Materials and Methods

Nanopores: Solid-state nanopores for these experiments were produced by using the highly focused electron beam of a transmission electron microscope (TEM) to drill a single pore in a low-stress silicon nitride (SiN) membrane of ~20nm thickness. The dimensions of this pore can be controlled subsequently using previously described methods[1]. For the experiments described here, we use nanopores with a diameter of 20-27 nm. An example TEM micrograph of one such pore is shown in Fig S1a.

After fabrication, the nanopore is mounted in a custom-made flow cell[2] that combines polydimethyl siloxide (PDMS) and polyether ether ketone (PEEK) components to allow independent delivery of solvent to both sides of the membrane while simultaneously permitting optical measurement. The assembled flow cell is then mounted above a high-NA objective (60x), through which the optical tweezers are focused and direct imaging of the sample is achieved. The optical tweezer apparatus consists of two co-aligned lasers. The first, a 2 W diode pump Nd:YAG laser (CrystaLaser, wavelength 1064 nm), is used to trap target beads. The second, a 5 mW red laser (Coherent, wavelength 650 nm), is reflected off of the trapped bead and onto a quadrant photodiode (QPD), which is used to precisely monitor bead position.

Electrical contact to either side of the membrane is made through agarose salt bridges and Pt connecting wires using small, enclosed reservoirs of 1M KCl with 100 mM potassium ferro/ferri cyanide as a redox agent. This prevents additional noise associated with the light sensitivity of conventional Ag/AgCl electrodes[2]. The buffer at the nanopore contains 10mM tris-HCl (pH 8.0) and KCl at a concentration of 100 mM, 600 mM or 1 M and is put through a 20nm syringe filter and degassed before use. Measured currents are

collected with a commercial patch-clamp amplifier (Axon 200B, Axon Instruments) and are low-pass filtered at 1 kHz prior to digitization at 20 kHz. The low-frequency filtering does not affect the data in these optical tweezer measurements, since the measured current changes are not transitory as in the case of free translocation.

Molecule-Bead Constructs: Bead constructs are produced as described previously[3]. Double-strand DNA (λ-phage, 47.7 kb) is end-labeled with a single biotin molecule using common molecular biology protocols and is subsequently attached to streptavadin-coated polystyrene beads (diameter ~2 μm, Polysciences) with a 1hr incubation at 37° C and in buffer conditions of 1 M KCl and 10 mM Tris-HCl (pH 8.0). The concentration of DNA is controlled relative to the beads in order to achieve attachment of 1-10 molecules per bead on average. This reduces the chance of having multiple molecules enter the pore simultaneously.

RecA-DNA nucleoprotein filaments are assembled on DNA already conjugated to beads as described above. First, 10 mM $MgCl_2$ is added to 10 μL of the bead solution, followed by RecA protein (New England Biolabs, Ipswitch, MA) and ATPγS (Roche, Switzerland) with final concentrations of 6.5 μM and 1.5 mM, respectively. ATPγS is a poorly-hydrolyzable equivalent of ATP and is used to prevent protein dissociation by hydrolysis[4]. The solution was finally allowed to incubate for 1 hr at 37° C before being diluted in clean buffer. RecA-dsDNA bead solutions were used immediately after formation.

Molecular Capturing and Force Spectroscopy: Clean buffer is introduced to both sides of the nanopore in the flow cell. The pore is electrically characterized through I-V curves and analysis of noise spectra. Only pores demonstrating linear I-V behavior and low noise[5] (<20pA rms) are used. Target beads with molecules attached are introduced to the nanopore at very low concentration (~0.001% w/v) to avoid multiple beads entering the optical tweezer focus simultaneously. Upon trapping a single bead, an excess of clean solvent is flowed through the chamber in order to remove all other beads and ensure equal buffer conditions on both sides of the pore. The system is allowed to settle for several minutes to reduce remnant flows that may perturb measurements. The bead is then brought close to the membrane surface (within ~3 μm, as judged by piezo controls and optical feedback[2]), directly below the location of the nanopore. A bias is applied across the membrane in order to pull a molecule electrophoretically through the pore. The appropriate size of this bias varies with salt concentration, but is generally 50-150 mV. Capture of a single molecule is manifested as a sudden, simultaneous change in both the measured trans-pore current and the position signal[2]. Only events that exhibit changes in both of these signals are considered successful molecular captures.

Upon insertion of a molecule, the applied bias is immediately reduced to a lower value of 10-20 mV ("holding bias"). This acts to keep the captured molecule inside the pore while being insufficient to introduce additional molecules. The bead can be moved freely toward and away from the membrane under these conditions. A final check is performed by moving the bead away from the membrane to a distance of ~6 μm, where its position is monitored during a series of different applied biases. A step-wise response ensures that

spurious interactions with the membrane surface are not confused for single-molecule capture events.

Force measurements are performed in a manner similar to those presented previously[3]. After a single molecule is captured in the nanopore, a holding bias is applied and the z distance between the membrane and the trapped bead is increased by 1 μm with a retraction speed of 100 nm/sec using piezo control. The QPD sum signal is recorded during this motion, resulting in a characteristic curve caused by interference of the red position laser (Fig. S1b, gray). This is used as a calibration curve for force measurements. At the end of this motion, a series of increasing voltages is applied across the nanopore in steps of 10-20 mV. The increasing electrophoretic force moves the bead step-wise out of its initial position and towards the pore. The QPD sum signal is recorded at each applied voltage (averaged over at least 2 seconds per position) and compared to the calibration curve in order to arrive at a physical z distance moved for each position (Fig. S1b, red). Within the linear region, the optical trap acts as a Hookean spring, such that for each applied voltage $F_{trap} = -\kappa \Delta z$, where κ is the trap stiffness as measured from power spectral density[2]. Typical trap stiffnesses for the presented measurements are 30-35 pN/μm.

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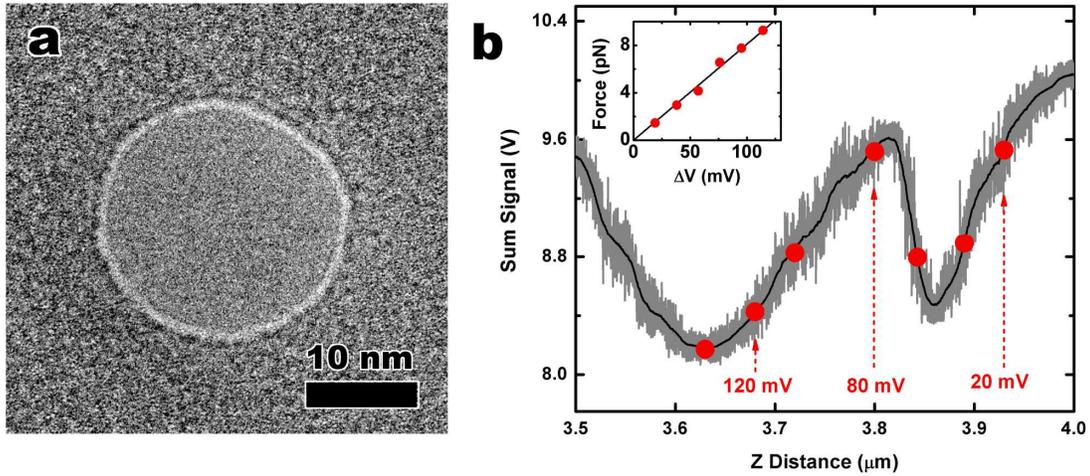
Figure S1 (a) TEM image of a nanopore used in the present experiments (diameter 21 nm). (b) Example of a QPD sum signal calibration curve (raw data: gray, smoothed: black) with overlaid force measurements, obtained by increasing the voltage applied on a captured molecule in a step-wise fashion (red). The inset shows the corresponding force vs. applied voltage relation for the given data points (solid line is a linear fit). Presented data are from measurements on a RecA-dsDNA filament in 1 M KCl.