Supporting Information

Fields and Cohen 10.1073/pnas.1103554108

SI Methods

Optics. Light from a 633 nm HeNe laser (Thorlabs HRP120) was expanded, passed through a linear polarizer, and guided through two electrooptic deflectors (EODs; ConOptics). A half-wave Fresnel rhomb (Thorlabs FR600HM) rotated the polarization of the light by 90° between the EODs. A series of relay lenses (Thorlabs) imaged the center of the first EOD onto the second, and the center of the second EOD onto the back aperture of an objective in an inverted microscope (Olympus IX71 with a 60X PlanAPO NA 1.45 oil-immersion objective). Illumination power was set to 250–900 μW depending on the experiment, but was held constant at 430 μW for experiments involving RecA. Fluorescence was collected through the same objective and separated from back-scattered excitation light by a dichroic mirror and a high quality emission filter (Chroma 49006). A tube lens focused the light onto a pinhole with a diameter of 300 μm (corresponding to 5 μm in the sample plane; Edmund Optics NT56-285). The pinhole diameter set the size of the trapping region and was chosen to just encompass the laser scan pattern. The light was then imaged onto an avalanche photodiode (APD) single-photon counting module (Perkin Elmer SPCM-AQRH-14). The video of trapped molecules was taken by splitting half of the fluorescence onto an Andor iXon DU-897 back-illuminated electron-multiplying CCD camera using a 50:50 beam splitter (Thorlabs CM1-BS013). For the FCS experiments, the laser was not scanned and the emission was passed through a 50 μm pinhole.

Device Design and Fabrication. The ABEL sample cell consisted of a shallow (approximately 600 nm) central trapping region flanked by four deeper (approximately 15 μm) channels etched within a 2.5 mm square piece of fused silica (1). Fused silica was selected due to its lower autofluorescence relative to glass or polydimethyl siloxane (PDMS). The dual depth design ensured that the electrical resistance was much larger within the shallow trapping region than elsewhere, focusing the electric field within the trapping region.

The fabrication scheme described here is similar to that of ref. 2, in which the pattern was constructed in two steps of photolithography, corresponding to the deep and shallow channels of the devices, respectively. Modifications to the published protocol include changes to the mask design and use of an approximately 60 nm chromium etch mask instead of silicon. One hundred devices were fabricated in parallel on a single 4" diameter, 500 μm-thick fused silica wafer. To enclose the channels, we irreversibly bonded each etched device to the center of a 1″ square fused silica cover slip (Esco R425025) using sodium silicate, following a published protocol (3).

We used a cast piece of PDMS to contain excess fluid around the sides of the fused silica device and to hold the feedback electrodes in place. Fluid leakage around the corners of the device allowed pressure equilibration without significant voltage loss. Damp tissue paper placed in an enclosed space with the device alleviated sample evaporation. We used platinum electrodes (Alfa Aesar 45058) to minimize electrochemical products.

Electronics. A field-programmable gate array (FPGA; National Instruments PCI-7831R) relayed voltages to two high-voltage amplifiers (Model 7602, Krohn–Hite) to control the beam deflections of the EODs. Concurrently, the FPGA counted and recorded the photons that the APD detected, using their precise arrival times to determine the beam position at the time of emission. The FPGA used a Kalman filter (described below) to calculate feedback voltages, which were amplified by two additional Krohn–Hite high-voltage amplifiers and applied to the trap. The FPGA continually relayed information to a personal computer for storage; this same computer also allowed the operator to set the feedback parameters on the FPGA.

Sample Preparation. Chemicals were purchased from VWR unless otherwise noted. 5′ C6-linked amino modified 30 nt ssDNA oligonucleotides were custom ordered from IDT and labeled with Alexa 647 NHS-ester (Invitrogen A-20196) according to the manufacturer protocol. Free Alexa 647 NHS-ester was recovered during purification. Devices were cleaned with piranha solution (a highly corrosive 3:1 mixture of concentrated sulfuric acid and 30% hydrogen peroxide). Prior to trapping samples containing RecA, devices were functionalized with polyethylene glycol (Vectabond Reagent SP-1800, Vector Laboratories, and MPEG-SVA-5000, Laysan Bio Inc.), according to manufacturer instruction. All experiments were performed in 10 mM Hepes buffer, pH 7.4, with fluorophore concentrations of approximately 2 pM. Oxygen was removed from solution using the protocatechuate 3,4-dioxygenase (Sigma–Aldrich P8279) and protocatechuic acid scavenging system as described (4). Triplet state quenching was achieved using 1 mM methyl viologen (Sigma–Aldrich 856177) and 1 mM ascorbic acid (Sigma–Aldrich A92902) (5). RecA and ATP were purchased (New England Biolabs M0355 and P0756) and included in indicated samples at 1 μM and 1 mM, respectively. 1 mM $MgCl_2$ was also included in the samples containing RecA.

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2. Cohen AE, Moerner WE (2008) Controlling Brownian motion of single protein molecules and single fluorophores in aqueous buffer. *Opt Express* 16:6941–6956.
3. Wang HY, Foote RS, Jacobson SC, Schneibel JH, Ramsey JM (1997) Low temperature bonding for microfabrication of chemical analysis devices. *Sens Actuators B* 45:199–207.
4. Aitken CE, Marshall RA, Puglisi JD (2008) An oxygen scavenging system for improvement of dye stability in single-molecule fluorescence experiments. *Biophys J* 94:1826–1835.
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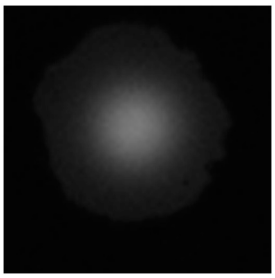

Movie S1 A series of Alexa 647 molecules are trapped until photobleaching or diffusional escape. The movie is shown in real time.

Movie S1 (AVI)