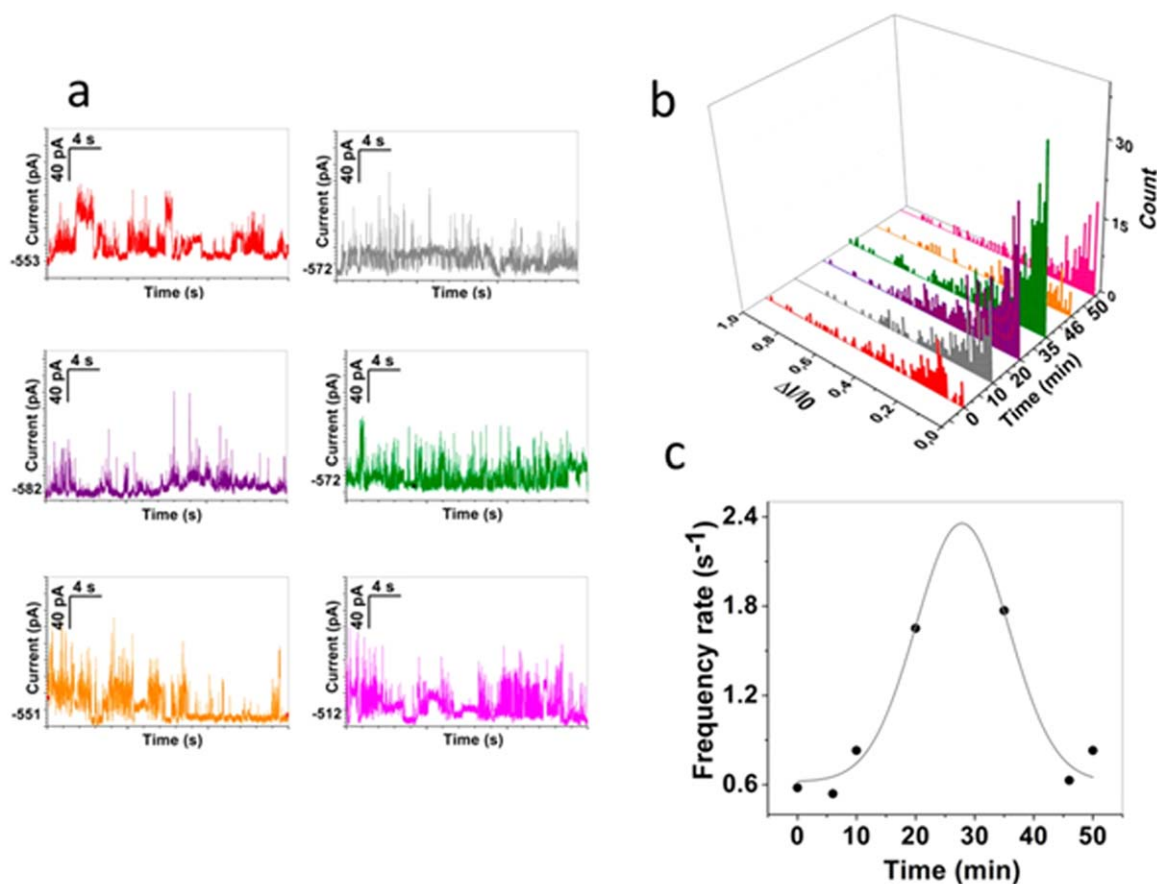


Figure 11. Real-time monitoring of protein amyloid degradation by trypsin. (a) Representative current traces, (b) The histogram of relative current blockade ($\Delta I/I_0$) and for 1.1 μM β -lactoglobulin amyloid, incubated 70 °C pH 2 during 3,3 h (red line) after pepsin addition at different time after 10 min (gray), 20 min (violet line), 35 min (green line), 46 min (orange line) and after 50 min (pink line). (c) The frequency rate of events from NP3 as a function of time at -200 mV under 1 M KCl, pH 2.7 and pepsin (380 nM). (Reprinted (adapted) with permission from Ref. ⁸⁴ (N. Giamblanco, D. Coglitore, A. Gubbiotti, T. Ma, E. Balanzat, J.-M. Janot, M. Chinappi, and S. Balme, *Analytical chemistry*, **90** (21), 12900–12908). Copyright (2018) American Chemical Society.)

L- and D-cysteine modified PI nanopores were used to detect BSA molecules based on chiral selectivity. The authors studied both the transport properties of BSA through modified multiporous membrane and individual translocation events of BSA through modified single nanopore membrane prepared by the same etching and modification methods. L-cysteine modified nanopores were more efficient in both cases with higher flux values and higher current-pulse frequencies. The resistive-pulse signals obtained from the translocation of BSA from L-Cys nanopore were 12.9 events per second, when only 1.6 events per second events were obtained from translocation from d-Cys nanopore. Therefore they concluded from the results that L-Cys modified nanopore had higher selectivity for BSA transport and it was modulated by chiral gate.

Another interesting application of single conical PET nanopore was by Giamblanco et al. where they showed the real-time enzymatic degradation of amyloid with peptase and characterized the intermediates produced by amyloid formation.⁸⁴ They first functionalized the PET surface with PEG to decrease the surface charge of the nanopore and prevent the adsorption of protein. They used the modified PET nanopore as an alternative to SiN nanopores for the characterization of β -lactoglobulin amyloid aggregates and protofibrils by the current blockade values. They used three nanopores with the diameters: 117.5 nm base, 12.1 nm tip (NP1); 300 nm base, 3.8 nm tip (NP2); 92.5 nm base, 2.7 nm tip (NP3) and they showed that the translocation was slower for NP2 and NP3. They found since the discrimination between protofibril and aggregates was possible at certain pH values, real-time monitoring of the degradation of β -lactoglobulin amyloid by pepsin enzyme was also possible (Fig. 11).

Sensing of particles.—Nanoporous polymers can also be implemented for sensing of nanoparticles. Pevarnik and co-workers reveal the inner structure of a cylindrical PET nanopore using the translocation behavior of polystyrene particles.⁸⁵ Although the main focus of the work is not resistive-pulse sensing, we included this work because it is an example of resistive-pulse sensing using cylindrical nanopores instead of widely implemented conical ones. In this work, they showed that the diameter of a cylindrical pore fluctuates along the length of the nanopore and these fluctuations could be analyzed by the corresponding resistive-pulses in the current. The irregularities in the diameter of the cylindrical nanopore were also revealed in the SEM images of the gold replicas obtained from multiporous PET membranes (Fig. 12f). In this work, spherical polystyrene particles with diameters of 220, 330, and 410 nm were translocated through the single PET nanopore and ionic current-pulses were shown (Fig. 12). It was given in Figs. 12a–12c, that the signal shapes were similar and had a characteristic pattern regardless of the particle size. Furthermore, the same pattern was reversed when the particles were driven in the opposite direction which meant that the fluctuations in the current were characteristic to that particular pore. Another use for this unique pattern was that it could allow to find out if the particle is completely translocated through the pore or entered to pore and exited back from the same side without completing its translocation. Scatter plots of current-pulse amplitudes vs current-pulse durations revealed that a mixture polystyrene particles could be easily discriminated by size and surface charge densities.

Similar to Pevarnik's work, Qui and co-workers characterized the spherical and rod shaped particles with comparable volumes using

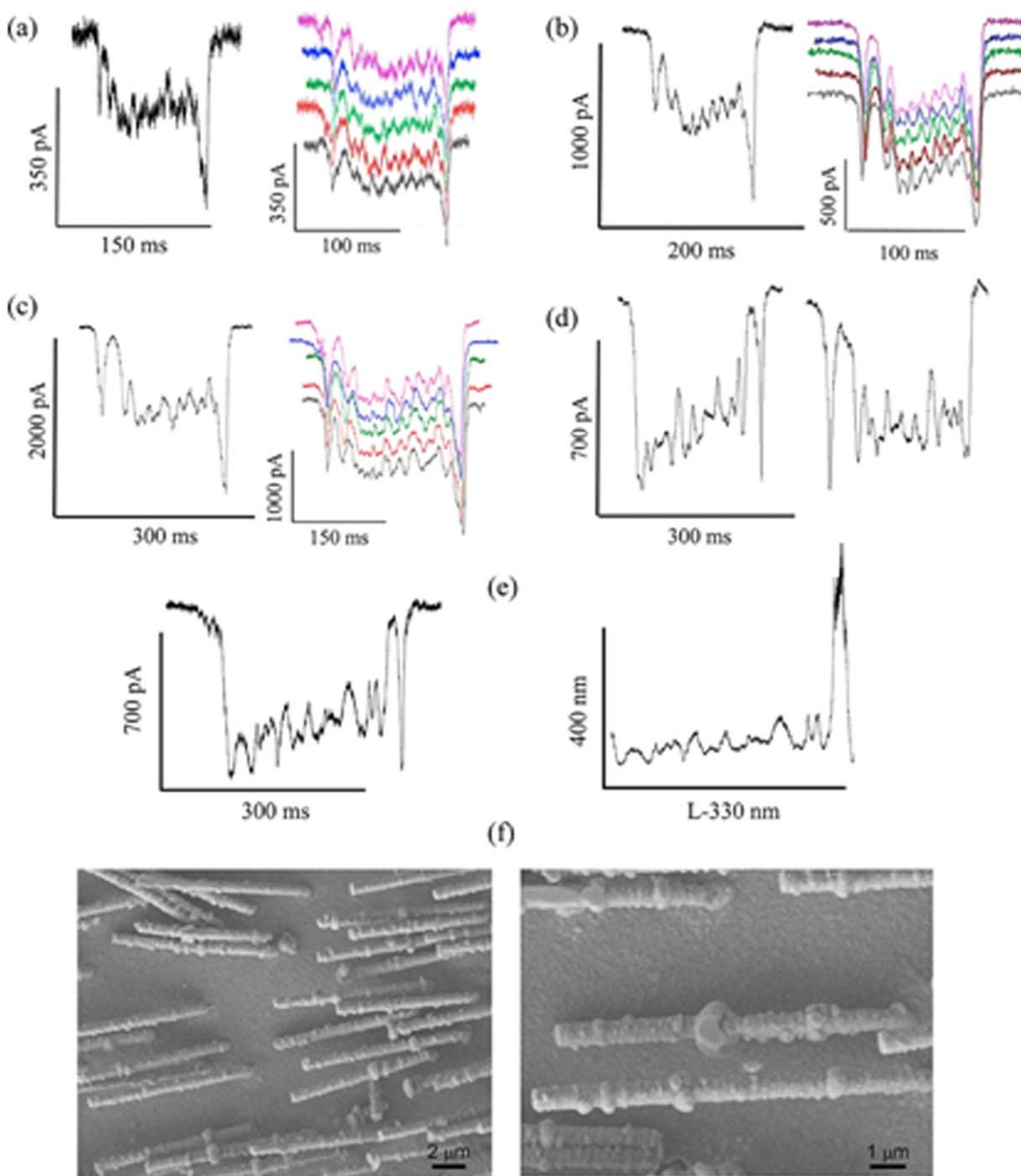


Figure 12. (a–c) Magnified views of the ionic current events for 220, 330, and 410 nm particles. The events are linearly stretched in the x-direction so that the beginning and end of each are aligned, to compensate for slight variations in the mean particle velocity. (d) Events for 330 nm particles passing in opposite directions through a different pore, showing that the pattern of variations is reversed for the opposite particle direction. (e) An example of a single 330 nm nanoparticle passing through the same pore together with the pore diameter profile. L is the pore length. (f) SEM images of gold replicas of similar PET pores. (Reprinted (adapted) with permission from Ref. 85 (M. Pevarnik, K. Healy, M. E. Toimil-Molares, A. Morrison, S. E. Létant, and Z. S. Siwy, *ACS Nano*, 6 (8), 7295–7302). Copyright (2012) American Chemical Society.)

their translocation behaviors through nanopores with longitudinal irregularities.⁸⁶ They performed the resistive-pulse sensing experiments with single cylindrical nanopores prepared in PET membrane. Spherical polystyrene beads and two types of rod-like silica particles were chosen as the analytes for this set-up. The current-pulses obtained for individual particles' translocation through 770 nm nanopore are given in Fig. 13. The undulations in the current with the translocation of the spherical analyte were found to be independent of the diameter of the polystyrene beads and this finding indicated that the pore diameter fluctuated along its length. The resistive-pulse signals were also evaluated by their pulse

amplitudes and durations. The authors found that short rods had much higher current pulse amplitudes than the predicted values and the pulses generated by spherical beads that have larger volumes. To explain this experimental behavior, they decided that the shorter rods rotated around the pore axis, so the effective volume for the rod was increased. In order to understand and show the effect of the fluctuations of the nanopore diameter on distinguishing the different shapes of analytes, they performed another series of experiments with single cylindrical polycarbonate (PC) pores. The reason was that the pores fabricated in PC had smooth surfaces and were nearly completely cylindrical. The current pulses obtained with PC

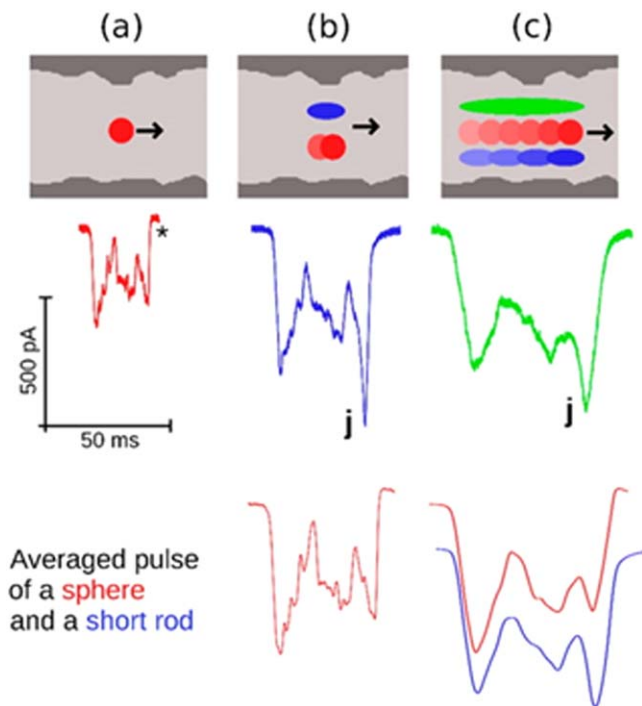


Figure 13. Ion current pulses data and simulated (averaged) traces for three types of particles. Current–time data are shown for (a) a 410 nm diameter sphere (red trace), (b) a 230 nm × 590 nm rod (blue trace), and (c) a 210 nm × 1950 nm rod (green trace). The red trace in (a) was averaged over 11 points to give the simulated red trace in (c). Similarly, the blue trace in (b) was averaged over 148 points to give the simulated blue trace in (c). The averaged pulses were scaled to facilitate comparison with the raw experimental pulses. The pore had an average opening diameter of 770 nm and a length of 11 μm . Current traces were collected at 20 kHz using 0.6 V in 0.1 M KCl at pH 8. (Reprinted (adapted) with permission from Ref. 86 (Y. Qiu, P. Hinkle, C. Yang, H. E. Bakker, M. Schiel, H. Wang, D. Melnikov, M. Gracheva, M. E. Toimil-Molares, A. Imhof, and Z. S. Siwy, *ACS Nano*, 9 (4), 4390–4397). Copyright (2015) American Chemical Society.)

nanopore yielded signals with rectangular shapes, confirming the cylindrical shape. Another finding was that once again the rods excluded a larger volume than their volumes which confirmed that the rods performed rotational moves. In PET pores, the rod amplitude was higher than the resistive-pulses obtained with the PC pore which indicated that pores with undulations in their diameter made distinguishing shapes of the passing objects possible.

Sensing of other analytes.—Heins and co-workers detected single porphyrin molecules using a single conical PI nanopore as the resistive-pulse sensor.⁸⁷ The chosen analyte, porphyrin, was not a particle or a macromolecule, but had a molecular structure. In order to detect porphyrin, the tip diameter of the pore needed to be comparable in size with the diameter of the molecule, therefore the nanopore was fabricated to have 4.5 nm tip diameter. Resistive-pulse sensing of the analyte with such nanopore yielded a threshold potential of 300 mV below which no current-pulses were obtained. This behavior was attributed to the electrostatic repulsion between the cation-selective nanopore and the anionic analyte, in addition to the entropic penalty the analyte needed to pay to enter such small nanopore. At higher potentials, the electrostatic and entropic barriers were overcome, and resistive-pulses were obtained (Fig. 14). The authors showed that the number of current-pulses were directly related to the analyte concentration and lower concentrations can be detected if higher transmembrane potentials were applied.

Another interesting study was performed by Zhou and co-workers to characterize the hepatitis B virus capsids using single nanopore PET membrane.⁸⁸ To minimize the surface charge density

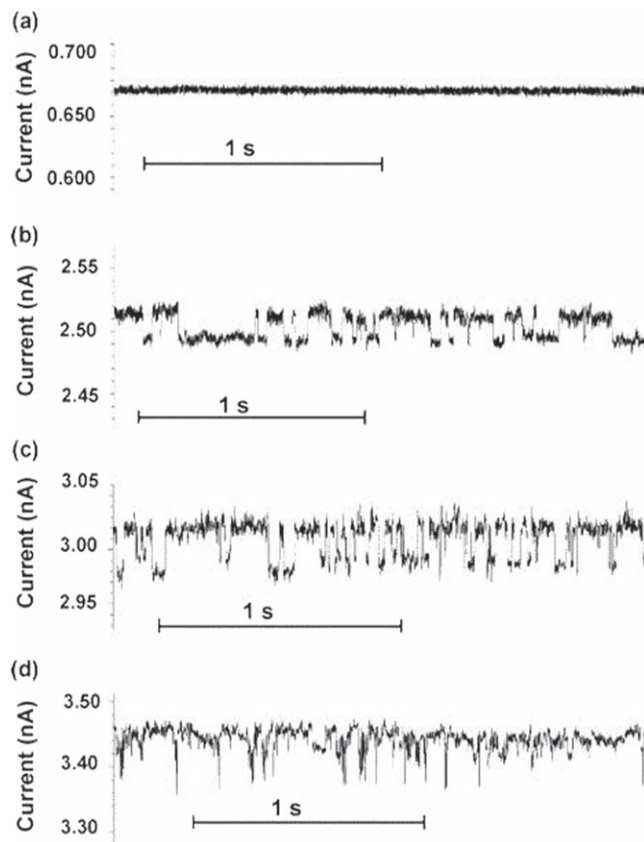


Figure 14. Current-time recordings for the conical nanopore in the presence of 60 nM TPPS. The transmembrane potential was (a) 100 mV, (b) 400 mV, (c) 500 mV, and (d) 600 mV. (Reprinted (adapted) with permission from Ref. 87 (E. A. Heins, Z. S. Siwy, L. A. Baker, and C. R. Martin, *Nano Lett.*, 5 (9), 1824–1829). Copyright (2005) American Chemical Society.)

and adsorption of virus capsids, the inner surface of the nanopore was modified with triethylene glycol. They showed that the duration of the covalent modification with triethylene glycol had an impact on the rectification behavior; nanopores with 4 h modification did not rectify the current whereas nanopores with 2 h reaction time had rectification ratios of 1.33 and 1.34. The virus domains were detected successfully, and discrimination between $T = 3$ capsid and $T = 4$ capsid was performed based on their pulse amplitudes (Fig. 15). However, no concentration dependence was observed and the effect of entropic barrier was highlighted.

Sensing Applications Based on Ion-Current Rectification (ICR)

Another way to use the track-etched membranes for sensor applications is utilizing single nanopores and their rectification behavior, especially those with asymmetric geometry. Due to the geometry and surface charge distribution of the nanopore, especially the conical nanopores show diode-like behavior and give passage to ions selectively. This is sometimes called as ‘pumping of ions’ in one direction and results with rectification of the ionic current. This mechanism is used in several studies in terms of rectification and its ratio as a function of concentration.

Chemical etching of the ion tracks in PET and Kapton membranes leaves carboxylate groups on the nanopore surface. These groups are negatively charged at neutral pHs which is above their isoelectric point (i.e. pH = 3). If the tip diameter is small enough (< 10 nm), these nanopores show ion current rectification. In this regard, PET and Kapton nanopores are cation selective which means that cations contribute to the ion current more. At this point, the geometry of the nanopore plays a critical role in that the cation transfer from tip to base is promoted for PET and Kapton. This

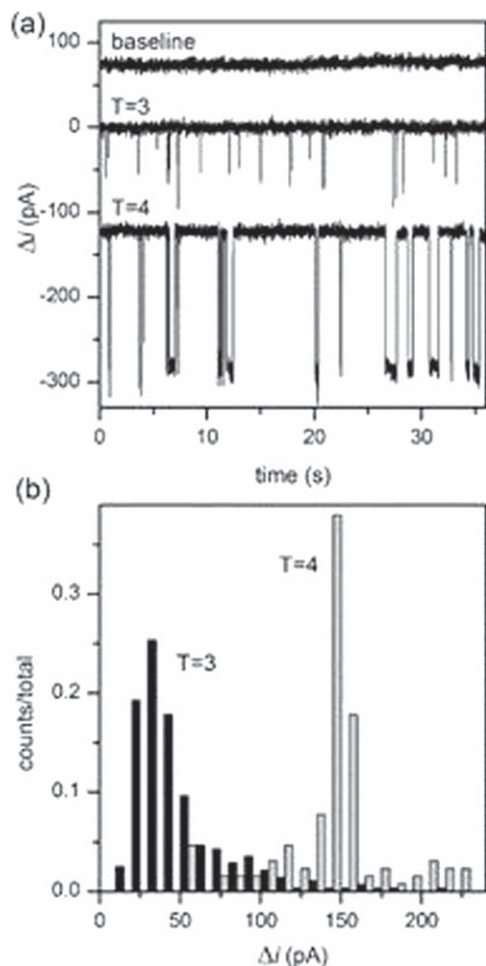


Figure 15. (a) Variation of Δi with time for no capsids (baseline), purified T = 3 capsids, and purified T = 4 capsids (d_t : 55 ± 13 nm, d_b : 590 ± 140 nm). The applied potential was 0.5 V. (b) Histogram of Δi for purified T = 3 and T = 4 capsids characterized with the same pore. (Reprinted (adapted) with permission from Ref. 88 (K. Zhou, L. Li, Z. Tan, A. Zlotnick, and S. C. Jacobson, *J Am Chem Soc*, **133** (6), 1618–1621). Copyright (2011) American Chemical Society.)

behavior is modeled on the electrostatic ‘ratchet’ which refers the asymmetry in their electrostatic potential. This asymmetry creates an electrostatic trap for cations in positive potentials and forms the ‘off’ state. When the potential is reversed the traps is phased out and the ‘on’ state is observed (see Fig. 16). In this part, we will give examples of studies using ion current rectification phenomenon for sensor applications that specifically uses track-etched single nanopore membranes as the sensor element. In addition, the functionalization of the surface plays a critical role to either manipulate the surface charge or add selectivity to the analyte. For this purpose, chemical reactions to couple the surface groups with selective molecules or atomic layer deposition are preferred to modify the nanopore surface in order to alter the current rectification behavior.

Ma and co-workers proposed a new strategy to detect over-sulfated chondroitin (OSCS) contaminants from heparin solution.⁹⁰ Poly-L-lysine (PLL) functionalized, conical PET nanopores were used to detect low heparin concentrations (25 ng ml^{-1} – $3 \text{ } \mu\text{g ml}^{-1}$) using ion-current rectification. The authors showed that when heparin was added to the tip side after modification with PLL, the I-V behavior was reversed because of the excess negative charge from heparin, proving that I-V curve could be used to detect heparin. For the sensing of OSCS, the main idea was that the OSCS could inhibit the degradation of heparin by heparinase enzyme and comparing the I-V curves could be used to detect the inhibition by

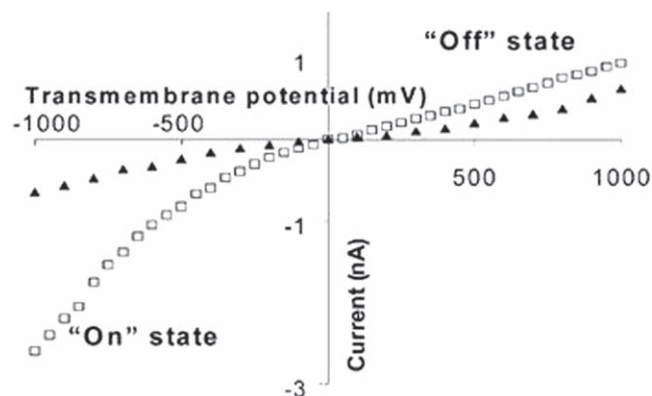


Figure 16. I-V curves for conical Au deposited PET nanopores in 0.1 M KCl (squares) and 0.1 M KF (triangles). Reprinted (adapted) with permission from Ref. 89 (Z. Siwy, E. Heins, C. C. Harrell, P. Kohli, and C. R. Martin, *J Am Chem Soc*, **126** (35), 10850–10851) (Copyright (2004) American Chemical Society.)

OSCS. It was observed that adding OSCS along with heparinase, somewhat inhibited the degradation reaction and decreased the rectification factor to 0.9.

Wang and co-workers designed a single nanopore (PET) coupled with DNA aptamers to detect cocaine molecules.⁹¹ By adding surfactant (i.e., dodecyl diphenyloxide disulfonate) to the etching solution, they prepared bullet-shaped nanopores as the sensing element. They were able to detect cocaine down to 1 nM by immobilizing capture DNA aptamers (C-aptamer) onto the nanopore walls which binds cocaine with target DNA aptamers (T-aptamer). The performance of the newly fabricated nanopore aptamer sensor was determined by investigating and comparing the I–V properties of the modified nanopore, with added cocaine and/or T-aptamer molecules. As shown in Fig. 17, detection and sensing of cocaine was only possible with T-aptamer. The data was obtained from the averages and standard deviations from five independent experiments.

Perez-Mitta and co-workers designed sugar-regulated pH responsive nanopore sensor (PC) by integrating an electropolymerized responsive layer of polyaniline derivative poly(3-aminephenylboronic acid) PAPBA onto gold deposited surface of the pore.⁹² Due to the zwitterionic nature of PAPBA, it’s possible to alter the surface charge of the pore, generating a different I-V response at different pH values (Fig. 18). The effect of sugar concentration on the pH responsiveness was also investigated in the presence of 100 mM fructose. This effect stemmed from the inclination of boronic acids to form complexes with diols. Since the unbounded boronate and the formed complex between boronates and sugars had different pKa values, the acid-base behavior of PAPBA would change depending on the pH value and the presence of fructose.

Lepoitevin fabricated a cylindrical PET nanopore, used atomic layer deposition to decorate the nanopore surface with $\text{Al}_2\text{O}_3/\text{ZnO}$ bilayer to reduce the nanopore size, and modified the surface with N-[3-(Trimethoxysilyl)propyl]ethylenediamine and finally biotinylized the nanopore surface.⁹³ Different steps of functionalization were monitored by the I-V responses generated by asymmetrical modification conditions. By bringing the attribute of diode-like behavior to the cylindrical nanopore that normally has a linear I-V response, the authors have shown that detection of proteins such as avidin/streptavidin, IgG and BSA is possible without the need of labelling. Their results showed that discrimination between avidin and streptavidin was possible at pH values below 9 and the amount of protein for detection was as low as 1 nM.

Ali et al. gave another example of acquiring a non-linear I-V response with a cylindrical nanopore by modifying the surface with a DNA aptamer.⁹⁴ The surface modification was confirmed through I-V curves of the modified and unmodified nanopore (Fig. 19). This specific aptamer was chosen for the selective recognition and

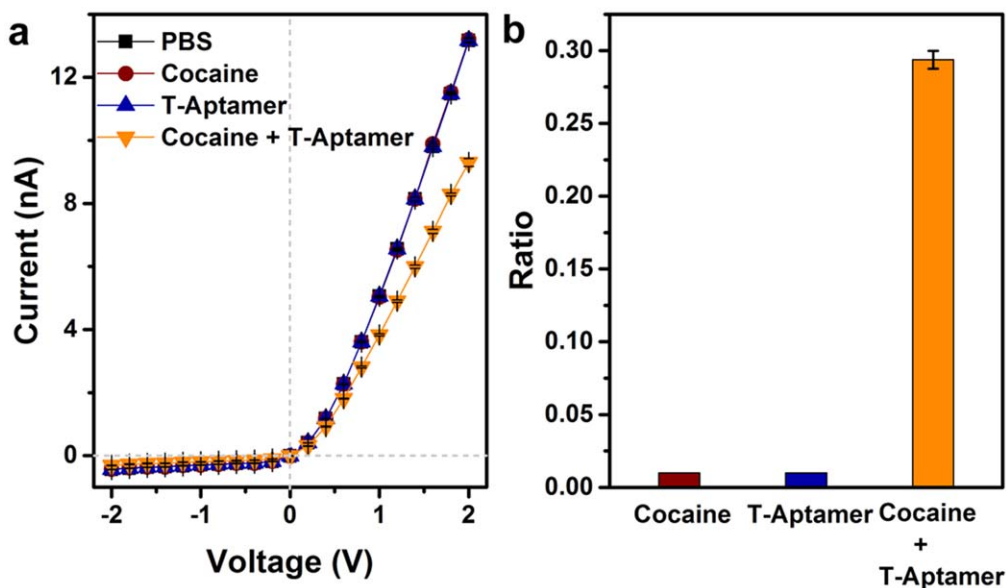


Figure 17. Discriminating property of 1 μM target cocaine in the single nanochannel-based platform without or with addition of 1 μM T-aptamer. (a) I–V curves measured in 0.01 M PBS (pH = 7.4) with or without T-aptamer at a scanning voltage varied from -2 to $+2$ V. (b) Comparison of the current change ratios, $(I_0 - I)/I_0$, recorded at $+2$ V with 1 μM target cocaine with or without -aptamer, where I_0 and I are the current measured in the absence and presence of target analytes. Reprinted (adapted) with permission from Ref. 91 (J. Wang, J. Hou, H. C. Zhang, Y. Tian, and L. Jiang, *ACS applied materials & interfaces*, **10** (2), 2033–2039) (Copyright (2018) American Chemical Society.)

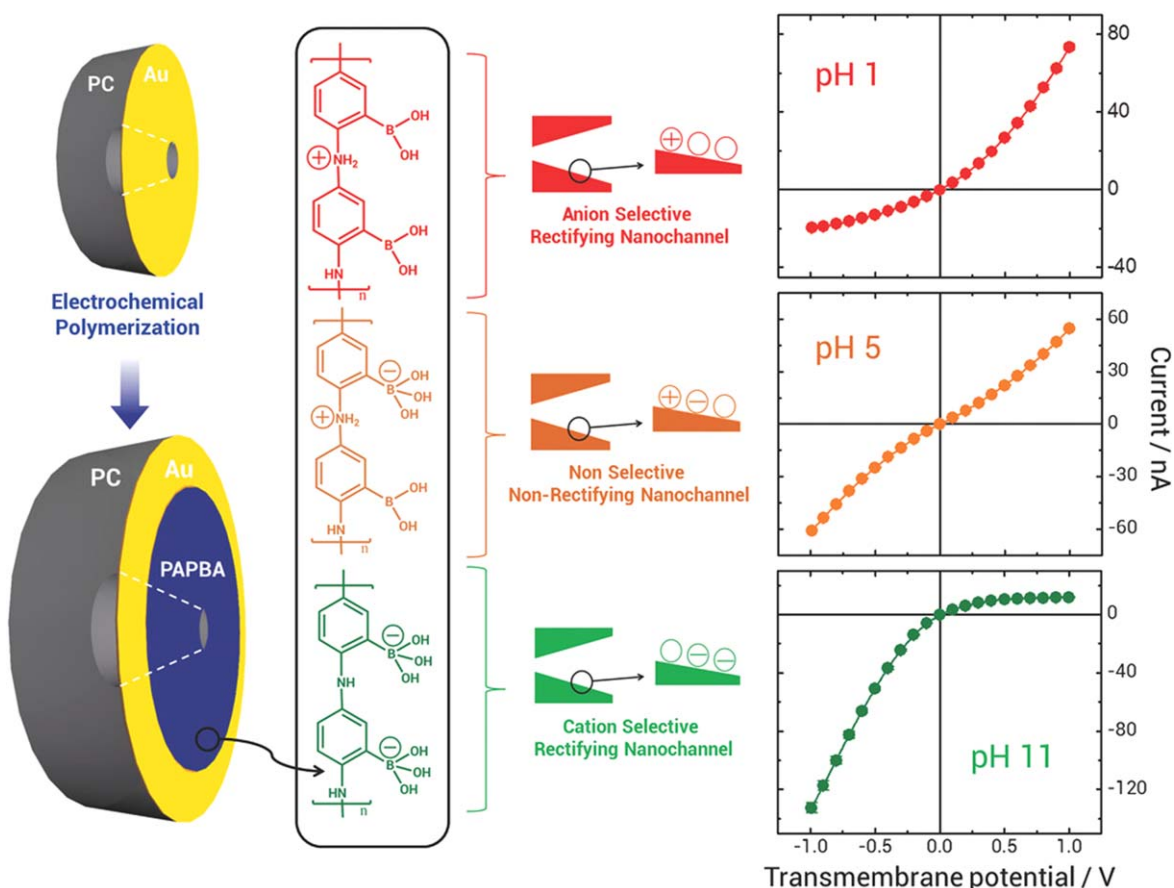


Figure 18. Scheme depicting the functionalization of a metallized polycarbonate nanochannel and the charge state of the channel related to I–V measurements for pH 1 (red), 5 (orange), and 11 (green) in 0.1 M KCl solution (Reproduced with permission from Ref. 92).

sensing of lysozyme protein through bioconjugation. When lysozyme was introduced to the modified membrane from one side, positively charged enzyme was bound to the aptamer, causing an

asymmetry in the surface charge of the pore. Increasing the concentration of lysozyme resulted in the reduction in the positive current. Furthermore, it was shown that the concentration of the

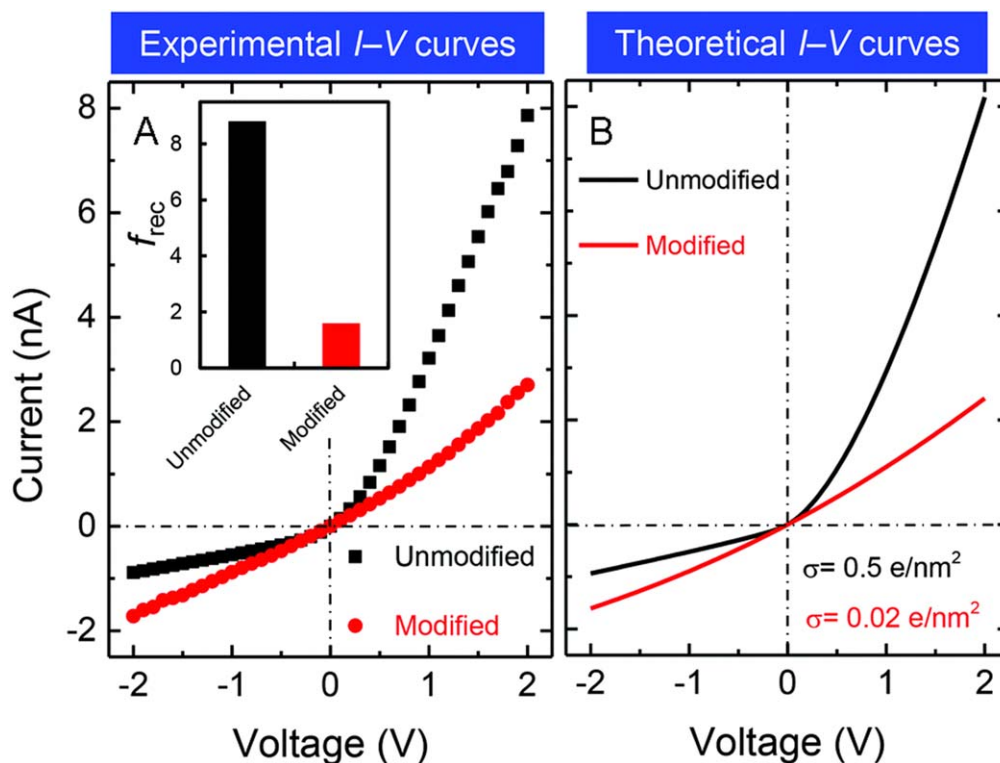


Figure 19. Experimental and theoretical I-V characteristics of the single conical pore before (black) and after (red) the immobilization of “caged” fluorescein moieties. The experimental I-V curves are recorded in 0.1 M KCl (tris-buffer, pH 7.6) solution. The radii of the small and large pore openings are $a_L = 10$ and $a_R = 250$ nm, respectively. The inset shows the rectification ratios (f_{rec}) before and after pore modification. The error bars of the experimental data are smaller than the symbol size. Reproduced with permission from Ref. 94.

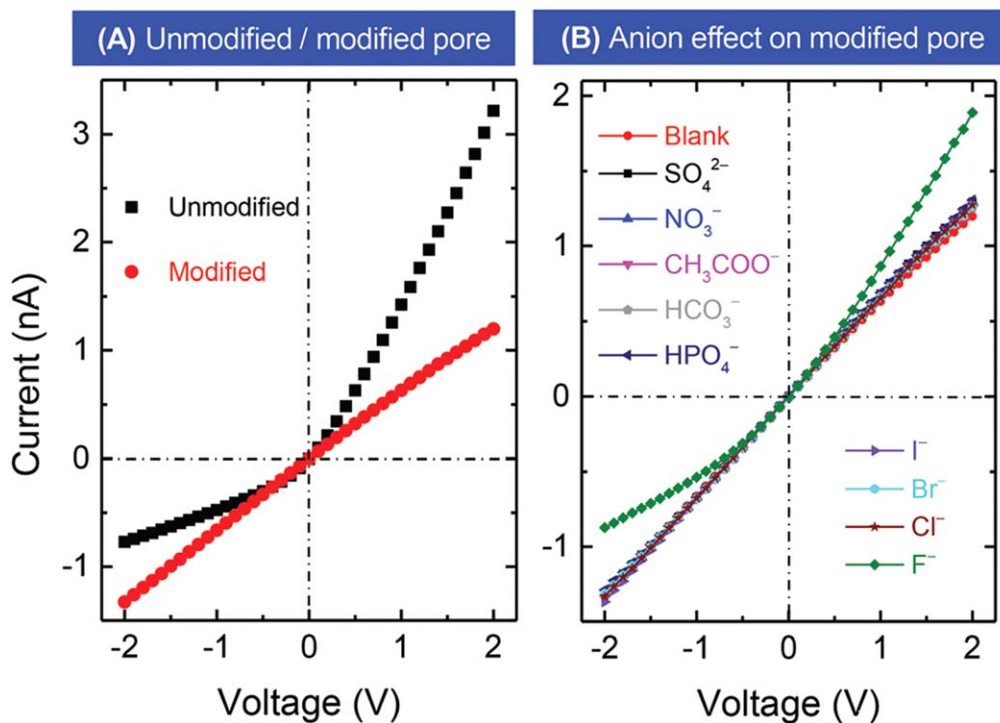


Figure 20. (A) I-V characteristics of the single conical pore measured in 0.1 M KCl (pH 7.6) solution before (black) and after (red) the immobilization of “caged” fluorescein moieties. The radii of small and large pore openings are 8 and 280 nm, respectively. (B) I-V characteristics of the modified pore before (blank) and after the addition of 1 mM concentration of various anions (sodium salts) in the electrolyte solution separately. The error bars of the experimental data are smaller than the symbol size. (Reproduced with permission from Ref. 95).

electrolyte solution had a direct effect on the ionic current rectification, and the rectification values were found to be 1.2, 5.0 and 20.0 for 1 M, 100 mM and 10 mM KCl, respectively.

Ali and co-workers also designed and used a single nanopore PET fluoride sensor by caging fluorescein moieties on nanopore surface.⁹⁵ With this purpose they synthesized the amine-terminated, fluorescein whose phenolic hydroxyl groups are protected with tert-butylidiphenylsilyl (TBDPS) moieties (Fc-TBDPS-NH₂). The modification of the surface caused a decrease on the pore surface charge due to the neutral TBDPS moieties and therefore yielding a more linear I-V response (rectification degree lowered from 8.8 to 1.6).

When the fluoride anions are introduced to the system, the nanopore wall goes from a neutral state to a charged state, this surface polarity along the conical nanopore leads to the electrical rectification observed in the I-V curve. In addition, the authors confirmed the selectivity of the nanopore towards fluoride by testing the pore's I-V characteristics after adding Cl⁻, Br⁻, I⁻, SO₄²⁻, NO₃⁻, CH₃COO⁻, HCO₃⁻ and HPO₄⁴⁻ in the electrolyte solution. No change was observed in the I-V curves except fluoride (Fig. 20b).

Conclusions

In this review, we discussed fundamentals of track-etching technique and several sensor applications of single nanopores track-etched in polymer membranes. We included the background for the tracking process and chemical etching technique for a better understanding of the background of this sensor paradigm. We especially focused on DNA, protein and nanoparticle sensors that work on the resistive-pulse principle. In the several studies that we cited in this paper, the detection and discrimination of the analytes were successfully executed by obtaining resistive-pulse signals under varying conditions of potential, concentration etc and commenting on the pulse amplitudes and pulse durations. We also gave examples of a different type of sensing that is based on the current-voltage characteristics of asymmetric nanopores. This behavior, which can be explained simply as the preferential flow of ions in an asymmetric pore, is completely dependent on pore surface charge and could be used for specific sensing of analytes. The track-etch technology, its applications of resistive-pulse and ion current rectification sensing is increasingly popular because of its robustness and ease of fabrication. Major limitations in track-etched nanopores are the non-uniform surface charge and shape, which affects the performance of sensor applications. We, as the authors, believe that there is still room for improvement and diversity in both theoretical and experimental studies on understanding transport phenomena in the field of resistive-pulse sensing or ion current rectification sensors. Customization of the sensing element could further enhance the selectivity which could lead to practical commercial applications in the future.

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