

ELECTRONIC SUPPLEMENTARY INFORMATION

Microfluidic capture and release of bacteria in a conical nanopore array

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A) Materials and methods

Poly(ethylene terephthalate) (PET) membranes, 12 μm thick, which contained $10^5/\text{cm}^2$ or $10^6/\text{cm}^2$ heavy-ion-induced damage tracks in random patterns, were obtained from GSI Helmholtz Centre for Heavy Ion Research GmbH (Darmstadt, Germany). SPR220-7 (positive photoresist) and silicon wafers for chip master molds were obtained, and used for fabrication, at the Stanford Nanofabrication Facility. Poly(dimethylsiloxane) (PDMS) RTV 615 was obtained from GE Silicones (Waterford, NY, USA). L7012 LIVE/DEAD® BacLight Bacterial Viability Kit was purchased from Invitrogen (Carlsbad, CA, USA). Cyanobacteria (*Synechococcus elongatus* strain 7942) and *Chlamydomonas reinhardtii* were generous gifts from Devaki Bhaya's laboratory in the Department of Plant Biology, Carnegie Institution for Science (Stanford, CA, USA). All other chemicals were purchased from Sigma-Aldrich Chemicals (St Louis, MO, USA). All chemicals were of reagent grade and used as received.

B) Fabrication of the conical nanoporous membrane

A polyethylene terephthalate (PET) membrane with damage tracks was mounted in a two-compartment Kel-F cell. In such a cell, a PET membrane separates two different solutions, the etching solution (NaOH aqueous solution) and the stopping solution (formic acid aqueous solution). The damage tracks in the PET membrane were chemically etched into a conically shaped pore as a result of asymmetric etching, created by the etching and stopping solutions. These conically shaped nanopores have two openings: the large-diameter (or base) opening at one face of the membrane and the small-diameter (or tip) opening at the opposite face (Fig. 1a). Base and tip diameters of conical nanopores are controlled by both the concentration of the etching solution and the etching time. For the conical nanopores used in this paper, the etching time is five hours with 5 M NaOH etching solution and 5 M formic acid stopping solution. To characterize the morphology inside the conical nanopores, gold was deposited in the etched pores using an electroless plating method. A plating time of 15 h was used. The PET was removed after plating to expose the gold replica of the pores. This was accomplished as described previously, by dissolving the PET in 1,1,1,3,3,3 hexafluoroisopropanol (HFIP).

C) Fabrication of the bacteria capture-and-release chip

Silicon wafer master molds, used to fabricate the PDMS chips, were fabricated in the Stanford Nanofabrication Facility (SNF) via standard photolithography techniques. Mask designs were printed by Fineline Imaging (Colorado Springs, CO, USA) at a resolution of 40640 dpi. Wafers were spin-coated with 20 μm SPR220-7 positive photoresist on a clean silicon wafer, which had been primed with hexamethyldisilazane (HDMS) in order to promote adhesion between the photoresist and the wafer. The wafer was exposed to UV light and developed with MF26A developer. Finally, silicon master molds underwent vapor deposition of methyltrichlorosilane (1 hour) in order to reduce wear and tear of the photoresist structures during multiple PDMS castings. From the master molds, two PDMS layers, a top and a bottom, were generated. For the top layer, 5 mm of thoroughly mixed 5:1 PDMS (RTV615 A: B) was poured onto a wafer mold.

For the bottomlayer, 20:1 PDMS was mixed thoroughly and 2mm was cast on another mold. Both layers were degassed under vacuum (2 cycles for 20 min under partial vacuum and then 1h under full vacuum) to remove air bubbles, then cured at 80°C for one hour. The chips were then cut out with a scalpel. To integrate the CNM with the PDMS chip, the top and bottom PDMS layers, and the four sides of the CNM were dipped into a PDMS:toluene mortar (1:4, v/v), which was spincoated on a glass slide and pre-cured for 4 min at 80°C (pre-curing prevents the mortar from clogging the channels). The conical nanoporous membrane was sandwiched between the top and bottom layers with alignment performed under a microscope. Base openings of the conical nanopores faced the top layer containing the inlet and side channels. The tip openings of the conical nanopores faced the bottom layer containing the outlet channel. The assembled chip was sealed with gentle pressure and cured overnight.

D) Culture of cyanobacteria and *chlamydomonas reinhardtii*

Cyanobacteria (*Synechococcus elongates* strain 7942) and *chlamydomonas reinhardtii* were generous gifts from Devaki Bhaya's lab in the Carnegie Institution for Science (Stanford, CA, USA). The cyanobacteria were grown in BG-11 medium at 30°C, illuminated at $130 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ by incandescent bulbs, and bubbled with 3% CO₂ in air. *Chlamydomonas reinhardtii* was grown and incubated in TAP medium at 22°C, illuminated at $80 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ by incandescent bulbs. Usually after 7-day growth, cyanobacteria and *chlamydomonas reinhardtii* were harvested and ready for analysis.

E) Supporting Figure

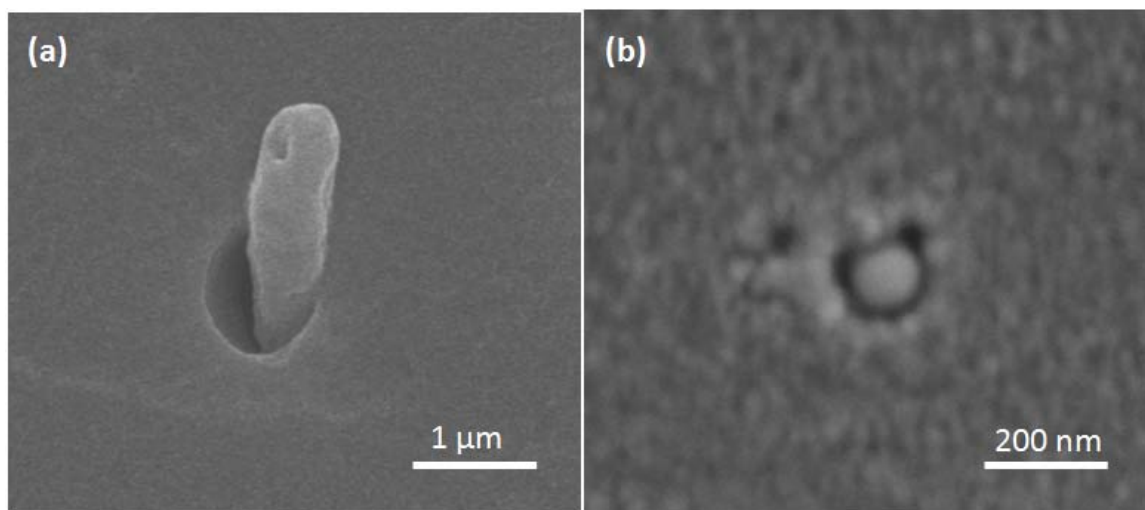


Fig. S1 SEM images of a) *E. coli* bacteria captured in a conical nanopore (base opening: 1 μm; tip opening: 270 nm) and b) virus-sized polystyrene nanoparticle captured in a conical nanopore (base opening: 150 nm; tip opening: 10 nm).

1. B.-han Chueh, D. Huh, C. R. Kyrtos, T. Houssin, N. Futai, and S. Takayama, *Anal. Chem.*, 2007, **79**, 3504-3508.