Supporting Information for

Modular Stitching to Image Single-Molecule DNA Transport

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Experimental Section

1. DNA sequences in this study

λ-DNA has two overhanging ends that are complementary: 5'–GGGCGGCGACCT–3' and 5'–AGGTCGCCGCCC–3'.The "stitching oligomer" has two strands that are annealed together. One strand is 5'–GGGCGGCGACCTATAATACTACCATGATCTAGTTCAAGTACAG –3' and the other strand is 5'–GGGCGGCGACCTCTGTACTTGAACTAGATCATGGTAGTATTAT–3'. The overhang sequence of the labeled module for end-labeling is 5'–AGGTCGCCGCCC–3'.

2. Miniature gel electrophoresis cell for imaging

A miniature gel electrophoresis setup was constructed to allow direct imaging in epi-fluorescence mode. Test chains were added to agarose suspension at 45 ºC at picomolar concentration for single molecule imaging. 1 ml solution was cast into an imaging sample chamber (Lab-Tek, bottom area 20 mm x 20 mm) at 45 ºC and allowed to solidify at room temperature for 20 min. Both sides of the gel pad were cut and removed to accommodate two Au electrodes running parallel along the gel sides. The Au electrodes were made by electron beam deposition of a 200 nm Au layer onto the glass substrate. The solidified agarose gel slab was immersed in 3 ml buffer in a so-called "submarine" mode. Electrophoresis buffer (1× TBE) was present to ensure electrical circulation and prevent electrolysis. A mixture of glucose, glucose oxidase, calatase and trolox was used to minimize photobleaching.The sample chamber was mounted onto a microscope stage. A DC voltage was applied across the two electrodes through a function generator (Wavetek) to generate an electric field up to 16 V/cm.

Supplementary discussion

1. Quantifying contribution of "hooking"

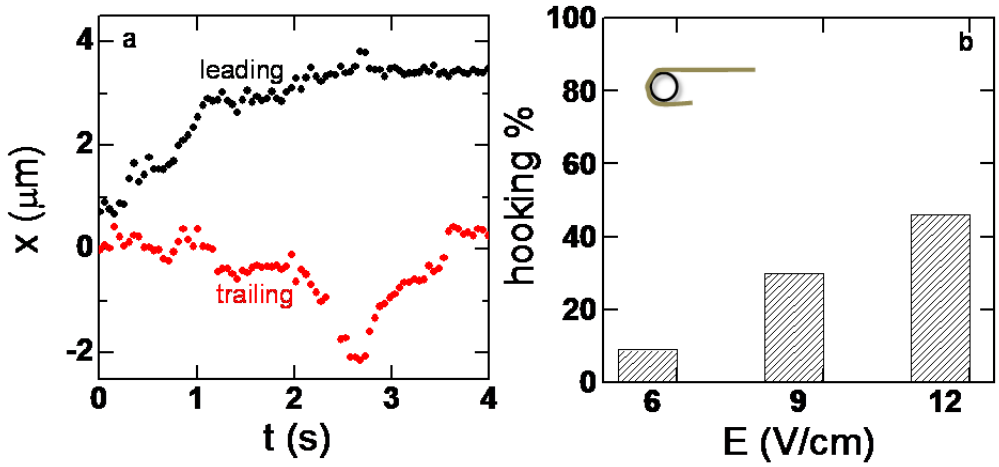

Figure S1: Hooking identified by submolecular imaging. (a) End positions are plotted against time during a hooking event. Comparing the leading (black) and trailing (red) ends of the molecule, one observes a dip for the trailing end denoting backwards motion. (b) The incidence of hooking is plotted against electric field strength.

In "hooking" (or "geometration") models, intermittency of transport and chain length fluctuation are considered to reflect chain hooking onto a gel fiber, becoming stretched by this event, and subsequently sliding off it. But we quantified the incidence of hooking by spotting characteristic backwards motion of the trailing end before recoil, akin to motion past a pulley as sketched schematically in (b), and it emerges that hooking is seen in 10% at 6 V/cm, 30% at 9 V/cm, and <50% at 12 V/cm of the total number of recoil events. Most recoil events, even when hooking is observed, seem to involve the pausing mechanism discussed in Figure 2d in the main text. Parenthetically, increased fraction of "hooking" events with increasing electric field is consistent with decreasing pausing duration (Figure 3c of the main text), both of which suggest easier end

protrusion at higher and higher field strength, as increased "hooking" fraction implies enhanced chance for both ends to protrude simultaneously from different sides of a gel hindrance.

Supplementary movies

Movie S1. A two-color epifluorescence movie showing a DNA chainmoving through the agarose gel network. Its two ends, marked by red crosses for easier visualization,are tracked simultaneously with the main chain, labeled with a different dye color as described in the main text.The migration pattern is plain: one end of a chain tends to stretch out and pulls slack from the still-quiescent remainder of the chain until the other end is yanked forward. Electric force (E = 9V/cm) points to the right. Field of view is $28\times5\mu m^2$. Movie plays in real time.

Movie S2. A representative DNA chainthat transiently hooks around and then slides off the gel fiber. Its two ends, marked by red crosses for easier visualization, are tracked simultaneously with the main chain, labeled with a different dye color as described in the main text. Electric force (E = 9V/cm) points to the right. Field of view is $25\times9\mu m^2$. Movie plays in real time.