Electronic Supplementary Information

Methods

Chip fabrication

Flow and control wafers were obtained from the Stanford Microfluidics Foundry, design name DTPAd, according to the 640-chamber MITOMI design from [1]. This design features 15 μm high flow channels, rounded by photoresist reflow, and a rectangular control channel cross-section for optimal valve closing operation. PDMS chips were fabricated as follows: For the control layer, a 5:1 mixture of Sylgard 184 (Dow Corning) base:curing agent was poured onto the wafer, degassed, partially cured at 80°C for 20 min. The flow layer wafer was spin-coated at 2500 rpm for 75 s with a 20:1 base:curing agent mixture of Sylgard 184 and partially cured at 80°C for 30 min. The control layers were cut out, holes were punched and control layers were aligned onto the flow layer PDMS on the flow layer wafer. Assembled chips were baked at 80°C for 90 min, cut, peeled off the flow layer wafer and holes were punched. After fabrication, PDMS chips were stored under Argon atmosphere for no more than 1 month.

Chip functionalization

The chip was bonded onto a clean glass slide at 80°C for 5 h. hydrochloric acid (12.5 %) was flushed into the chip for overnight storage in humid atmosphere. Silanization was prepared by flushing with ethanol for 20 min. A mixture of 70 % 3-Aminopropyldimethyl-ethoxysilane (ABCR), 5 % H_2SO_4, 25% ddH_2O was prepared, stirred for 1 h, mixed 1:4 with EtOH, and flowed through the chip for 30 min, followed by 10 min rinsing with ethanol. The silanization was consolidated by baking the chip at 80°C after peeling it off the glass slide. PEGylation was prepared by 30 min pre-incubation in borate buffer (50 mM sodium borate, pH 8.5) and accomplished by incubating the chip with 0.25 mg/ml NHS-PEG-Maleimide (MW 5000, Rapp Polymere), dissolved in borate buffer, and covered with a glass slip. The chip was then rinsed with ddH_2O and bonded for 30 min at 80°C onto a clean glass slide. For DNA functionalization, strand 1 (oligo sequences below) at 2 μM in coupling buffer (50 mM sodium phosphate pH 7.2, 50mM NaCl, 10 mM EDTA) was first flowed through the chip for 60 minutes, with the unused back chambers of the flow chambers sealed off using the corresponding valve. DNA oligos 2 and 3 were pre-incubated in 5x phosphate buffered saline (PBS) for 2 hours at concentrations of 2 μM (strand 2) and 4 μM (strand 3) and then flushed through the chip for 60 min. In parallel, a #1 thickness cover slip was prepared to serve as stamping counterpart: Therefore, it was amino-silanized (30 min), treated with NHS-PEG-Biotin (60 min, 0.25 mg/mL in Borate buffer, MW 3000, Rapp Polymere), and incubated with Neutravidin (60 min, 1 mg/mL in PBS), and rinsed with ddH_2O. Finally, the chip was rinsed and prepared for quick transfer onto the Neutravidin-presenting glass slide with a solution of 30 mM Trehalose and 5 ppm Tween in PBS allowed for short-term wetting and stabilization of the PDMS-surface-bound DNA oligomers. For all of these steps, flow layer pressure was kept constant at 4 psi.

Oligomer sequences

All oligomers were purchased from IBA GmbH. 5'-GAA TTC-3' is the palindromic recognition sequence of EcoRI and is displayed in the duplex of strands 1 and 2. For the all-DNA probe experiments, the following three strands were used:

Strand 1: (SH)-5'-ttttttttttt-CTG CAG GAA TTC GAT ATC AAG CTT ATC GAT-3'

Strand 2: 3'-GAC GTC CTT AAG CTA TAG TTC GAA TAG CTA C-ttttttt-5'-(Cy5)-5'-ttttttt-C GAC GTC CTT AAG CTA TAG TTC GAA TAG CTA-3'

Strand 3: Biotin-5'-ttttttttttt-TAG CTA TTC GAA CTA TAG CTT AAG GAC GTC-(Cy3)-3'

For the multiplexing experiments, the following strands were used in various combinations:

Strand 1-consensus: (SH)-5'-ttttttttttt-TAGACCGGAATGAATTCGCTTATCT-3'

Strand 1-star: (SH)-5'-ttttttttttt-TAGACCGGAATGAATTGGCTTATCT-3'

Strand 2-consensus: 3'-ATCTGGCCTTACTTAAGCGAATAGA-ttttttt-5'-(cy5)-5'-ttttttt-TTAGTAAGGGAGCATATTGCATACGTTGAGGACTTATCAG-3'

Strand 2-star: 3'-ATCTGGCCTTACTTAACCGAATAGA-ttttttt-5'-(cy5)-5'-ttttttt-TTAGTAAGGGAGCATATTGCATACGTTGAGGACTTATCAG-3'

Strand 3-25bp: Biotin-5'-ttttttttttt-CTGATAAGTCCTCAACGTATGCAAT (Cy3)-3'

Strand 3-30bp: Biotin-5'-ttttttttttt-CTGATAAGTCCTCAACGTATGCAATATGCT (Cy3)-3'

Strand 3-35bp: Biotin-5'-ttttttttttt-CTGATAAGTCCTCAACGTATGCAATATGCTCCCTT (Cy3)-3'

Strand 3-40bp: Biotin-5'-ttttttttttt-CTGATAAGTCCTCAACGTATGCAATATGCTCCCTTACTAA (Cy3)-3'

Prior to each experiment, Thiol-containing strands were reduced with 5 mM TCEP (Thermo Fischer Scientific), purified by ethanol precipitation and resuspended in coupling buffer. EcoRI was purchased from New England Biolabs and flushed into the chip at a concentration of 10 nM in a buffer solution (pH 7.6) containing 10 mM HEPES, 170 mM NaCl, 1 mM EDTA, 50 μM DTT, and 100 μg/mL BSA.

Contacting mechanism

The functionalized chip, bound to the Neutravidin glass slide, was flushed with 1x PBS at a reduced pressure of 0.5 psi prior to button actuation ("stamping") and measurement. The button valve pressure was linearly increased from 0 psi to 15 psi over 150 s. After a contact time of 10 min, the button was retracted by lowering the pressure to 0 psi linearly over 150 s.

Readout

Data acquisition was performed chamber-by-chamber, scanning the microfluidic chip. Confocal stacks of up to 25 images at a vertical distance of 1 μm were recorded

for the cy3 and cy5 channels using a spinning disk unit (Yokogawa) and an EMCCD Camera (Andor) on an inverted microscope through a 40x / 1.3 NA oil immersion objective (Carl Zeiss). The chip was scanned using hybrid DC/piezo motors (Physik Instrumente) for x- and y-directions, an objective piezo positioner (Physik Instrumente) for z-direction and a custom-designed scan software (Labview, National Instruments). Excitation lasers at 532 nm (cy3 channel) and 640 nm (cy5 channel) were used in combination with emission filters at 593 nm and 676 nm (AHF Analysentechnik). The exposure time was set to 100 ms to balance data quality and experiment duration. Thus, a 640-chamber chip can be scanned and imaged in 60-90 minutes. If time constraints apply, reducing the exposure time and/or increasing the confocal slice-to-slice distance will speed up readout at the expense of raw data signal-to-noise and fit quality.

Analysis

The recorded stacks of confocal fluorescent images were analyzed by choosing two distinct circular regions of interest (ROI): Region 1 corresponds to the button valve contact area, while region 2 does not overlap with the contact area. Vertical mean intensity profiles were plotted for each region and fluorescent channel. These profiles were fitted with the sum of two Gaussian functions and a constant background offset in the case of contacted region 1, and the sum of a single Gaussian and a constant background in the case of no-contact region 2. These Gaussian fit data yield integrated intensity values for each region, channel, and location (top/bottom). From these, relative rupture probabilities are computed, namely the value of normalized fluorescence. Its value between 0 and 1 denotes the fraction of probes under load, which rupture at the bottom bond.

Bell-Evans model

The multiplexing application data can be fitted with a Bell-Evans model simulation, as described in high detail previously [2]. In short, both the probe and reference bonds can be described by a two-state rupture probability depending on force f, force loading rate f', potential width Δx and rate k_{off}:

$$p(f,f') = \frac{k_{off}}{f'} \cdot \exp\left(\frac{f \cdot \Delta x}{k_B T}\right) \cdot \exp\left(-\frac{k_{off}}{f'} \cdot \int_0^f du \cdot \exp\left(\frac{u \cdot \Delta x}{k_B T}\right)\right)$$

Δx and $\log(k_{off})$ are assumed to be linear functions of oligomer length n:

$$k_{off} = 10^{\alpha - \beta \cdot n}\, s^{-1}$$

$$\Delta x = (t + n \cdot m) \cdot 10^{-10}\, m$$

α, β, t and m are the independent fit parameters. For dsDNA, previous experimental studies have found: $\alpha = (3 \pm 1)$, $\beta = (0.5 \pm 0.1)$, $t = (7 \pm 3)$, $m = (0.7 \pm 0.3)$[3]. The loading rate of 10^5 pN s^{-1} was estimated experimentally from PDMS stamp separation velocity, effective spring constants of the PEG linkers and the functionalization density [4]. The oligomer length dependence of normalized fluorescence is then given by:

$$NF\left(f',n_{ref},n_{probe}\right) =$$

$$= \frac{1}{2}\left[1 - \int_{fA}^{\infty} df\left(p_{ref}\left(f,f',n_{ref}\right) - p_{probe}\left(f,f',n_{probe}\right)\right) - \int_{fB}^{\infty} df\left(p_{ref}\left(f,f',n_{ref}\right) - p_{probe}\left(f,f',n_{probe}\right)\right)\right]$$

with the fA and fB, the points of equal rupture probability, defined by:

$$p_{ref}\left(fA,f',n_{ref}\right) = p_{probe}\left(fA,f',n_{probe}\right)$$

$$p_{ref}\left(fB,f',n_{ref}\right) = p_{probe}\left(fB,f',n_{probe}\right)$$

If only one force fA > 0 fulfills this requirement, then fB = 0. The fit routine yields values in consistency with literature values: α = 3.73, β = 0.355, t = 5.24, m = 0.415.

References

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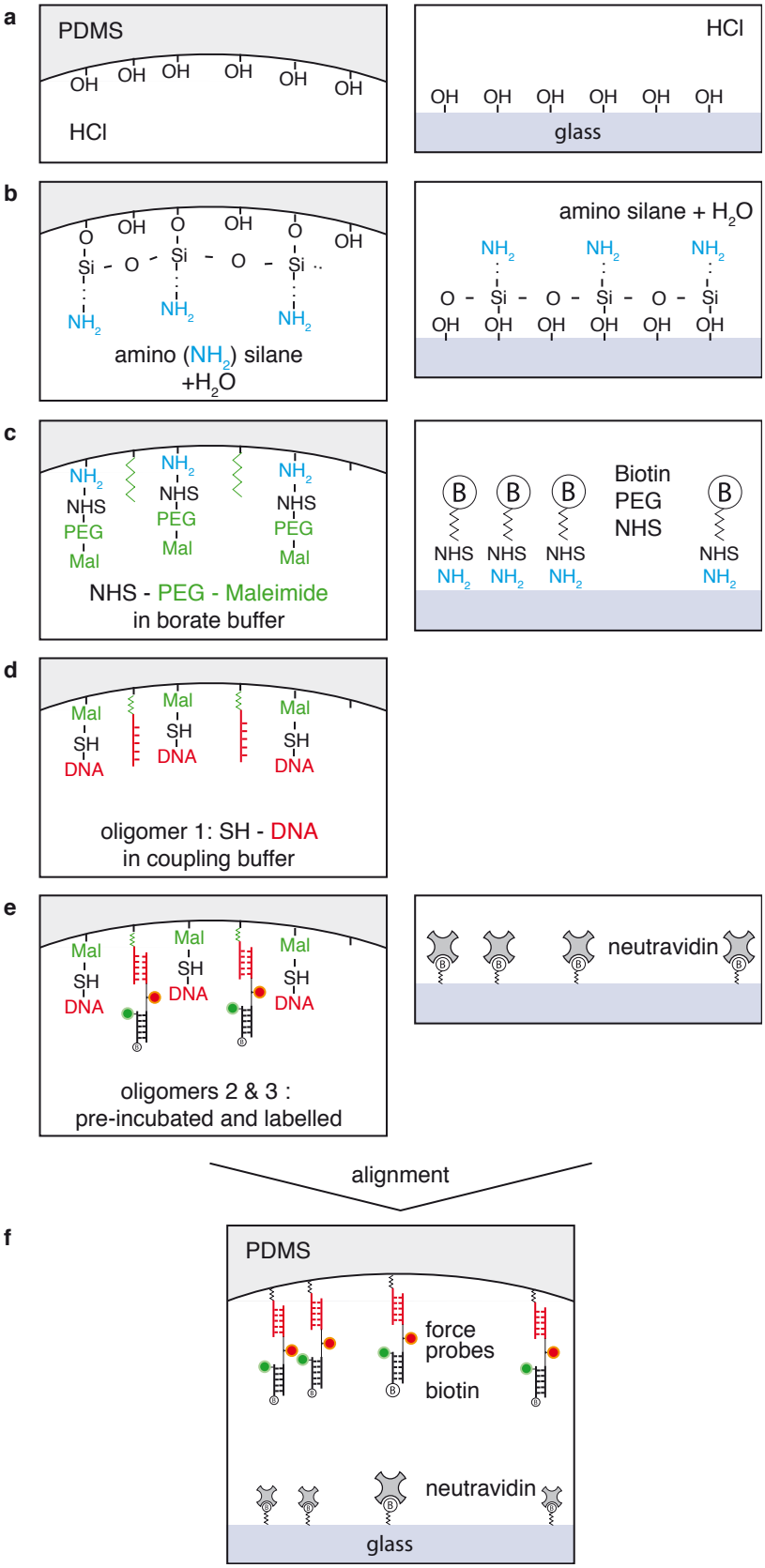
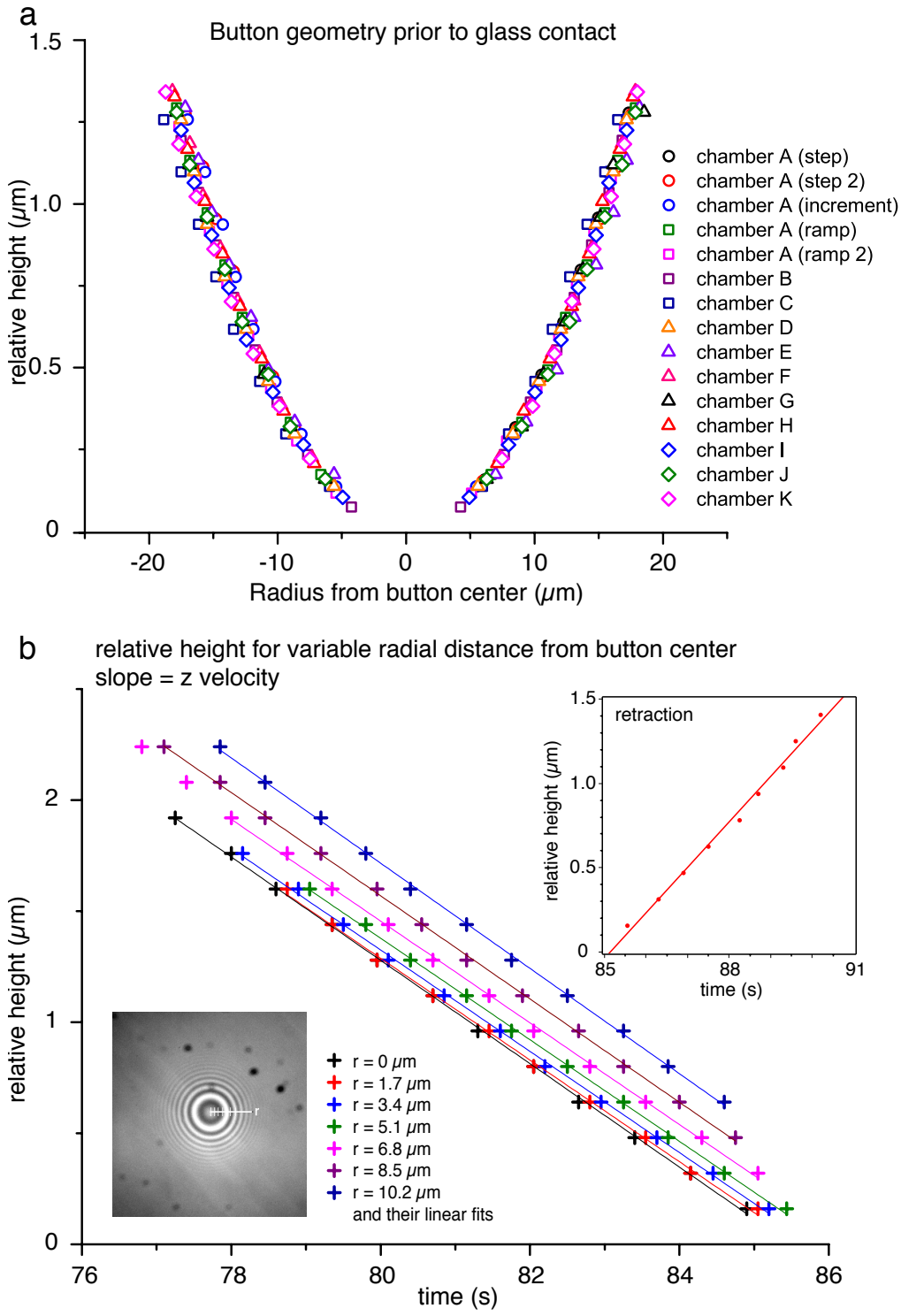
3. Strunz, T., Oroszlan, K., Schäfer, R. & Güntherodt, H.J. Dynamic force spectroscopy of single DNA molecules. *P Natl Acad Sci Usa* 96, 11277-11282 (1999).

4. Albrecht, C.H., Neuert, G., Lugmaier, R.A. & Gaub, H.E. Molecular force balance measurements reveal that double-stranded DNA unbinds under force in rate-dependent pathways. *Biophys J* 94, 4766-4774 (2008).



Supplemental Figure 1

Chip and slide functionalization. (a)-(e) schematically summarize the multistep functionalization procedure of chip and slide as described in the online method section. (f) shows the resulting setup after chip and slide alignment.



Supplemental Figure 2

Button geometry and dynamics. (a) Button geometry just prior to glass contact in multiple chambers, determined by interference patterns between glass and PDMS in microscopy. (b) PDMS-Glass distance plotted against time. Various radial distances from button valve center (cf. bottom-left inset) are plotted for approach dynamics (main graph) and compared to a typical retraction curve (top-right inset). These data are collected from time-course interference pattern changes. Data (crosses) are fitted with linear functions (lines). The slope of each fit allows for the determination of approach or retraction velocities.