

Understanding the paradoxical mechanical response of in-phase A-tracts at different force regimes

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Supplementary Information

Supplementary Methods

Theory Model

Worm-Like Chain (WLC) Model

It is helpful to start with the simpler case of the WLC model. Here we will follow the notation and the derivation from refs (1,2). According to the WLC model, the bending energy of the polymer is given by

$$E = \frac{Pk_B T \theta^2}{2L} \quad (1)$$

where P is the persistence length; θ is the angle between the vectors tangent to the curve at two points separated by a distance L ; T is the temperature; and k_B the Boltzmann constant. From this expression, the probability of finding a given bending angle is simply

$$\mathcal{P}(\theta) = \sqrt{\frac{P}{2\pi L}} e^{-\frac{P\theta^2}{2L}} \quad (2)$$

From here one can easily obtain the mean cosine of the bending angle, which is given by the following integral

$$\langle \cos \theta \rangle_{WLC} = \int_{-\infty}^{\infty} d\theta \cos \theta \mathcal{P}(\theta) = e^{-L/2P} \quad (3)$$

and then the mean squared end-to-end distance

$$\langle R^2 \rangle = \int_0^L ds \int_0^L ds' \langle \cos(\theta(s, s')) \rangle = 2 \int_0^L ds \int_0^s ds' e^{-\frac{(s-s')}{2P}} = 4PL \left[1 - \frac{2P}{L} (1 - e^{-L/2P}) \right] \quad (4)$$

Intrinsically-bent WLC

For an intrinsically bent molecule, the minimum energy does not correspond to zero bending angle. Thus, we will assume that the intrinsic bending describes a circle of radius R_0 and curvature $a \equiv 1/R_0$. In this case, the energy reads

$$E = \frac{P_0 k_B T (\theta - aL)^2}{2L} \quad (5)$$

Then, the angle probability is

$$\mathcal{P}(\theta) = \sqrt{\frac{P_0}{2\pi L}} e^{-\frac{P_0(\theta - aL)^2}{2L}} \quad (6)$$

And the mean cosine can be obtained as

$$\langle \cos \theta \rangle = \sqrt{\frac{P_0}{2\pi L}} \int_{-\infty}^{\infty} d\theta \cos \theta e^{-\frac{P_0(\theta-aL)^2}{2L}} = \sqrt{\frac{P_0}{2\pi L}} \int_{-\infty}^{\infty} d\theta \cos(\theta + aL) e^{-\frac{P_0\theta^2}{2L}} = \cos(aL) e^{-\frac{L}{2P_0}} \quad (7)$$

This expression is shown in the main text and was used to fit the A-tracts AFM data. The formula for the mean squared end-to-end distance can be derived in the same way as done for the WLC. In this case, one would need to solve the following integral

$$\langle R^2 \rangle = 2 \int_0^L ds \int_0^s ds' \cos(a(s-s')) e^{-\frac{(s-s')}{2P_0}} \quad (8)$$

This integral is more cumbersome than for the WLC case, but can also be solved analytically yielding the expression shown in the main text

$$\langle R_{s,s+L}^2 \rangle = \frac{2}{a^2 + b^2} \left\{ bL + \frac{1}{a^2 + b^2} [(a^2 - b^2)(1 - \cos(aL)e^{-bL}) - 2ab\sin(aL)e^{-bL}] \right\} \quad (9)$$

with $b \equiv 1/(2P_0)$. Notice that by making $a=0$, one recovers the expression of the WLC, as required.

However, there is a more elegant solution to the problem. Realize that

$$\langle \cos \theta \rangle = \mathcal{R}e \left\{ e^{-\frac{L}{2P^*}} \right\} \quad (10)$$

Where $\mathcal{R}e$ denotes the real part and P^* is a complex number given by

$$P^* = \frac{P_0}{1 - 2iP_0a} \quad (11)$$

The mean squared end-to-end distance is then obtained in a straightforward way, following the same rationale employed for the integrals of the WLC

$$\langle R^2 \rangle = \int_0^L ds \int_0^L ds' \mathcal{R}e \left\{ e^{-\frac{|s-s'|}{2P^*}} \right\} = \mathcal{R}e \left\{ \int_0^L ds \int_0^L ds' e^{-\frac{|s-s'|}{2P^*}} \right\} = \mathcal{R}e \left\{ 4P^*L \left[1 - \frac{2P^*}{L} (1 - e^{-\frac{L}{2P^*}}) \right] \right\} \quad (12)$$

This expression reveals that the mean squared end-to-end distance in the IBWLC is simply the real part of the one from the WLC when a complex persistence length is used. We will express this as

$$\langle R_{IBWLC}^2 \rangle = \mathcal{R}e \langle R_{WLC;P \rightarrow P^*}^2 \rangle \quad (13)$$

Expanding Eq. (12) one arrives at Eq. (9).

Comparing the low-force response of A-tracts with the Tompitak et al. model (3)

To the best of our knowledge, the only model that explicitly contemplates the effect of A-tract curvature on the force response of DNA is the one by Tompitak et al (3). The main assumption

of the model is that intrinsically curved DNA is forming a spring-like structure (from now on a superhelix) with radius R and a helical rise per base pair s_0 . External forces stretch the superhelix similar to a spring, reducing the radius of the superhelix and increasing the extension:

$$F = \frac{(k_B T)^2}{\tilde{A}} \left[\frac{1}{4(1 - \frac{x}{L} + F/k)^2} - \frac{1}{4} + \frac{x}{L} - \frac{F}{k} \right]$$

where k is the effective constant of the superhelical backbone given by

$$k = k_B T \tilde{\gamma} - (k_B T)^2 \frac{\tilde{g}}{\tilde{C}}$$

In these expressions, \tilde{A} , \tilde{C} and $\tilde{\gamma}$ are, respectively, the effective bending, twisting and stretching moduli of the superhelix; and \tilde{g} is the effective twist-stretch coupling. These parameters are given by

$$\begin{aligned} \tilde{A} &= \frac{2rAC}{A + C - (A - C)r^2} \\ \tilde{C} &= r(A - (A - C)r^2) \\ \tilde{\gamma} &= \frac{r(C + (A - C)r)}{k_B T R^2} \\ \tilde{g} &= \frac{(A - C)(b^2 - s_0^2)r^2}{bRk_B T} \end{aligned}$$

where A and C are the persistence length and twisting modulus of the DNA; s_0 is the superhelical rise of the superhelix; and $r = s_0/b$, being b the distance between base pairs.

We tested this model against our data taking reference values considering the parameters in (3): $b=0.34$ nm, $R = 8$ nm, $s_0 = 0.15$ nm, $A = 50$ nm and $C=100$ nm. As shown in **Figure S6**, the model failed to describe the force-extension response over the full range of forces (**Figure S6A**). Only for forces lower than 0.1 pN we observed a reasonable description (**Figure S6B**). This limitation of the model is in agreement with the findings reported in the literature (3). Therefore, we conclude that, so far, there is no model that captures the force response of our A-tracts sequences over the range of forces studied.

Supplementary Tables

Supplementary Table S1. DNA primers used in this work.

| Fragment | Oligonucleotide | Sequence |
|----------------------------------|--------------------------|---|
| Intron | 58.F Bam-Xho-Psi intron4 | GCGTAAGTGGATCCCTCGAGTTATAACAGGTAGCGGAGAAATTG AAG |
| | 59.RApa-Eco-Sal intron4 | ACTTACGGGGCCCGATATCGTCGACGGTCCCTGTAAGAAATTT TAAAGG |
| Control tweezers substrate | 89.F lambda 4002 XhoI | GCGTAAGTCTCGAGCCGGATGCGGAGTCTTATCC |
| | 90.R lambda 45263 ApaI | GCGTAAGTGGGCCCCGCAAGGATTGCCCGCATG |
| Digoxigenin tailed oligos | XbaI-A | [pho]CTAGACCCGGGCTCGAGGATCCCC |
| | 88.XbaI C ApaI | GGGGATCCTCGAGCCCGGGTCTAGGGCC |
| Biotin tailed oligos | 27P-XhoI-A | [pho]TCGAGCCCGGGCCATGGGATCCCC |
| | 26XhoI-B | GGGGATCCCATGGCCCGGGC |

Supplementary Table S2. Sequence of DNA fragments used in this work. Groups of two or more consecutive adenines were highlighted in green and groups of two or more thymines are shown in red. All sequences are written from the 5' end.

| Fragment | Size (bp) | Sequence |
|-----------------------------|-----------|--|
| A-tract AFM substrate | 2636 | TCGAGTTATAACAGGTAGCGGAGAAATGAAGAAAAATGGTCAAAAAATCGATGAAAAAC CCGAAAAACTCAGAAAAATTCATTAATAATCTTAAAAATGCTAGAAAAATTCGGAAAAATGC CCAAAAATTTAGAAAAATGTTTAAATAATCCTGAGAAAAATGTCAAAAATACCGAAAAATTTAT TAGTAAAAATCTTGAAAAATTTGCCAAAAATCTGGAAAAACCTGGAAATTTTCGACTTTTTT TTTCTAAAAGTTTCAAAAATCCCAAATAATCATTCAAAAAATAAACTTTCTAAAAATC TCTAAAAATTTTGGCGAAAAATTTGAAAAATCACTAAAAATGAAATATCCCGGAAA TTTCGAGAAAAATCCAAAAATTTTCGGAAAAATACACAAAAATACCGGTTTTTTCGTGAAAAAG CACAGAAATTTTGGACAAAAATTTAAAAATCTGTAAAAATCCCCAAAAATTTTCAAAAT TTTTTCTCGAAAAATTTGCAAAAAATGTAATAATTTCCCCAAATAATCCGAAAAATCCAAAA AAATTAACATAAAAAATCGGAAAAATTTGAAAAATCCCTGAAAAATGTCGTAAAAATACCA AAAAATTCGCTAAAAATCTGAAAAATTTGCTAGAAAAATTCGAAAAAATAAGTTTAAAAATTT AGAAAAATGTTAAAAATTTCCAAAAAATCATGTAAAAATCTTGAGAAAAATGTCAAAAATACCG AAATTTATAGAAAAATCTGAAAAATCACTGACATTTTTCGAAAAATTCGAAAAATTTGGT TAAAAATTTTTTAAAAAATCCTGGAATTTTCCAAATTTTTTTTCTAAAAATAAAAAA TTCCGAAAAATCCTTTAAAAATTTTACAGGGACCGTCGAGTTATAACAGGTAGCGGAGA AATTTGAGAAAAATGGTCAAAAAATCGATGAAAAATCCGAAAAATCAGAAAAATTCATT AAAATCTTAAAAATTTGCTAGAAAAATTCGAAAAATGCCAAAAATTTTAGAAAAATGTTTAAA TATTCCTGAGAAAAATGTCAAAAATACCGAAAAATTTATTTAGTAAAAATCTTGAAAAATTTGCC AAAAATCTGGAAAAATCTGGAATTTTCGACTTTTTTTTTTTCTAAAAATTTCAAAAATTTCCCA AATAATCATTCAAAAAATAAACTTTCTAAAAATCTCTAAAAATTTTTGCGGAAAAATTTG AAAAATCACTAAAAATTTGAAATATCCCCGGAAAAATTCGAGAAAAATCCAAAAATTTTCG GAAAAATACAAAAATACCGGTTTTTGTGAAAAAGCACAGAAATTTTTCGACAAAAATCCT TTAAAAATCTGTAAAAATCCCCAAAAATTTTCAAATTTTTTCTCGAAAAATTTGCAAAAA TGTAAAAATTTCCCCAAATAATCCGAAAAATCCAAAAAATTTACCTAAAAATCGGAAAAAT TTTGAATAATCCCTGAAAAATGTCGTAAAAATACCAAAAAATTTTCGCTAAAAATCTGAAAA ATTTGCTAGAAAAATTCGAAAAAATGCTTAAAAATTTTCAAAAAATGTTAAAAATTTCCAAAA AACATGTAATAATCTTGAGAAAAATGTCAAAAATACCGAAAAATTTATAGAAAAATCTGAAAA CTCACTGACATTTTTCGAAAAATTTCCGAAAAATTTGGTAAAAATTTTTTTAAAAAATCCTG GAATTTTCCAAATTTTTTTTTTTCTAAAAATTTAAAAATTTCCGAAAAATCCTTTAAAAATTT TTACAGGGACCGTCGAGTTATAACAGGTAGCGGAGAAAAATGAAGAAAAATGGTCAAAAA TCGATGAAAAATCCGAAAAATCAGAAAAATTCATTAAAAATTTAAAAATTTCACTAAAAAATGCTAGAAA TCGAAAAATGCCAAAAATTTAGAAAAATGTTTAAATAATCCTGAGAAAAATGTCAAAAAT ACCGAAAAATTTATAGTAAAAATCTTGAAAAATTTGCCAAAAATCTGGAAAAATCCTGGAATTT TTTCGACTTTTTTTTTTTCTAAAAATTTCAAAAATTTCCAAATAATCATTCAAAAAATAAACT TTCTAAAAAATCTCTAAAAATTTTTCGCGAAAAATTTTGAAAAAATTTCACTAAAAAATTTGAAA TATCCCCGAAAAATTCGAGAAAAATCCAAAAATTTTCGAAAAATACACAAAAATACCGGTTTT TTGCTGAAAAATGACAGAAATTTTTCGACAAAAATTTAAAAATCTGTAAAAATCCCCCAA AAATTTTCAAATTTTTTTCTCGAAAAATTTGCAAAAAATGTAATAATTTCCCCAAATAATCC GAAAAATCCAAAAAATTAACCTAAAAATTCGGAAAAATTTTGAATAATTTCACTAAAAATTTGTC GTAAAAATACCAAAAAATTTTCGCTAAAAATCTGAAAAATTTGCTAGAAAAATTCGAAAAAAT GCTTAAAAATTTTCGAAAAATGTTAAAAATTTCCAAAAAATCATGTAATAATCTTGAGAAAAATG |

| | | |
|----------------------------|------|---|
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| Control tweezers substrate | 5316 | <p> GGGGATCCCATGGCCCGGGcTCGAGCCGGATGCGGAGTCTTATCCGTGAAAAATCAACGC GCCTACTGGCTGGTTACCAACCTGTATCAGAACATGCGGGCCAAATGCGCTTACTGATGC GGAATTTACGCCGTAGGGCCGAGATGAGCTTGTCCATATGACTGCGAGAAATTAACCGTGG TGAGGCGATCCCTGAAACAGTAAAACAACTTCTGTCTATGAGGACCTGAAATCG TGCACAGGCTCTGGCAGGATCGCAGAAATCAAGCTAAGTTTCGACTGAAAGGAGCAAG TGTATGACGGGCAGAGGCAATTAATTCATTTACCTGGGGACCCATAATAGCTTCTGTGCG CCGGACGTTGCCGCGCTAACAGGCGCAACAGTAACAGCATAAATCAGGCCGCGGCTAAA ATGGCACGGGAGGTCTTCTGGTTATCGAAGGTAAGGCTGTGGCGAACGGGTGATTTACCGG TTTGTCTACAGGGAAGAACGGGAAGGAAAGATGAGCACGAACCTGGTTTTTAAGGAGTGT CGCCAGAGTGCCTGATGAAACGGGTATTTGGCGGTATATGGAGTTAAAGATGACCATCT ACATTTACTGAGCTAATAACAGGCCTGCTGGTAAATCGCAGGCCTTTTTATTTGGGGGAGAG GGAAATCATGAAAAAATCAACCTTTGAAATTTTCGATCTCCAGCATACGAAAAATCGTAT TCACGCAGTACAGCAATCCCTTCAGACCCAAACCAAACTCGTAGTAACCATTCAGGA </p> |

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Supplementary Table S3. *eWLC mechanical parameters of control and A-tracts at 100mM NaCl using different fitting ranges.* The 100 mM NaCl OT data was analyzed in the context of the eWLC using different fitting ranges.

| Fitting Range | Molecule | P_{eWLC} (nm) | S (pN) |
|---------------|----------|-----------------|----------------|
| 10-45 pN | A-tract | 44 ± 3 | 2400 ± 220 |
| | Control | 47 ± 4 | 1540 ± 90 |
| 10-40 pN | A-tract | 43 ± 3 | 2470 ± 230 |
| | Control | 41 ± 3 | 1710 ± 110 |
| 5-45 pN | A-tract | 36 ± 2 | 2960 ± 360 |
| | Control | 44 ± 2 | 1530 ± 90 |
| 5-40 pN | A-tract | 36 ± 2 | 3080 ± 370 |
| | Control | 43 ± 1 | 1600 ± 100 |

Supplementary References

1. Rivetti, C., Guthold, M. and Bustamante, C. (1996) Scanning force microscopy of DNA deposited onto mica: equilibration versus kinetic trapping studied by statistical polymer chain analysis. *J Mol Biol*, **264**, 919-932.
2. Rivetti, C., Walker, C. and Bustamante, C. (1998) Polymer chain statistics and conformational analysis of DNA molecules with bends or sections of different flexibility. *J Mol Biol*, **280**, 41-59.
3. Tompitak, M., Schiessel, H. and Barkema, G.T. (2016) Force responses of strongly intrinsically curved DNA helices deviate from worm-like chain predictions. *EPL (Europhysics Letters)*, **116**, 68005.

Figure S1

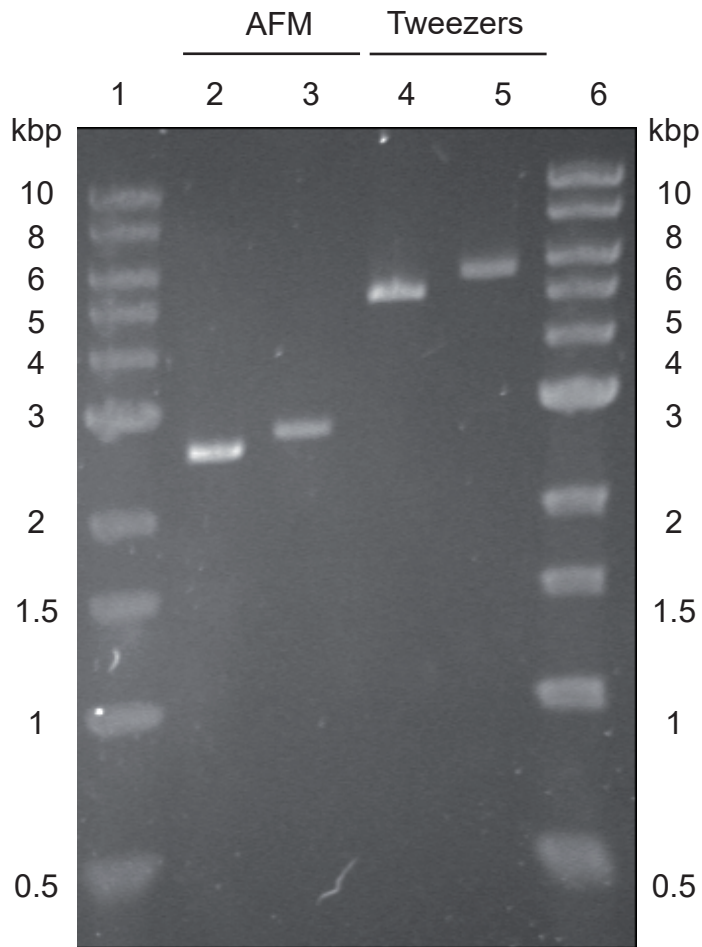


Figure S1. Migration of control and A-tracts substrates in agarose gels. 0.8 % agarose gel electrophoresis of AFM substrates (control DNA (2645 bp) lane 2 and A-tracts (2636 bp) lane 3), and central insert before ligation of the tailed oligos of magnetic and optical tweezers substrates (control, lane 4 and A-tracts, lane 5 (5272 bp)) were run 30 ng/lane). Lanes 1 and 6 correspond to 1 kbp DNA ladder. The electrophoresis was run at room temperature for 50 min at 90 V in the absence of intercalator and the gel was later stained with SYBR[®]Safe. The substrates containing the A-tracts migrated slower than one would expect on the basis of its length.

Figure S2

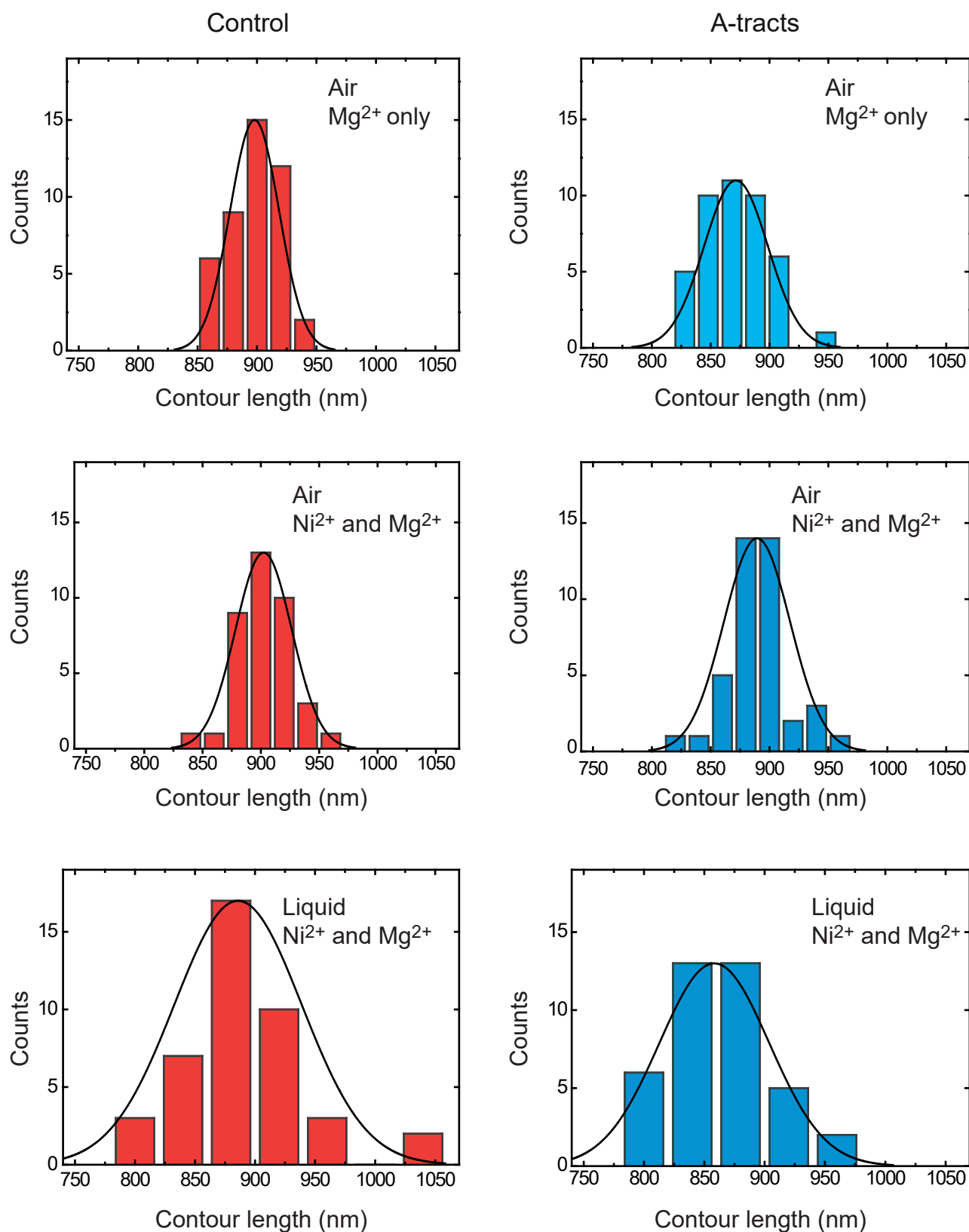


Figure S2. Distribution of contour lengths of control and A-tracts molecules. The data sets were fitted to Gaussian distributions. Mean values, number of molecules and errors are shown in **Table 1**.

Figure S3

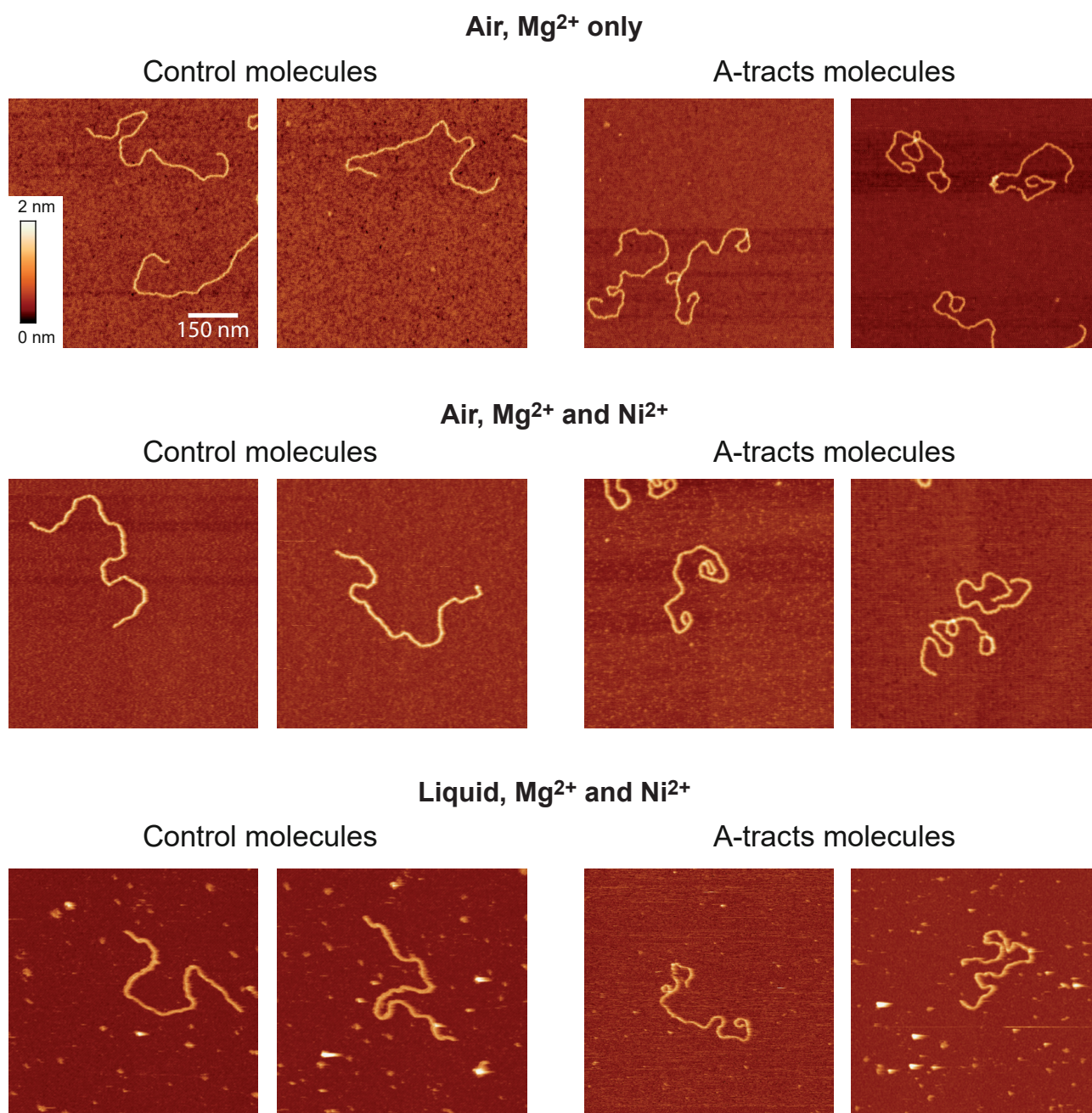


Figure S3. Representative images of the control and A-tracts molecules using air AFM imaging with two different adsorption conditions, and liquid AFM imaging. The Z and XY-scales are the same for all the images and are indicated in the image located at the upper left corner.

Figure S4

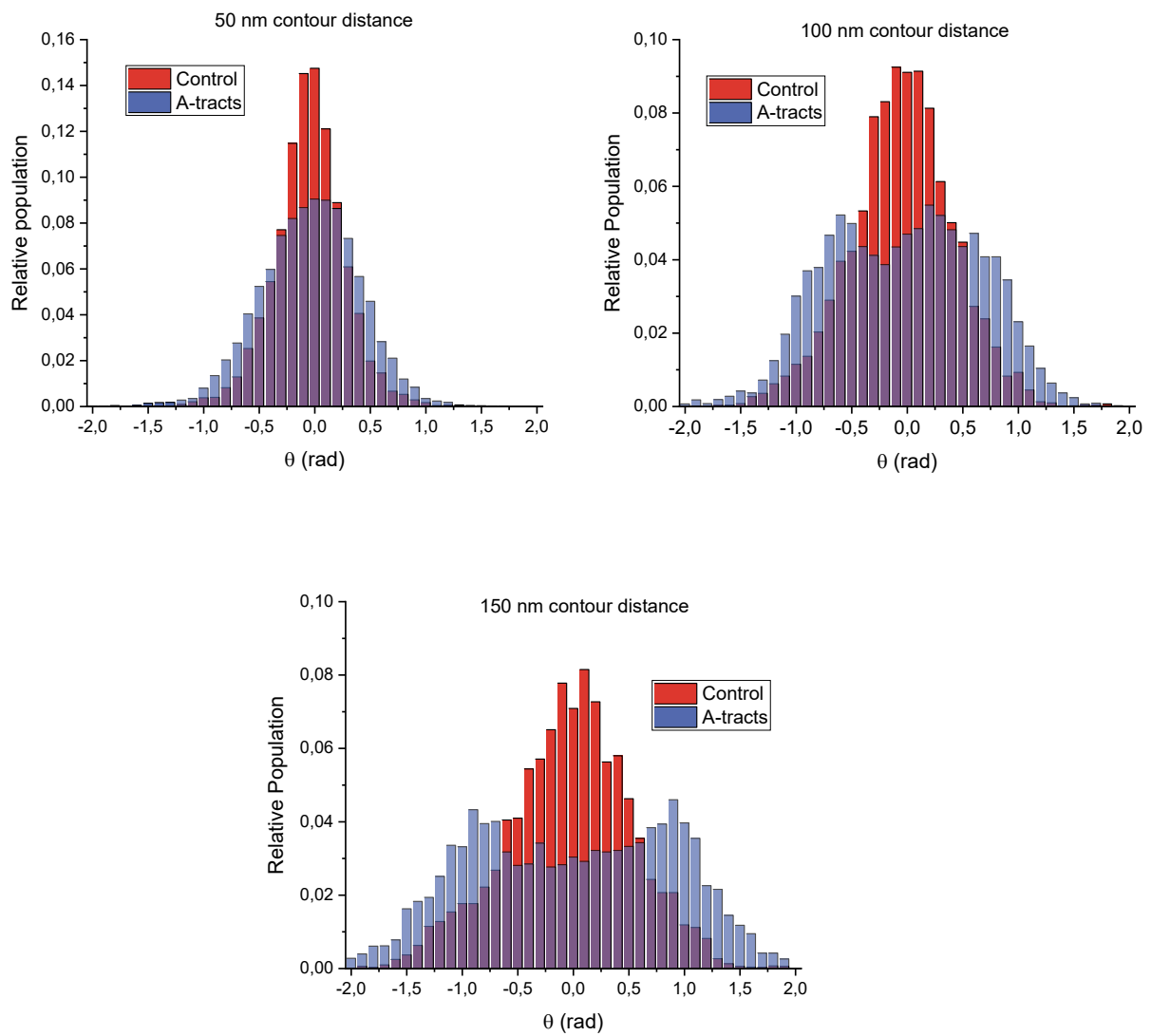


Figure S4. Distribution of bending angles at different contour lengths computed for control and A-tracts molecules.

Figure S5

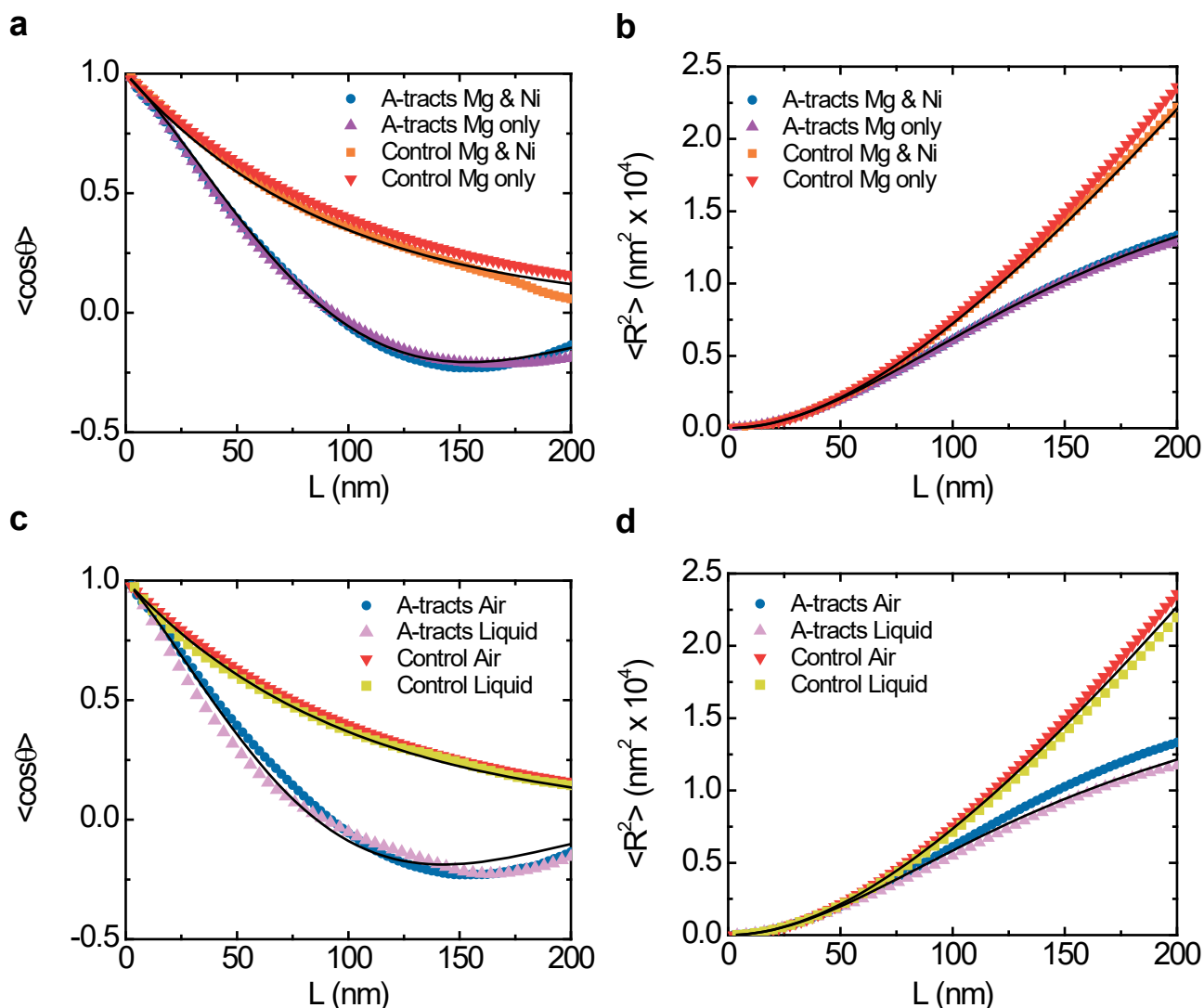


Figure S5. Comparison of the mechanical properties of control and A-tracts obtained using two different adsorption conditions in air AFM imaging (a, b) and using air and liquid AFM imaging (c, d). (a) $\langle \cos\theta \rangle(L)$ data for air AFM imaging using Mg²⁺ to adsorb the molecules (same data as in Figure 2d, main text) and using both Mg²⁺ and Ni²⁺. Only the fits of the Mg²⁺ & Ni²⁺ data are shown here (the fits to Mg²⁺ can be found in Figure 2d, main text). The black lines are the fits of the control and A-tract to the WLC and IBWLC models, respectively. The values of the fitting parameters are shown in Table 1, main text. (b) Comparison between air AFM imaging $\langle R^2 \rangle(L)$ data using Mg²⁺ and using Mg²⁺ & Ni²⁺. The black line is the theoretical expression for $\langle R^2 \rangle(L)$ (WLC for the control and IBWLC for A-tracts) using the parameters extracted from the fits in (a). (c, d) Same as (a, b) but comparing air (Mg²⁺ only) with liquid AFM imaging. For sample preparation, ions concentration and AFM imaging details see Materials and Methods section, main text.

Figure S6

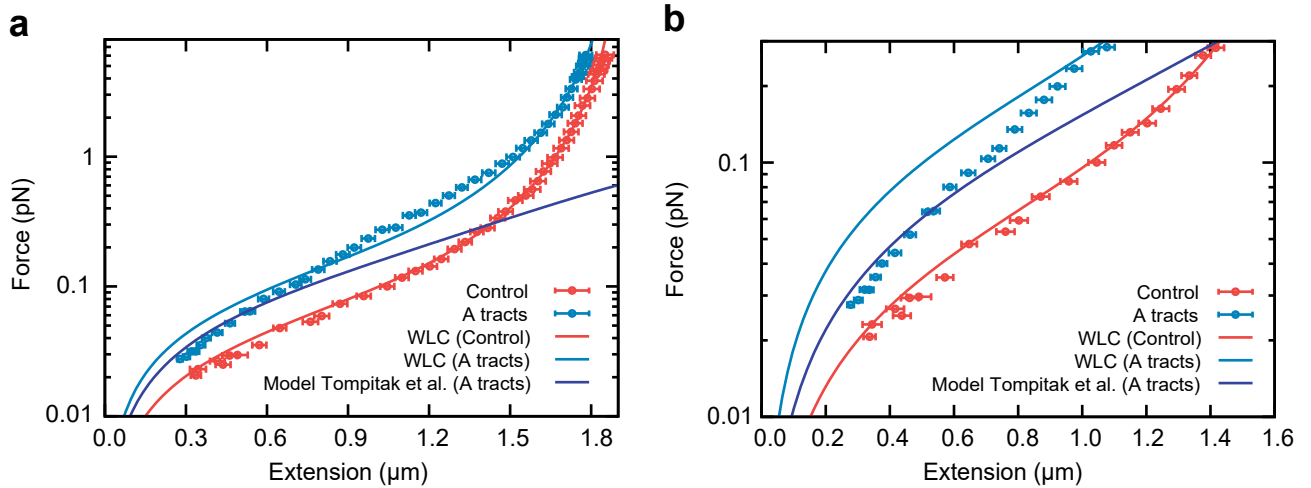


Figure S6. Comparison of the WLC model and the Tompitak model for describing our magnetic tweezers force-extension data. a) Experimental A-tracts and control data and fits to the WLC model (Eq. 7 main text) and to the Tompitak model (3). b) Detail of (a) at low forces. The improvement of the Tompitak model over the WLC was observed only at very low forces (< 0.1 pN). The curve for the Tompitak model considered the following values: superhelical radius $R = 8$ nm, superhelical rise $s_0 = 0.15$ nm, persistence length $A = 50$ nm and twisting modulus $C=100$ nm.

Figure S7

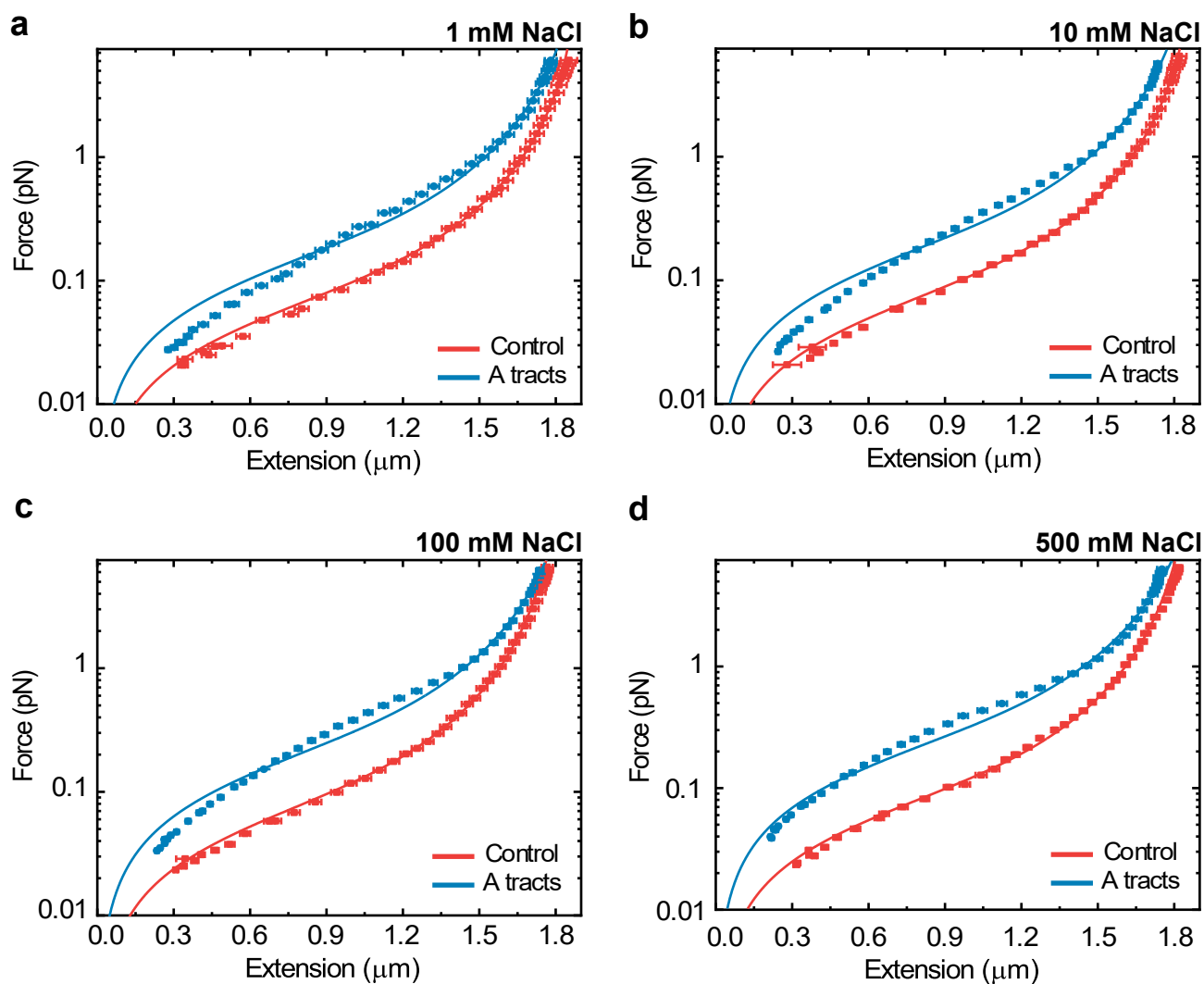


Figure S7. Average magnetic tweezers force-extension curves of A-tracts and control molecules at different NaCl concentrations. Average force-extension curves of A-tracts and control molecules obtained at different concentrations of NaCl. Error bars are standard error of the mean. The data were fitted to the WLC formula (Eq. 7, main text). The values of the fitting parameters are represented in **Fig. 3b, c** main text.

Figure S8

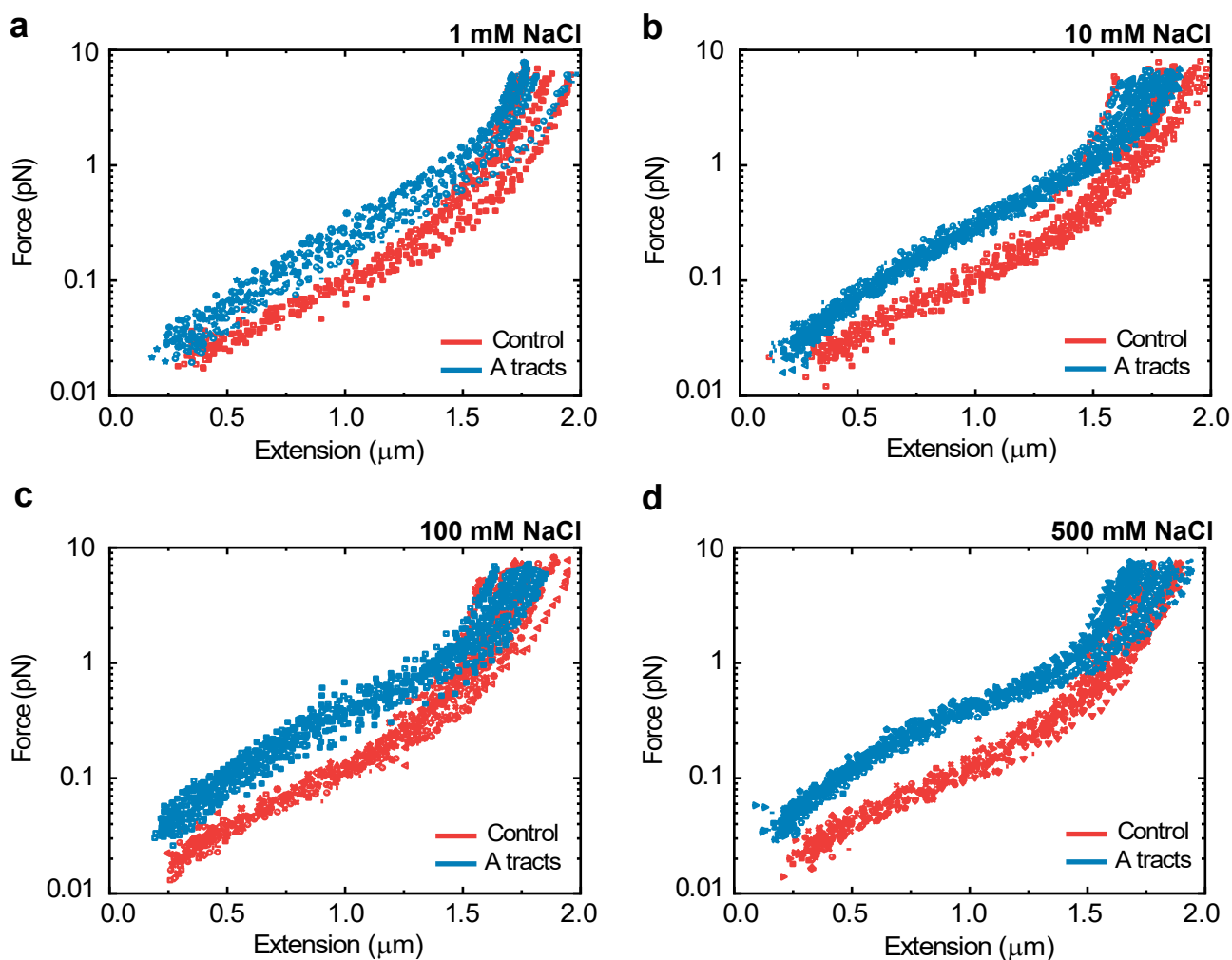


Figure S8. Raw magnetic tweezers force-extension curves of A-tracts and control molecules at different NaCl concentrations. Blue data correspond to A-tracts DNA molecules. Red data correspond to control DNA molecules. The number of molecules represented for each condition are indicated in **Table 1**.

Figure S9

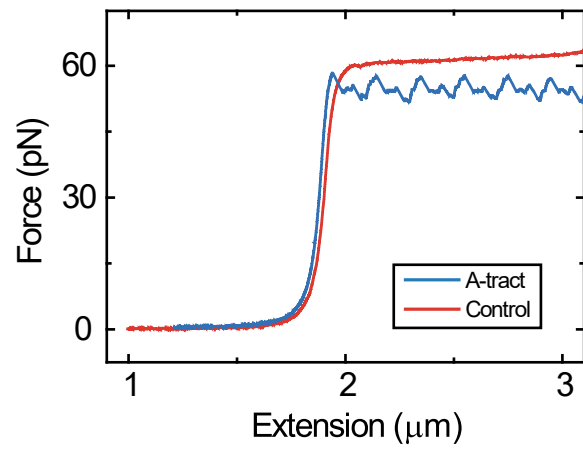


Figure S9. *Overstretching transition of control and A-tracts molecules.* Representative force-extension curves showing the overstretching transitions of control (red) and A-tracts (blue) at forces ~ 60 pN.

Figure S10

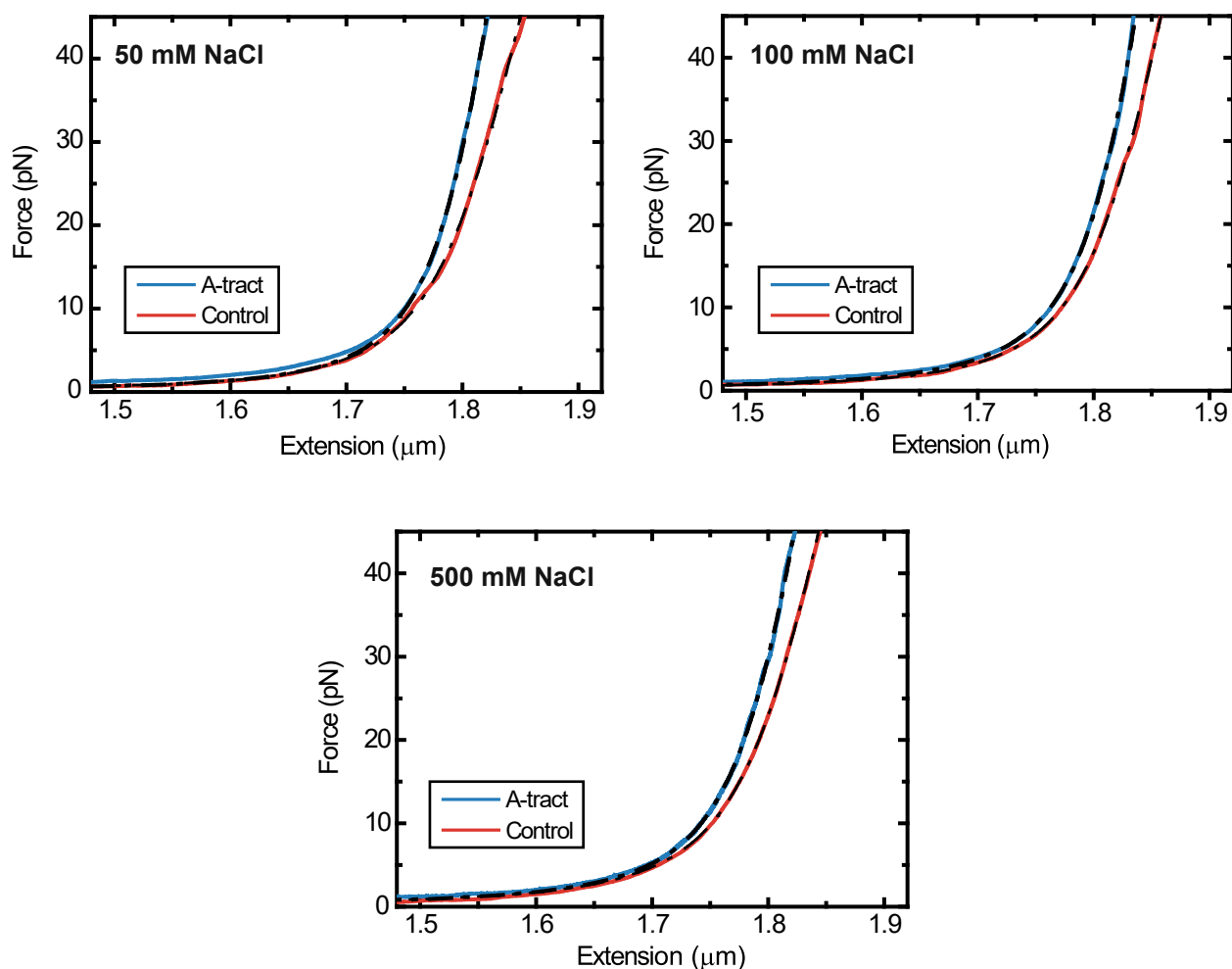


Figure S10. Average optical tweezers force-extension curves of A-tracts and control molecules at different NaCl concentrations. Raw data was averaged using a running window of 100 points. All data sets were then fitted to the extensible WLC (eWLC) in the 10-45 pN force range. The data and the fit at 100 mM NaCl is the same as in **Fig. 4a**, main text. The values of the fits shown at 50 mM NaCl were $L_0 = 1834$ nm, $P = 45$ nm, $S = 1440$ pN and $L_0 = 1828$ nm, $P = 48$ nm, $S = 2545$ pN for the control and A-tracts, respectively; and the ones at 500 mM NaCl were $L_0 = 1841$ nm, $P = 34$ nm, $S = 1675$ pN and $L_0 = 1833$ nm, $P = 35$ nm, $S = 2286$ pN for the control and for the A-tracts.

Figure S11

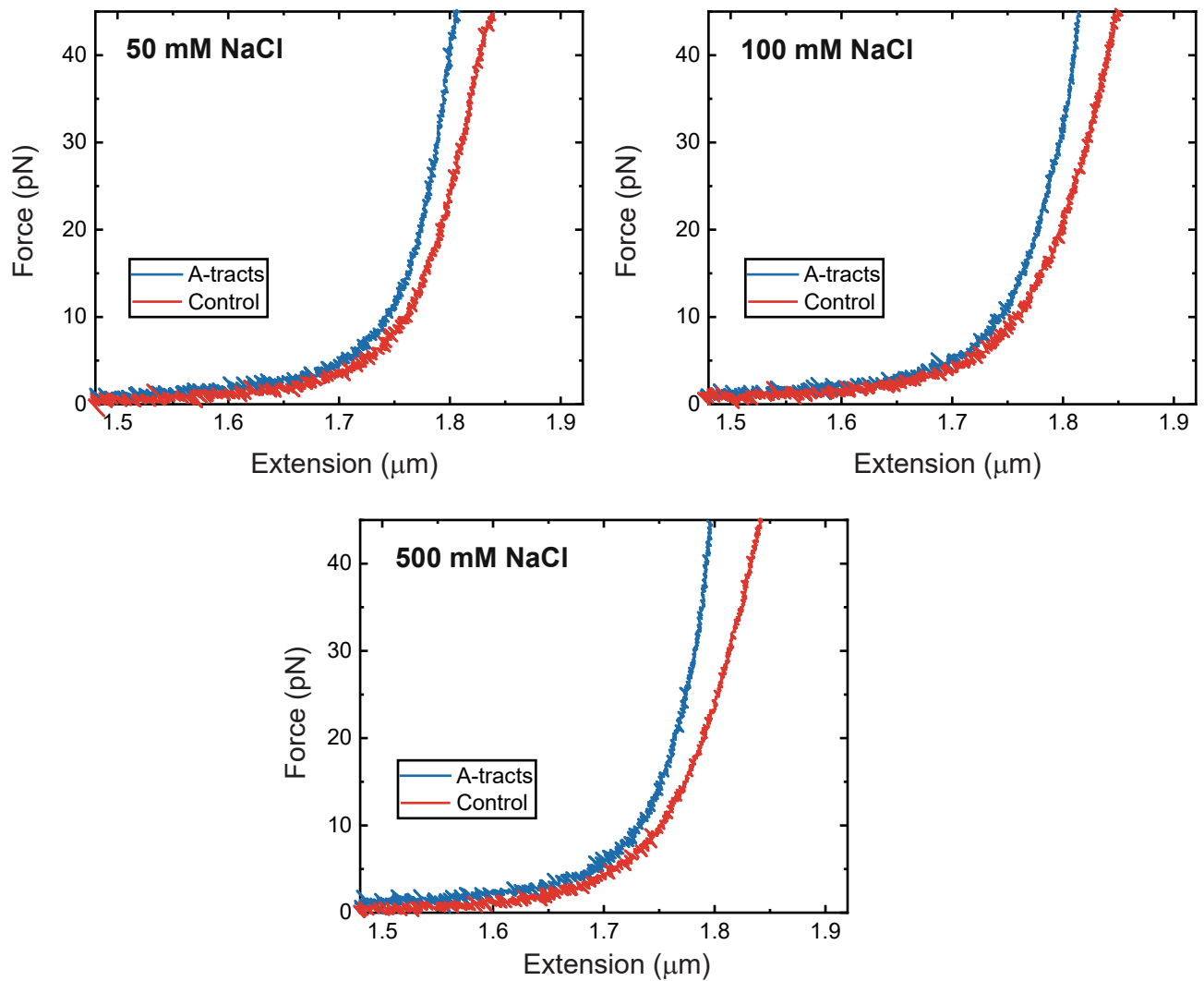


Figure S11. Raw optical tweezers force-extension curves of A-tracts and control molecules at different NaCl concentrations. Blue data correspond to A-tracts DNA molecules. Red data correspond to control DNA molecules. The number of molecules represented for each condition are indicated in **Table 1**.