The effect of thermal fluctuations on elastic instabilities of biopolymers

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Chapter 8

Comparison with experiments and conclusions

To test the validity of the model over an extensive range of parameters, use has been made of a series of measurements performed by the Seidel group in Dresden. For combinations of forces from 0.25 pN to 4 pN and salt concentrations from 20 mM to 320 mM the turns extension curves have been measured for chains of approximately 600 nm contourlength. The experimental data have been smoothed out with a moving average algorithm. To correct for the geometry of connection to the beads and substrate, the effective contour length of the chain has been obtained by fitting the 0 turns extension to the ideal not torsional restricted worm like chain. Up to lowest order this should be equivalent to the torsionally constrained 0 turns configuration. The effective chains thus obtained have a length that varies between 570 nm and 630 nm. A batch of measurements under varying forces, but constant salt concentration has been performed on one chain allowing us to verify that the effective chain length stays more or less constant once the geometry of the chain attachment is fixed. Only for forces below 1 pN the effective chain length decreases. This is partly due to the bend chain attachment, combined with too wildly fluctuating chains for our perturbative model.

The minimization procedure was initiated as follows: starting from the bifurcation point, lk_{cr}, the applied linking number per length was set to $\nu = \mathrm{lk}_{cr} + 0.2$. The parameters of the model were set to $\mathrm{lk} = 0.8\,\mathrm{lk}_{cr}$, $\alpha = 1$, $\sigma_r = 1/(2\kappa)$, and $R = 1/\kappa$. The free energy for a single chain was minimized after which the obtained values were used to set ν to $(\omega + \mathrm{lk}_{\mathrm{str}} + 2\,\mathrm{lk}_{cr})/2$, while keeping the other parameters unchanged. After this the minimization was repeated. In that way the applied linking number is approximately halfway in between the critical value and the maximal value. The reasoning is that with a linking number close to the bifurcation point the influence of an incomplete description of the end loop becomes too strong, while a linking number too far from the transition might underestimate the influence of multi plectoneme configurations. The precise value is not very important. Too close to the bifurcation point the chain collapses before the transition in low salt condition.

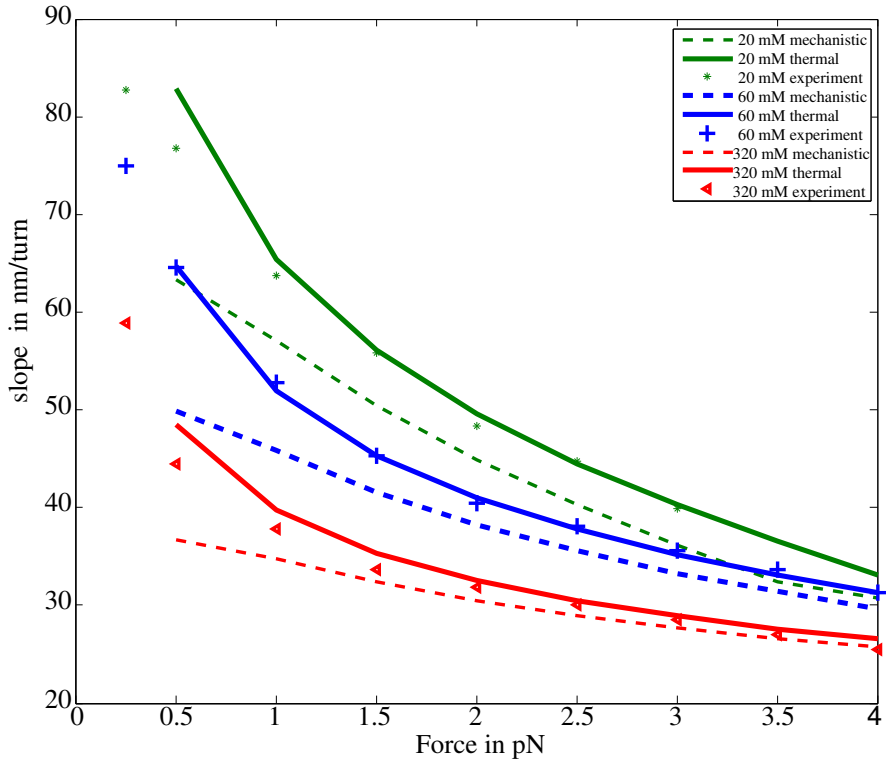

Figure 8.1: Slopes with and without thermal contributions. The dotted curves were calculated from the model up to Chapter 5, Equations eqs. (5.28) and (5.29). The solid thermal curves include multiplectonemes and were calculated using the method outlined in the text.

The reason is not so much the influence of the loop but a K^2 value that gets too low. Of course any prediction based on the model for K^2 values below 3 is unreliable. The generation of the force extension behavior is based on plectoneme energies from this minimization. The whole procedure is very fast.

As a first check of our model we compare predicted plectonemic slopes to those determined in experiments. Note that the choice of where to measure the slope is not always obvious in both theory and experiment. Whenever there was a clear constant slope visible it was taken as "the slope", otherwise the first slope after the transition was taken. Especially for the short 600 nm chains it was not always clear what to take as slope. This is especially true for low salt, 20 mM to 60 mM, conditions. Nonetheless the slopes for the full range indicated a nice agreement between experiment and model. The results for 20, 60, and 320 mM are in Figure 8.1. The influence of the multi plectoneme phase is clearly visible for low salt concentrations. There is also a clear improvement in the low force range, although there the value of K^2 of around 2 at the transition makes the agreement mere coincidental. The turn-extension plot at 20 mM and 3 pN in Figure 8.2 shows the details. The transition happens in both cases at a lower linking number than in the experiment, presumably because it is

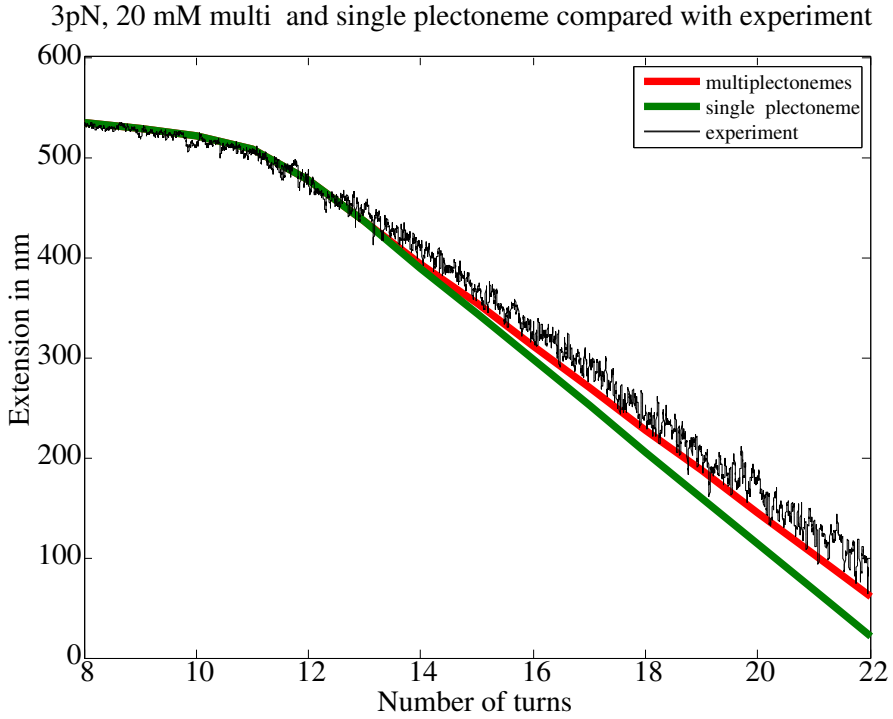

Figure 8.2: Influence of the multi-plectoneme phase on the turn extension slope. The black curve is a single plectoneme curve, the red curve when multiplectonemes are taken into consideration

too close to the bifurcation point. To produce the plots the torsional persistence length was lowered to 90 nm from 110 nm to 120 nm to get the transition point close to the experimental. In Figure 8.3 The number of plectonemes is set out against the number of turns for these conditions.

The curves for 20 mM and 320 mM are shown in Figure 8.4 and Figure 8.5. For most cases our model predicts the experimental curves extremely well. The 20 mM measurements show an exceptional behavior at 3.5 pN. It is possible that the chain undergoes a phase transition as has been suggested [150]. Another possibility is that because plectoneme formation is relatively expensive, starting plectonemes are extremely unstable. That can explain the sawtooth behavior with signs of attempts at plectoneme nucleation.

A set of experiments performed on a 3850 nm chain, in a 320 mM solution with the same setup shows a longer clear slope in Figure 8.6. The transition point suggests a 120 nm torsional persistence length.

Another test of the model is the analysis of the plectoneme torques. The torque can be easily obtained by dividing the increase of the free energy by the rotation angle that caused it. It is commonly believed that the linear slope of the curves coincides with a state of constant torque [180, 152]. This makes it attractive to use the DNA plectoneme as a source of constant torque in the study of molecules that interact with DNA like topoisomerase and helicase.

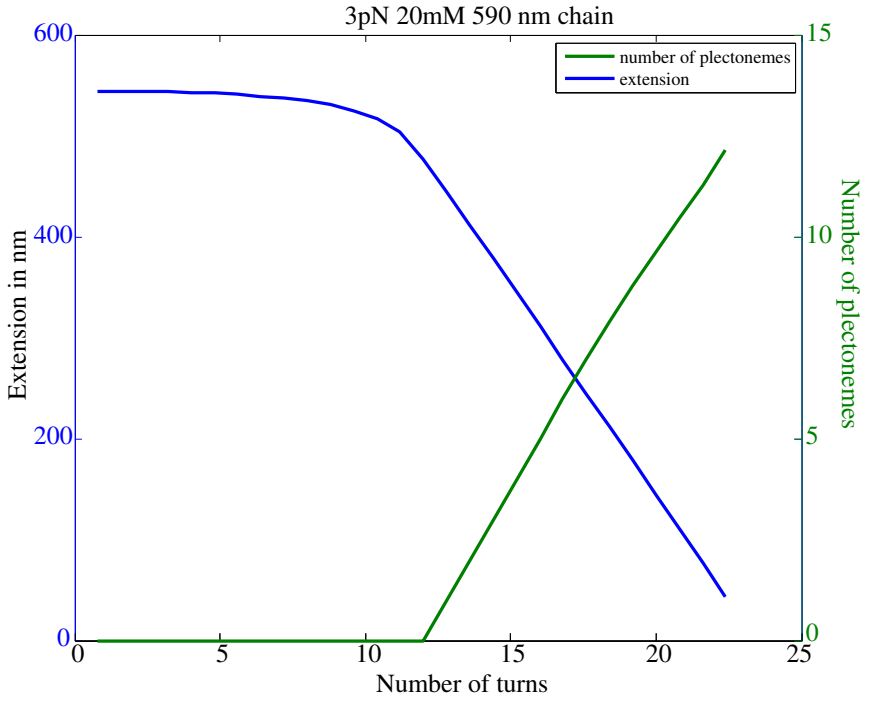

Figure 8.3: Number of plectonemes at 3 pN and 20 mM salt in green combined with the corresponding turns extension plot in blue

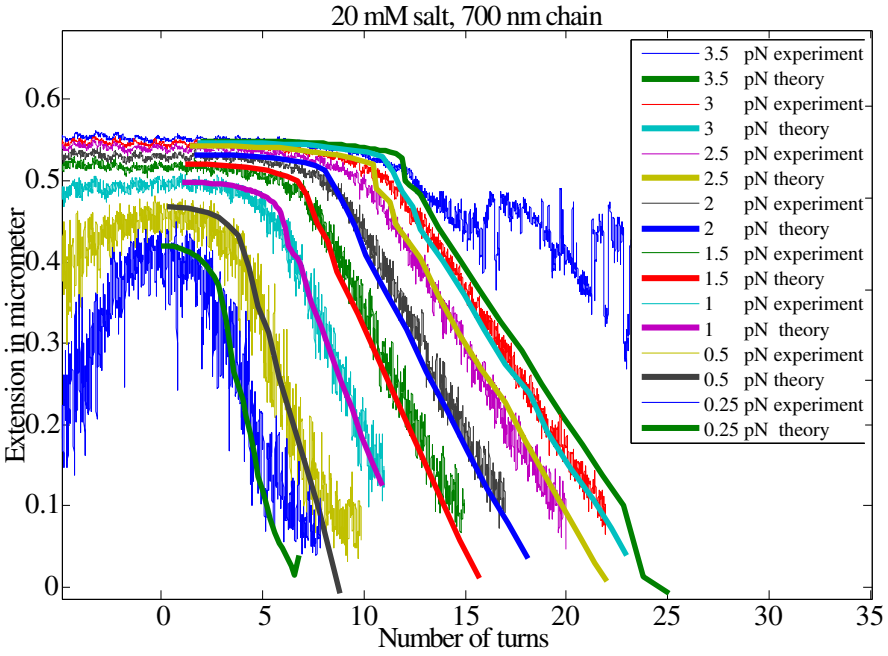

Figure 8.4: Turns versus extension plots for 20 mM salt concentrations. Pc was set to 90 nm to get a good agreement, but as explained in the text it might be a calculational artifact

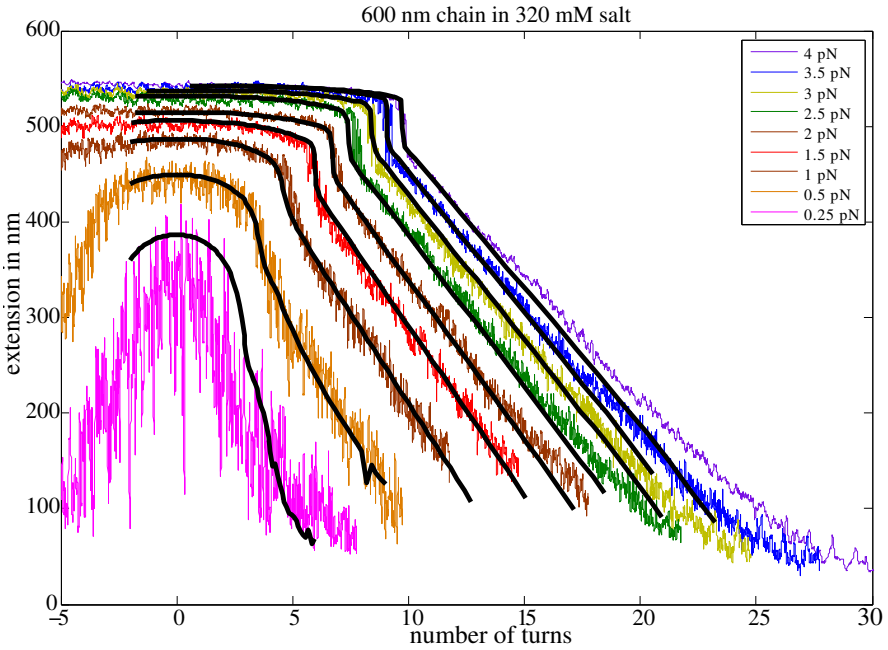

Figure 8.5: Turns versus extension plots for a 600 nm chain under varying tension in 320 mM salt. P_c =120 nm

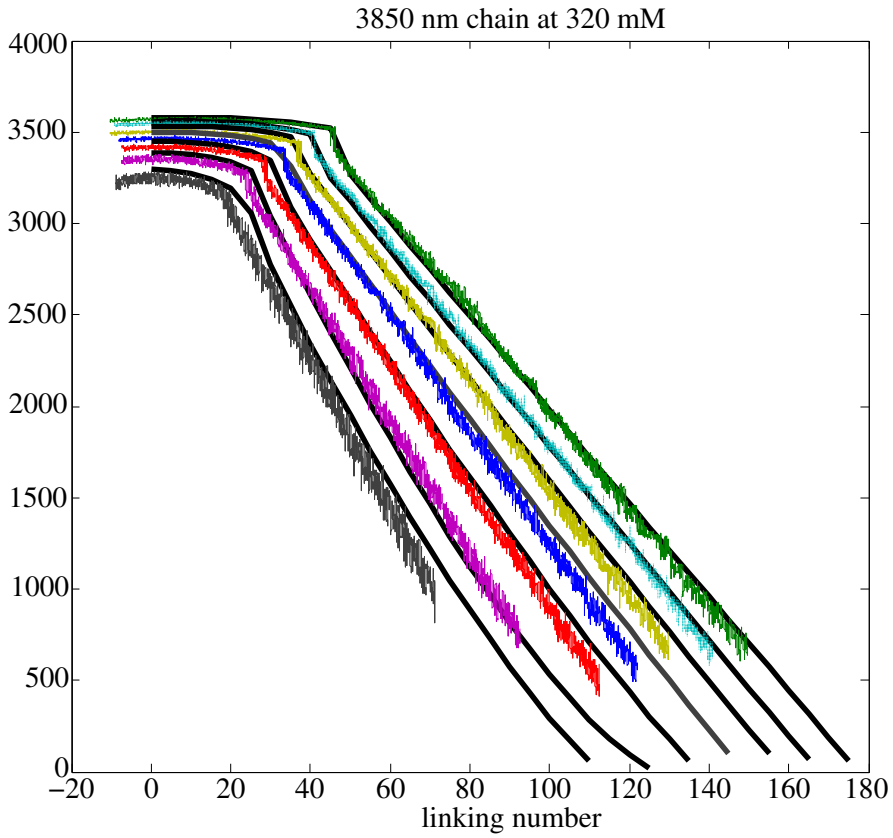

Figure 8.6: Turns versus extension plots for a chain of 3540 nm with tension from 1 pN to 4 pN in 320 mM salt. A torsional persistence length of 120 nm was used.

Table 8.1: Indirect torque measurements using Maxwell relations [147] compared to the theoretical values from our model

Salt (mM)	Force (pN)	Exp. Torque (pN nm)	Theor. Torque (pN nm)
10	2.86	28.1	35
	2.53	26.2	32
50	3.66	29.6	34.7
	3.23	27.4	32.4
100	3.33	24.4	30.1
	2.61	20.7	26.3
500	4.33	22.3	29.6
	3.80	20.2	27.5

One way to measure this plectoneme torque is by using a specially nanofabricated quartz cylinder in conjunction with an optical tweezer [181]. The setup seems to be very promising enabling the measurement of torque at the same time as force and extension. A small set of measurements was used with relatively short chains of 700 nm [145]. Another method makes use of the constant torque in the plectoneme region combined with Maxwell relations between torque/linking number and force/extension as free energy parameters to calculate the plectoneme torque over a large range of forces based on an approximately linear linking number torque relation before the transition at a force in the upper range. Making use of the assumption of a constant torque after plectoneme formation, the torque for a large range of data can be calculated just from the turn extension plot. This is the setup from Mosconi et al. [147]. The resulting torques in the measurements [145, 147] seem to differ. One suggestion is that the salt concentrations perhaps differ too much. It is interesting to compare the torques our model predicts with those of reference [147]. To our surprise the torques we calculate differ from their measurements substantially enough to doubt the validity of our model, see Table 8.1. This was perhaps somehow to be expected, since the torque data were the main driving force for Maffeo et al [150] to incorporate a charge reduction factor into their model. What is somewhat mysterious is that the force extension curves themselves are in good agreement with our model. If the torque only depends on the shape of that curve, it must be that there is somewhere a wrong assumption made.

Comparing our torque predictions with the direct torque measurements from the older optical tweezer measurements [145] reveal however a remarkable good agreement as is shown in Figure 8.7, where the torques are shown as a function of the supercoiling density defined as the ratio of the linking number density to the linking number density of the two strands of the

double helix when the chain is straight and relaxed. This last density is of course $1/\text{helical repeat} = 1/3.6\ \text{nm}^{-1}$.

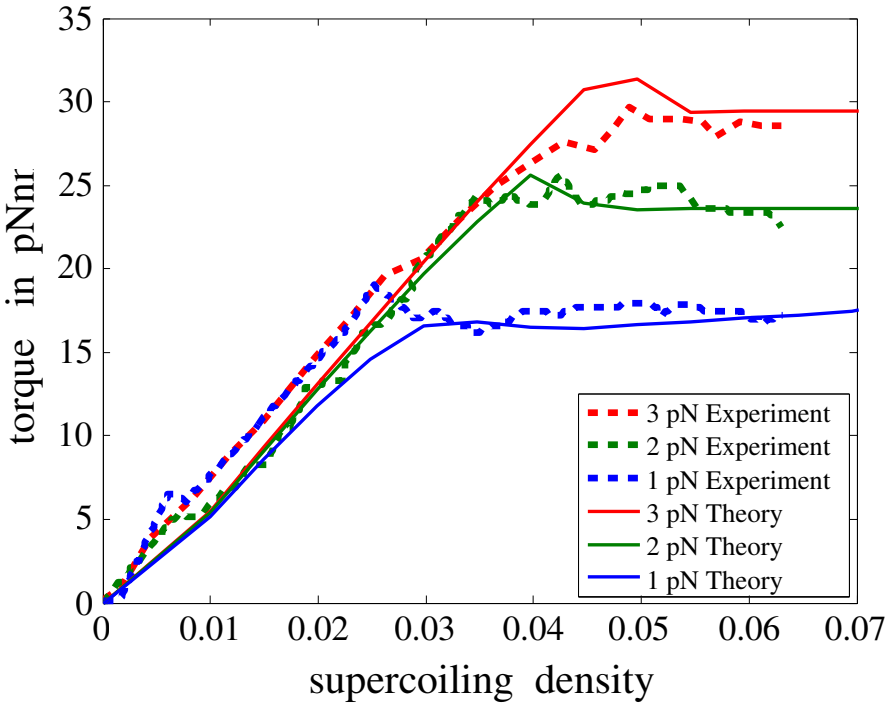

Figure 8.7: Comparison between experimental results from an optical tweezer experiment using a quartz cylinder to directly measure the torque [145] and our theoretical model. The DNA has a contourlength of 725 nm, monovalent salt concentration 150 mM

The culprit is readily revealed as the multiplectoneme phase. In extracting the torque from the force extension measurements an essential assumption is that the torque in in the linear slope is indeed constant. That almost presupposes that the slope is a one plectoneme slope. Lacking a method to verify this assumption it had to be accepted on face value. In reality the torque is not constant at all for lower forces. Thanks to the fast increasing number of plectonemes along the chain the torque is almost linearly *increasing* spoiling the calculations. When we take this increase into account, the resulting torque values agree again wonderfully well with the predictions from our model.

As an example we borrow the calculations from Mosconi et al. [182]. The relevant curves are in Figure 8.8. The Maxwell relation calculations are performed over the path as shown in the figure on the left. The resulting torque for 3.67 pN is 27 pN nm. But if we examine the torque as calculated from the model the result is higher, around 34.9 pN nm. Though the torque is constant for the high-tension slope, the path from B to C in Figure 8.8 is one of decreasing torque thereby resulting in a too low estimate for the plectoneme torque.

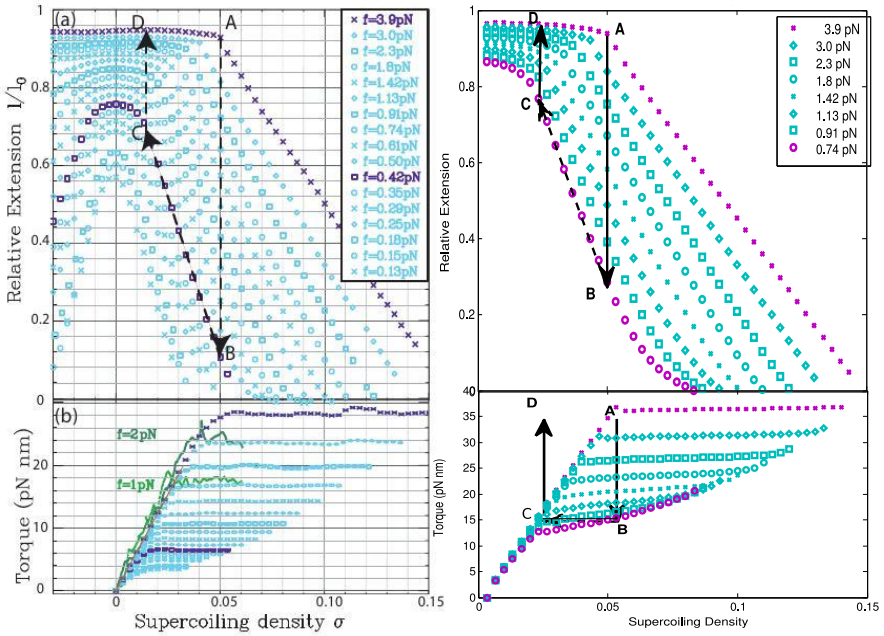

Figure 8.8: Magnetic tweezer measurements from Mosconi et al [182] as basis for indirect torque measurements. On the left are data from the actual measurements, the torques were determined assuming them to be constant. On the right the data as calculated from the theory for tensions where fluctuations are small enough using the criterium $K^2 \leq 3$. The contourlength is 5.6 µm, monovalent salt concentration of 100 mM

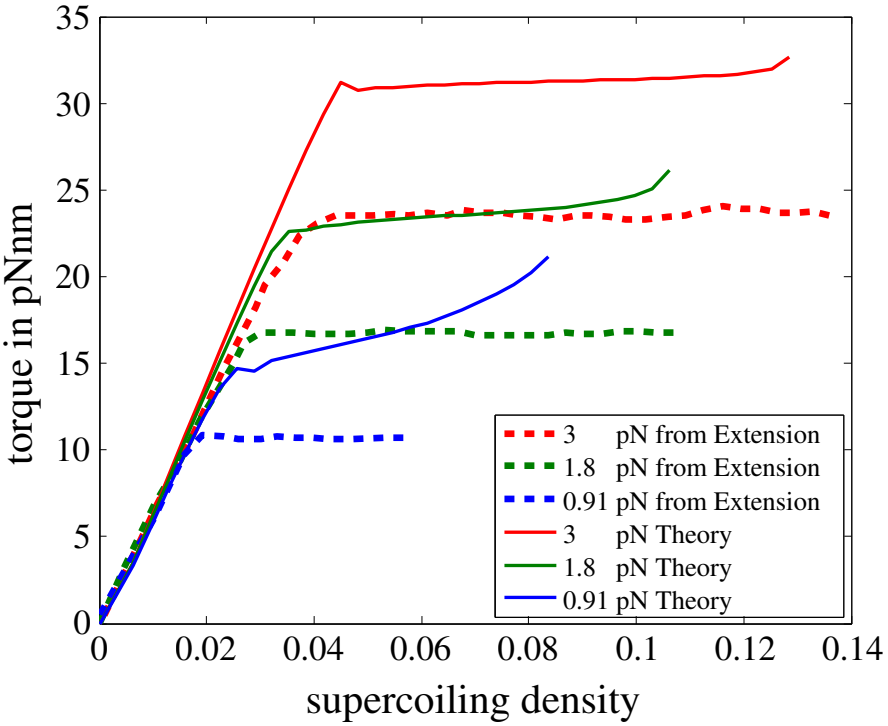

Figure 8.9: Torque as function of supercoiling density for three different tensions calculated from the model compared to the indirect determination of the torque using Maxwell relations under constant plectoneme torque assumption [182]. The conditions are the same as in Figure 8.8

8.1 Conclusions

Plectoneme formation in DNA can be thought of as a tool to study short distance interactions between DNA molecules in a reasonably controlled way. It seems to be important to acquire a good understanding of all the facets of the possible interactions. It was my intention to partly open the way to a detailed description of the mechanical, electrostatic and thermal forces that act on DNA in the cell. There are still many open questions of which I would like to mention a few:

- How is thermal writhe influenced by a writhing groundstate? The separation of the thermal writhe from its writhing background in the plectoneme as done in Appendix C.1 is a rough approximation, what are the effects of co- or anti-writhing paths?
- Is it possible get insight into possible charge correlations that might be important for homological recombination ?
- Do van der Waals forces play any role in DNA-DNA interactions in the cell ?
- Is the instability at low salt and high tensile forces a sign of a transition to PDNA, or is it a plectoneme nucleation problem ?
- What is the dependence of moduli other than the persistence length on the salt concentration?
- Does a crowded environment allow for larger torques ?