Supporting Material

S.1. Additional experimental data

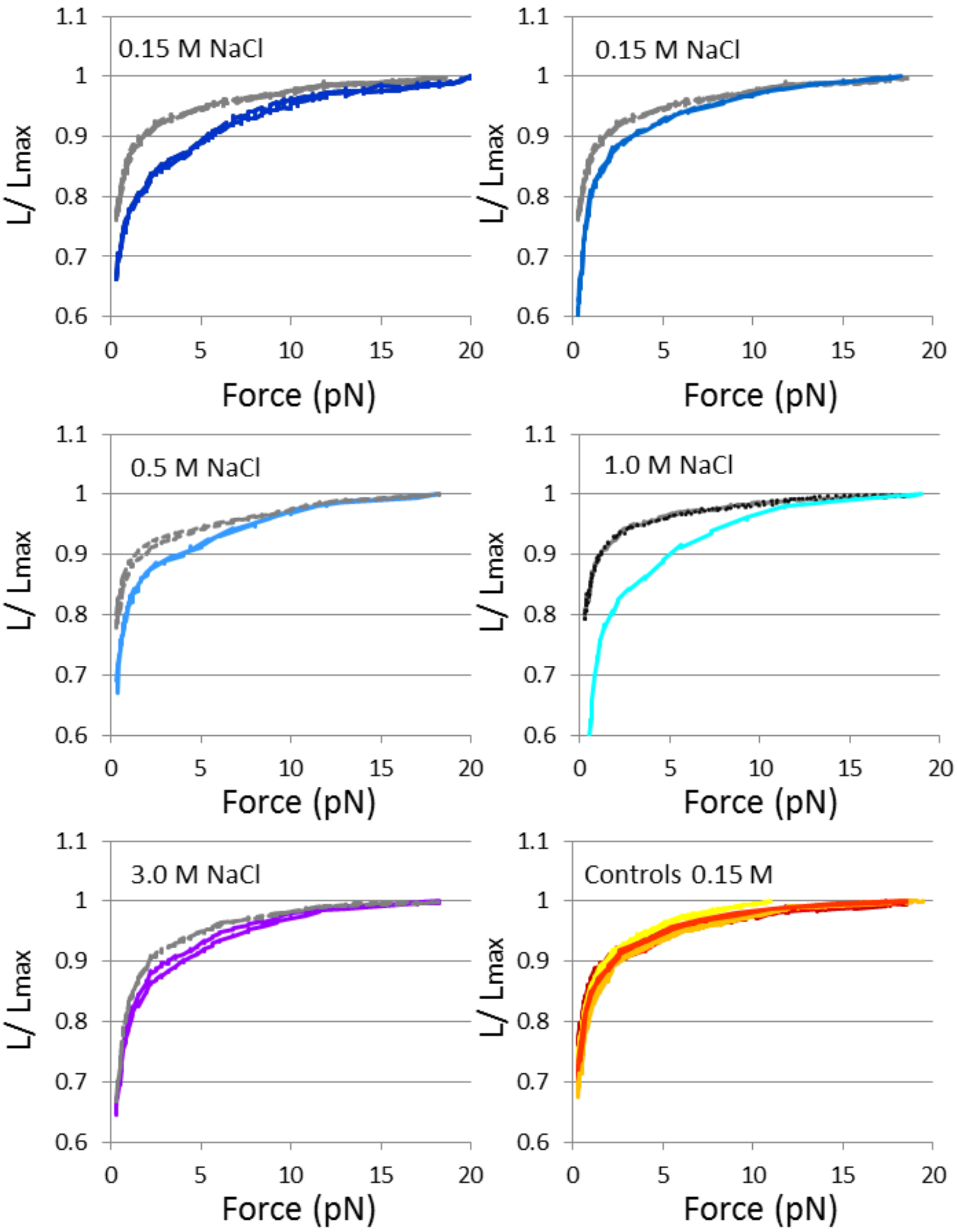


Fig. S.1. Additional representative results showing the extension vs. force curves (increasing and decreasing force) in 0.15, 0.5, 1.0, and 3 M NaCl and 50 mM phosphate buffer at 24°C for a dsDNA construct (coloured curves) and a control curve for dsDNA without homologous regions in the corresponding ionic conditions (dashed gray with an additional black points for a second control experiment). Five control curves obtained with different beads at 24°C and phosphate buffer- 0.15 M NaCl are shown in yellow, orange, light red, red, and dark red (bottom right).

First, we present unprocessed experimental data (at 24°C) for other experimental runs in addition to what is shown in Fig.1 of the main text. In the top two panels we present the results of two separate controls and construct experiments done at the same conditions. We see that the difference between the construct and control experiments is present in both experiments. Though, its magnitude varies somewhat, leading to a large uncertainty in the fitting parameters as seen below.

We also show that this difference persists with changing salt concentrations between 0.15 M and 3M. Last of all, we show that the variation in the curves of multiple control experiments is much smaller than the differences seen between construct and control curves at a given salt concentration. This is first seen by looking at the middle right panel where we plot two control curves against one construct; clearly the biggest difference is between the construct and the two controls that lie close to each other.

Furthermore, we show in the bottom right hand panel the curves of multiple control experiments for 0.15 M NaCl, again the difference is much smaller than observed differences between constructs and controls.

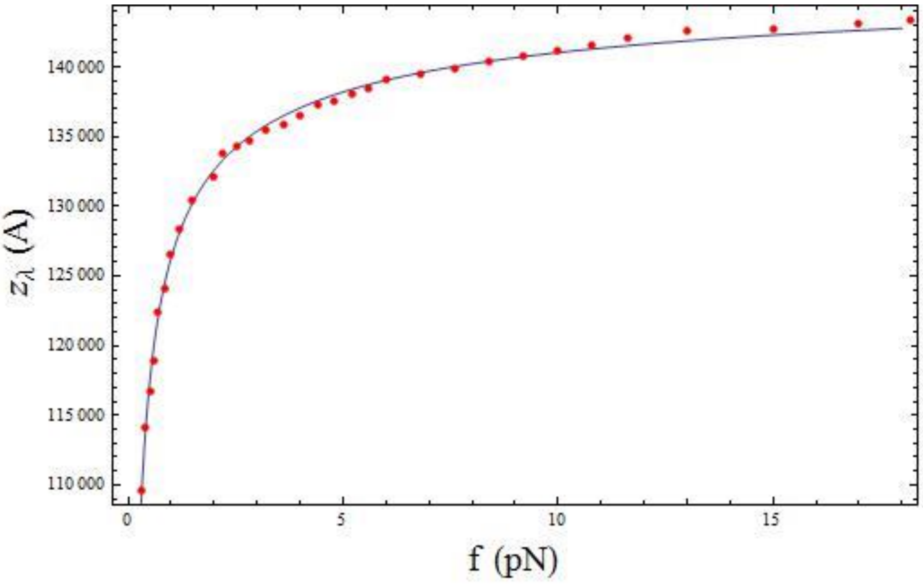


Fig. S.2. WLC fit to the control experiment. In this figure we show a fit to the data for the control DNA in 0.15M NaCl aqueous solution to the worm like chain extension formula. Using Eq. (3) of the main text with $L_{loop} = 0$, the fitted value of bending persistence length is $l_p = 636.57$ Å.

In Fig. S.2 we show a typical fit of the bending persistence length to control experimental data. Fits were done on control experiment runs and those values were used in fitting Δz. Though the fit is quite good, the value of $l_p = 636.57\text{Å}$ should really be considered as an apparent value bending persistence length as its value is larger than the commonly accepted value of $l_p \approx 500\text{Å}$. This value is due to the fit being performed at relatively large values of the pulling force. In fact, there is quite a large spread of values in l_p about $l_p \approx 500\text{Å}$, when we fitted them to available experimental data.

This is because the uncertainty in the extension (for repeated runs) for larger force values affects l_p more than extensions at lower forces, due to the non-linear nature of the extension formula. A fit containing more low force values would likely give a range of values closer to $500\,\text{Å}$. Importantly, we demonstrate that difference between construct and control lies outside this uncertainty in extension values (see Fig S.1).

Fitting extension curves over the range of force values considered reflects, however, better the true nature of these curves and the uncertainty in extension values. One could introduce DNA extensibility into the wormlike chain formula, which would be a small correction at these force values, but this seems rather pointless as the fits to the control curves are adequate.

S.2. Model 1: formulating the non-linear term

We will use polymer scaling arguments to deduce what the form of the non-linear term in the free energy of the loop, supposing that a non-linear term comes from different parts of the loop interacting in an attractive manner. We may write down for the average interaction attractive energy

$$E_{\text{int}} = -\iint_{\Gamma} d\mathbf{r}\, d\mathbf{r}' \rho(\mathbf{r}) V(\mathbf{r}-\mathbf{r}') \rho(\mathbf{r}'). \tag{S.1}$$

Here, $\rho(\mathbf{r})$ is the local looped DNA density at position $\mathbf{r}$ and $-V(\mathbf{r}-\mathbf{r}')$ is an attractive interaction energy between two parts of the loop at positions $\mathbf{r}$ and $\mathbf{r}'$. Both volume integrals are taken over average volume of space, Γ, that the loop occupies. If we suppose that the interaction is short ranged such that $V(\mathbf{r}-\mathbf{r}') \approx V_0 \delta(\mathbf{r}-\mathbf{r}')$ and we assume the density $\rho(\mathbf{r})$ to be uniform in the volume taken up by the loop, i.e. $\rho(\mathbf{r}) = \bar{\rho}$, then

$$E_{\text{int}} \approx -\bar{\rho}^2 V_0 \Gamma. \tag{S.2}$$

Now since the density should go as $\rho \propto L_{loop} / \Gamma$ we have that

$$E_{\text{int}} \sim L_{loop}^2 \Gamma^{-1}. \tag{S.3}$$

We now use polymer scaling arguments to deduce the dependence of E_{int} on L_{loop}. Supposing that volume taken up by the loop goes as $\Gamma \sim R_g^3 \sim L_{loop}^{3\nu}$, so that we have

$$E_{\text{int}} \sim L_{loop}^{2-3\nu}. \tag{S.4}$$

We will choose the value $\nu = 1/2$. We cannot assert that this value is exact; as for excluded volume polymer it is known to be close to 3/5 in the absence of sufficiently strong attraction between the polymer segments [M.Doi, *Introduction to polymer physics,* Clarendon Press, Oxford, 1996]. In our rather complex system there may well be a balance of the attractions between different parts of the loop and electrostatic repulsions, allowing for a lower value of ν. For simple estimates we take the value of ν of a simple random walk, thus behaving as a polymer in a 'θ-solvent', which fits the experimental data well. Thus when the loop is large we suggest the form

$$E_{\text{int}} = -aL_{loop}^{1/2}. \tag{S.5}$$

An additional factor $\exp\left(-b / L_{loop}\right)$ is introduced to take account of the bending free energy cost to form a loop that hinders these interactions. This yields the form for F_{loop} presented in Eq. (4) of the main text.

S.3. Equations for the length of the loop

For Model 1, the length of the loop, obtained by numerical minimization of Eq. (4) of the main text, can be well approximated by

$$L_{loop} = \left(\frac{a}{\varepsilon_{loop} - 2g_{WLC}(f)}\right)^2 H\left(\left(\frac{\varepsilon_{loop} - 2g_{WLC}(f)}{a}\right)^2 b\right), \tag{S.6}$$

where

$$H\left(\tilde{b}\right) = \left(0.249 + 1.85\tilde{b} - 12.50\tilde{b}^2 + 46.35\tilde{b}^3 - 76.82\tilde{b}^4\right)\theta(0.315 - \tilde{b}). \tag{S.7}$$

The value of the argument of the function H, $\tilde{b} = (\varepsilon_{loop} - 2g_{WLC}(f))^2 b / a^2$, increases as one increases both b and the pulling force f. One should note that when $\tilde{b}$ exceeds the value 0.315 the $L_{loop} \neq 0$ state is no longer stable, the only stable one is then the $L_{loop} = 0$ state. Thus, we have allowed for the $L_{loop} \neq 0$ state to be metastable. The latter assumes that the energy barrier is sufficiently large for the molecule complete unfolding of the loop, i.e. emergence of the $L_{loop} = 0$ state. Hence, thermal fluctuations are not expected to cause a significant unfolding. We plot this function and the resulting expression for z in Fig.2 and use it to fit the experimental data shown in Fig. 5 (both in the main text), with the additional constraint that the maximum value of L_{loop} is $L_{\max} = 34000\text{Å}$.

For Model 2 the minimization is simple and we obtain

$$L_{loop} = L_0 + \beta g_{wlc}(f), \tag{S.8}$$

with the requirement that $L_{\max} \geq L_{loop} \geq 0$ We plot this function (and the resulting expression for z) in Fig.3 and use it to fit experimental data in Fig 6 of the main text.

S.4. Additional parameter plots for Model 1

Here, we show additional plots for the readers to get a better feeling of how Model 1 works. In Fig. S.3 we plot the L_{loop} depending part of the total free energy, $F_{loop}(L_{loop}) - 2g_{wlc}(f)L_{loop}$, *vs* L_{loop}. We see that decreasing a, as well as increasing either $\varepsilon = \varepsilon_{loop} - 2g_{wlc}(f)$ or b destabilizes the free energy minimum at $L_{loop} \neq 0$.

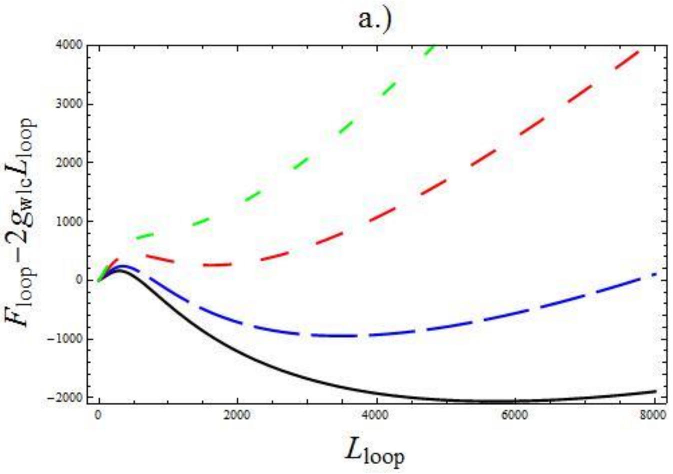
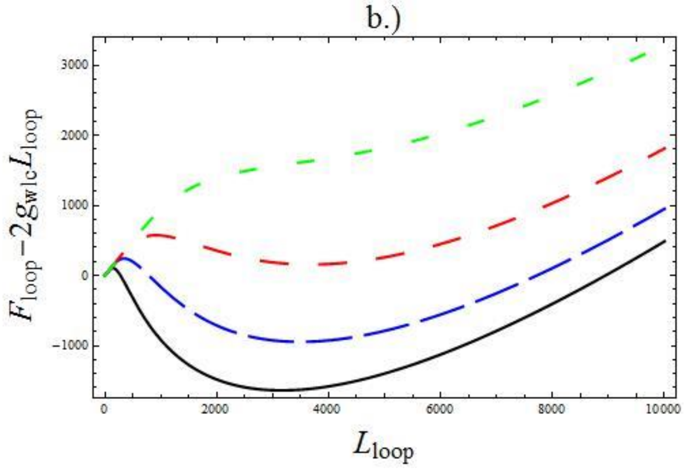
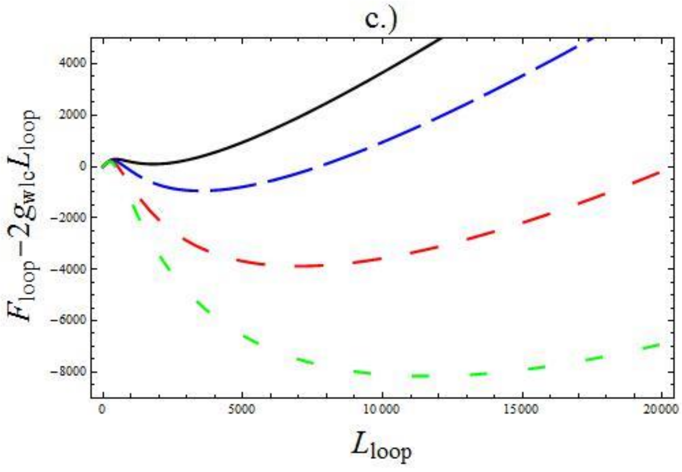


Fig. S.3. Plots of the free energy contribution $F_{loop}(L_{loop}) - 2g_{wlc}(f)L_{loop}$ for various values of the parameters a, b and $\varepsilon = \varepsilon_{loop} - 2g_{wlc}(f)$.

a.) We fix $b = 1000\text{Å}$ and $a = 100 k_B T / \text{Å}^{1/2}$ The thick solid (black), long dashed (blue), medium dashed (red), and short dashed (green) lines correspond, respectively, to the values $\varepsilon = 0.75, 1, 1.5, 2\ k_B T / \text{Å}$



b.) We fix $a = 100 k_B T / \text{Å}^{1/2}$ and $\varepsilon = 1 k_B T / \text{Å}$. The thick solid (black), long dashed (blue), medium dashed (red), and short dashed (green) lines correspond to the values $b = 500, 1000, 2000, 4000\ k_B T / \text{Å}$.



c.) We fix $b = 1000\,\text{Å}$ and $\varepsilon = 1 k_B T / \text{Å}$. The thick solid (black), long dashed (blue), medium dashed (red), and short dashed (green) lines correspond to the values $a = 70, 100, 150, 200\ k_B T / \text{Å}^{1/2}$.

In Fig S.4 we show the effect of changing b on the value of $L_{loop} / L_{\max}$. Here we see that the shape of the continuous part of the curves where $L_{loop} \neq 0$ is not affected much by changing b, but increasing b reduces the force at which we see a sudden jump down to $L_{loop} = 0$, as well as the magnitude of that jump.

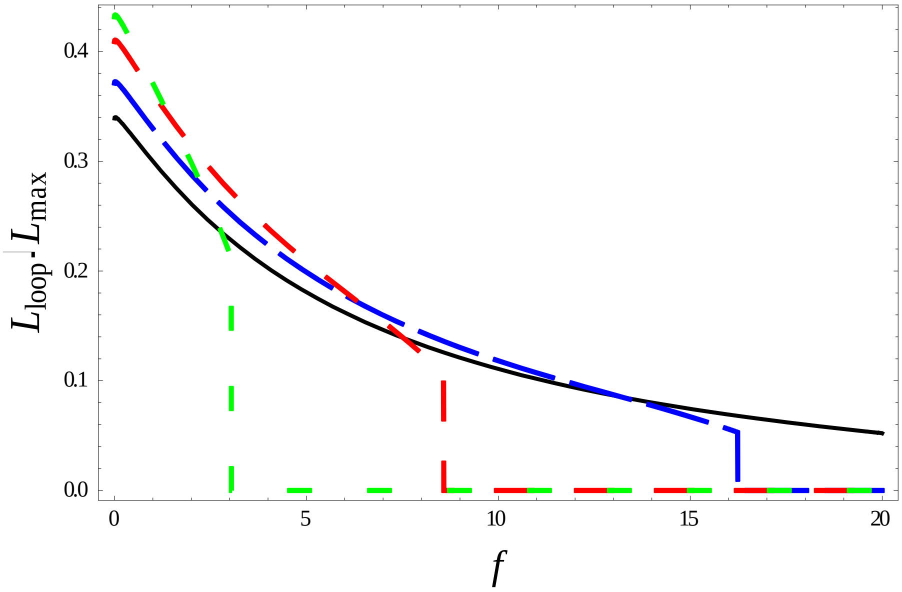


Fig. S.4. The ratio of loop length to maximal extension as a function of the pulling force: the effect of Kuhn length. Here we fix $\varepsilon_{loop} = 0.5 k_B T / \text{Å}$ and $a = 100 k_B T / \text{Å}^{1/2}$, and plot curves for different values of Kuhn length: $b = 1000\,\text{Å}$ (black solid line), $b = 2000\,\text{Å}$ (blue long dashed line), $b = 4000\,\text{Å}$ (red medium dashed line) and $b = 8000\,\text{Å}$ (green short dashed line).

S.5. Model parameter fit values

Here, we present values for model parameters fitted to various experimental runs. Shown in Fig. S.5 and Fig. S.6 are the fitted values of Model 1 and 2, respectively, to the available experimental data. The fitted values, averaged over experimental runs done at the same conditions, are also presented in Tables S.1 and S.2. Some of the data seem to indicate that the degree of pairing first increases with salt concentration, before reducing. However, the data of multiple experimental runs have large scatter; the uncertainty on the fitted values is large, so the existence of this trend remains uncertain.

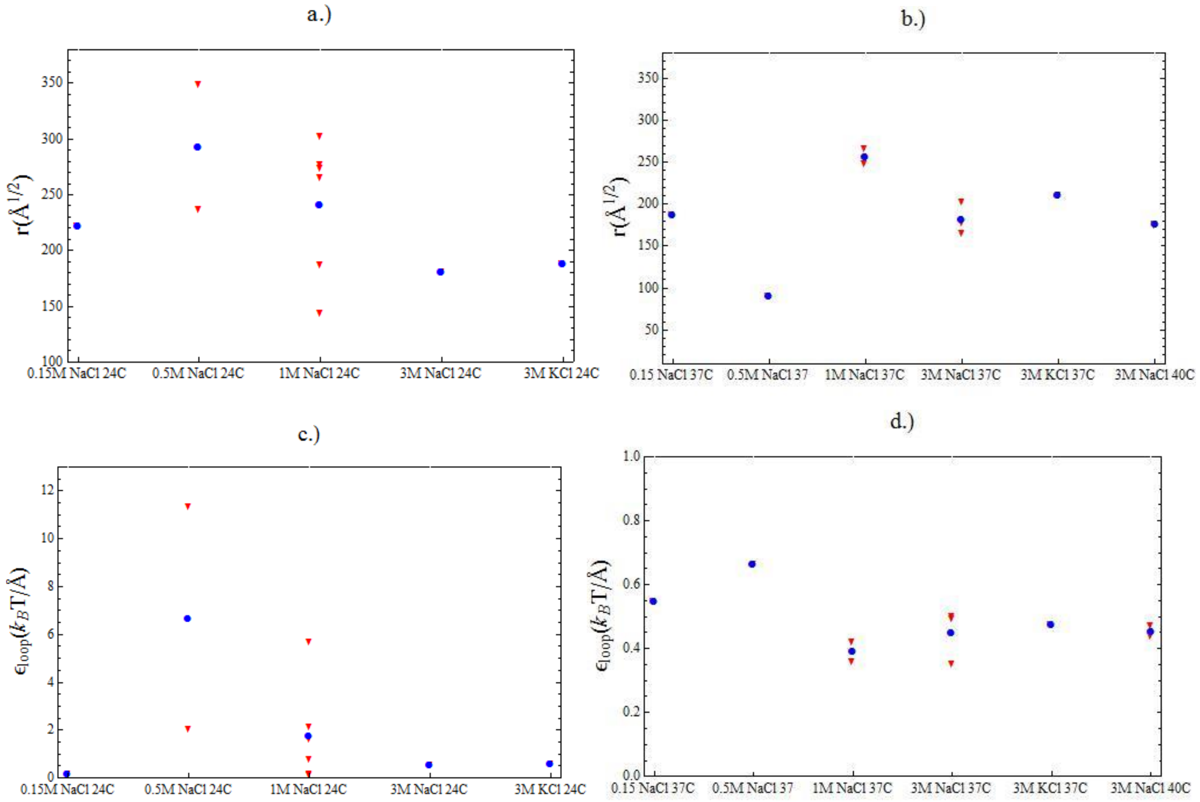


Fig S.5. The fitted parameter values of Model 1, r $(= a/\varepsilon_{loop})$ and ε_{loop} , for different temperatures and ionic conditions. Panels a.) and b.) show the fitted values for the ratio r, while c.) and d.) display the values of ε_{loop}. As indicated in the labels on the x-axis, a.) and c.) correspond to data at the lower temperature value 24° C, while b.) and d.) correspond to the higher temperature values of 37° C and 40° C. The red triangles correspond to individual experimental runs, whereas the blue circles correspond to the averaged values.

Temperature	Ionic conditions	Mean Fitted values of $r = a/\varepsilon_{loop}$	Mean Fitted values of ε_{loop}
24°C	0.15M NaCl	222 $\text{Å}^{1/2}$	0.167 $k_BT/\text{Å}$
24°C	0.5M NaCl	293 $\text{Å}^{1/2}$	6.68 $k_BT/\text{Å}$
24°C	1M NaCl	241 $\text{Å}^{1/2}$	1.75 $k_BT/\text{Å}$
24°C	3M NaCl	181 $\text{Å}^{1/2}$	0.547 $k_BT/\text{Å}$
24°C	3M KCl	188 $\text{Å}^{1/2}$	0.571 $k_BT/\text{Å}$
37°C	0.15M NaCl	187 $\text{Å}^{1/2}$	0.547 $k_BT/\text{Å}$
37°C	0.5M NaCl	90.6 $\text{Å}^{1/2}$	0.664 $k_BT/\text{Å}$
37°C	1M NaCl	256 $\text{Å}^{1/2}$	0.390 $k_BT/\text{Å}$
37°C	3M NaCl	182 $\text{Å}^{1/2}$	0.450 $k_BT/\text{Å}$
37°C	3M KCl	210 $\text{Å}^{1/2}$	0.476 $k_BT/\text{Å}$
40°C	3M NaCl	176 $\text{Å}^{1/2}$	0.454 $k_BT/\text{Å}$

Table S.1. The average values of the fitted parameters of Model 1, $r = a/\varepsilon_{loop}$ and ε_{loop} for the various ionic conditions and temperatures probed in the pulling experiments.

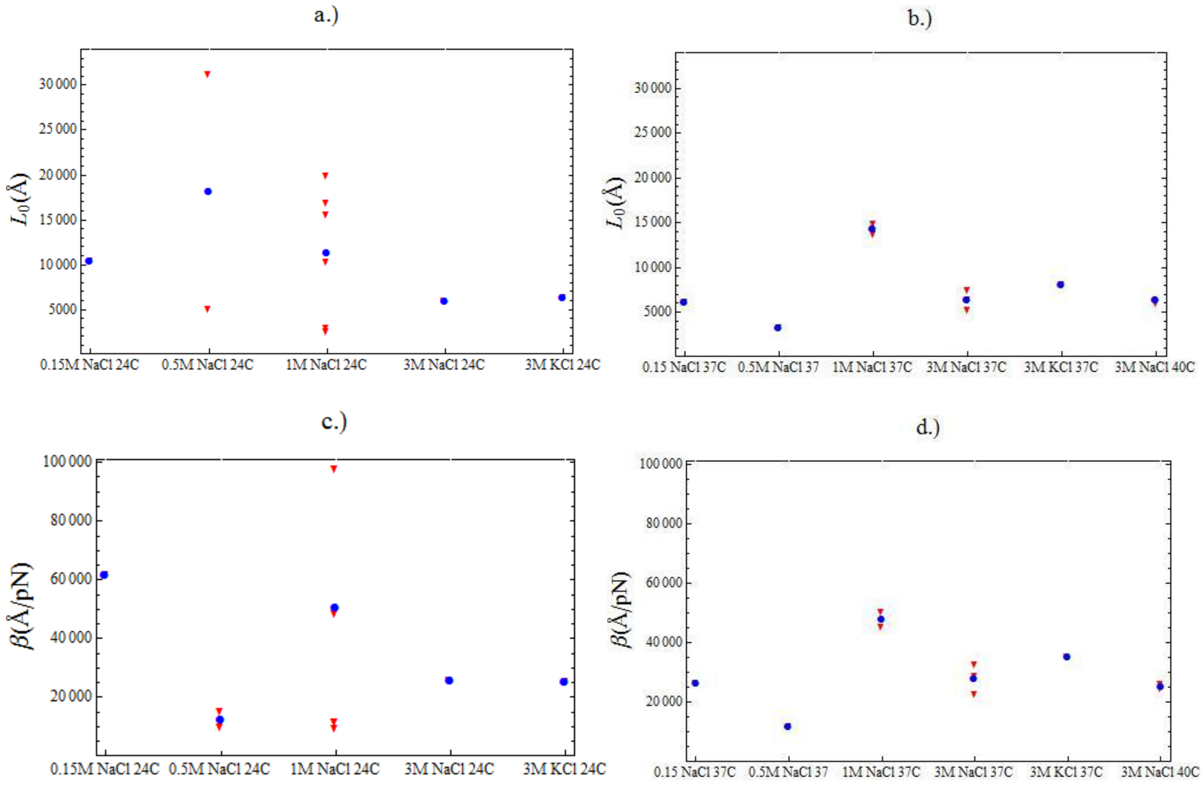


Fig. S.6. The fitted parameter values of Model 2, L_0 and β, for different temperatures and ionic conditions. The fitted values for L_0 are presented in a.) and b.) while c.) and d.) display the values of β. As indicated in the labels on the x-axis, a.) and c.) correspond to data at the lower temperature value 24° C, while b.) and d.) correspond to the higher temperature values of 37° C and 40° C. The red triangles correspond to individual experimental runs, whereas the blue circles correspond to the averaged values.

Temperature	Ionic conditions	Mean Fitted values of L_0	Mean Fitted values of β
24°C	0.15M NaCl	10500Å	61600 Å/pN
24°C	0.5M NaCl	18100Å	12300 Å/pN
24°C	1M NaCl	11300Å	50600 Å/pN
24°C	3M NaCl	5880Å	25500 Å/pN
24°C	3M KCl	6790Å	23400 Å/pN
37°C	0.15M NaCl	6090Å	26200 Å/pN
37°C	0.5M NaCl	3200Å	11600 Å/pN
37°C	1M NaCl	14800Å	47700 Å/pN
37°C	3M NaCl	6340Å	27900 Å/pN
37°C	3M KCl	8010Å	35100 Å/pN
40°C	3M NaCl	6340Å	25200 Å/pN

Table S.2. The average values of the fitted parameters of Model 2, L_0 and β, for the various ionic conditions and temperatures probed in the pulling experiments.

S.5. The DNA coding of the homologous segments in the construct DNA

The construct molecules were made by adding an additional DNA segment to the Lambda DNA. This segment was made by PCR reaction amplifying between positions 16322 and 26598 on lambda DNA. The segment was attached so that base pair text (the Lambda DNA base pair sequence from positions 16322 to 26598) ran in the opposite direction to that of the Lambda DNA. The sequence between positions 16322 and 26598 reads as:

```
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16381 gcggcggatg tggataaatg ggcgctgtat gtcatcggcc agtactgcga ccagtcagtg
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16621 cgcagtaatg tggtgatgcc ggatgatggc gcgccgttcc gctacagctt cagcgccctg
16681 aaggaccgcc ataatgccgt tgaggtgaac tggattgacc cgaacaacgg ctgggagacg
16741 gcgacagagc ttgttgaaga tacgcaggcc attgcccgtt acggtcgtaa tgttacgaag
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