**ION PUMP**

“Uphill” cation transport: A bioinspired photo-driven ion pump

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Biological ion pumps with active ionic transport properties lay the foundation for many life processes. However, few analogs have been produced because extra energy is needed to couple to this “uphill” process. We demonstrate a bioinspired artificial photo-driven ion pump based on a single polyethylene terephthalate conical nanochannel. The pumping process behaving as an inversion of zero-volt current can be realized by applying ultraviolet irradiation from the large opening. The light energy can accelerate the dissociation of the benzoic acid derivative dimers existing on the inner surface of nanochannel, which consequently produces more mobile carboxyl groups. Enhanced electrostatic interaction between the ions traversing the nanochannel and the charged groups on the inner wall is the key reason for the uphill cation transport behavior. This system creates an ideal experimental and theoretical platform for further development and design of various stimuli-driven and specific ion–selective bioinspired ion pumps, which anticipates wide potential applications in biosensing, energy conversion, and desalination.

**INTRODUCTION**

The flow of ions across the ion channels and ion pumps embedded in cell membrane is a prerequisite for life processes (1). Different from the biological ion channels with passive ionic transport property, ion pumps with active ionic transport properties can transport ions against the electrochemical potential across cell and organelle, which lay the foundation for many vital activities, such as nerve conduction (2), muscle contraction (3), and secondary transport (4). Examples include the sodium-potassium pump [Na⁺- and K⁺-dependent adenosine triphosphatase (Na⁺,K⁺-ATPase)] (5) in animals and the proton pump (H⁺-ATPase) (6) in plants and fungi (Fig. 1, left). These two “uphill” pumping processes are both realized by coupling an energy-yielding reaction: hydrolysis of adenosine triphosphate.

In the past decade, the efficiency of these ion pumps to fulfill the uphill transport has been a source of inspiration for scientists to mimic such processes using artificial smart nanochannel system, which has elicited considerable interest in the interdisciplinary fields of chemistry, materials science, bioscience, and nanotechnology (7–10). Siwy and Fülsöti (11) fabricated a synthetic electric field–driven ion pump based on a conical nanochannel to pump potassium ions against the concentration gradient. Queralt-Martín et al. (12) also demonstrated an electrical pumping phenomenon of potassium ions in a reconstituted OmpF ion channel. Compared with the electrical field, light is a particularly charming energy source because it is a remote noncontact stimulus that can be controlled both spatially and temporally with precisely regulated wavelength, direction, illuminated area, and intensity, which provides more flexibility of the as-prepared devices in various complicated application areas (13, 14). During the past few years, scientists have developed the analogs of the photo-driven ion channels by either directly using light-responsive materials or integrating diverse light-responsive molecules into the inner surfaces of the nanochannel wall (15–17). However, as for the photo-driven ion pumps, few analogs have been produced (18).

Here, we establish a bioinspired nanoscale photo-driven ion pump to realize this energy-coupled uphill cation transport process based on a single polyethylene terephthalate (PET) conical nanochannel. As shown in Fig. 1 (right), with the concentrated solution placed in the small opening (defined as the tip side), the pumping phenomenon that behaved as an inversion of zero-volt current appeared when we applied ultraviolet (UV) irradiation from the large opening (defined as the base side). Under UV irradiation, enhanced dissociation of the benzoic acid derivative dimers existing on the inner walls will create more mobile carboxyl groups (19). Both experimental and theoretical results have proved that enhanced electrostatic interaction between the ions traversing the nanochannel and the charged groups (–COO⁻) is the key reason for the observed pumping phenomenon. This intelligent system may provide inspiration for constructing the energy conversion devices that convert energy from assorted sources (such as light, temperature, and mechanical forces) to potential energy stored in an electrochemical gradient (20).

**RESULTS**

**Fabrication of the conical nanochannel**

We fabricated a single conical nanochannel embedded in a commercial PET membrane using the well-developed track-etching method (fig. S1) (21). In all samples, the diameters of the bases and the tips are ~1.5 μm and 6 to 14 nm, respectively. We choose the polyester material PET because it is suitable to generate a charged surface with carboxyl groups (mainly benzoic acid and its derivatives) whose pKₐ (where Kₐ is the acid dissociation constant) is about 3.8 (22). When the nanochannel is exposed to solutions with a pH value above 3.8, the inner surface becomes negatively charged, which can make the system cation-selective (23). Notably, the partial association of carboxyl to dimers leads to the formation of hydrogen bonds (24), which will inhibit the dissociation of carboxyl groups (25), although the hydrogen-bonded complex dissociation induced by photon irradiation has been widely reported in the past years (26–29). In our photo-driven system, the carboxyl groups on the nanochannel inner surface remain in a dissociation equilibrium with a certain amount of carboxyl dimers in the very beginning state. As the dissociation equilibrium would change because of the extra energy, the excess mobile carboxyl produced under UV irradiation may result from the UV-driven carboxyl dimer dissociation to monomers (19). The benzoic acid derivative dimers have strong UV photoabsorption due to the strong...
irradiation, which could be ascribed to the enhanced electrostatic interaction between the ions traversing the nanochannel and the charged groups (−COO−) on the inner walls of the nanochannel (31). After we remove the UV irradiation, the inner environment inside the nanochannel will return to the original state because of the thermodynamic dissociation equilibrium.

**Experimental observation of the pumping phenomenon**

We studied the pumping property through measuring the ionic current that was generated by the flowing ions inside the nanochannel with anode placed at the base side (fig. S2). Because the pumping process is our main focus, we monitor the weak current across the nanochannel wall. ADP, adenosine diphosphate; Pi, inorganic phosphate.

Electronic transitions associated with aromatics and the charge transfer (30). Besides potassium chloride (KCl), we also test the pumping phenomenon using sodium chloride (NaCl) and lithium chloride (LiCl). The UV light could also make the Na⁺ and Li⁺ migrate against the concentration gradient when we irradiate the system from the base side. The corresponding zero-volt currents are lower than that of the K⁺, which is ascribed to their different ion mobility (32). To better understand the photo-driven pumping property, we adopt a different concentration gradient of electrolyte (KCl) solutions. Cb remains at 0.1 M, and Cb gradually decreases from 0.09 to 0.01 M. Figure 2B shows the relationship between the concentration gradient (C0/Ct) and the zero-volt currents. Before irradiation, as the concentration gradient increases, the zero-volt currents increase and all the zero-volt currents are negative. In this case, the cation diffusion is driven by the concentration gradient, and a higher concentration gradient results in a larger zero-volt current. After irradiation from the base side, the absolute values of zero-volt currents first become smaller. Then, the zero-volt currents turn to the opposite direction and become larger, in which case the cation transport is against the concentration gradient.

**Fig. 1. From biological ion pump to bioinspired ion pump.** The diagram at the top left corner is the natural proton pump (H⁺-ATPase) in plants and fungi. The proton pump transports H⁺ against concentration gradient to extracellular environment to maintain its acid environment, which is essential to the secondary transport process related to cellular chemical species exchange. The diagram at the bottom left corner refers to the natural sodium-potassium pump (Na⁺,K⁺-ATPase) in animals, and it can transport K⁺ and Na⁺ against their electrochemical gradient, which lay the foundation for many life processes, such as nerve conduction and muscle contraction. These two biological ion pumps are both implemented by coupling the uphill pumping process to an energy source, such as adenosine triphosphate (ATP). Inspired from various biological ion pumps, the bioinspired photo-driven ion pump system is shown at the right side. The single cation-selective PET nanochannel can pump cations from low concentration to high concentration under UV irradiation, which could be ascribed to the enhanced electrostatic interaction between the ions traversing the nanochannel and the charged groups (−COO−) on the nanochannel wall. ADP, adenosine diphosphate; Pi, inorganic phosphate.
Numerical simulation of the pumping phenomenon

Our further theoretical simulation based on solving the PNP (Poisson and Nernst-Planck) equations (see Materials and Methods) also quantitatively supported the aforementioned experimental pumping phenomenon (33, 34). To obtain an affordable computation scale, we simplify the system model to a 1000-nm-long conical nanochannel (tip, 10 nm; base, 250 nm; fig. S4). The effect of UV irradiation on the PET surface is presented as the increase in surface charge density (σ). Figure 3A shows the ratchet-type electrical potential profile along the axis of the PET nanochannel with a surface charge density of −0.06 C/m² when integrating asymmetric electrolyte concentration (C₁ = 0.1 M; C₀ = 0.075 M) (35). There exists an obvious electrical potential difference (ΔV) from the base side to the tip side, which can be considered as the driving force to accomplish the pumping process (36). As the surface charge density increases, ΔV increases accordingly (Fig. 3B), indicating that the increase in surface charge may enhance the electrical field.

In our photo-driven system, ΔV drives cation transport from the base side (high potential) to the tip side (low potential), termed electrostatic flow (Fig. 3C, purple arrow), whereas the cation transport driven by the concentration gradient, termed concentration flow (Fig. 3C, blue arrow), is from the tip side (high concentration) to the base side (low concentration). When the concentration flow is larger than that of the electrostatic flow, the direction of transmembrane ionic transport is dominated by the concentration gradient. When the system approaches steady state, a concentration gradient from the tip side to the base side inside the nanochannel will be established, as visually shown in the calculated cation distribution along the axis of the PET nanochannel (Fig. 3C, black line). On the contrary, if the electrostatic flow is dominant, then an inverse concentration gradient from the base side to the tip side will be set up inside the nanochannel when the system reaches steady state (Fig. 3C, red line). Here, we defined the calculated concentration difference under steady state inside the nanochannel to be C₀b, where C₀b = C₀(x = 1000 nm) − C₀(x = 0 nm). The C₀b with positive and negative values represent the negative zero−volt current and the positive zero−volt current, respectively. Figure 3D shows the relationship between the C₀b and the surface charge density. As the surface charge density increases from −0.14 to −0.16 C/m², the C₀b turned from positive values to negative values, implying that the internal cations undergo a process of changing from down the concentration gradient transport to against the concentration gradient transport. A slight variation in the surface charge may contribute to the pumping behavior. The experimentally observed pumping phenomenon can be rationally explained.

Effect of the electrolyte pH on the pumping property

We also studied the pumping property by measuring the current-voltage (I-V) data. Figure 4A shows I-V properties of the nanochannel when integrating asymmetric electrolyte concentration (C₁ = 0.1 M; C₀ = 0.075 M). It exhibits linear I-V curves at low pH value, such as 2.6 (triangle), in which case the carboxyl groups are unionized and the nanochannel is neutral. Asymmetric I-V curves are observed when we change the pH values from 2.6 to 6.1 (circle) and 10.5 (square), which means that the original nanochannel can rectify at high pH values with voltage-gating property (8). It is the asymmetric geometry and the negative surface charge (−COO⁻) that cause the asymmetric ionic transport property (37, 38). Under UV irradiation, compared with the ionic currents measured in darkness, the values measured at pH 6.1 and 10.5 increase much more than the values measured at pH 2.6. This is because of the effect of the increased surface charge density under UV irradiation (39, 40). These changes can also be shown by the zero-volt currents. As shown in Fig. 4B, all the zero-volt currents measured in darkness at different pH values are negative, and the absolute values increase as the pH values increase (black columns). After UV irradiation, all the zero-volt currents turn to the positive direction, but only the current that measured at pH 6.1 is positive (yellow-green columns). At pH 2.6, the nanochannel is electrically neutral and does not have the pumping ability, and at pH 10.5, the enhanced electrostatic flow under UV irradiation does not have sufficient driving force to reverse such a large current (−15 pA).

Effect of the charge polarity on the pumping property

For comparison, we also conducted the control experiments with ethylenediamine (EN)—modified (positively charged) and platinum-sputtered (neutral) nanochannels (41). Figure 5A shows I-V properties of these three kinds of nanochannels when integrating asymmetric electrolyte concentration (C₁ = 0.1 M; C₀ = 0.075 M) at pH 6.1. Both the negatively
charged nanochannel (triangle) and the positively charged nanochannel (square) show the same trend: that the ionic currents measured under UV irradiation are larger than those measured in darkness owing to the increased surface charge density. The platinum-sputtered nanochannel (circle) has distinct results: that the currents measured both in darkness and under UV irradiation have approximately the same values, implying that the UV irradiation does not have influence on the neutral nanochannel (42). In Fig. 5B, we can figure out the zero-volt currents under these three conditions. In the original condition with concentration gradients in darkness, the zero-volt current of the positively charged nanochannel is positive (+5.6 ± 1.4 pA), whereas the zero-volt current of the negatively charged nanochannel is negative (−12.3 ± 3.6 pA). The directions of the current values are decided by the charge polarity. Under UV irradiation, both the zero-volt currents turn to the positive direction (yellow-green columns). Notably, the zero-volt currents of the neutral nanochannel are about +0.21 ± 0.8 pA (in darkness) and +0.62 ± 0.6 pA (under UV irradiation), which are both within a margin of error, implying again that the neutral nanochannel does not have the pumping ability.

**Effect of light intensity and wavelength on the pumping property**

To better understand the pumping property generated by UV irradiation, we studied the dependence of zero-volt currents on UV (313 nm) intensity. As the light intensity increases, the zero-volt currents become positive until the light intensity achieves approximately 250 mW/cm² and then markedly increase (Fig. 6A, yellow-green circles). The zero-volt current reaches the maximum value of +6.4 ± 1.7 pA at the light intensity of 281 mW/cm². However, without UV irradiation, the zero-volt currents are almost the same with values around −8.5 pA (Fig. 6A, 40).
This observation indicates that UV with enough intensity can reverse the cation flow inside the nanochannel and promote the pumping process. Figure 6B shows the relationship between zero-volt currents and the wavelength of the light. The zero-volt current achieves the maximum value of $+6.3 \pm 2.4 \, \text{pA}$ with the wavelength of $313$ nm (Fig. 6B, yellow-green circles). For comparison, we took the absorption spectrum of PET and plotted it as a solid line in Fig. 6B. The benzoic acid derivative dimers have strong photoabsorption in the UV region due to the strong electronic transitions of aromatics. The PET membrane shows a strong absorbance around the wavelength of $313$ nm. Apparently, the maximum zero-volt current generation is basically at the same position. This agreement illustrates that the changes of zero-volt current stems from the changes of inner surface groups.

**DISCUSSION**

In summary, we have experimentally and theoretically demonstrated a bioinspired photo-driven ion pump system based on a single track-etched PET conical nanochannel, in which the cations flow against the electrochemical gradient powered by the light energy. Because of the strong photoabsorption of benzoic acid derivative dimers in the UV region, the irradiation can induce the dissociation of the hydrogen...
bonds in carboxyl dimers existing on the inner surface of the nanochannel, which consequently produces more mobile charged groups. We conclude that this uphill cation transport phenomenon is ascribed to the enhanced electrostatic interaction under UV irradiation between the cations traversing the nanochannel and mobile charged groups (−COO−) on the nanochannel wall. This system creates an ideal experimental and theoretical platform for further development and design of various stimuli-driven and specific ion–selective bioinspired ion pumps. Specifically, concerning the phototoxicity of UV light, an optimized visible light–driven system can function in a durable and friendly way is highly anticipated, which has wide applications in biosensing, energy conversion, and desalination (43).

MATERIALS AND METHODS

Materials
PET membrane (Hostaphan RN12; 12 μm thick), N-hydroxysulfosuccinimide (NHSS), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), EN, KCl, NaCl, and LiCl were purchased from Sinopharm Chemical Reagent Beijing Co. Ltd. All the experimental solutions were prepared using degassed Milli-Q water (18.2 megohms·cm).

Nanochannel fabrication
A single conical nanochannel embedded in the PET membrane was prepared by the well-developed ion track–etching technique (fig. S1). One side of the membrane was in contact with the etchant (9 M NaOH), and the other side was in contact with the stopping solution (1 M KCl + 1 M HCOOH). The etching was carried out at 30°C, and a constant voltage of 1.0 V was applied to monitor the process. The etching process was stopped at a desired current value corresponding to a certain tip diameter. In all samples, the diameters of the bases were about 1.5 μm, and the diameters of the tips were about 6 to 14 nm.

Modification
To modify the nanochannel with EN, we first immersed the PET membrane with a single conical nanochannel in an aqueous solution of EDC (15 mg/ml) and NHSS (3 mg/ml) for 1.5 hours. Then, we immersed the treated membrane in an aqueous solution of 10 mM EN at room temperature for 2 hours to accomplish the covalent surface coupling process. Finally, we cleaned the as-prepared membrane with Milli-Q water and dried it by N2.

Platinum deposition
Platinum films were deposited onto the inner walls of the nanochannel by ion sputtering with a Pt target (99.99%) by using an ion sputtering system (SBC-12, KYKY Technology Development Ltd.) in a vacuum, which was described in detail in our previous studies (42).

Ionic current recordings
The pumping property was studied by measuring ionic current through the single conical nanochannel. The ionic current was measured by a picoammeter (Keithley Instruments; fig. S2). The PET membrane with a single nanochannel in the center was mounted between two chambers of conductivity cell, which can be irradiated from the sidewall. Ag/AgCl electrodes were used to apply a transmembrane potential across the membrane.

Numerical simulation
The theoretical simulation was based on the coupled two-dimensional PNP equations within the commercial finite element package COMSOL 4.4 script environment. The simulation model is shown in fig. S4. To obtain an affordable computation scale, we simplified the simulation system to a two-dimensional 1000-nm-long conical nanochannel. The tip side and the base side were set to be 10 and 250 nm, respectively. An electrolyte reservoir was introduced to the tip to reduce the effect of the exit mass transfer resistance. The model was similar to the classical simulation model previously reported (33, 37). The effect of UV irradiation was simplified to be the increase in surface charge.

SUPPLEMENTARY MATERIALS
Supplementary material for this article is available at http://advances.sciencemag.org/cgi/content/full/2/10/e1600689/DC1

Nanochannel fabrication
Ionic current recordings
The cycling performance
Numerical simulation model
REFERENCES AND NOTES


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