Electronic Supplementary Material (ESI) for Soft Matter

Supplementary Information

Direct Observation of Single Flexible Polymers using Single Stranded DNA

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PDMS Microfluidic Device

Hybrid polydimethylsiloxane (PDMS)/glass microfluidic devices were fabricated using standard soft-lithography techniques. A schematic of the device is shown in **Figure S1**. Microfluidic devices were used to stretch ssDNA in extensional flow.[1] The fluidic layer was patterned in PDMS using replica molding. The mold for the device was prepared by spin coating negative photoresist (SU-8) onto a silicon wafer (3" diameter) and patterning with UV exposure using a high-resolution transparency film as a mask. Typical photoresist layer thicknesses range from 10-50 μm depending on the spin conditions and photoresist used. The molds were developed using propylene glycol methyl ether acetate (PGMEA) and treated under vacuum with trichlorosilane vapor to prevent adhesion of cured PDMS. Next, PDMS with a 10:1 base:crosslinker ratio (w/w) was cast on the mold with a thickness of ~5 mm. The cast was cured for 2 hours at 70°C. After curing, the PDMS replica is peeled off the silicon mold, and inlet and outlet ports are introduced using a blunt needle. The PDMS replica with access ports is bonded to a glass coverslip via plasma oxidation to yield a functional device.

DNA molecules were held near the stagnation point in an extensional flow generated in a cross-slot device. In this way, ssDNA molecules stretch to near full extension in free solution using an extensional flow, which is an effective flow field for polymer stretching and orientation. Manual trapping of single ssDNA molecules was performed using a micrometering valve connected to one of the outlet tubes.[2]Manual adjustment of the micrometering valve allows for real-time positioning of the stagnation point, thereby enabling trapping of single molecules. Both outlets combine into a single outlet using a Y-junction and are directed into a waste tube.

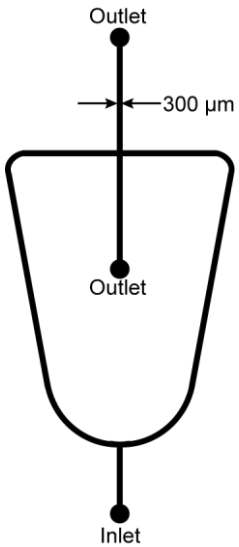

Fig S1 Schematic of the PDMS device used for stretching ssDNA. The width of the channels was 300 μm and the channel height was 50 μm.

Gel Electrophoresis

Figure S2 shows alkaline agarose gel electrophoresis forssDNA samples containing variable ratios of aa-dUTP:dTTP. Alkaline agarose gel electrophoresis was performed using standard protocols in molecular biology, and the denaturing conditions allow foraccurate determination of molecular weight. The results shown in Figure S2 are consistent with those in Figure 4b in that ssDNA samples are relatively monodisperse, with the general absence of broad molecular weight distributions; however, as the fraction of aa-dUTP is increased, some amount of ssDNA product exhibits inhibited mobility in the denaturing gel, presumably due to the modified aa-dUTP nucleotide and the chemical nature of the additional primary amine. Single molecule visualization directly reveals that after dye labeling, ssDNA molecules from these samples (Sequence 1) stretch to reveal clean, linear and pristine ssDNA polymer backbones.

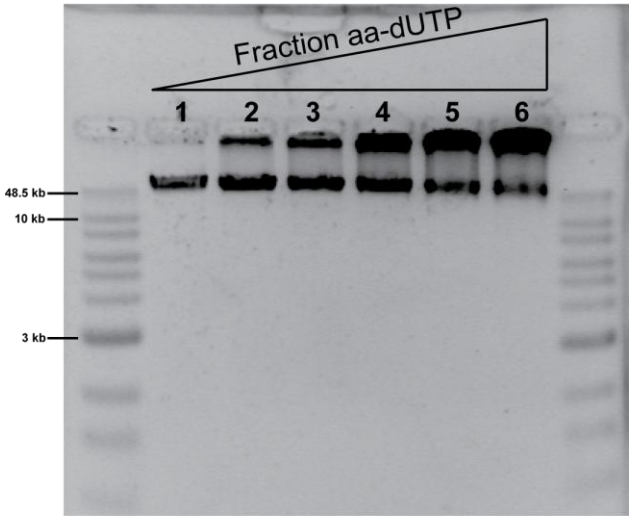

Fig S2 Denaturingalkaline gel electrophoresis for replicated ssDNA products (Sequence 1) with varying dTTP:dUTP ratios. The dTTP:dUTP ratios varied between 1:0, 4:1, 3:2, 2:3, 1:4, and 0:1 in Lanes 1-6, respectively. The lower band corresponds to ssDNA product, which shows similar molecular weight as a function of increasing dUTP. As the dUTP content was increased, some of the ssDNA product showed inhibited mobility within the gel, however, the gel conditions were denaturing, which fully prevents base pairing interactions. The gel was a 0.6% alkaline agarose gel and was run at 60V for 1 hour.

Figure S3 shows native and alkaline agarose gel electrophoresis for pyrimidine-rich products compared to purine-rich products (Sequence 1). In contrast to ssDNA products from Sequence 1 templates, pyrimidine-rich products showed low reaction yields and failed to migrate in native agarose gels. In alkaline gels, pyrimidine-rich products migrated efficiently, but showed band smearing over a wide range of lengths, indicative of an increase in polydispersity compared to purine-rich ssDNA products.

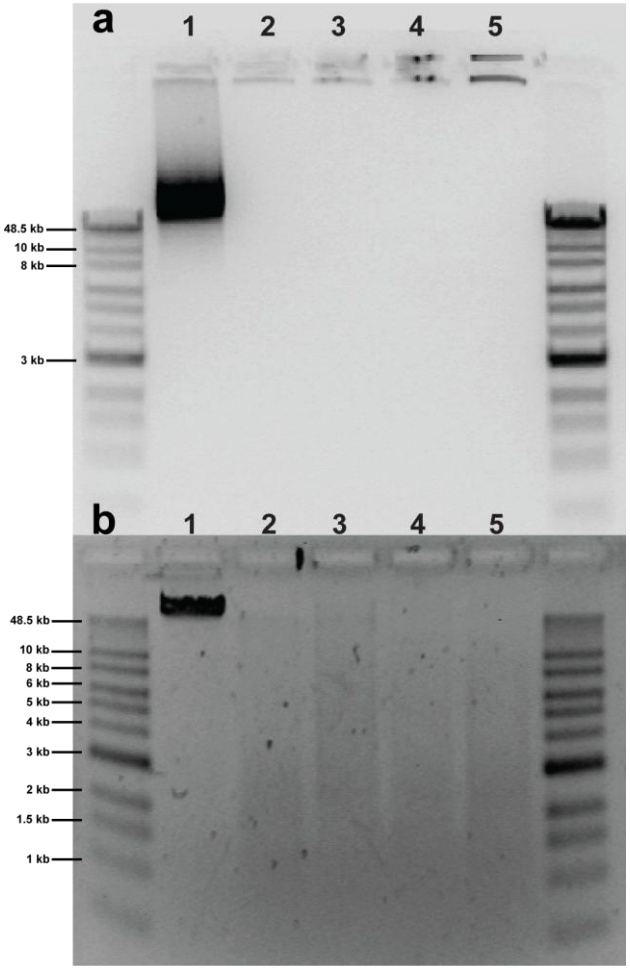

Fig S3RCR products for Sequences 1, 4, 5, 6, and Pyr 6 in Lanes 1-5, respectively, were run in both (a) native and (b) alkaline agarose gel electrophoresis. Sequence 1 yields large ssDNA product and was used in single molecule visualization studies. Sequence 1 migrates at nearly the same mobility in native and denaturing gels, which suggests the absence of base pairing interactions. For other sequences (4, 5, 6),the gels illustrate the sequence-dependent product mobility in native agarose gel electrophoresis. In general, we observed pyrimidine-rich sequences to migrate slowly through native gels, which presumably does not arise due to base pairing, because nearly homopolymeric (dT) ssDNA also did not migrate efficiently in native gels (Lane 5, Pyr 6). Finally, the gels highlight differences in product length distributions for purine-rich (Sequence 1) and pyrimidine-rich (Sequences 4, 5, 6, Pyr 6) sequences.

References

(1) M. Tanyeri, E.M. Johnson-Chavarria and C.M. Schroeder, *Appl. Phys. Lett.*,**96**, 224101 (2010).
(2) C. M. Schroeder, H. P. Babcock, E. S. G. Shaqfeh and S. Chu, *Science*, **301**, 1515-1519 (2003).