Recapturing and trapping single molecules with a solid-state nanopore

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Published online: 2 December 2007; doi:10.1038/nnano.2007.381

The development of solid-state nanopores[1–7], inspired by their biological counterparts[8–15], shows great potential for the study of single macromolecules[16–21]. Applications such as DNA sequencing[6,22,23] and the exploration of protein folding[6] require control of the dynamics of the molecule's interaction with the pore, but DNA capture by a solid-state nanopore is not well understood[24–26]. By recapturing individual molecules soon after they pass through a nanopore, we reveal the mechanism by which double-stranded DNA enters the pore. The observed recapture rates and times agree with solutions of a drift-diffusion model. Electric forces draw DNA to the pore over micrometer-scale distances, and upon arrival at the pore, molecules begin translocation almost immediately. Repeated translocation of the same molecule improves measurement accuracy, offers a way to probe the chemical transformations and internal dynamics of macromolecules on sub-millisecond time and sub-micrometre length scales, and demonstrates the ability to trap, study and manipulate individual macromolecules in solution.

In this letter, we present a detailed view of the dynamics of single molecule capture by a solid-state nanopore on millisecond timescales and sub-micrometre length scales. We monitor the current through a nanopore and detect blockages in the current when DNA passes through the pore, partially obstructing the current path. After translocating a solid-state nanopore, a single DNA molecule is allowed to continue to move under the influence of the pore's proximal electric field and diffusive forces for a pre-set time period. The electric force is then reversed to bring the same molecule back to, and then through, the nanopore. Both passages are detected by a blockage of the ionic current through the pore. In previous work with solid-state nanopores[16,17,19–21,25,27], the capture dynamics could not be studied directly because the location of a molecule was unknown until it entered the nanopore. Here, the molecule is known to be inside the pore at both ends of a measured time interval, the length of which directly reveals the essential characteristics of the molecular motions involved.

We studied a 5 nm × 7 nm nanopore in a ~20-nm-thick SiN membrane (Fig. 1a) that joined two reservoirs of aqueous 1 M KCl maintained at pH 8 by 10 mM Tris, 1 mM EDTA buffer. Electrical contact to the reservoirs was made with Ag/AgCl electrodes. An equimolar mixture of 6 and 4 kilobase-pair (kbp) double-stranded DNA (dsDNA) fragments was added to the reservoir contacted by the ground electrode. The other reservoir was biased at +120 mV. Ionic current blockages were monitored to detect the passage of DNA through the pore[16]. After a molecule was detected passing through the pore (Fig. 1b), the bias voltage was maintained at 120 mV for a programmed time, t_{delay}, between 2 and 32 ms (Fig. 1c), then reversed to −120 mV for 500 ms (Fig. 1d,e). The voltage was then returned to +120 mV, regardless of when or if a molecule translocated in the reverse direction (see Supplementary Information for details on methods and materials). Fast voltage switching has previously been used to probe the escape of single-stranded DNA (ssDNA) from a protein pore[13].

Figure 1f shows a representative current trace. A molecule is detected translocating the pore in the forward direction by an ionic current blockage (B), 2 ms are allowed to elapse (C), then the voltage is reversed (D), and the molecule is seen to translocate the pore in the reverse direction, made evident by a second current blockage (E). Immediately after translocating the pore and before the voltage reversal, the molecule is driven away from the pore by the near-pore electric field and random thermal forces. We varied t_{delay}, the time between the first translocation and the voltage reversal, and measured $t_{capture}$, the time until the molecule re-enters the pore after voltage reversal, to probe the behaviour of the molecules at different distances from the nanopore.

All electronic signals due to forward and reverse passages of the molecule through the pore are analysed individually. They show a characteristic blockage current and unfolded translocation time that scales appropriately with the length of the molecules[16]. Based on the structure of these signals (see Supplementary Information for details), we discriminate between 4 kbp and 6 kbp molecules[16,21]. Within the limits imposed on length discrimination by the statistical spread in translocation times and the sticking of molecules to the pore during translocation, we verify that if a 4 kbp molecule passes the pore in the forward direction, the recaptured molecule is also 4 kbp, and likewise for 6 kbp molecules.

Figure 2a shows the rate at which molecules are captured by the pore versus time after voltage bias reversal for $t_{delay} = 2$ ms. In the forward direction, the capture rate of molecules is suppressed just after the voltage bias is switched from negative to positive because the molecules near the pore have been repelled by the reversed voltage for the previous 500 ms. In contrast, in the reverse direction, 87% of returning molecules arrive within 50 ms

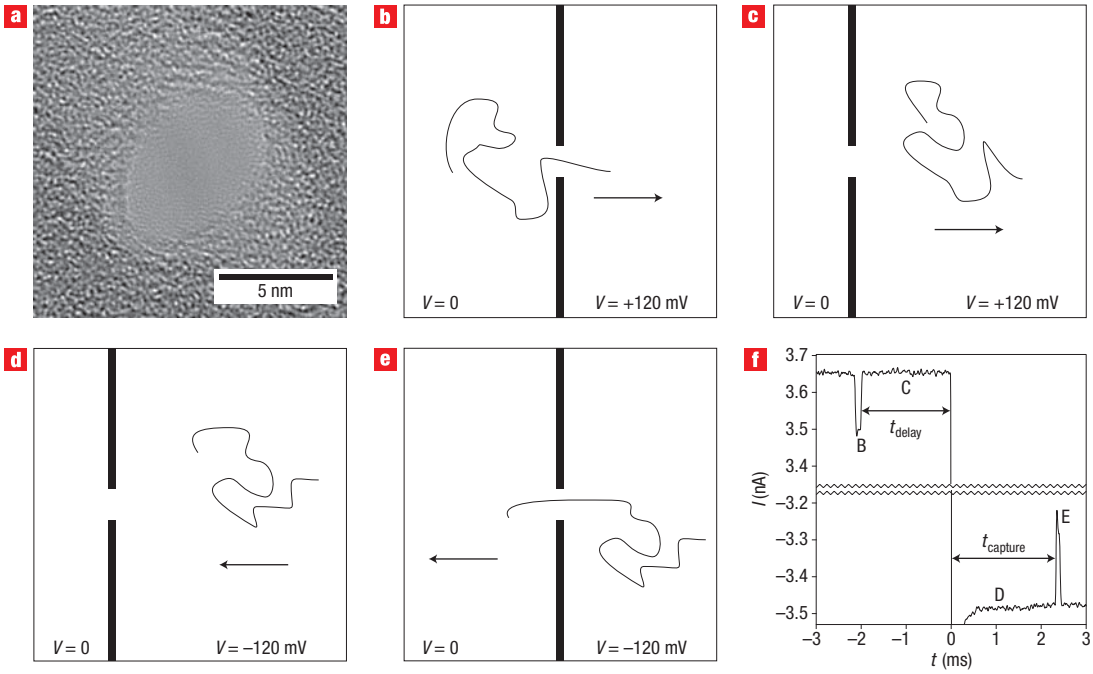

Figure 1 Overview of the recapture experiment. a, Transmission electron micrograph of the SiN nanopore used. **b**–**e**, Schematic representation of the experiment. The arrow represents the direction of the electric force on the DNA molecule. A single DNA molecule passes through the nanopore in the forward direction (**b**). After passing through the pore, the molecule moves away from the pore under the influence of the electric field for a fixed delay time (**c**). The field is reversed, and the molecule moves towards the pore (**d**). The molecule passes through the pore in the reverse direction (**e**). **f**, A representative current trace for an experiment with a 2 ms delay before voltage reversal. A gap of 6.6 nA is omitted from the middle of the trace. The letters mark the correspondence between the current trace and the schematic illustrations of molecular motion (**b**–**e**). Molecules cannot be detected passing the pore during the first 300 μs after voltage reversal while the capacitance of the nanopore/flow cell system charges.

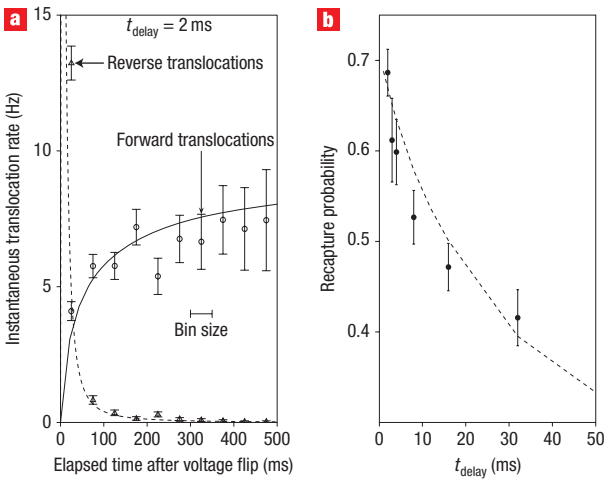

Figure 2 Capture rates and recapture probabilities. a, Instantaneous capture rates when a reverse voltage is applied at $t_{delay} = 2$ ms after the molecule is first detected entering the pore. Each point represents the average rate at which molecules entered the pore within a 50 ms time interval after voltage reversal (for example, the point at 25 ms represents the rate within the interval 0 and 50 ms after the voltage flip). The solid (forward-biased capture) and dashed (recapture) lines represent the predictions of the drift-diffusion model discussed in the text. **b**, Fraction of molecules recaptured within 500 ms of voltage reversal, as a function of time delay between forward translocation detection and voltage reversal. The dashed line represents the prediction of the drift-diffusion model discussed in the text. On both plots, the error bars represent the uncertainty due to counting statistics.

after the bias is turned negative. The high recapture rate just after the voltage is reversed is due to the return of the molecule that previously passed the pore in the forward direction and triggered the voltage reversal. Molecules that pass through the pore and are not rapidly recaptured form a background recapture rate two orders of magnitude lower than the rates discussed above.

Figure 2b shows the recapture success rate, the fraction of forward translocations followed by a reverse translocation at any $t_{capture}$ within the 500 ms voltage reversal window, as a function of t_{delay}. Figure 3 shows histograms of $t_{capture}$ for each t_{delay} collected for many events. For $t_{delay} < 4$ ms, most molecules arrive at the pore and are translocated through in less than 10 ms. Both the distribution of return times and the overall recapture success rate depend strongly on the delay before reversal.

We compare our observations with a theoretical model in which the DNA's motion is determined by an electric force on the charged phosphate backbone and random thermal forces due to collisions with water molecules. Competition between thermal and electrical forces leads to a characteristic length beyond which the latter dominates the former.

On average, diffusion drives a molecule away from the pore, located at $r = 0$. We can define the radial diffusion velocity $v_d(R, t)$, as $E[dr/dt|r(t) = R]$, the expectation value of the rate of change of a diffusing molecule's distance from the pore. This is equivalent to

$$v_d(R,t) = \lim_{\Delta t \to 0} \frac{E[r(t+\Delta t)|r(t) = R] - R}{\Delta t} \quad (1)$$

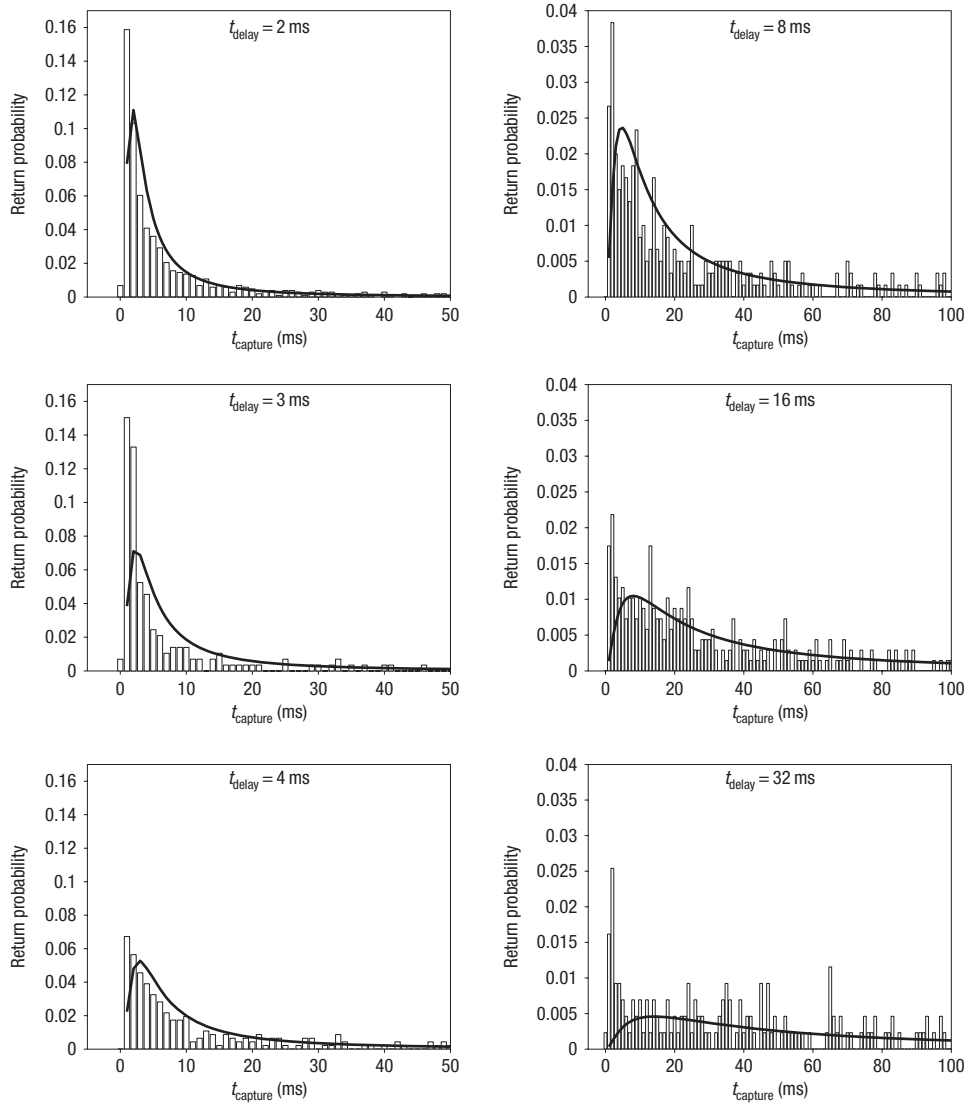

Figure 3 Capture time histograms for returning molecules for different delays before voltage reversal. Each bar represents the fraction of forward translocated molecules recaptured in the 1 ms interval centred about the corresponding time. Note the axes have different scales for the left and right histograms. The bold lines represent the predictions of the drift-diffusion model discussed in the text.

where

$$\mathrm{E}[r(t+\Delta t)|r(t)=R] = \int \rho^2 \mathrm{d}\rho\, \mathrm{d}\Omega \sqrt{R^2+\rho^2+2R\rho\cos(\theta)} \times \frac{\exp(-\rho^2/4D\Delta t)}{(4\pi D\Delta t)^{3/2}} = R + \frac{D\Delta t}{R} \quad (D\Delta t \ll R^2) \tag{2}$$

so

$$v_{\mathrm{d}}(r) = D/r \tag{3}$$

where D is the DNA's diffusion constant.

An electrical current density $\mathbf{J}$ results from an electric field $\mathbf{E}$, given by Ohm's law, $\mathbf{J} = \sigma\mathbf{E}$, where σ is the electrical conductivity of the ionic solution. At distances much greater than the diameter of the pore, the current density and electric field will be (hemi)spherically symmetric, and related to the experimentally observed current I through the biased nanopore by $\mathbf{E}(r) = (\mathbf{J}(r)/\sigma) = (I\mathbf{r}/2\pi r^2\sigma)$.

DNA in free solution is known to move with a constant electrophoretic mobility μ[28,29]. If we ignore the conformational degrees of freedom of the DNA molecule and assume its charge is distributed symmetrically about its centre of mass, located at r, the radial electrophoretic velocity $v_{\mathrm{e}}(r, t)$ is given by $v_{\mathrm{e}} = \mu I/2\pi r^2\sigma$.

Comparing v_{e} to v_{d}, we see there is a characteristic distance $L = (|\mu I|/2\pi\sigma D)$ beyond which the average velocity away from the pore due to diffusion is greater than the electrophoretic velocity[30]. For our experimental conditions, this length is 940 nm for 4 kbp dsDNA and 1.2 μm for 6 kbp dsDNA. (In contrast, for 100 bp ssDNA and a, for example, protein pore with 100 pA of current, this length is less than 1 nm, and we do not expect recapture of recently translocated molecules by voltage reversal would be possible.)

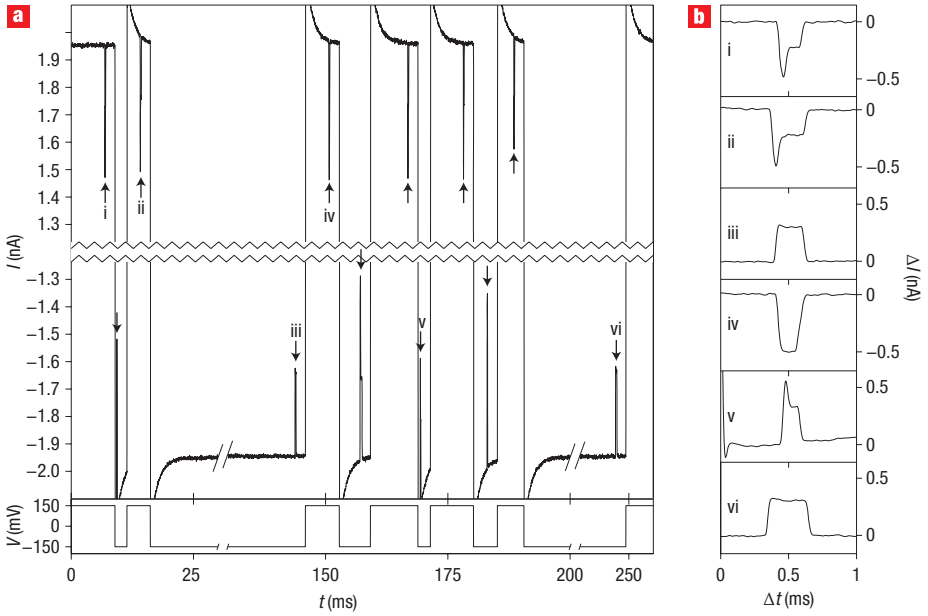

Figure 4 Current versus time traces from a single-molecule trapping experiment. a, A single 10 kbp dsDNA molecule passes the pore 12 times over 250 ms. The main panel shows the current through the pore versus time. For clarity, 2.4 nA are excised from the centre of the current axis, and the time axis has also been compressed. The short pulses (marked with arrows) show current being blocked as the molecule passes through the pore. At 2 ms after each passage, the voltage bias (plotted below the current) is reversed. As in Fig. 1, the molecule is initially captured at positive voltage bias. The exponential settling at the beginning of each transition results from charging of the membrane capacitance. **b**, Expanded current traces resulting from separate passages of the molecule through the pore. Each is labelled from i to vi to identify the portion of the current trace in **a** from which it was taken.

Assuming a (hemi)spherically symmetric distribution of non-interacting dsDNA molecules, the volume concentration $c(r, t)$ of DNA obeys the drift-diffusion transport equation

$$\frac{\partial c(r,t)}{\partial t} = \frac{1}{r^2}\frac{\partial}{\partial r} r^2 \left(\frac{\mp |\mu I|}{2\pi\sigma r^2} c(r,t) + D\frac{\partial c(r,t)}{\partial r} \right) \qquad (4)$$

where the minus sign is used when the electrical force is directed away from the pore and the plus sign when this force is towards it. This equation can be solved numerically, with appropriate boundary and initial conditions, to model the voltage reversal experiment (see Supplementary Information). With no free parameters, this drift-diffusion model predicts the correct ratios between recapture success rates at different t_{delay} (Fig. 2b) and the relative distributions of $t_{capture}$ for all t_{delay} (Fig. 3), but overstates the actual number of molecules recaptured at all $t_{capture}$ and t_{delay}. A single parameter fit, which scales the number of recaptures predicted by an overall factor of 70% for all $t_{capture}$ and t_{delay}, makes a good match to the observed recapture success rates and capture time distributions. The dashed lines in Fig. 2 and solid lines in Fig. 3 represent this fit, with no other free parameters. In the forward direction, the same equation models the capture of molecules initially driven away from the pore by an electric force generated by the reversed voltage. The solid line in Fig. 2a is a single-parameter (the steady-state flux of molecules through the pore) fit of the drift-diffusion model to the observed forward capture rates.

Approximations made in the model, including assuming a spherically symmetric electrical field on all length scales[31] and ignoring the possibility of nonspecific binding of the DNA to the membrane surface, could account for the missing 30% of returning molecules. At long values of t_{delay}, we see a higher return rate at short times than predicted by the model. This could be due either to molecules that stick briefly to the membrane surface and are not driven as far away, or to extended configurations of molecules that leave parts of them far closer to the pore than their centres of mass. We have disregarded effects of electro-osmotic flow, which, due to the negatively charged surface of the pore, would oppose the DNA's electrophoretic motion.

Numerical analysis of the drift-diffusion equation shows that of the molecules that return to the pore from distances less than the characteristic distance L, discussed above, most do so within a time $L^2/2D$ (here, 220 ms for the 4 kb DNA and 450 ms for the 6 kb DNA) A molecule that starts at $0.4L$ (400–500 nm) has an 85% chance (neglecting the overall 70% pre-factor) of translocation in this time (see Supplementary Information for further details of the calculations).

We also probed the time required for DNA to enter the nanopore. The factor $t_{capture}$ consists of t_{return}, the time it takes a molecule to arrive at the pore, and t_{ent}, the time it takes a molecule to enter after arriving. t_{return} is predicted by the drift-diffusion model discussed above, and its distribution depends strongly on t_{delay}. t_{ent} is not included in the drift-diffusion model and does not depend on t_{delay}, as it involves the behaviour of the molecule after it has already reached the pore. The histograms of $t_{capture}$ presented in Fig. 3 depend strongly on t_{delay}, in the same manner as the drift-diffusion calculations of t_{return}, which indicates the recapture time is determined mainly by t_{return}. The recapture rate is also highest immediately after the voltage is reversed, which is inconsistent with the notion[24,32] that the molecule requires a significant amount of time post-arrival to enter the pore. Hence, upon arriving at the pore, the typical molecule in this experiment translocates in less than a millisecond.

Besides exploring molecular dynamics there are other advantages and applications to recapturing molecules that have passed the pore in the forward direction, including convincing evidence that an electronic signal corresponds to a molecule translocating the pore. This provides a way to distinguish molecular signals from background noise on a single-molecule basis and is valid even for polydisperse samples and analytes[17,18] for which no sensitive assay like the polymerase chain reaction exists. Recapturing the molecule would also allow one to measure changes in molecules (such as hybridization changes, changes in protein conformation, stripping of binding proteins) induced by passage through the nanopore. Immediate voltage reversal can also be used to study the conformational dynamics of a polymer. The Zimm relaxation time[33] for 4 kb dsDNA is 300 μs and 610 μs for 6 kb dsDNA. In this experiment the configuration of the molecule during the reverse translocation was not influenced by the previous translocation. However, with an increase in viscosity[20] and/or molecule length, the relaxation time can be extended sufficiently to enable us to probe and possibly manipulate the non-equilibrium conformation induced in the molecule by passage through the nanopore and to explore the influence of a molecule's initial conformation on translocation through the pore.

Extending the single recapture experiments presented so far to repeatedly recapture the same molecule realizes a new kind of single-molecule trap based on nanopore technology. Figure 4 presents the electronic signals from such a trap (see Supplementary Information for details of the setup). In this particular experiment (in a new nanopore), a single 10 kbp dsDNA molecule from a mixture of 5.4 and 10 kbp molecules was passed back and forth 12 times over a period of 250 ms. (Single molecules have so far been trapped for as many as 22 passes over 500 ms.) Current blockage induced by the passage of the molecule through the nanopore revealed information about the molecule (length, conformation and interaction with the pore[16]) and, with triggered voltage reversals, provided the feedback mechanism to maintain the trap. Thus, biologically interesting molecules can be trapped, detected and analysed in free solution without any labels or chemical modifications. Repeated electronic interrogation of a single molecule potentially provides a means for greatly enhancing the accuracy with which each molecule can be characterized by a nanopore and allows measurement over time of dynamical properties such as the molecule's conformation and chemical state.

Received 10 August 2007; accepted 23 October 2007;
published 2 December 2007.

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Acknowledgements

This work was supported by NIH/NGRI grant no. 5 R01 HG00370302. Some fabrication was carried out at Harvard University's Center for Nanoscale Systems, with the assistance of D. Bell, Yuan Lu and J.D. Deng. We thank E. Brandin for preparing the molecules for the trapping experiment, S. Coutreau and P. Testa for machining assistance, Jiali Li, D. Branton, S. Bezrukov, D. Hoogerheide and D. Vlassarev for useful discussions, and M. Biercuk for valuable suggestions regarding the manuscript.
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Supplementary information accompanies this paper on www.nature.com/naturenanotechnology.