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Supporting Material

Massively parallel single-molecule manipulation using centrifugal force

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Massively parallel single-molecule manipulation using centrifugal force: Supplemental materials and methods

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INSTRUMENT DESCRIPTION

The Centrifuge Force Microscope consists of an optical system mounted perpendicularly to the rotation axis of a computer controlled rotary stage (ADRT-150, Aerotech and CP20, Soloist). The optical system consists of an LED lamp (LED528E, Thorlabs), 20x plan objective (NA 0.4), and a 5 megapixel CCD camera (GC 2450, Prosilica; maximum frame rate 15 fps). The sample holder is an acrylic chamber sealed with a coverslip and an o-ring. It can be translated (i.e. focused) using an adjustment screw (AJS100-02H, Newport) for coarse adjustment and a piezo for fine adjustment (AE0505D08F, Thorlabs). Data and power transmission between the rotating optical system and external devices is accomplished with a fiber optic rotary joint (MJX-155-28-SC, Princetel) integrated into an electrical slipring (SRF24, Princetel). Two media converters (855-10734 and 855-10735, IMC Networks) convert the gigabit ethernet signal from the camera to fiber optic and back to gigabit ethernet for connection to a computer (Precision T5400, Dell). Power for the camera, media converter, LED, and piezo are transmitted through the electrical slipring, allowing dynamic control while spinning. A counterweight placed opposite to the optical system balances the load. With a top speed of 600 rpm and a rotation arm length of 385.5 mm, the current instrument can generate a force field of ~1-155 g. The specifications of the rotary stage were chosen to enable angular acceleration from stationary to full speed within 1 s.

EXPERIMENTAL DETAILS

Digoxigenin labeled DNA was prepared by labeling the cohesive ends of 48kB lambda phage DNA (N3013S, New England Biolabs) with biotin (biotin-14-dATP and biotin-14-dCTP, Invitrogen) using Klenow polymerase (M0212S, New England Biolabs), followed by purification (QiaQuick purification kit, Qiagen). The biotinylated DNA was then cut almost exactly in half with the XbaI restriction enzyme (R014SS, New England Biolabs) and re-purified. The overhanging ends of the 24kB DNA were filled in and labeled with a single digoxigenin (dig-11-dUTP, Roche) or plain nucleotides for dig-labeled or unlabeled DNA. Finally, the DNA was re-purified and the dig-DNA was mixed with unlabeled DNA in a 1:4 ratio before reacting with highly monodisperse streptavidin labeled beads (Dynabeads M-270, Invitrogen, measured CV = 1.7%) for use in the experiment.

Functionalized coverslips were prepared by base washing a glass coverslip (immersed for 5 minutes in boiling solution of 1 part 30% H_2O_2, 4 parts NH_4OH, and 19 parts d-H_2O), followed by adsorption of anti-digoxigenin (11094400, mouse monoclonal, Roche). The coverslip was then washed with blocking buffer comprised of PBS with 0.1% Tween 20 and 1mg/ml dephosphorylated alpha-casein (C8032, Sigma). Experiments were performed in the same blocking buffer to decrease non-specific adhesion to the surface.

For DNA overstretching experiments, streptavidin was adsorbed onto 25 μm polystyrene beads (Thermo Scientific NIST traceable size standards 4225A, 24.61 ± 0.22 μm mean diameter, CV = 1.1%), then biotinylated lambda DNA was added. Streptavidin was adsorbed onto a glass coverslip, and experiments were performed in a buffer of 150 mM NaCl, 10 mM Tris, 1 mM EDTA. Density tables were used to find the buffer density of 1.0043 g/cm^3 (1). The bead density was measured to be 1.0481 g/cm^3 by centrifuging beads suspended in fluids of known density and examining which way they settled (beads floated in 6.98% NaCl solution but sunk in 6.95% NaCl solution).

To observe the overstretching transition, the angular velocity of the stage was increased in stepwise increments of 20 degrees per second while the variance of each bead image was measured. Overstretching was easily detected by a dramatic change in the variance (i.e. focus) of the beads. The beginning of this transition occurred at 213 rpm, which corresponds to 66 pN.

DATA ANALYSIS

Analysis of videos recorded at 10 frames per second was performed by identifying the locations of fully tethered beads at a frame near the beginning of the movie (once full speed was reached) and analyzing small regions of interest at each location in subsequent frames to determine the time of bond rupture. Finding the bead locations was done in ImageJ by first performing a background subtraction, then making a binary image and using the analyze particles tool to find the center positions and the variance of a region of interest around each bead. The image variance is important because stuck and tethered beads can easily be distinguished from the variance, and stuck beads were excluded from analysis in this way. This information is

then fed to a custom Matlab program, which analyzes subsequent frames of the video to find the mean, variance, and centroid in the small regions of interest. The rupture time for each bead was identified by a dramatic drop in the measured variance to near zero, corresponding to a grey, bead-free image. In rare cases where multiple drops in the variance were observed, the bead was excluded from analysis, as this typically indicated a multiple tether.

While high-resolution detection was not necessary for these studies, we nonetheless verified that displacements less than 100 nm could easily be measured with this simple variance technique (using the Physik Instrumente P-601 closed-loop piezo translator). Higher resolution can be achieved with higher numerical aperture optics, or by using more sophisticated approaches (2-5). For our current imaging setup, the depth of field is approximately 6 microns. In order to observe bead motions larger than this, translation of the focus is required.

Regarding temporal resolution, this is largely governed by the camera frame rate and the angular acceleration of the system. In particular, for constant force experiments, rupture events that occur during the angular acceleration of the sample will reduce the number of acquired statistics. The acceleration time can be reduced by using heavier particles and spinning to lower speed, by decreasing the moment of inertia of the imaging arm, or by using a higher-torque motor.

Once rupture times were collected, histograms were made and this data was fit with a decaying exponential with no offset. The resulting time constants and associated fitting errors were used to determine the off-rate at each force level using a simple Arrhenius approach with force-dependence modeled in the standard way by a single, sharp barrier.

The force imparted on the beads due to centrifugation was calculated as $F = m\omega^2 R$, where m is the mass of the bead (minus the mass of the medium displaced), ω is the magnitude of its angular velocity, and R is its distance from the axis of rotation. In our case, R = 385.5 mm, and m = 6.9 x 10-12 g (calculated using manufacturer's bead specifications of 2.8 μm diameter and 1.6 g/cm^3 density). The weight of the bead acts perpendicularly to the centrifugal force, and was added in quadrature when calculating the net force on the tether.

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