Electronic Supplementary Material (ESI) for Nanoscale.

Supporting Information for

Nanomechanics of self-assembled DNA building blocks

*Michael Penth[1,2], Kordula Schellnhuber[1,2], Roland Bennewitz[1,2], Johanna Blass[1]**

[1] INM – Leibniz-Institute for New Materials, Campus D2 2, D-66132 Saarbrücken

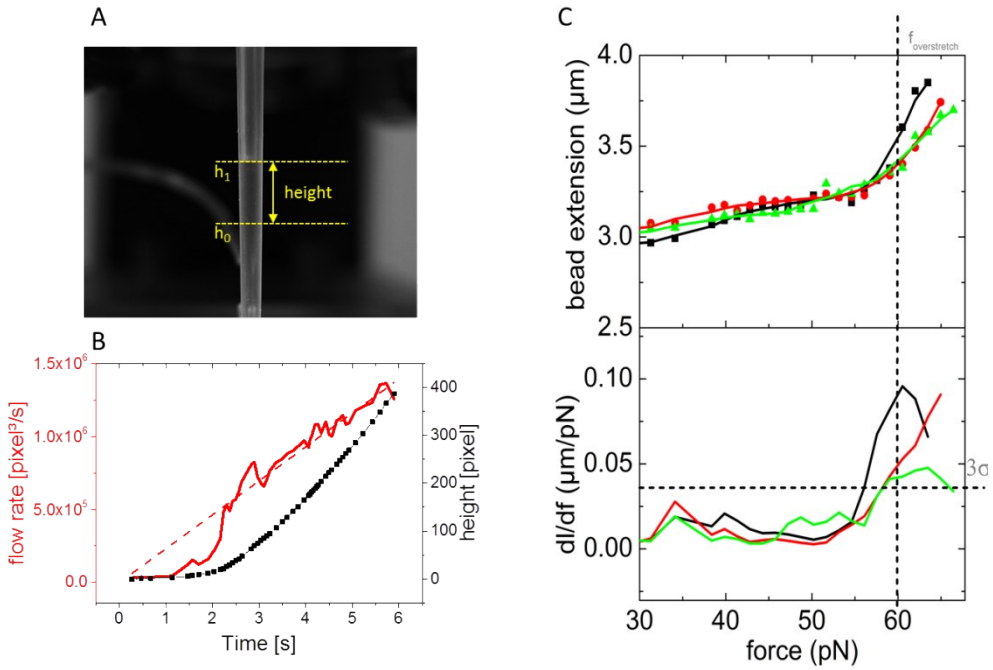
[2] Physics Department, Campus D2 2, Saarland University, D-66123 Saarbrücken

*johanna.blass@leibniz-inm.de

ESI note 1: Force calibration

The force acting on the micro bead is controlled by the fluid flow rate in the microfluidic channel according to Stoke's law $F=6\pi\eta Rv$ where η is the viscosity of the fluid, R the radius of the bead and v the fluid flow rate in the channel. In force-spectroscopy experiments, the flow rate is ramped up linearly to increase the force acting on the bead. Particularly for high loading rates of 7 pN/s, we observed that the actual flow rate in the channel deviates from the expected linear ramping profile (ESI Figure1B). To directly determine the actual flow rate in the channel, we observed the height (h_1) of the fluid column in the reservoir pipet with respect to the initial height before starting the experiment (h_0) (ESI Figure1A). The first derivative of the height reveals the flow rate that was converted to force in a second step.

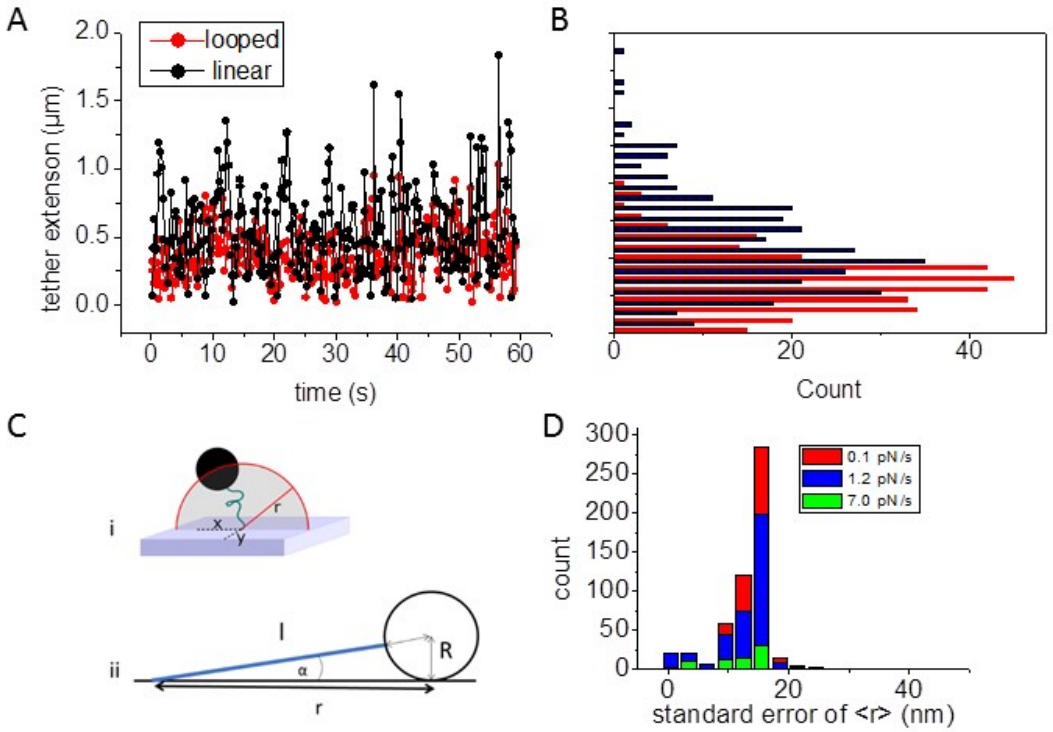
To convert the fluid flow rate in the channel to the force acting on the bead in the microfluidic channel, we used the beginning of the overstretching transition. We identified the onset of overstretching as a sudden elongation of the DNA, defined as a deviation from the position expected from a local fit to the curve by more than three times the standard deviation in the linear region as illustrated in Supplementary Figure 1C. The force at this onset was set to 60 pN.[1]



ESI Figure 1: **Force calibration of FlowFM.** (A) Image of fluid height in reservoir pipet used for in-situ force calibration for the flow rate for the high loading ramp of 7 pN/s. (B) Fluid flow rate as a function of time calculated from the measured fluid height in (A) and the geometries of the reservoir pipet. The dotted line reflects linear increase of flow rate. The fluid flow rate was converted to force using the overstretching plateau as shown in (C). (C) Bead deflection as a function of force for three different beads recorded with a loading rate of 7 pN/s. The solid lines are 5-point moving averages. (B) Derivative of moving average of bead deflection in (A) versus force. The beginning of overstretching transition was defined as the point where the derivative exceeds three times the standard deviation of the linear regime between 30 pN and 50 pN.

ESI note 2: Tethered particle motion

Before starting a force-spectroscopy measurement, we recorded the motion of the tethered beads for 1 minute and analyzed the root mean square of the movement for each single-tethered bead (Supplementary Figure 2). The tether extension l was calculated from the bead displacement in the in-plane coordinates x and y with a trigonometric function $= \frac{R}{sin(\arctan\left(\frac{R}{r}\right))} - R$, where R=0.5 µm is the diameter of the bead and r is the projected tether length in 2D with respect to the center of gyration $r = \sqrt{(x-\bar{x})^2 + (y-\bar{y})^2}$ (see Supplementary Fig. 2C). Typical tether extensions and the respective histogram are shown in Supplementary Figure 2A and B for a linear and looped tether. The mean of the tether extension in (A) is reflected as RMS in Figure 4. To estimate the error of the RMS value, the histogram of the standard error of the mean is shown in Supplementary Figure 2D. From the histogram we can estimate an error the RMS value of below 20 nm.



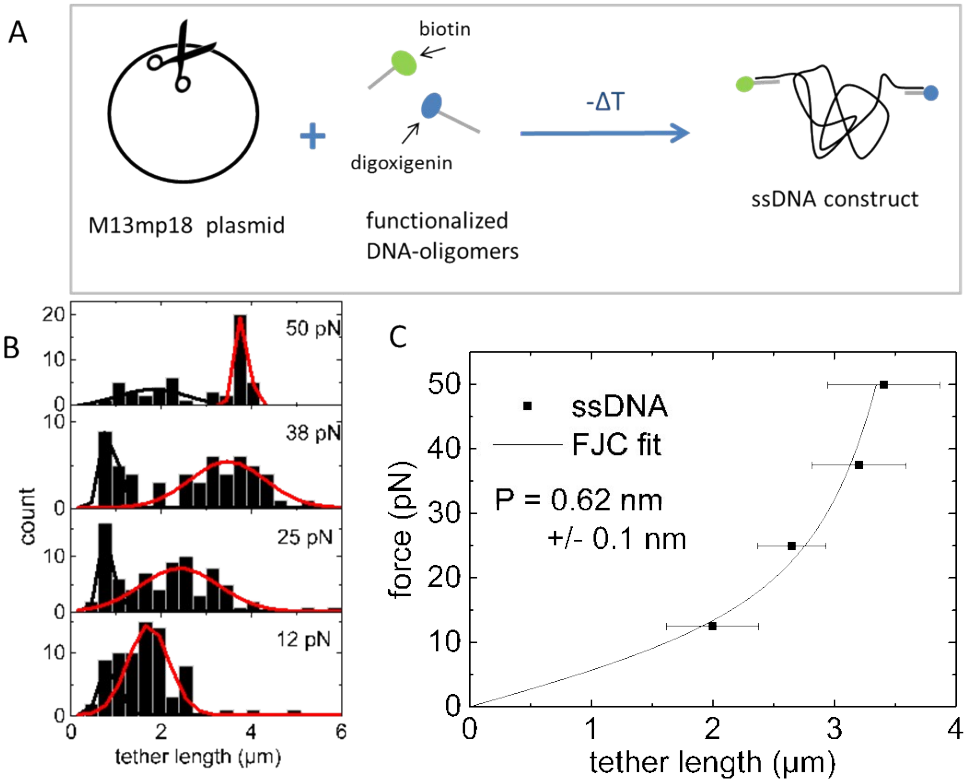
ESI Figure 2: **Experimental details of RMS of linear and looped DNA tethers.** (A) Typical signal of the root mean square displacement of the beads attached to the channel wall via linear and looped tether. (B) Histogram of the displacement in (A). (C) (i) Illustration of a tethered particle in microfluidic channel without flow; its deflection r is tracked by the projected coordinates x and y. (ii) Tethered bead stretched under hydrodynamic flow in the microfluidic channel. Changes in the tether extension l will appear as the displacement of the bead (r). (D) Histogram of the standard error of the mean for RMS value revealed from the 60 s without flow prior to force-spectroscopy experiments with three different loading rates.

ESI note 3: Stretching ssDNA in FlowFM

The persistence length of the single-stranded DNA that we used as a starting point for our self-assembled DNA construct was determined in FlowFM experiments. Therefore, the DNA plasmid was linearized and the two oligomers required for surface and bead attachment were hybridized onto the DNA scaffold (see ESI Figure 3A). All other oligomers were omitted from the hybridization protocol to leave the construct single stranded.

FlowFM experiments were carried out in constant force mode for four different forces. A constant force was applied for 20 s in order to break stacked domains that can lead to randomized coiling of the ssDNA chain.[2] The distribution of the tether length was included in histograms for statistical analysis in ESI Figure 3B. Two peaks are observed where one is shifting to higher extensions with force. The mean value of the gaussian fit of the second peak was plotted into the force-extension diagram in ESI Figure 3C and fitted with a freely jointed chain (FJC) model. The FJC fit reveals a persistence length of 0.62 nm ± 0.1 nm in good agreement with literature values for long ssDNA molecules.[3–5]

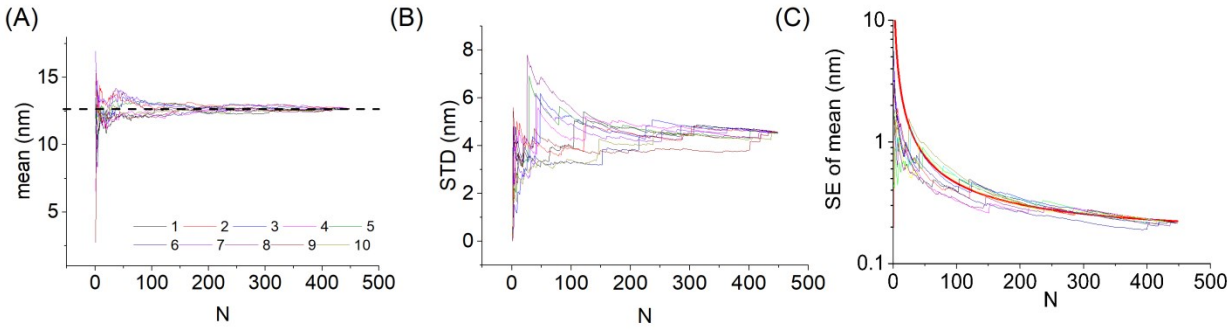
The second peak observed for forces below 35 pN may originate from preserved coiling of the ssDNA chain or is due to unspecific tethered beads and was not included in further analysis.



ESI Figure 3: **Stretching ssDNA in FlowFM**. (A) Sketch of assembly of single stranded DNA construct from M13mp18 plasmid and two functionalized oligomers. (B) Histograms of tether length at different pulling forces, the distribution follows a Gaussian distribution with two peaks where the mean value of the second maximum (red) for the four different forces was included in the force-extension plot in (C). (C) Force versus tether length obtained from histograms in (b); Freely jointed chain model reveals a persistence length of P=0.62 nm for ssDNA construct, the error bars are the deviation of two independent measurements.

ESI note 4: Statistics on sample size of FlowFM measurements

We have studied 1615 single molecules in nine independent experiments to obtain the most probable persistence length in Figure 3. In order to evaluate how many investigated molecules are needed for a statistically sound data set, we studied the mean value, standard deviation (STD) and the standard error (SE) of the mean as a function of the number of investigated molecules *N*. We included the persistence length obtained from 449 individual molecules recorded in three independent experiments with different flow force rates. To calculate the statistical quantities, the order of persistence length values was randomized in ten different trials resulting in ten curves for each statistical quantity. In ESI Figure 4, we plot the mean value, the standard deviation and the standard error of the mean as a function of the number of molecules. The mean and the standard deviation show significant variation for a small number of N and approach stable values several hundred of different molecules. Moreover, above N=100 the SE of mean is small with <0.5nm and decays further with $1/\sqrt{N}$ indicating that the mean obtained from 100 molecules is already close the mean of the true population.



ESI Figure 4: (A) Mean value, (B) STD and (C) SE of mean as a function of the number of different molecules (total N=449) for ten trails with randomized order. The red line in (C) reflects the correlation SE of mean ~ $1/\sqrt{N}$ fitted to N>100.

ESI note 5: Python code for applying the model of Rivetti et al. to our construct

```
# written by Roland Bennewitz and Johanna Blass
# cite by []
# inspired by the Publication of Rivetti et al.


import numpy as np

length_ds=0.34
length_ss=0.57
P_ds=50.0
P_ss=50

# number of loose bases at each end of segements due to thermal unbinding
loose_bases=2


# read in list of segments
pfad =
```

```
dateiname = datei = pfad + dateiname
list=np.genfromtxt(datei, dtype=float, delimiter=' ',skip_header=1)
N=len(list)
print("number of DNA sections: ", N)

# number of bases that are single or double stranded
bases_ss = list[:,0]*list[:,1]
bases_ds = list[:,0]*list[:,2]

N_ss = bases_ss.sum(axis=0)
N_ds = bases_ds.sum(axis=0)

l_ds=(bases_ds-2*loose_bases*list[:,2])*length_ds
l_ss=bases_ss*length_ss+2*loose_bases*list[:,2]*length_ss

L_c=(N_ds-2*loose_bases)*length_ds+(N_ss+2*loose_bases)*length_ss
print('contour length: ', L_c)


#repeated expression calculated once
bracket_ds=(1-np.exp(-1*l_ds/P_ds))
bracket_ss=(1-np.exp(-1*l_ss/P_ss))

r2_sum=0.0
r2_sum_ss=0.0
r2_sum_ds=0.0

# first term
r2_ss = l_ss*P_ss*(1-P_ss/(l_ss)*(1-np.exp(-1.0*l_ss/P_ss)))
r2_ds = np.nan_to_num(l_ds*P_ds*(1-P_ds/l_ds*(1-np.exp(-1.0*l_ds/P_ds))))
r2_sum_ss = r2_ss.sum(axis=0)
r2_sum_ds = r2_ds.sum(axis=0)
r2_sum_1 = r2_sum_ss+r2_sum_ds


# second term
r2_sum_2 = (P_ss*P_ds*(1-np.exp(-1.0*l_ds[:-1]/P_ds))*(1-np.exp(-1.0*l_ss[:-1]/P_ss))).sum(axis=0)

#third term

# list with persistence length
P_list = []
P_list_ss = P_ss*list[:,1]+P_ss*list[:,2]
P_list_ds = P_ds*list[:,2]
x = 1
while x <= len(P_list_ds)-1:
    P_list.append(P_list_ds[x])
    P_list.append(P_list_ss[x])
    x += 1

# list with segment length
l_list = []
x = 1
while x <= len(l_ds)-1:
    l_list.append(l_ds[x])
    l_list.append(l_ss[x])
    x += 1

#remove zeros from lists and create arrays
l_zero = np.array(l_list)
l = l_zero[l_zero != 0]
P_zero = np.array(P_list)
```

```
P = P_zero[P_zero != 0]

P_arr_ss = np.array(P_list_ss)
P_arr_ds = np.array(P_list_ds)
l_arr_ss = np.array(l_ss)
l_arr_ds = np.array(l_ds)

r2_sum_3=0
n=1
while n<=N-1:
    m=n+2
    while m<=N+1:
        factor=1.0
        j=n+1
        while j<=m-1:
            factor=factor*np.exp(-1.0*l[j]/P[j])
            j+=1
        r2_sum_3+=P[n]*P[m]*factor*(1-np.exp(-1.0*l[n]/P[n]))*(1-np.exp(-1.0*l[m]/P[m]))
        m+=1

print('effective persistence length by summation after third term:',(r2_sum_1+r2_sum_2+r2_sum_3)/L_c)
```

ESI note 5: List of single (ss) and double stranded (ss) sections of our DNA construct

#bases	ss	ds
60	0	1
60	0	1
60	0	1
5	1	0
60	0	1
60	0	1
60	0	1
60	0	1
60	0	1
60	0	1
60	0	1
60	0	1
37	0	1
30	0	1
60	0	1
60	0	1
52	0	1
40	0	1
60	0	1
60	0	1
60	0	1
60	0	1
60	0	1

#bases	ss	ds
60	0	1
60	0	1
60	0	1
60	0	1
60	0	1
60	0	1
60	0	1
4	1	0
20	0	1
60	0	1
60	0	1
60	0	1
60	0	1
60	0	1
60	0	1
60	0	1
60	0	1
60	0	1
54	0	1
4	1	0
50	0	1
60	0	1
60	0	1

#bases	ss	ds
60	0	1
60	0	1
60	0	1
60	0	1
60	0	1
60	0	1
60	0	1
57	0	1
23	1	0
60	0	1
60	0	1
60	0	1
60	0	1
60	0	1
60	0	1
60	0	1
60	0	1
60	0	1
60	0	1
10	1	0
60	0	1
60	0	1
60	0	1
53	0	1
20	0	1

#bases	ss	ds
60	0	1
60	0	1
35	0	1
4	1	0
60	0	1
60	0	1
60	0	1
60	0	1
60	0	1
60	0	1
60	0	1
60	0	1
60	0	1
60	0	1
54	0	1
60	0	1
29	1	0
60	0	1
60	0	1
60	0	1
60	0	1
60	0	1
60	0	1
60	0	1
60	0	1
41	0	1

#bases	ss	ds
60	0	1
60	0	1
60	0	1
60	0	1
60	0	1
60	0	1
60	0	1
60	0	1
60	0	1
60	0	1
60	0	1
60	0	1
60	0	1
60	0	1
60	0	1
60	0	1
22	0	1
50	0	1

#bases	ss	ds
29	1	0
60	0	1
60	0	1
60	0	1
60	0	1
60	0	1
60	0	1
60	0	1
60	0	1
60	0	1
60	0	1
60	0	1
60	0	1
60	0	1
60	0	1
60	0	1
55	0	1

References

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