

## **Supporting Information**

### **Velocity of DNA during translocation through a solid state nanopore**

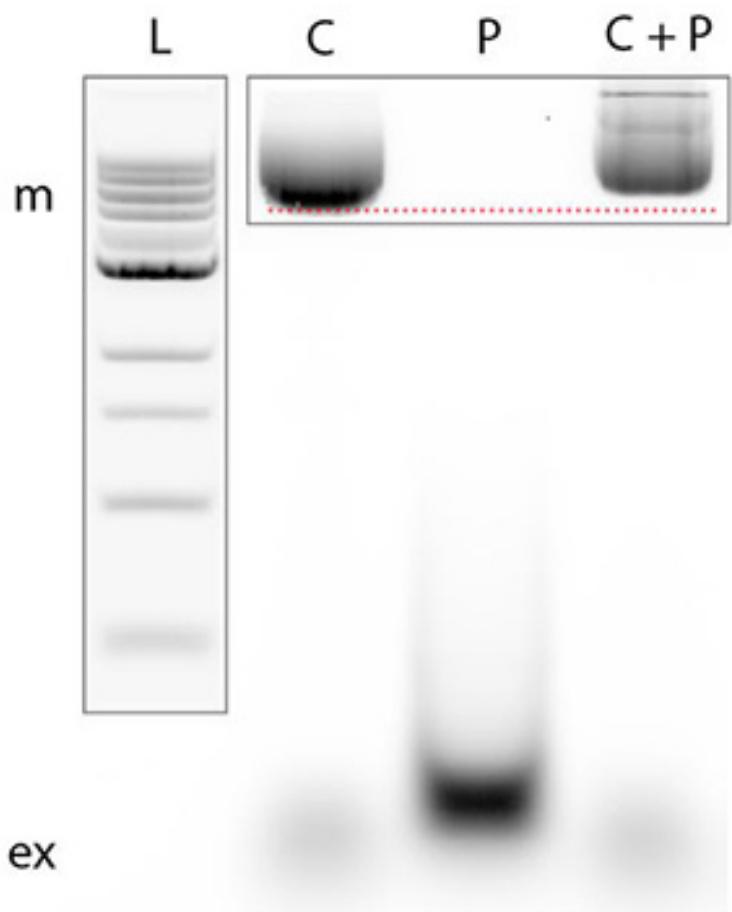
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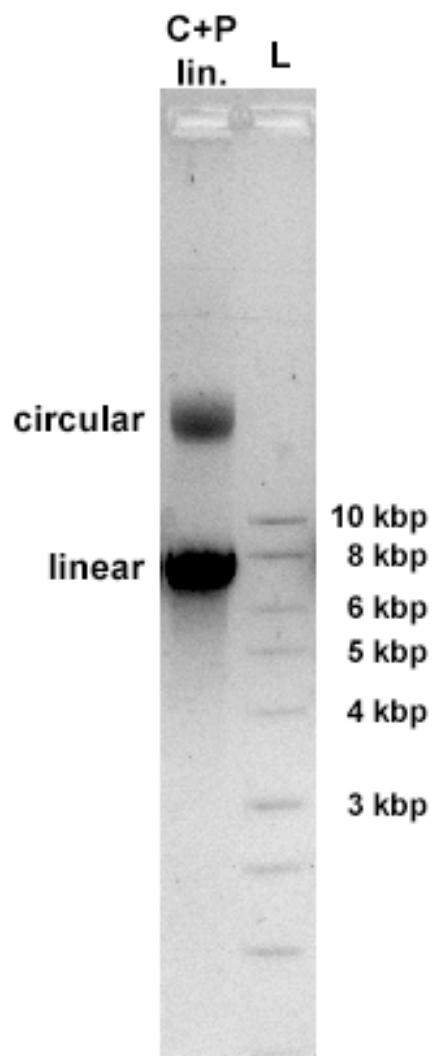
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## **1. Construct Characterization**

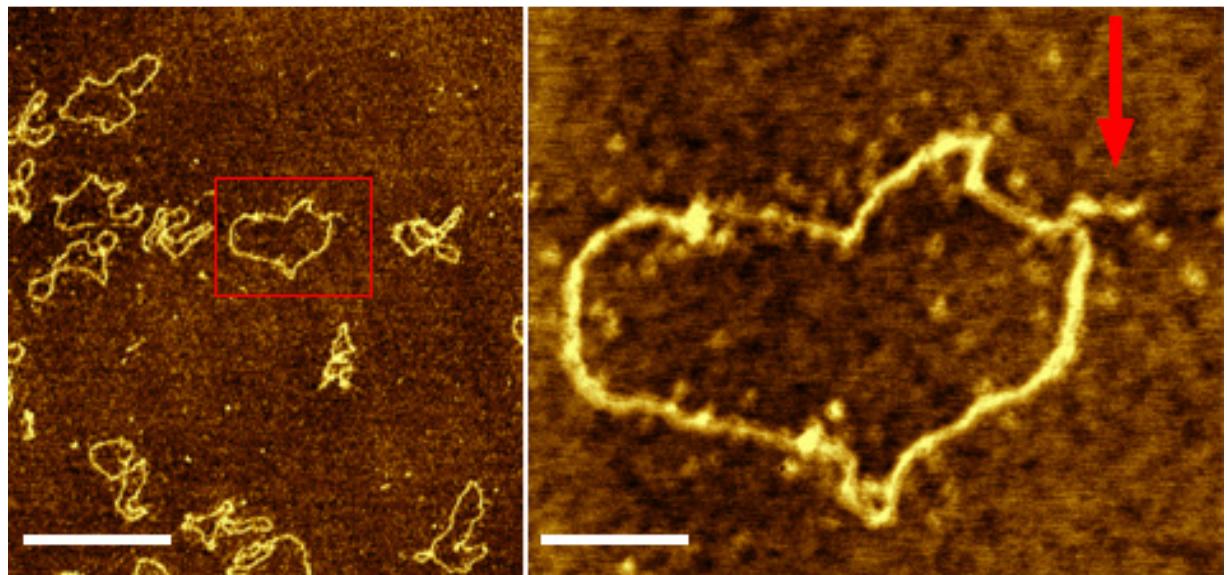
Our synthetic DNA constructs were characterized using gel electrophoresis, atomic force microscopy (AFM), and nanopore measurements. Figure S1 shows the assembly of a DNA construct and the resulting shift in mobility after the attachment of the protrusion. Figure S2 shows that the migration speed of a linearized DNA origami construct is similar to a dsDNA molecule of equal length. This indicates no significant difference in the persistence length due to the presence of the nicks that are present every 42 bp in the origami constructs, as any significant reduction in the persistence length would cause the construct to migrate faster in a gel.



**Figure S1** – Assembly of circular DNA construct with asymmetric protrusion. Labels: **L**, New England Biolabs 1kB DNA ladder; **C**, folded circular DNA object without protrusion; **P**, pre-annealed protrusion; **P+C**, mixture of P and C in 1:1 after incubation at RT for 1 day; **m**, folded objects; **ex**, non-integrated excess staple strands. Marked regions of interest were auto-leveled. The dashed red line assists to note the mobility shift after addition of the protrusion.

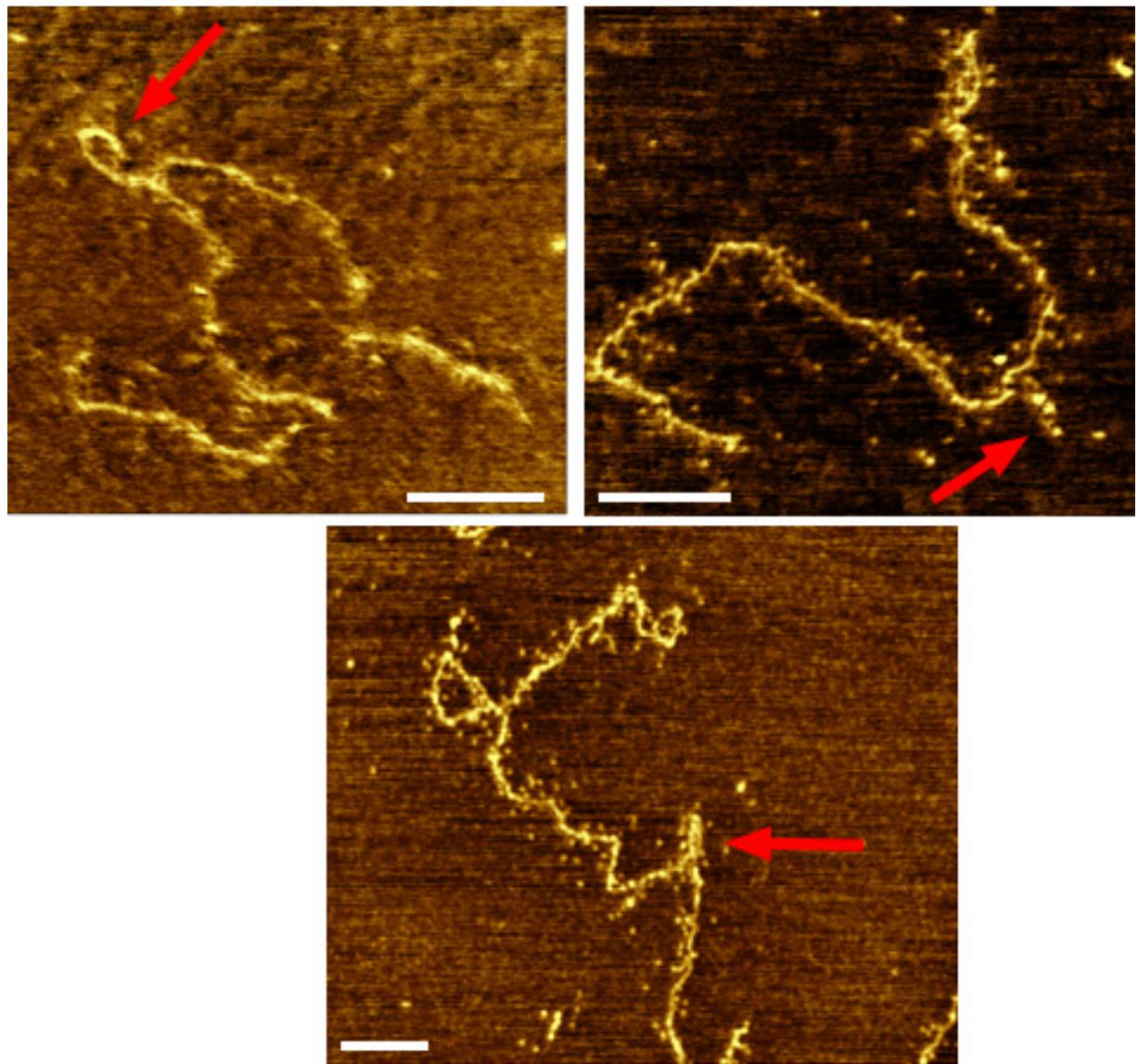


**Figure S2** – The 7560 bp asymmetric DNA construct after linearization (lane **C+P lin.**) alongside a Promega BenchTop 1 kbp DNA ladder (lane **L**). The linear DNA origami construct migrates accurately at the expected size for equivalent-length dsDNA molecules, indicating no significant reduction in the persistence length compared to dsDNA.

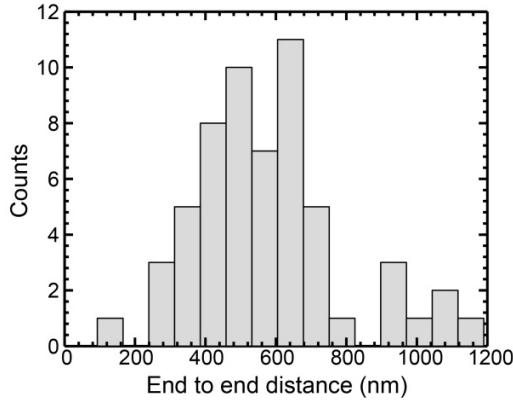


**Figure S3** – AFM scans of some DNA origami constructs before linearization. Arrow indicates the protrusion. Scale bar denote 1  $\mu\text{m}$  (left) and 200 nm (right).

AFM was used to image the DNA origami constructs as shown in Figure S3 and S4. Protrusions are marked with a red arrow. Samples with 10 mM  $\text{Mg}^{2+}$  were incubated on freshly cleaved mica for several min, after which they were rinsed with water and dried with nitrogen gas. Imaging was carried out with a Nanoscope IV in tapping mode with Olympus AC160TS tips.



**Figure S4** – AFM scans of some DNA origami constructs after linearization. Arrows indicate the protrusion. Scale bars denote 200 nm.



**Figure S5** – The end-to-end distance measured for 58 DNA construct molecules after linearization. We find a root-mean-square end-to-end distance of 612 nm.

In order to investigate the persistence length of the DNA origami constructs, we measured the end-to-end distances of 58 molecules, as shown in Figure S5. It has previously been shown that this approach can be used to estimate the persistence length of dsDNA on a 2D surface, given that DNA molecules have been found to re-equilibrate on the surface in AFM measurements in the presence of Mg<sup>2+</sup>.<sup>1</sup> We find a root-mean-square end-to-end distance (RMSD) of 612 nm. We compare this with several values given by the worm-like-chain (WLC) and freely-jointed-chain models, as shown in Table S1. We can determine the 2D end-to-end distance of a WLC polymer on a 2D surface using

$$\langle R^2 \rangle_{2D} \approx 4L_p L \left(1 - \frac{2L_p}{L}\right) \quad \text{for } L \gg L_p \quad , \quad (\text{S1})$$

where  $L$  is the contour length and  $L_p$  is the persistence length of the molecule<sup>1</sup>. Assuming WLC behavior and using Eq. S1, we thus find a persistence length of 38 nm for our DNA constructs. This value is not significantly lower than the value expected for dsDNA (50 nm), while it is very much larger than values that can be expected for a freely-jointed chain model, both if one would

insert a persistence length of ~1nm as expected for ssDNA, or 14 nm for a freely hinging chain of 42bp segments. Indeed, our DNA constructs do not behave like freely-jointed chains that would result in much smaller values for the RMSD (Table S1). These experimental data clearly indicate that the synthetic DNA constructs used should recapture the same translocation behavior as regular dsDNA molecules.

**Table S1** – A comparison of the measured root-mean-square end-to-end distance (RMSD) with the values predicted by several models for  $L = 2570$  nm.

AFM measured RMSD for DNA constructs on a mica surface	612 nm
Worm-like chain 2D RMSD using Eq. S1 with $L_p = 50$ nm	703 nm
Worm-like chain 3D RMSD with $L_p = 50$ nm	866 nm
Freely-jointed chain 3D RMSD with segment length of 50 nm	507 nm
Freely-jointed chain 3D RMSD with segment length of 14 nm (42 bp)	192 nm

## 2. Yield

The gel shown in Figure S1, shows that the initial assembly reaction went to completion. We quantified the final yield using nanopore measurements, as shown in Table S2. The value of the yield is given as the percentage of unfolded translocation events with a protrusion out of all unfolded translocation events. We observe yields ranging from 29% to 85%. Several factors affect the final yield. **i)** The protrusion is first assembled through the hybridization of six oligomers. Any excess oligomers from this mixture may interfere with the binding of the protrusion if they hybridize to the M13 scaffold strand before a fully assembled protrusion does. **ii)** Since the protrusion is mixed with the scaffold strand at a 1:1 ratio, the yield may be affected by uncertainties in the stoichiometry of the oligomers and the presence of some protrusions which are not fully assembled. **iii)** We observe that purifications, which are required to remove

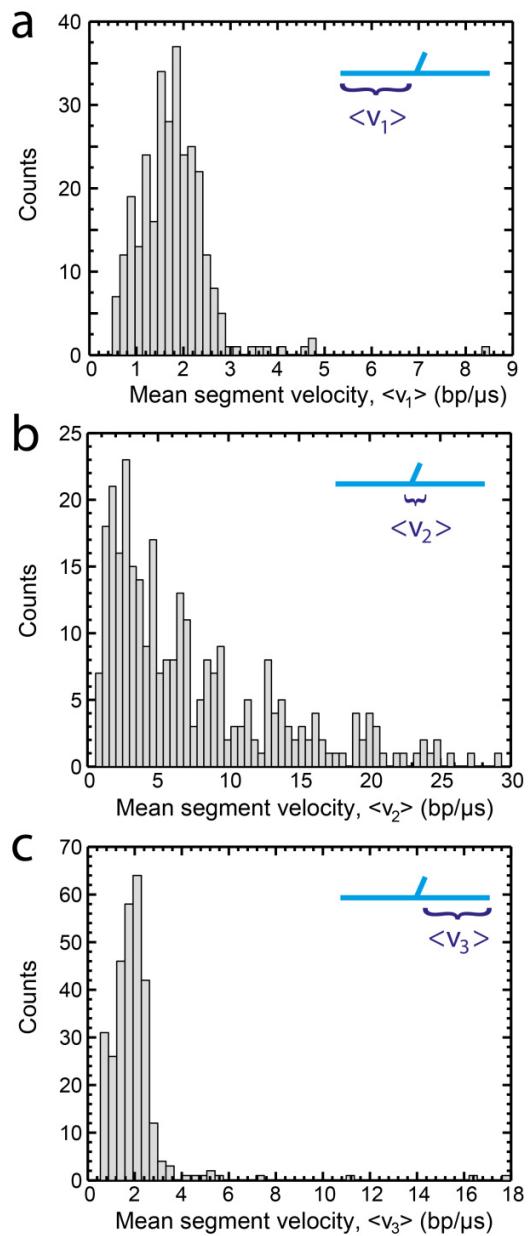
excess oligomers and restriction enzymes, reduce the final yield (Table S2). These factors all contribute to the final observed yield.

**Table S2** – The final yield of the linear DNA construct with a side protrusion, as determined using nanopore measurements.

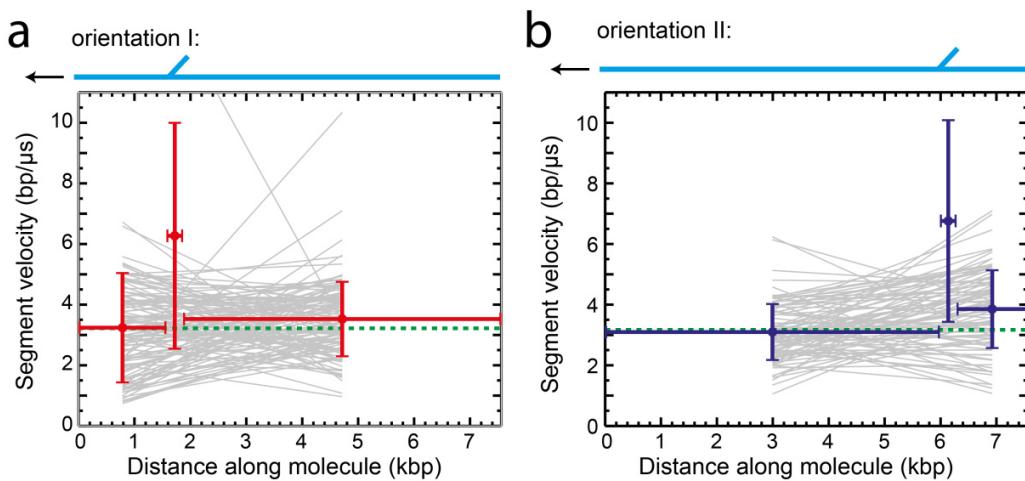
Assembly #	Construct	Yield (%)	Number of purifications after assembly
1	asymmetric	38%	2
		29%	
2	asymmetric	53%	1
3	symmetric	84%	1
		85%	

### 3. Velocity Data

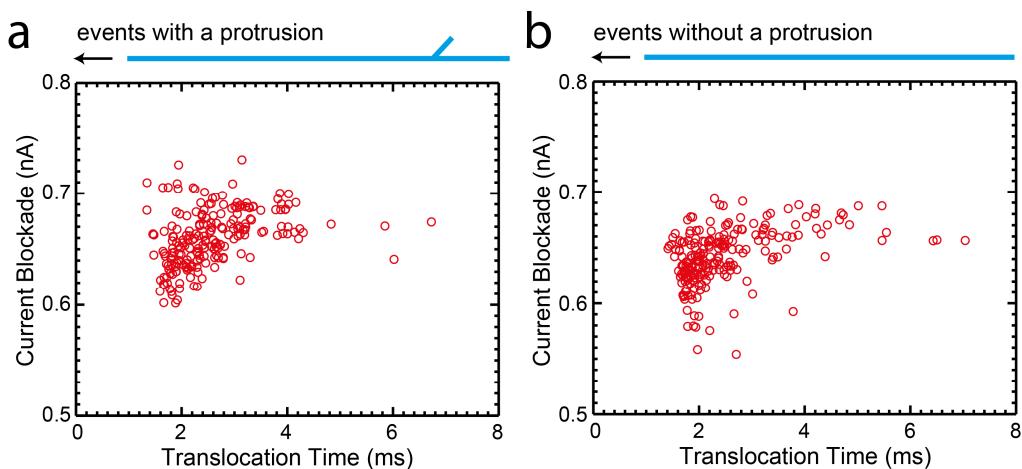
Figure S6 shows histograms of the translocation velocities measured for each of the three segments of the symmetric construct. The most probable translocation velocity is similar for all three with a value of around 2 bp/ $\mu$ s. A small shift towards higher velocities is seen in the last segment relative to the first segment which can be attributed to the speed up at the end of the translocation process. Figure S7 shows the mean local translocation velocity over various segments of the asymmetric DNA construct, including the protrusion. Figure S8 shows scatter plots, with the current blockade versus total translocation time, for the asymmetric construct for both events with and without the protrusion.



**Figure S6** – The segment velocities observed for each of the three segments of the symmetric construct.

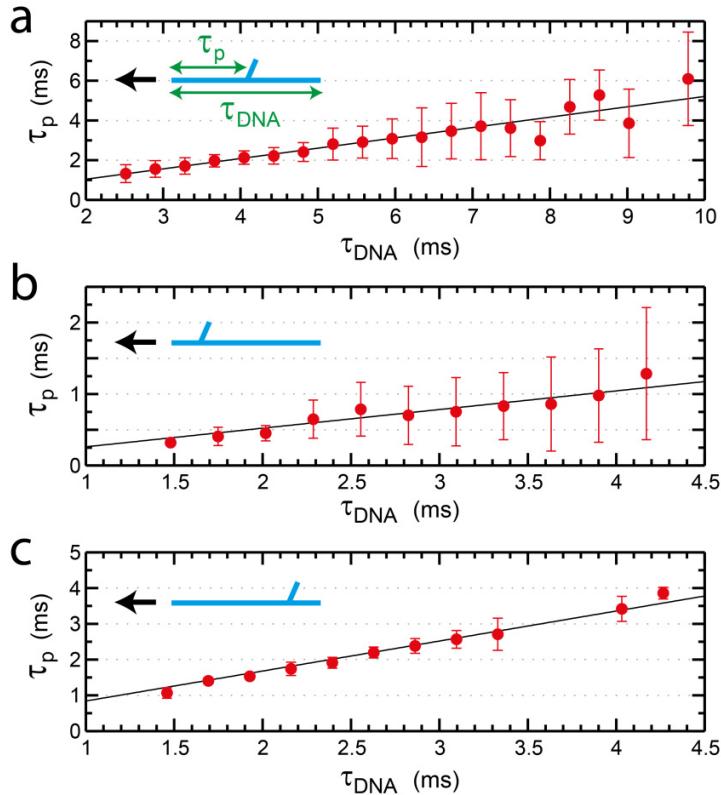


**Figure S7** – The mean translocation velocity over various segments of the asymmetric DNA construct, for each orientation. Each horizontal line indicates the length of the segment that was used to elucidate its average speed, while the vertical line indicates the standard deviation for that segment. The dashed green line is the mean translocation velocity of all data in the dataset. Changes in the local velocity in the first and last segment are overlaid as grey lines where each line is the data for each molecule.



**Figure S8** – Scatter plots showing the current blockade and total translocation time for the asymmetric DNA construct, showing both events with and without a protrusion.

#### 4. Scaling of $\tau_p$ of with $\tau_{DNA}$



**Figure S9** – The linear scaling observed between the time required to reach the protrusion  $\tau_p$  and the total translocation time of the event  $\tau_{DNA}$  for the **a)** symmetric construct **b)** asymmetric construct in orientation I, and **c)** asymmetric construct in orientation II. Solid lines are linear fits with an intercept of zero and slopes of **a)** 0.521, **b)** 0.261, and **c)** 0.839.

## 5. Protrusion Oligomer Sequences

**Table S3** – The following oligomers were used to assemble the protrusions.

Construct	Oligos Used
Symmetric construct (protrusion in center)	2251_PK 2252_PK 1917_PK - 1920_PK
Asymmetric construct (protrusion at side)	1631_PK 1916_PK - 1920_PK

**Table S4** – Oligomer sequences, bold sequences indicate regions bound to the M13 scaffold.

Oligo ID	Sequence
1631_PK (140 bases)	<b>AAACATGCCATTAAAAATACCGAACGAACCACCA</b> GCAGAAGAAG ATCCAGCTTATGCTGAACACTGGTCTGATGATGTTAGAAGCGCTAGC GCAATAGGGACTTAACCT <b>TAATTGGATGTCGATAGGATTCCGTGACTC</b>
1916_PK (110 bases)	AGGTTAAGTCCCTATTGCGCTAGCGCTTCTAACATCATCAGACCAGTG TTCAGCATAAGCTGGATCTTATAAAACAGAGGTGAGGCGGTAGTA <b>TTAACACCGCCTGCAA</b>
1917_PK (100 bases)	<b>ACTGTAGATCCACTTGC</b> GACCTTCGCTCGAGTCCCAGGAGGTGGATGTT AAA <b>ACTCAGCAGGGACTTATGGAGAGTCACGGAATCCTATCGACATCC</b> AATTAA
1918_PK (100 bases)	<b>TCCATAAGTCCCTGCTGAGTTAACATCCACCTCCGGACTCGAGCGA</b> AGGTGCAAGTGGATCTACAGT <b>CGAGCTGAATTAAATCAAGATCGCGA</b> <b>CCGTC</b>
1919_PK (130 bases)	CCTTATAACATGAGGTTGGAGTCTGGACCATGGGCTAACGTGCTAC GGGCACGTGCGTTAGCCACTCCACTATTAGATCCACCAAGTT <b>GCATGACGGTCGCGATCTGATTAATTCAAGCTCG</b>
1020_PK (100 bases)	<b>ATGCAACTGGTGGATCTAACATAGT</b> GAGGATGGAGTCGGCTAACGCACG TGCCCGTAGCACGTTGAGCCCATGGTCCAGACTCCAAACCTCATGTA TAAGG
2251_PK (119 bases)	<b>ATTTGTACAA</b> TCAATAGAAAATTAAAGATCCAGCTTATGCTGAAC ACTGGTCTGATGATGTTAGAAGCGCTAGCGCAATAGGGACTAACCT <b>TAATTGGATGTCGATAGGATTCCGTG</b>
2252_PK (94 bases)	AGGTTAAGTCCCTATTGCGCTAGCGCTTCTAACATCATCAGACCAGTG TTCAGCATAAGCTGGATCTCATATGGTTACCAGCGCAAAGACAA

## 6. M13 Backbone Oligomer Sequences

The following table lists all of the oligomers used to hybridize the 7560 base M13 ssDNA scaffold. The HincII cut site used to linearize the molecule, leaving a blunt end, is highlighted in yellow. The control construct which did not contain any protrusion used all of the oligos in the table. In the case of the symmetric and asymmetric constructs, certain oligos were left out (as noted) so the protrusion could be attached. Green marked oligos are not used for the asymmetric construct with protrusion at side. Red marked oligos are not used for the symmetric construct with protrusion in center. An additional oligomer (2253\_PK) was added for symmetric construct with center protrusion:

2253\_PK – AAGGGCGACATTCAACCGATTGAGGGAGGGAA

**Table S5** – M13 oligomer sequences.

AAAAATTTAGAACCTCATATTTAAATGCAATGCCTG
AAACAAACATCAAGAAAACAAAATTAAATTACATTAAACAATT
<b>AAACATGCCATTAAAAATACCGAACGAACCACCCAGCAGAAG</b>
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AAACGCTCATGGAAATACCTACATTTGACGCTCAATCGTCT
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AAATCATAGGTCTGAGAGACTACCTTTAACCTCCGGCTTA
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AACCTACCATATCAAAATTATTCACGTAAAACAGAAATAA
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## **References**

- (1) Rivetti, C.; Guthold, M.; Bustamante, C. J. Mol. Biol. 1996, 264, (5), 919-932.