# Electrical, Optical, and Docking Properties of Conical Nanopores

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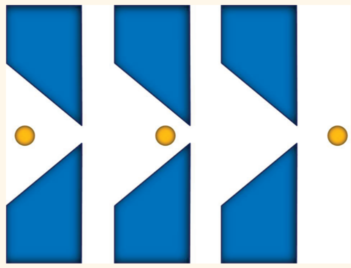
**ABSTRACT** The diffusion-influenced translocation behavior of individual nanoparticles upon passage through a conical nanopore has been elucidated by using a pressure-reversal, resistive-pulse technique, as reported by Lan and White in this issue of *ACS Nano*. We outline here some recent progress in conical nanopore analysis, and we present some prospects for future developments. Compared to cylindrical nanopores, the geometric change brought about by tapered nanopores causes a dramatic difference in electrical and optical properties. Such conical nanopores may also be integrated into microfluidic chips to capture cells or nanoparticles, one per nanopore, and then to release them. These advances hold the promise of making conical nanopores useful as highly efficient actuators and sensors.



Pores connecting an inside compartment to the surrounding outside exist throughout nature. Pores in living organisms, such as ion and water channels, play important roles in maintaining normal physiological functions and serve as "smart" gates to ensure proper transport of ions and water and regulation of the cell's internal environment.

Nanopores have played an important role in various applications such as biosensing platforms, DNA sequencing, drug delivery, and mimicry of biological channels. With the development of nanotechnology, synthetic nanopores have become a subject of intense interest owing to their superior robustness, controllable shape, and modifiable interior surface.[1,2]

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The simplest nanopores are just cylindrical holes. Though they are easily prepared, the relatively large transmembrane potential needed to initiate transport across the nanopore increases the noise of current measurements, thus limiting their sensing capability. Nanopores that have a conical shape, wider on one side than the other, have exhibited unique properties including enhanced transport, lower resistance, and improved sensor performance.[3,4]

**Nanopore Fabrication.** Typically, conical nanopores are made from polymer, glass, or silicon-based materials.[5] Conical nanopores embedded in a polymer membrane can be fabricated by chemical etching. In 2001, Apel *et al.*[6] prepared a conical-shaped polymeric nanopore by means of an asymmetric track-etch method. The resulting conical nanopore had its larger side facing the etch solution and the smaller side facing the stopping solution. Later, the Martin research group[4] developed a new two-step etching process to gain better control over the tip diameter of the nanopore. The second step is an isotropic process in which the entire length of the conical nanopore is uniformly etched. The preparation of a conical nanopore embedded in a glass membrane is quite different, and the White research group[7] has developed a simple electrochemical etching procedure for this purpose. In this method, a platinum wire with an electrochemically sharpened tip is sealed into a glass capillary and the glass surface proximate to the tip is then polished until the tip is exposed. Next, the platinum wire is etched and pulled out to obtain a single, tapered glass nanopore.

**Asymmetric Current Pulse Shape.** When a molecule or particle passes through a nanopore of comparable size under the action of a

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transmembrane potential, the ion current is reduced and a resistive pulse is observed when the current is recorded as a function of time. The electric field strength in a conical nanopore tip is strong, and the resulting resistive pulse measurement is well-suited for sensing applications.[3,8] In this issue of *ACS Nano*, Lan and White[9] present an interesting result on the passage of individual nanoparticles through a conical-shaped nanopore using a pressure-reversal technique in which the current is monitored. They apply a cycle of negative and positive pressures to a conical glass nanopore membrane to detect polystyrene nanoparticles (ref 9, abstract graphic). It is observed that a particle passes through the nanopore back and forth multiple times at low particle concentration and low pressure, suggesting that the same particle is sampled many times (ref 9, Figure 1B). Moreover, particle identity can be determined in a mixed particle solution even though the diffusional motion of the particles induces a stochastic influence on the particle translocation time (ref 9, Figure 3).

The pressure-driven, resistive-pulse technique provides a useful tool to study the electrical properties of analytes in conical nanopores. White and co-workers have pioneered the field of pressure-driven nanoparticle[9,10] and microgel[11] transport across conical glass nanopores. They demonstrated that the translocation frequency not only depends linearly on nanoparticle concentration but also is proportional to the applied transmembrane pressure.[10] Moreover, the magnitude of the resistive pulse approximately scales proportionally to the particle volume. Surprisingly, they found that pulse widths induced by a nanoparticle transporting across a conical nanopore are nearly independent of the radius of the nanopore, as long as the ratio of the diameter of the nanoparticle to the nanopore opening is less than 0.5. The flow velocity of a particle across a conical nanopore is proportional to the tip radius, whereas the flow velocity in a cylindrical nanopore increases with the square of the pore radius.

The work of Lan and White demonstrates that the size of a particle and the direction of translocation across a pore can be determined from the amplitude and asymmetric shape of the current pulse (ref 9, Figure 3). A mirror image relationship exists between pulse shapes for particles going back and forth through the nanopore. Consequently, one can distinguish the particle translocation direction from the shape of the current−time pulse. When driven with pressure, the translocation of a particle across a nanopore can be operated at lower transmembrane potential than when driven with an electric field. This ensures a lower baseline current and a better resistive pulse signal for pressure-driven flow.

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Lan and White have further investigated the stochastic influence of thermal diffusion on particle trajectories during translocation events. Brownian motion of the nanoparticle causes a difference in the transit times for the two different directions. A finite-element simulation based on a convective diffusion model was used to predict the stochastic particle translation event, and the computational results agree closely with the experimental data. The novel pressure-reversal resistive-pulse technique opens new avenues for detecting and sizing particles. The finding that the same particle can be passed through a nanopore multiple times in the analysis suggests a significant increase in the reliability for the information gained from analyzing pulse shapes in current−time recording.

**Ion Current Rectification.** The structure of a synthetic conical nanopore is more similar to a biological nanopore than is a cylindrical pore. Consequently, the property of ion current rectification existing in a conical nanopore makes it particularly useful as a biomimetic ion channel.[1] Apart from the resistive-pulse method widely used to measure the electrical properties of nanopores, ion current rectification is becoming a popular technique to measure biomimetic stimuli responses and to detect biomolecules.[12,13] Ion current rectification has been studied in detail for conical polymer nanopores. Recently, conical glass nanopores have gained increasing attention, thanks to their facile and inexpensive preparation, chemical stability, and mechanical robustness.[7−11] In particular, the silica hydroxyl-terminated interior surface of a glass nanopore channel can be readily coated with self-assembled silane monolayers and further functionalized by covalent modification. The functional groups can help tune the ionic transport properties to achieve stimuli response.[14,15] The Li research group[14] reported pH and thermal dual-stimuli-actuated ion gating with a single glass conical nanopore. A smart homopolymer that undergoes both pH- and temperature-induced conformational transitions is integrated into this nanodevice. The ratio of ionic currents between the "on" state and the "off" state is as large as 188, indicating the achievement of high gating efficiency in the homopolymer-modifed

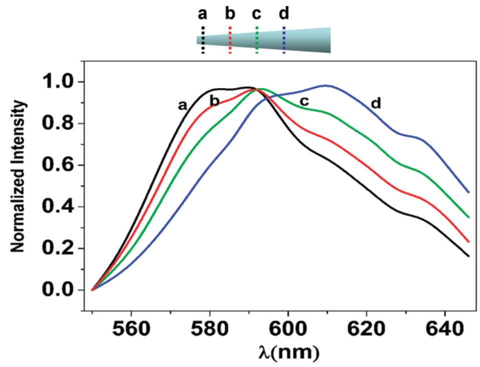


**Figure 1. Confocal fluorescence spectra of Nile red dye at different optical sections of a single glass conical nanopore (~50 nm tip radius). Spectra were obtained at different optical sections of the nanopore at a radius of (a) ~50 nm, (b) ~210 nm, (c) ~520 nm, and (d) ~1.6 μm (the inset is a schematic of the optical sections within the nanopore). Reproduced with permission from ref 17. Copyright 2011 Springer.**

glass nanopore channel. Lan *et al.*[16] investigated pressure-dependent ion current rectification in a charged conical glass nanopore. As the pressure-driven flow rate of the electrolyte (low ionic strength) increases, the ion current rectification decreases. The applied pressure can therefore regulate ion current rectification for conical nanopores with radii of ~180 nm or larger.

**Optical Measurements.** The optical properties of nanoparticles in tapered nanopores have been much less explored than have the electrical properties, but optical properties can serve as excellent probes of the behavior of molecules in a variety of confined spaces. This is simply accomplished by moving the observation volume to different positions in the nanopore channel.[17] Confocal fluorescence microscopy makes it possible to probe different sections of an intact conical nanopore, revealing the localized optical properties of molecules or nanoparticles in a range of confinement geometries. Nile red is a sensitive fluorescence dye that can be used to gauge local environmental differences. As shown in Figure 1, the confocal fluorescence spectrum of Nile red displays an apparent red shift from the tip to the base of the conical nanopore.

Fluorescence recovery after photobleaching (FRAP) is a good approach for monitoring molecular diffusion at different depths of the conical nanopore. The FRAP measurement of fluorescein isothiocyanate revealed that the diffusion of this dye confined in the conical nanopore was much slower than that in the bulk solution. As expected, it was slower in the tip exit and faster at the base entrance of the tapered nanopore. Thus, a single conical nanopore can be used as a convenient platform to investigate the confinement effects of space on the behavior of analytes. Using fluorescence microscopy, the pressure-dependent structure of lipid bilayers suspended across conical nanopores has been imaged.[18]

**Docking Experiments.** In recent work by the Zare research group,[19] conical nanopores were used to capture and to release single nanoscale analytes inside a microchip. In the procedure, an array of conical nanopores in a polymer membrane is incorporated into a microfluidic device to dock bacteria (Figure 2) using a three-layer microfluidic design.[20]

Careful design of the dimensions of the conical nanopores to fit the size of the docking bacteria made the experiment possible. Diameters of the base and tip of the conical nanopores were controlled to be 1.3 μm and ~270 nm, respectively. The analyte, cyanobacteria, has a rod-like morphology with diameters of 800 nm and lengths of 4–6 μm. These dimensions did not allow two cyanobacteria to be trapped within one conical nanopore, thus ensuring single bacterium analysis. Furthermore, the shape of the conical nanopore restrains the "loaded" rod-like bacteria to be oriented only vertically with respect to the nanopore membrane. With negative and positive pressure-driven flow, the docking and undocking of a single bacterium in a conical nanopore was realized, as demonstrated by fluorescence microscope images (Figure 2b). These conical nanopores can selectively capture cyanobacteria from a mixture containing chlamydomonas, which possesses a spherical shape and is much larger than cyanobacteria. Additionally, capture within a conical nanopore generates little effect on cell viability during a 1 h hold time. Compared with current single-cell trapping techniques, the conical nanopore capturing method is simple, inexpensive, and seems to be particularly well-suited for cells or small particles.

## OUTLOOK AND FUTURE CHALLENGES

Conical nanopores provide excellent platforms to investigate the transport of individual molecules, particles, or cells, as well as events occurring within confined geometries. Nanoscale analysis is facilitated with a large opening that tapers to a nanoscale geometry. In addition to being used as containers to capture and to hold a target, nanopores may also be used as reactors with functionalized interior walls for the study of physical and chemical processes at the nanoscale occurring *in situ*.

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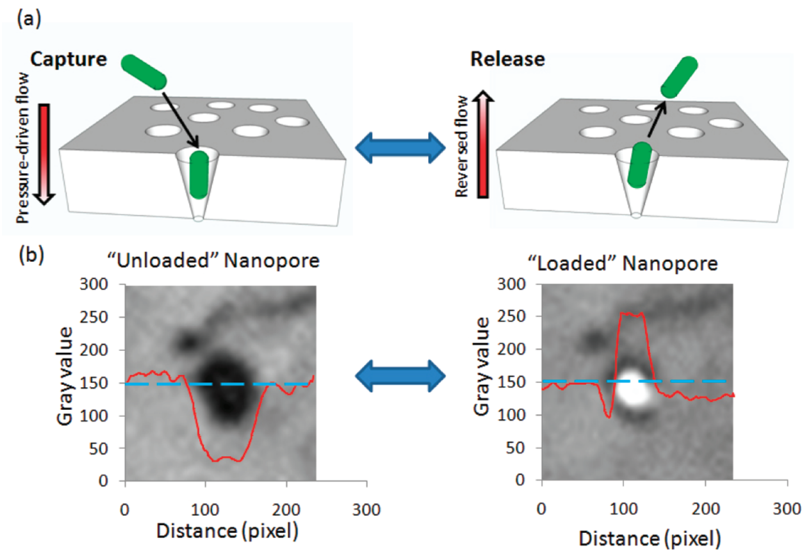


**Figure 2. Mechanism of capture and release of a bacterium within a conical nanopore. (a) Scheme of capture (left) and release (right). (b) Fluorescence microscope images of one conical nanopore unloaded (left) and loaded (right) with one cyanobacterium. The brightness of a loaded nanopore is significantly higher than that of an unloaded one, owing to the strong fluorescence intensity from the trapped cyanobacterium. Reproduced with permission from ref 19. Copyright 2012 The Royal Society of Chemistry.**

Though conical nanopores have demonstrated unique electrical and optical properties, there is still much to be done to improve our understanding of the dynamics and interactions that can occur within them. They also hold the promise of practical applications to a range of diverse fields.

While there are various methods for the fabrication of conical nanopores, the reproducibility and range of controlled geometries can still be improved. The production of ordered arrays of conical nanopores would enable high-throughput single-particle analyses. Glass conical nanopores are more mechanically robust and easier to functionalize than are polymer conical nanopores, making them excellent starting candidates for the development of biomimetic channels with multiple stimuli.

More attention should be paid to the unique optical properties of conical nanopores. Quantitative spectroscopic exploration of conical nanopores with high sensitivity and spatial resolution remains a great challenge. The combined use of optical and electrical measurements would enable increased understanding of analytes inside confined geometries.

*Conflict of Interest:* The authors declare no competing financial interest.

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