

Theory and Simulation of DNA Damage and Supercoiling



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Abstract

We employ an array of methods including statistical mechanics and computer simulation to better understand how both damaged and undamaged DNA respond to linear and torsional mechanical stress and the effect of damage on supercoiled phenomena.

Via the use of an all-atom thymine dimer residue, damaged linear and circular DNA were simulated atomistically for various biologically relevant systems using the AmberTools molecular dynamics package. Local structural parameters and global properties are characterised and shown to agree well with experimental data.

Using oxDNA, a nucleotide level coarse-grained model of DNA, we make use of another model for a thymine dimer to probe larger systems and for longer times, investigating a 336-bp minicircles and its negatively supercoiled topoisomers as well as a 160-bp minicircle. We show that the presence of a thymine dimer causes a sharp local increase in curvature as well as increase in negative writhe for the under-twisted systems - potentially helping to suppress bubble formation.

A planar model for damaged DNA via a piecewise construction of worm-like chains with different bending moduli is developed. This model is then applied to curls, plectonemes and minicircles. A more general twist-storing model of plectonemes is also developed and shown to reproduce phase diagrams in accordance with experiments and simulations.

The theory of thermal fluctuations through a polymeric path integral is developed for a ribbon-like model of DNA in the fixed linking number ensemble. This theory is then applied to several systems such as straight chains and solenoids, successfully reproducing some well-known results. Finally we apply the theory to a previously intractable system of non-trivial saddle points, with the general systematic methodology elucidated.

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Introduction

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1.1 Nucleic Acids and their Structure

Deoxyribonucleic acid (DNA) was first isolated by Miescher [1] in 1869 when investigating the chemical composition of the nucleus. Miescher noticed that this substance differed from proteins and due to its abundance in the nuclei, he initially named the substance *nuclein*. These nucleins were found to be acidic and usually surrounded by proteins [2]. Altmann [3] then succeeded in removing the proteins from nuclein to leave an acidic molecule which he named *nucleic acid*.

The composition of nucleic acids was not elucidated until 1891 when Kossel [4] determined that they were comprised of a sugar and five distinct nitrogenous nucleobases: Adenine (A), Thymine (T), Guanine (G) and Cytosine (C) and Uracil (U). These can be further split into *pyrimidines* (CTU) and *purines* (GA). The identification of nucleic acids with different compositions lead to the introduction of two families of nucleic acids: yeast and thymus nucleic acids. The distinction between the two groups pertained to two observations. First was that the constituent bases for both acids differed. While both contained A, G and C bases, thymo-nucleic acids second pyrimidine base was shown to be thymine, compared with uracil for yeast nucleic acids. Second was that the sugar component of each acid appeared to differ.

The yeast variety of nucleic acid was the first to have its sugar identified when Levene showed it to have a ribose sugar [5]. Following this Levene postulated a polynucleotide theory [6] for nucleic acids. The base-unit of this supposed biopolymer was conjectured following further advancements in the understanding of the structure of yeast nucleic acid [7]. Levene proposed that the monomeric unit of nucleic acids consisted of a sugar, a phosphate and a nucleobase. This unit was called a *nucleotide*.

With the ribose sugar now apparent in yeast nucleic acids, the identity of *ribonucleic acid* (RNA) had been established. The sugar associated with the thymus nucleic acids eventually followed with Levene determining that it in fact had a 2-deoxy-D-ribose sugar [8]. This is where the story of *deoxyribonucleic acid* (DNA), under the name now universally recognised, began. Due to the discovery of roughly equal quantities of the four bases in both DNA and RNA, a tetra-nucleotide model of nucleic acids remained popular for some time, with Levene being a strong proponent.

Even after the full characterisation of the primary structure of DNA, its secondary structures remained unclear. In 1951, Franklin ascertained that the phosphate groups were on the outside of DNA and established the existence of crystalline and hydrated forms of DNA which she termed A-form and B-form DNA, or simply A-DNA and B-DNA, respectively. This led to a major breakthrough when Franklin's now famous Photo 51 [9] was observed by James Watson who recognised the image to be the diffraction pattern of a helix thanks to his partnership with Francis Crick who had done work on this topic. This helped the pair devise their now famous model of B-DNA [10] which goes as follows: two poly-nucleotide strands wrap around each other to form a right-handed double helix. The nucleobase on each nucleotide is hydrogen-bonded to a base on the opposite strand, forming a *base-pair* (bp). These bonds cause the bases to point into the helix, exposing the sugar-phosphate backbone to the outside as observed by Franklin, see Fig. 1.1. Not only this but due to a proposed specificity of base-pairing between pairs (AT) and (CG), the information of the double helix was contained in just one of the strands, with one strand said to be *complementary* to the other.

A-DNA was also verified to be helical, albeit a shorter, more compact right-handed double helix than B-DNA. Only one other double-stranded structure is known in biological systems. Named Z-DNA, it is a narrower, more elongated left-handed double helix than the A or B forms and is relatively rare. It is thought that the vast majority of DNA in the body is B-DNA.

Outside of biological systems, many other DNA structures have been observed or postulated such as L-DNA that can form in the presence of large amount of under-twist where the DNA is highly denatured [12–14].

1.1.1 B-DNA

B-DNA will form the central object of study in this thesis and its structure is further expanded upon here. As established by Watson and Crick, base-pairs are bonded via hydrogen bonding: purines form hydrogen bonds with pyrimidines, with the AT base-pair only containing two hydrogen bonds, compared to CG which has

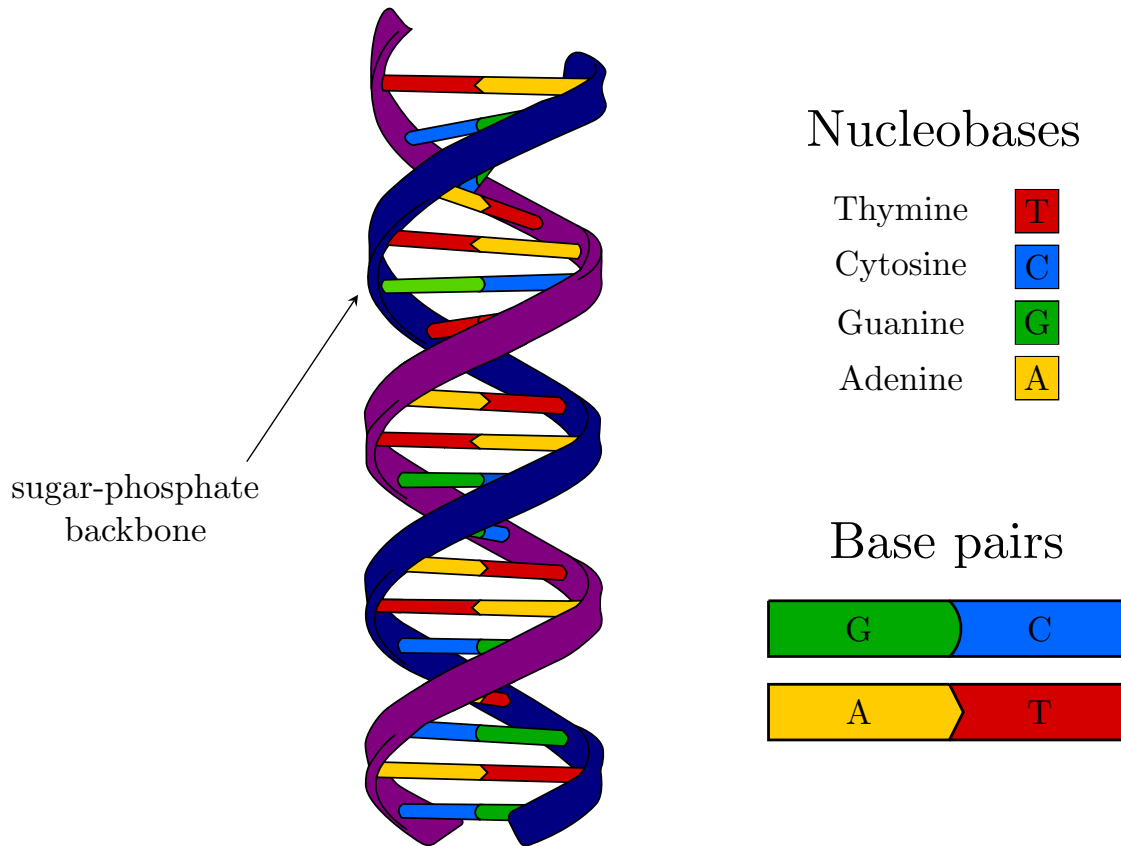


Figure 1.1: Diagram of Watson Crick model of B-DNA (adapted from [11]).

three, see Fig. 1.2. It was found that the sugar-phosphate backbones of the two strands in B-DNA are not directly opposite each other in the double-helix. Because the glycosidic bonds that attach the bases to the sugar in the backbone are at an angle (relative to the interface between the AT or GC pairs), one of the “faces” of the base pair is larger than the other. This leads to *grooves* in the double-helix forming their own helical structure. The larger of these two grooves is called the *major groove*, and the smaller the *minor groove*.

Another property of B-DNA is the rate at which the base-pairs rotate around its helical axis. We define the helical *pitch* as the distance along its helical axis required to complete one full turn. The pitch of B-DNA is approximately 10.5 bps, this corresponds to rotation of about 34° per base-pair. See Fig. 1.3 for an overview of the structure of B-DNA.

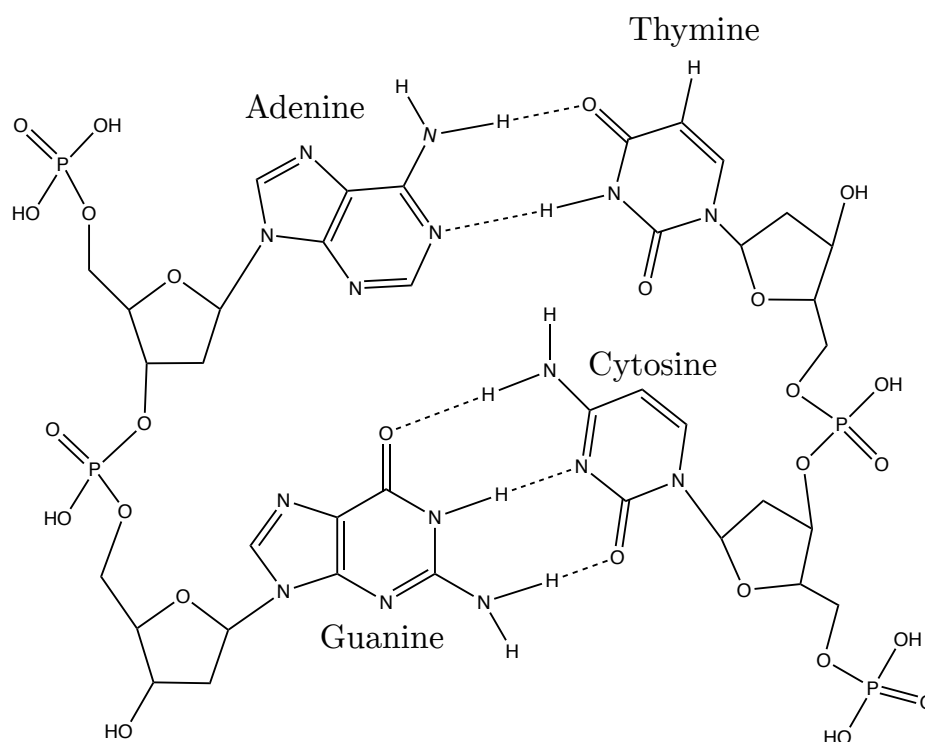


Figure 1.2: Chemical bonding diagram and nucleotide structure of the four nucleobases of DNA.

1.1.2 Biological Processes and Inheritance

Ever since the work of Mendel [15] on inheritance, scientists sought to understand the physical carrier of genetic information. The ability for DNA to perform this role had been quashed by the prevailing tetra-nucleotide theories of the time. This was until Avery *et al.* [16] demonstrated how DNA was responsible for the induction of virulence in bacteria. Following the postulate from Schrödinger [17] that genetic information may be carried by some “aperiodic crystal”, Watson and Crick incorporated these ideas into the rationale behind their model. Their notion of complementary base-pairs explained the observed equal levels of AT/CG while allowing for diverse sequences and hence the possibility that DNA could carry genetic information.

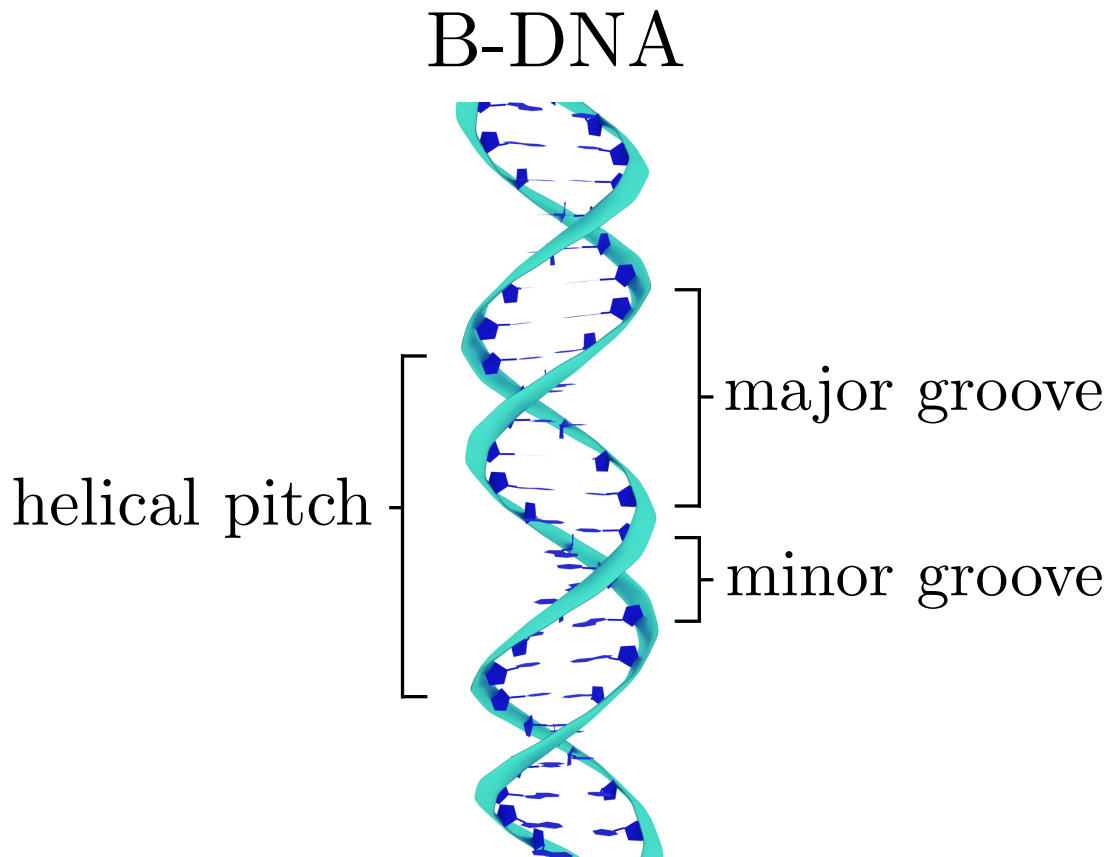


Figure 1.3: Schematic of linear B-DNA with major and minor grooving and helical pitch labelled.

Sequences that inscribe this information are said to be *coding*, with *non-coding* sequences aiding this functionality. Despite coding regions of the human genome providing these useful instructions to the body, only between 1% and 2% of all DNA in eukaryotes is coding [18]. This is in stark contrast with prokaryotes whose coding regions typically take up 88% of its genome [19]. Why the human genome contains so much non-coding DNA is still unclear.

Although this non-coding DNA has sometimes been misleadingly called “junk” DNA, it has been shown to be involved in an increasing number of processes such as gene expression and DNA replication. There are, however, non-coding regions for which no functionality is yet apparent. For example, it is not apparent why fragments of viral DNA are incorporated into the genome. Even if some non-coding DNA were to have no function, it is still known for new genes to evolve from

non-coding DNA [20]. All of this helps explain why the energetic cost of removing this non-coding DNA has not been undertaken during evolution.

One way in which DNA has been more recently postulated to encode information is through a “mechanical code” [21]. This mechanical code refers to the variations in mechanical properties within the genome that encode information used in biological processes involving mechanical forces acting on DNA. These variations in mechanical properties can influence DNA-protein interactions, gene expression, DNA replication, repair, and other important cellular processes [22–24]. One of the key aims of this thesis is to further develop the understanding of DNA’s response to mechanical stress, with the expectation that any mechanical processes that aid cellular survival would be utilised by the genome.

1.1.3 Transcription and Replication

In the years since the discovery that DNA encodes our genetic information, much has been found about how this information plays a fundamental role in cellular function. Firstly, DNA is used in *transcription*, a process where RNA uses DNA as a template. This RNA can then perform specific tasks in the cell such as encode protein sequences which are essential for the proper functioning of cells in living organisms.

During transcription, the enzyme *RNA polymerase* binds to the region of DNA to be copied and forms a transcription bubble via melting the duplex in said region. The RNA polymerase then adds RNA nucleotides to form a complementary sequence to that of the DNA. Once this RNA strand has been completed, it may detach from the DNA and fulfil its purpose.

Another essential function of DNA is the ability to make a copy of itself. The existence of base-pairing rules ensures an explanation as to how DNA would make a copy of itself, a process called *replication*. This is achieved in a somewhat similar way to transcription, however, replication involves the use of DNA polymerase instead of RNA polymerase.

Transcription and replication both require untwisting in order for the polymerase to create a copy sequence. In eukaryotes, for replication this unwrapping is

performed by DNA *helicase* which breaks hydrogen bonds as it moves along the chain allowing for the separation of the strands. It's important to note that while DNA replication helicase is a dedicated enzyme specifically involved in DNA replication, the helicase activity of RNA polymerase during transcription is an auxiliary function of the enzyme. The primary function of RNA polymerase is to catalyze the synthesis of RNA, but its helicase activity facilitates the process by unwinding the DNA strands temporarily.

1.1.4 Structure in the Cell

If long strands of DNA are subject to sufficient torsional stress, they can wrap around each other and loop to form coiled superstructures. This phenomenon is hence referred to as *supercoiling*. Examples of such a structure is when a chain wraps around itself, forming a loop at the top of a braided region. We shall refer to such structures as *plectonemes*. Plectonemes, like helices, can be either left or right-handed.

We now discuss the larger structures formed by DNA in both eukaryotic and prokaryotic cells. In eukaryotes, DNA chains are wrapped around structural proteins called *histones* to form *nucleosomes*, much like a thread wrapped around a spool. Hewish [25] determined that these units must be of length approximately equal to 200 bp. Finch [26] then showed that a nucleosomal DNA strand of length 145 bp wraps almost twice around a histone octamer forming a left-handed supercoil.

Many of these nucleosomes then form *chromatin* via the assembly of the nucleosomes into a higher-order structure through interactions with a scaffold protein framework. This organised chromatin structure plays a crucial role in regulating various cellular processes by modulating DNA accessibility and gene expression. This series of processes is designed to help tightly pack the DNA into the nucleus. This results in a compaction factor of about 10,000 compared to a simple double helix of the same length.

Prokaryotic cells contain a single DNA molecule which forms a closed chain which is called a *nucleoid*. We shall refer to closed chains as simply being *circular*.

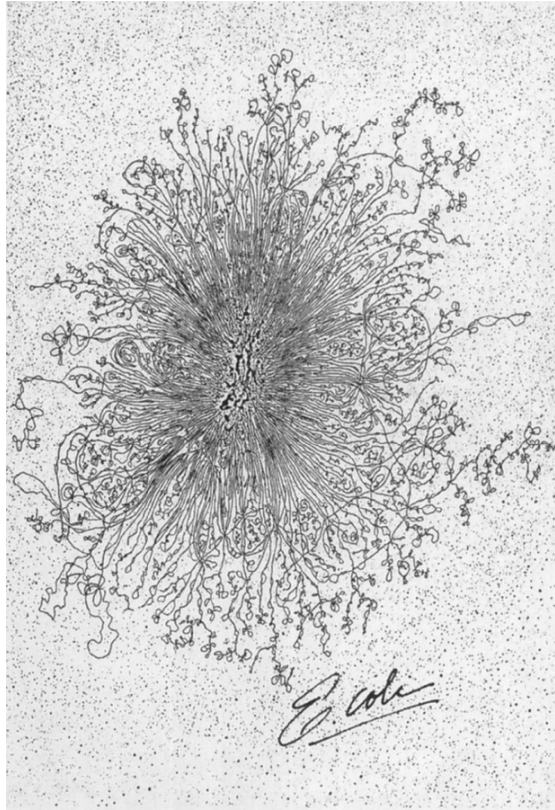


Figure 1.4: Image of a DNA nucleoid for the *E.coli* bacterium obtained via electron microscopy by Kavenoff [27]. Note that this image was taken after the cell had been lysed and the DNA allowed to expand.

A nucleoid is negatively supercoiled and forms a super-plectonemic structure (see Fig. 1.4) with nucleoid associated proteins (NAPs) restraining about half the plectonemes. This negative supercoiled structure is possible due to DNA *gyrase*, a specific topoisomerase that can induce negative supercoiling which makes it the only topoisomerase to do so. It has been shown by Sinden [28] that in *E.coli*, the plectonemic supercoiled structure organises into regions with specific topological domains.

1.1.5 Properties of B-DNA

B-DNA is a relatively rigid biopolymer. It has three significant degrees of freedom: bending, twisting, and compression. The latter of these is biologically the least significant and is neglected in many models. The bending and twisting degrees of freedom can be characterised by their respective stiffnesses. These parameters

quantify the energetic cost of deviating from some minimum energy state. These stiffnesses are often defined in relation to corresponding persistence lengths. Strictly speaking, the *persistence length* of DNA is the contour length over which the time-averaged orientation of the polymer becomes uncorrelated by a factor of e . I.e. for a polymer of length L with unit tangent $\hat{\mathbf{t}}$, the bending persistence length ℓ_p satisfies

$$\langle \hat{\mathbf{t}}(0) \cdot \hat{\mathbf{t}}(L) \rangle = e^{-L/\ell_p}. \quad (1.1)$$

Being able to prescribe a tangent to double-stranded DNA (dsDNA) requires mapping it onto a one-dimensional chain. The bending stiffness A , for a WLC with no bending anisotropy and twist-bend coupling is trivially related to ℓ_p via [29]

$$A = \ell_p k_B T. \quad (1.2)$$

I.e. the bending stiffness and the persistence length are equivalent up to a Boltzmann factor. This will not always be the case with the inclusion of bending anisotropy and groove-asymmetry leading to different formulae. For example, introducing bend anisotropy results in an effective persistence length that is the harmonic mean of the hard and soft bending rigidities, i.e. $A_{\text{eff}} = \ell_p k_B T$, where $A_{\text{eff}} = (1/A_1 + 1/A_2)^{-1}$ [30]. Defining A_{eff} as an effective bending stiffness simply allows us to say that the system behaves like a WLC with bend isotropy with this new modified elastic constant.

We may similarly define the twist persistence length via an exponential decay in the average auto-correlation of the unit vectors that define some twistable ribbon. Some texts do not make the distinction between persistence lengths and stiffnesses given that in some models they are trivially related. In practicality, the persistence length is usually more directly accessible from simulation data as it only requires the definition of the tangent. In general, extracting elastic moduli from data is more involved and requires several assumptions that may not always be valid, e.g. polymers only behave like WLCs on long length scales [31]. The bending persistence length of B-DNA is approximately 50 nm, with the twist persistence length being closer to 100 nm, with values varying with solvent conditions.

The existence of groove-asymmetry leads to an energetic coupling between the twisting and bending degrees of freedom. Recent theoretical studies have demonstrated that “twist-waves” and regions of higher curvature are the result of including twist-bend coupling [32, 33]. This coupling has been shown to reduce the effective torsional stiffness of DNA [34].

1.2 Experimental Methods

The vast array of cellular components make *in vivo* experiments of DNA very challenging when trying to investigate certain properties of DNA. Hence, we may often look to *in vitro* experiments for more insight into such systems where we may carefully control the environment in which the DNA is immersed. An example of an experiment of this class is the famous Franklin experiment that elucidated the double-helical nature of B-DNA [9]. The technique used in the experiment is called X-ray crystallography, however, it has the drawback that only ensemble averages may be observed, making dynamical properties inaccessible. This led to the development of single molecule experiments which allow for the study of mechanical and dynamical properties of DNA.

1.2.1 Molecular Tweezer Experiments

Molecular tweezers are the key experimental method for probing the mechanical response of DNA to external forces and torques. The key principle of this technique is to bind one end of the molecule to a bead. External forces and torques can then be applied to the bead thus imparting this into the DNA. The most popular examples include Optical tweezers (OT) and Magnetic tweezers (MT). In the case of optical tweezers the position of a dielectric bead can be carefully controlled with a laser which creates an optical trap using radiation pressure. This makes quantities such as end-to-end extension easily measurable [12]. This technique has also been extended to allow the application of torque [35, 36].

Magnetic tweezers conversely use a magnetic bead to induce linear and torsional stress on the duplex. This method was first utilised by Strick *et al.* [37] to

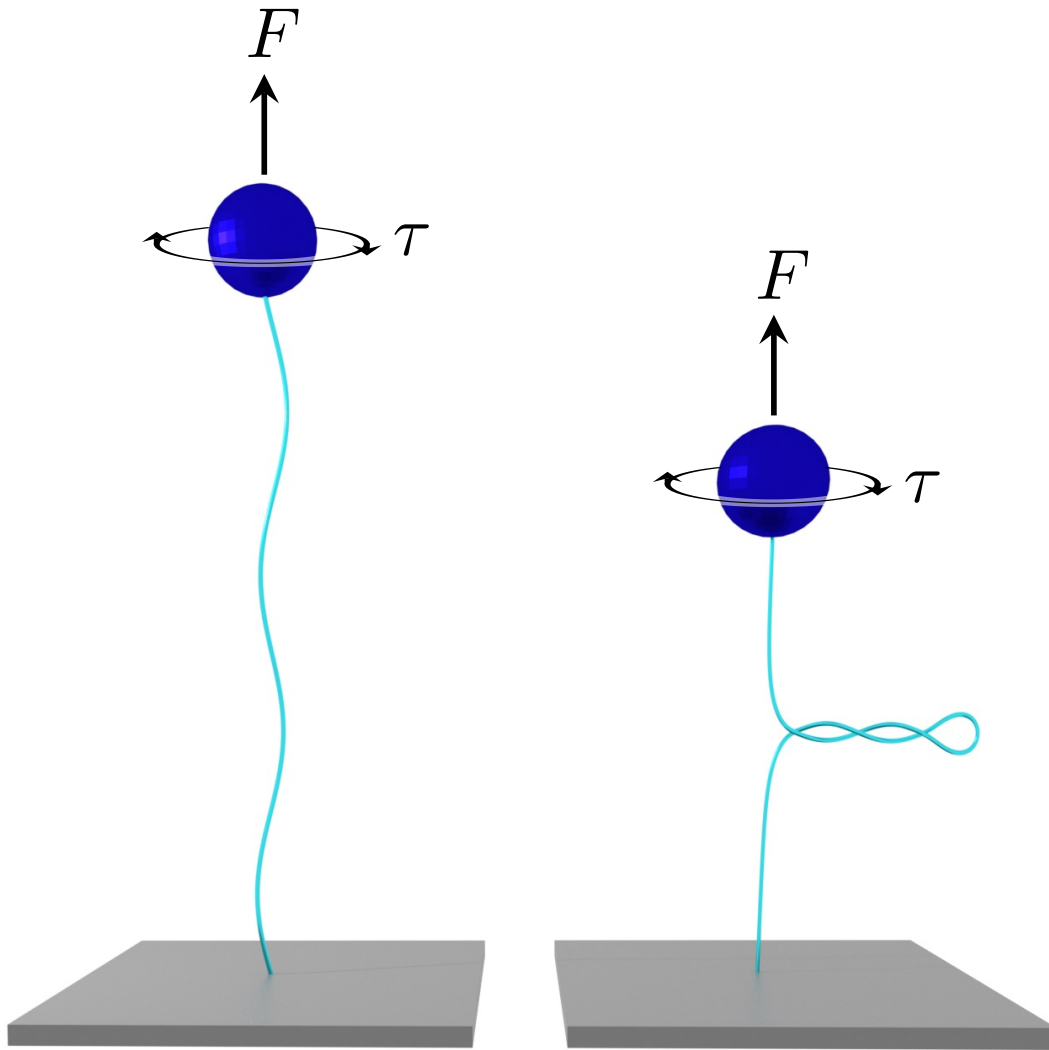


Figure 1.5: Diagram demonstrating the method of molecular tweezer experiments. The bead (dark blue) is subject to a force F and a torque τ . If a sufficient torque has been applied, decreasing the force will cause an extended configuration (left) to buckle to form a supercoiled configuration (right).

investigate the elastic properties of λ -bacteriophage but has since been used to measure the mechanical properties of DNA in various solvent conditions [38], plectonemic buckling transitions [39] and bubble phases [40, 41].

1.3 DNA Damage, Mutations and Repair

DNA damage is simply any mechanism that distorts the chemical structure of DNA. This is distinct from a mutation which is where the DNA sequence itself is altered. DNA often becomes damaged through physical interactions with ultraviolet (UV)

light and other mutagens. These damages can accumulate and lead to failures in the transcription of the damaged region, leading to mutations. These changes can severely affect gene function and gene expression leading to the development of cancerous cells.

1.3.1 Repair Mechanisms

To prevent damages and mutations from accumulating completely unchecked, evolution has created two main ways to repair DNA damage. The first is direct reversal of the damage reaction, returning the DNA to its original state. The other is removing the damaged region and replacing the corrupted information via an excision. This second repair method illustrates the main biological advantage of having a double-stranded carrier of genetic information: if one strand becomes damaged, then there exists a complementary strand that ensures the relevant information is not lost or corrupted.

There exist three main excision based pathways to rectify such errors. These include nucleotide excision repair (NER), base excision repair (BER) that repair damage, and DNA mismatch repair (MMR). BER is used for repairing small (typically 1-6 bps), non-helix-distorting lesions, usually in response to damage caused by endogenous mutagens. NER, however, is capable of repairing segments of DNA up to 30 base-pairs in length and can hence repair much larger DNA adducts. These damages are structurally more significant and are often a result of exogenous mutagens such as exposure to ultra-violet (UV) light. Both pathways rely upon enzymes such as DNA *polymerase* to resynthesise the excised region and DNA *ligase* to reattach the strand.

1.3.2 Thymine Dimers

In this thesis we mainly consider a particular type of damage known as the cis-syn cyclobutane thymine dimer (CPD) or simply the thymine dimer (TD) (a subclass of the cis-syn pyrimidine dimer or CPD). This is a mutagenic photo-product of two adjacent thymine bases and UV light. The reaction is a 2+2

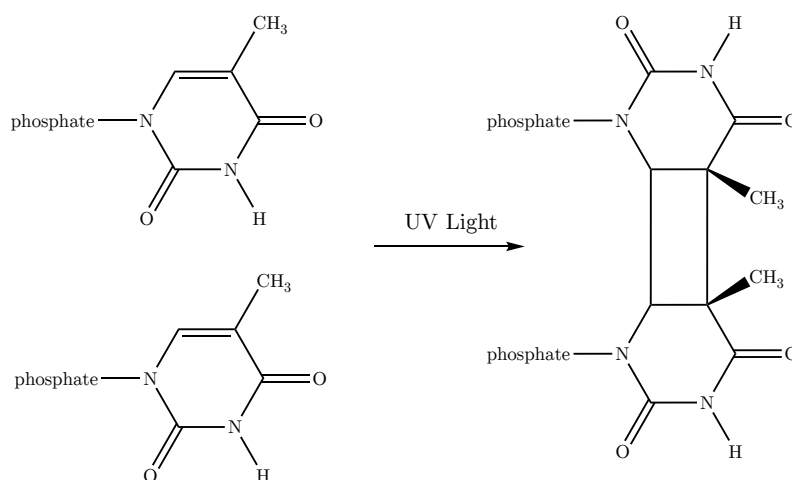


Figure 1.6: Chemical reaction describing the creation of a thymine dimer between two adjacent thymine bases and UV light.

cycloaddition which turns the two carbon-carbon double bonds into a square single bonded arrangement, see Fig. 1.6. This chemical alteration distorts the structure of the DNA and can hinder and potentially prevent transcription or replication occurring beyond the damage site.

For some eukaryotes and prokaryotes, this dimerization may be reversed via absorption of blue-light in a process called *photoreactivation*. An enzyme called *photolyase* is activated by the light and performs the repair. Unfortunately, this enzyme isn't used by placental mammals, where instead the damage is repaired by the far less efficient NER pathway. The reasons to why this enzyme mechanism has been lost in evolution has been discussed by Lucas-Lledó and Lynch [42] who propose weak natural selection as the cause.

1.3.3 Bubbles

DNA denaturation bubbles or simply *bubbles* refer to the process by which the double-stranded structure of DNA is disrupted, causing the two complementary strands to separate from each other. This is often referred to as *melting* the DNA. Bubble formation is used for transcription with a transcription bubble formed when a RNA polymerase enzyme binds to a promoter and causes two DNA strands to detach.

The process of DNA denaturation is initiated by the introduction of heat or some other denaturant, a change in pH or torsional stress. As the temperature rises or the concentration of denaturants increases, the hydrogen bonds that hold the two complementary strands of DNA together begin to break, creating bubbles. In this thesis we will mostly be concerned with bubble formation due to torsional stress. Bubble formation often occurs if a critical amount of under-twist is applied to the DNA. Bubbles are significantly easier to twist than regular DNA, with the enthalpic cost of breaking the hydrogen bonds being compensated by their ability to absorb twist.

1.3.4 Enzyme Search Problem

We have discussed the various repair mechanisms available to organisms in order to rectify defective DNA, but the natural question arises, how do the enzymes know where the damage is? This is the well-known *enzyme search problem* or *protein search problem*, a large unsolved problem in biology.

Naively, we could consider two diffusive location mechanisms. The first and most crude is three-dimensional (3D) diffusion, and the second is one-dimensional (1D) diffusion along the chain. Both of these methods, however, are simply too inefficient. If diffusion were the only mechanism involved, environmental and metabolic factors would damage the DNA faster than the cell could repair it, inevitably leading to cell death [43]. Mirny *et al.* [44] demonstrated that a combination of 1D and 3D diffusion can speed up the diffusion significantly. Much work has been done on the protein-DNA search problem but a great deal of it neglects the intricate structure of DNA in prokaryotes and eukaryotes.

Theoretical studies by Matek *et al.* [45], and Lim *et al.* [46] indicated bubbles and thymine dimers preferentially locate to the tip of plectonemic structures. It has hence been proposed that this fact could be utilised, perhaps in conjunction with diffusive methods, to greatly reduce search times.

1.4 Features of Thymine Dimer

We now outline the known structural and energetic effects that TDs have been observed to induce in experiments and simulation. Much has been done experimentally in the last 40 years including studies involving NMR and crystal structure experiments [47–49].

In contrast comparatively little has been done in regards to the simulation of TDs. An earlier example is the atomistic simulations by Miaskiewicz *et al.* [50], with more recent investigations coming from Knips and Zacharias [51, 52].

1.4.1 Induced Bending

It has been observed that the presence of a TD will naturally bend DNA; this was most notably seen in the electrophoretic mobility experiments of Husain *et al.* [47]. Husain *et al.* estimated a bending angle of approximately 30° for a 160 bp sequence with 5 TDs using a circularisation assay. This method however, fails to distinguish preferential bending from increased duplex flexibility. Preferential bending is the postulate that linear DNA with a TD will have some natural rest angle at which the DNA always bends. Increased flexibility is just the notion that the duplex will pay less energy for axial bending at the damage location, which is possible through the reduction in local elastic parameters. In reality it is possible that a TD will have a combination of these effects.

Wang and Taylor estimated an angle of roughly 7° [48] using nuclear magnetic resonance (NMR) experiments. Their method was based on calculating the ratio of the damaged and undamaged lengths of small poly(dA:dT) tracts, allowing the determination of the induced curvature due to the TD. Park *et al.* [53] later reported an angle of 30° toward the major groove through experiments yielding crystal structures of a linear duplex. Miaskiewicz reported a kink of approximately 10° from atomistic simulations [50]. With these values ranging to such an extent it is hard to determine a reliable value for this bending.

There seems to be little doubt that the TD increases the local average curvature of DNA, but it remains unclear if there is truly a biased direction in which the

duplex wishes to bend or whether it is simply more flexible in those regions and what the contribution of increased flexibility might be.

1.4.2 Structural Parameters

For short duplexes the evidence suggests that a TD remains in an intra-helical state and that the effect of the damage is limited only to the neighbouring bases. NMR experiments by Wenke *et al.* [54] and Rumora [55] *et al.* as well as crystal structures by Rumora *et al.* agree with this hypothesis.

Several studies provide details on the effect of TDs on the base-pair step parameters. Park *et al.* [53] report a roll of 22° and tilt of -17.8° and an unwinding of 9° for a TD containing duplex. The NMR structure of McAteer *et al.* [56] reports a reduction in tilt of 10° , an increase in roll of 22° and a reduction in twist of 15° for a damaged duplex relative to the native sequence. The atomistic simulations of Miaskiewicz [50] *et al.* displayed a large increase in roll and decreases in twist and tilt.

The large deviations in roll account for and support the idea that the duplex is naturally bent at the damage site as any axial bending can locally be decoupled into a contribution from rolling and tilting.

1.4.3 Melting Temperature Drop

Melting experiments of varying sequences indicate that the presence of a TD causes a drop in the melting temperature. NMR melting experiments [57] and UV melting experiments by Taylor *et al.* [49], McAteer *et al.* [58] and Barone *et al.* [59] all measure changes in enthalpy and entropy for the duplex and melted states. From this data one can compute the associated melting temperature, T_m , of each state from the formula

$$T_m = \frac{\Delta H}{R \ln(2c/x) + \Delta S}, \quad (1.3)$$

where $x = 1$ for self-complementary strands and $x = 4$ otherwise [60]. The predicted melting temperature drops for the various experiments at a salt concentration of

Experiment	Experiment Type	n_{bp}	ΔT_m (K) at 1 mM
Kemmink <i>et al.</i> [57]	NMR melting	8	12
Taylor <i>et al.</i> [49]	UV melting	10	8
Barone <i>et al.</i> [59]	UV melting	21	9.6
McAteer <i>et al.</i> [56]	UV melting	12	8

Table 1.1: Melting temperature drop for various experiments of varying sequence lengths (n_{bp}) calculated from Eq. 1.3 at a salt concentration of 1 mM. $\Delta T_m = T_{m,0} - T_{m,\text{TD}}$ where $T_{m,0}$ is the melting temperature of the native duplex and $T_{m,\text{TD}}$ is the melting temperature of the sequence containing the thymine dimer.

$c = 1$ mM are given in Table. 1.1. These experiments put the estimated melting temperature drop at 1 mM between 8 and 12 degrees.

This melting drop is likely in-part due to local weakening in hydrogen bonding as reported by McAteer *et al.* [56], Rumora *et al.* [55] and Wenke *et al.* [54].

1.5 Models

In this thesis we employ several models of significantly differing detail. All models have their strengths and weaknesses and often it will be important to consider what questions can be probed with what machinery. The more simplified models can be handled analytically while more detailed models require implementing numerically, with more complex models coming at a higher computational cost. Theoretical models can give us insight into systems and phenomena that are currently inaccessible to experiments.

In this thesis, we shall divide all theoretical models into three categories: *continuum*, *coarse-grained* and atomistic or *all-atom* models.

1.5.1 Continuum Models

At the lowest end of this spectrum of detail are continuum methods. These analytical models neglect most of the microscopic detail of DNA, usually modelling DNA as a homogeneous chain. These methods are often used to model systems much larger than those possible with molecular simulation due to their simplicity and computational efficiency. The most famous continuum model is that of the *worm-like chain* (WLC) [61]. In its most fundamental form it parametrises DNA simply

by its elastic parameters. This model was then expanded to deal with stretching forces [62], and later twisting forces [63].

Due to the inherently local nature of this formalism, electrostatics and self-avoidance are more challenging to implement in a WLC. Several models have also been developed to describe supercoiled structures such as plectonemes, rings and curls [64–66]. As well as modelling different configurations, additional energetic contributions from twist-bend coupling have also been incorporated [34].

Some efforts have been made to adapt the WLC for heterogeneous polymers with white noise heterogeneity [67–69]. Comparatively little work has been done on what we shall call *inhomogeneous* DNA, DNA with non-random stiffness fluctuations, with one study investigating the effect of forming a “kinked” loop configuration [70]. This is something we aim to address in this thesis. Finally some work has been done on incorporating twist-bend coupling to the WLC and its effect on loop energetics [71] and minicircles [33].

More general polymer models that include the twistable elastic polymer (TEP) [72] can also be applied to DNA. A rod-like model was devised to incorporate inhomogeneous DNA by Goyal [73], but again, little work seems to have been done in this field.

1.5.2 Coarse Grained Simulation

Offering a compromise between the computationally expensive all-atom description and the over-simplified continuum models, coarse-graining a system into simpler “chunks” which can be well described as single objects can often provide useful insight into systems not suitable for the other two descriptions. This level of coarse-graining depends on the size of these chunks, with more simplified coarse-grained models grouping together more components.

Notable coarse-grained models include the Santa-Lucia model [74] which is discretised at the base-pair step and is a free-energy model that can be used to predict the melting temperature of an oligonucleotide. Another model is the Benham model which predicts the denaturation probability of supercoiled duplexes [75, 76].

Another interesting model is the one devised by Peyrard and Bishop [77], which through a simple lattice model predicts denaturation probabilities.

Another popular model is oxDNA, a nucleotide level coarse-graining of DNA [78–80]. OxDNA will be the coarse-grained model of choice for this thesis. Methods of modelling thymine dimers within oxDNA have already been investigated by Lim *et al.* [46] and Randisi [81].

1.5.3 All-Atom Simulation

Atomistic or all-atom simulations represent some the more detailed methods available to theoretical molecular research. They have the ability to model individual atoms and their corresponding interactions meaning the scope for structural detail is relatively large. The most notable examples of all-atom software packages include Amber [82], GROMACS [83] and LAMMPS [84]. These all prescribe unique atoms a position and determine their interactions with other atoms according to a chosen molecular potential. This however, comes with a significant computational cost due to the relatively large number of particles.

The continual improvement in computation power coupled with the ever improving numerical efficiencies of computational algorithms have lead to the ability to simulate larger and larger systems for longer and longer times. Specifically the developments made in graphics processing units (GPUs) and parallel computing have allowed molecular simulators the ability to achieve time-scales and system sizes that were previously inaccessible.

The main theoretical limitation for all-atom simulations is the fact that full QM calculations are far too computationally expensive. This has lead to the development of semi-empirical methods which describes the forces experienced by atoms in molecular systems. This set of forces or *force field* (FF) acts as the central quantity in MD, with accurate determination of force field parameters crucial in order to achieve sensible dynamics. The most popular families of force fields for biological macromolecules include Amber [85], Charmm [86], Gromos [87] and OPLS [88]. The Amber force fields have recently diverged from parmbsc0 [89] into two

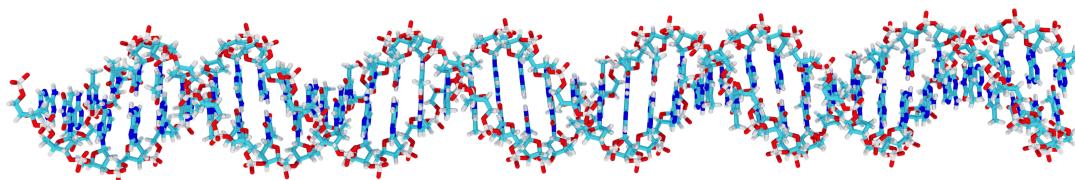


Figure 1.7: All-atom representation of a 40 bp A-tract of B-DNA.

streams, with the resultant force fields labelled OL15 [90] and bsc1 [91]. Both forks aim to specifically model B-DNA.

The development and improvement of atomistic force fields remains an active field of study. Since the inception of molecular mechanical methods, force fields have been created and used until they were shown to require improving. For example AMBER parm94-99 force fields were popular until severe artefacts were revealed when multi-nanosecond simulations of DNA became possible. This led to the development of parmbsc0, which improved upon its predecessors before too succumbing to inaccuracies after the microsecond time-scale.

1.6 Thesis Outline

The key questions we wish to address in this thesis are that of the equilibrium properties of inhomogeneous DNA, as well as the effects of thermal fluctuations on mean-field configurations.

Chapter 2 establishes the methods and models utilised in this work. First, we cover the basis of molecular simulation and molecular dynamics including descriptions of the oxDNA model and the Amber MD package. We then detail

the mathematical machinery required in this thesis which includes definitions of elliptic functions as well as some theory on differential operators. The rest of the chapter discusses the topology and geometry of DNA and how we calculate associated quantities for physical systems.

Chapter 3 outlines the atomistic methods and protocol as well as an all-atom thymine dimer model. This model is applied to linear and circular DNA with the structural parameters of DNA probed and compared with experiment. Duplex flexibility and curvature are also determined as well the simulation of a range of T-shaped structures called T-motifs.

Chapter 4 details the application of an oxDNA thymine dimer model to several open and closed systems. Duplex flexibility and curvature are investigated for linear DNA and circular supercoiled systems are simulated with twist and writhe partitioning studied for minicircles and their topoisomers. The correlation between bubble formation and writhe is also investigated.

Chapter 5 establishes the well-known worm-like chain model and its applications to DNA. Piece-wise models of damage are defined and their effects on chain energetics are calculated. A planar plectoneme model is also devised with the various damage models applied to the case of tip-localised damage. The twistable worm-like chain is also introduced and a fully three-dimensional plectoneme model is outlined and compared with theory and experiment.

Chapter 6 introduces the notion of a polymeric path integral and how this theory has been used to quantify the effect of thermal fluctuations on elastic minima. This theory is then extended to a ribbon-like model of DNA with the resulting fluctuation operator applied to straight and solenoidal systems recovering some well-known results. A systematic method of applying this theory is detailed and work is done on expanding the set of solvable systems to the non-trivial saddle point solutions of the twistable worm-like chain.

Finally, Chapter 7 reports the conclusions, summarising the thesis and discussing possible directions for future work.

2

Methods and Models

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2.1 Theory of Molecular Simulation

As discussed in Section 1.5, the ability to describe the physics of molecular systems coupled with the growing computational power readily available has led to the development of a wide-range of sophisticated numerical methods that can be used to gain insight into experimentally impenetrable areas. The most prevalent of such techniques include *molecular dynamics* and *Monte Carlo* simulations.

2.1.1 Molecular Dynamics

Molecular dynamics (MD) simulation is a powerful technique to compute thermodynamical and dynamical properties of molecules based on classical mechanics. If we can describe the potential energy of an individual particle in a molecule, we can compute the force it experiences and hence evolve the positions and velocities of the particle according to Newton's second law of motion. This propagation of the particles through space and time allow us to construct a *trajectory*. Assuming ergodicity, this trajectory can then be used to compute ensemble averages and observe predicted dynamics.

Consider a set of N particles whose total internal energy is V . The positions of the particles are given by $\{\mathbf{r}_i\}_{i=1,\dots,N}$ and the i^{th} particle has mass m_i . The force on particle i is

$$\mathbf{f}_i = -\nabla_{\mathbf{r}_i} V(\mathbf{r}_1, \dots, \mathbf{r}_N). \quad (2.1)$$

Newton's second law states

$$\mathbf{f}_i = m_i \frac{d^2 \mathbf{r}_i}{dt^2}. \quad (2.2)$$

Hence via equating Eq. 2.1 and Eq. 2.2, we may obtain the positions of every particle as a function of time via integration of the resulting differential equation.

Countless methods have been devised to numerically integrate the equations of motion of a system of interacting particles. Two popular examples of which are the Verlet [92] and velocity Verlet [93] integration schemes. These involve discretising

the time-variable into segments of length Δt , where Δt is the *time-step*. Since we choose Δt to be small, we may use Taylor expansions to determine the local evolution of the positions and/or velocities. For example, the Verlet algorithm states that

$$\mathbf{r}_i(t + \Delta t) \simeq 2\mathbf{r}_i(t) - \mathbf{r}_i(t - \Delta t) + \frac{\mathbf{f}_i(t)}{m_i} \Delta t^2 + \mathcal{O}(\Delta t^4). \quad (2.3)$$

Whereas the velocity Verlet in its simplest form reads

$$\mathbf{r}_i(t + \Delta t) \simeq \mathbf{r}_i(t) + \mathbf{v}_i(t) \Delta t + \frac{\mathbf{f}_i(t)}{2m_i} \Delta t^2 + \mathcal{O}(\Delta t^3), \quad (2.4)$$

$$\mathbf{v}_i(t + \Delta t) \simeq \mathbf{v}_i(t) + \frac{\mathbf{f}_i(t) + \mathbf{f}_i(t + \Delta t)}{2m_i} \Delta t + \mathcal{O}(\Delta t^3). \quad (2.5)$$

The velocity Verlet has the advantage that it is a “symplectic” algorithm, i.e. it is area preserving. This is desirable as classical mechanics formally demands this conservation of phase-space area due to Liouville’s theorem and so any reasonable approximation should maintain this condition. Furthermore, symplectic algorithms possess global stability.

Choosing a large time-step allows for the exploration of longer time scales. However, we must ensure that this time-step remains much smaller than the modes of the molecular simulation. For models where the fastest oscillatory modes are on the order of femtoseconds we need to balance having a small enough time-step to capture all physical phenomena, while not sacrificing computational efficiency. This can be challenging and so there have been algorithms such as SHAKE [94], which when used with the Verlet algorithm, restrains the fastest modes and solves the equations of motion for the slower modes. SHAKE achieves this by fixing the bond lengths rather than allowing them to oscillate. Whilst non-physical, it is thought that the suppressed fluctuations in bond lengths do not contribute to the longer time-scale dynamics of the system. Implementation of SHAKE requires a modification to the Verlet algorithm to incorporate the effect of these constraints. In this thesis SHAKE was only utilised for the all-atom simulations, constraining the bond lengths of the hydrogen atoms only.

Given the vast detail of many molecules and the associated high levels of computation power required to simulate them, it is often advantageous to devise

a simplified model that captures the inherent physics of a system. One method of achieving this is by grouping particles together that often evolve approximately as one cluster. This group is then treated as an individual particle in the new representation. This notion is called *coarse-graining* and we discuss this more at detail in Section 2.3 when discussing the oxDNA model.

2.1.2 Thermostats and Boundary Conditions

As the above MD algorithm is simulating the classical mechanical equations of motion in the absence of an external force, it does not produce the NVT ensemble, allowing only access to a micro-canonical ensemble (NVE). A method to achieve constant temperature was proposed through the creation of a *thermostat*. A thermostat is a method of controlling the temperature of MD, the first of which proposed was the Nosé-Hoover thermostat [95] but many more thermostats have since been created. In this thesis we make use of two thermostats: the Langevin thermostat and the Berendsen thermostat. The Langevin thermostat introduces thermal agitations in the form of stochastic white noise whose average properties ensure the system maintains its temperature. Specifically we introduce two new terms to Eq. 2.6. Now, Newton's second law reads

$$\mathbf{f}_i - \mathbf{\Gamma}_{ij} \frac{d\mathbf{r}_j}{dt} + \eta_i = m_i \frac{d^2\mathbf{r}_i}{dt^2}. \quad (2.6)$$

The first additional contribution is the damping term which dissipates energy via its coupling with particle velocity. $\mathbf{\Gamma}_{ij}$ is the drag tensor which when contracted with the particle velocity determines the drag on each individual particle. This drag tensor is the inverse of the mobility tensor, which is the preferred object in the literature. The second new term is η_i which is the stochastic white noise. We demand that this noise has zero mean and decorrelates instantly in time and with particle index. The equipartition theorem then allows us to compute the noise autocorrelation:

$$\langle \eta_i \rangle = 0, \quad \langle \eta_i(t) \otimes \eta_j(t') \rangle = 2k_B T \mathbf{\Gamma}_{ij} \delta_{ij} \delta(t - t'). \quad (2.7)$$

When used in this way the drag term mimics the effects of solvent viscosity and the noise term mimics momentum transfer from collisions with solvent molecules,

i.e. it allows us to capture solvent effects *implicitly*. This is an advantage as it does not add to the number of particles that need to be simulated. There are several choices for mobility tensor including the Oseen [96] and Rotne-Prager [97] tensors, however, they are still relatively expensive as they incorporate long-range interactions, i.e. each particle is subjected to the hydrodynamic flow of the other $N - 1$ particles. For this reason it is often preferable to simply use a diagonal drag tensor which assumes each particle experiences a linear Stokes' drag.

Physically well justified drag coefficients often over-damp the system resulting in slow-dynamics and so it is common to favour a smaller drag coefficient to effectively speed up the dynamics and explore more of phase-space. This makes the already difficult task of determining the physical time to which a simulation trajectory corresponds even more opaque. While the crude method of computing the total time as the number of steps multiplied by the time-step is still available to us, it will often not be truly reflective of the effective time-scales simulated. In this thesis, the total *simulation time* will be given by Eq. 2.8

$$t_{\text{total}} = N_{\text{steps}} \Delta t. \quad (2.8)$$

If physical times need to be extracted then a proper time-scale mapping must be made. This often involves comparison with some easily predicted or well-known time-scale such as diffusion. To map the diffusive time scale to real-world time scales, one must compare the simulation results to experimental measurements or theoretical predictions for similar systems.

For explicit solvent methods, a common way to maintain a system's temperature is through rescaling the velocities. An example of a thermostat which employs this method is the Berendsen thermostat [98]. The previously mentioned Nosé-Hoover thermostat instead uses an imaginary particle that couples to the system to replicate a heat bath.

MD simulations attempt to mimic a physiological molecular environment with a finite number of particles. A major issue is that the explicit modelling of the solvent molecules can quickly lead to a computationally intractable number of particles

that need to be simulated in order to replicate a realistic system. Therefore we must limit the spatial extent of our simulations to make them feasible. This can be achieved through putting our system inside a simulation box. Once spatially constrained, our system has the possibility to diffuse to edge of said box and hence, we must choose how we should deal with these scenarios.

We can choose for the edge of the box to be “wall like” which means that atoms will simply bounce back off the edge of the box. However, we can also choose for particles to leave the box. The initial issue with the latter choice is that we still want to simulate these “escaped” particles. The most sensible way to achieve this is to use *periodic boundary conditions* (PBCs). Very simply, if a particle leaves the simulation box, it will reappear on the opposite side of the box. The box itself need not be cubic but does need to tile in three dimensions. This rules out the use of a spherical unit cell, however, truncated octahedral and rhombic dodecahedral boxes are popular alternatives. An example of a system with PBCs in a truncated octahedral simulation box and how it fills 3D space is shown in Fig. 2.1.

A consequence of PBCs is that there arise situations in which certain particles interact with themselves, or with several copies of another particle. Hence, the choice of PBCs is normally accompanied with the use of an image convention so that we may deal with these scenarios. For example, in the the minimum image convention, a particle only experiences at most just one image of every other particle in the system. In practice it is beneficial to choose a box as small as possible without the possibility of the largest molecule (in our case usually the DNA) interacting with itself via the PBCs in order to reduce the computational effort spent simulating the solvent.

2.1.3 Force Fields

Bar some practical caveats which we have already discussed, the inherent complexity of the problem remains that if we may describe the forces experienced by a particle in a system, we can compute its evolution through time and space. This, however, presents another monumental challenge, namely accurately determining these forces. In practice the contributions to the potential energy of the system are first defined

Truncated Octahedral Simulation Box

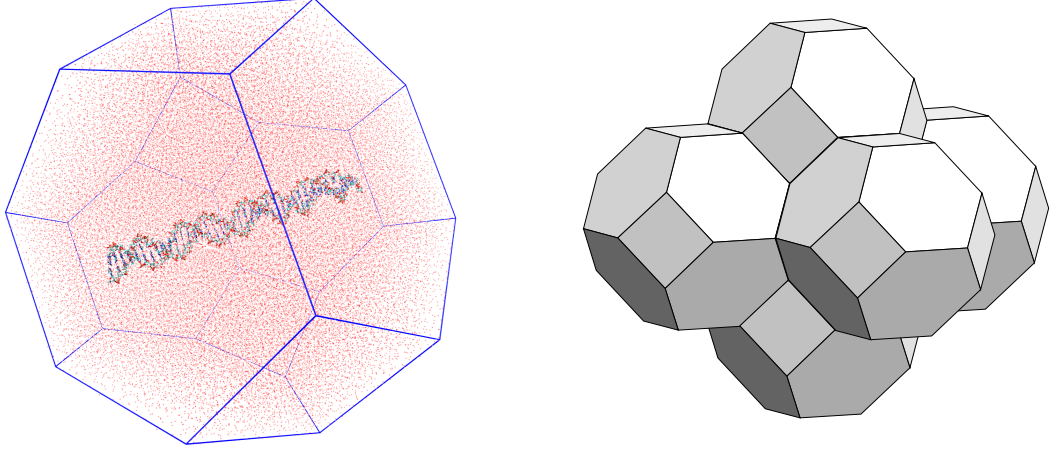


Figure 2.1: Left: An example of a truncated octahedral simulation box for a 40 bp poly(dA:dT) tract. Right: A schematic showing the 3D tessellation property of the truncated octahedron.

and then its derivatives calculated to obtain the forces. The collection of these potential energy functions and their coefficients is called the force field. The quality of the force field will directly contribute to the accuracy of the simulated results.

The creation of a molecular force field is a highly non-trivial procedure with the continuous improvement and creation of new force fields still a major field of research. Most force fields used in biomolecular simulations employ a semi-empirical approach by choosing physically motivated potentials with free parameters for each interaction and fitting these parameters to reproduce experimental data.

We may decompose the potential energy V as the sum of the potential energy of all bonded interactions and that of all non-bonded interactions between particles, i.e.

$$V = \sum V_{\text{bonded}} + \sum V_{\text{non-bonded}}. \quad (2.9)$$

The bonded potential may be split further into three contributions: bond stretching, bending at an angle, and rotation about bonds (dihedral angle). The bond stretching

and bending terms are often represented by simple harmonic forms:

$$V_{\text{bond}} = \sum_{\text{bonds}} \frac{k_{\text{bond}}}{2} \|\mathbf{r} - \mathbf{r}_0\|^2 \quad (2.10)$$

$$V_{\text{angles}} = \sum_{\text{angles}} \frac{k_{\text{angle}}}{2} (\theta - \theta_0)^2, \quad (2.11)$$

where $\mathbf{r}_0$ and θ_0 are equilibrium values. The dihedral potential, however, is usually a sum of cosines

$$V_{\text{dihedral}} = \sum_{\text{torsions}} \sum_n \frac{V_{\phi,n}}{2} [1 + \cos(n\phi - \zeta_n)], \quad (2.12)$$

where ζ_n is the phase angle. The phases are usually constrained at 0° or 180° so that the energy surface of achiral molecules is symmetric and so that enantiomers have the same energetic properties.

The non-bonded interactions are often represented as the sum of the potential energies of electrostatic (attractive and repulsive) forces, van der Waals (vdW) forces and steric repulsion. Electrostatic interactions can be defined by the the Coulombic potential, i.e.

$$V_{\text{Coulomb}} = \sum_{ij} \frac{q_i q_j}{4\pi\epsilon_0\epsilon_r r_{ij}}. \quad (2.13)$$

where q_i is the partial charge on particle i and $r_{ij} = \|\mathbf{r}_i - \mathbf{r}_j\|$ is the distance between particles i and j , ϵ_0 is the permittivity of free space and ϵ_r is the relative permittivity or dielectric constant of the medium. Van der Waals and steric forces are often modelled through the Lennard-Jones (LJ) potential, which is of the form

$$V_{\text{LJ}} = 4 \sum_{ij} \epsilon_{ij} \left[\left(\frac{\sigma}{r_{ij}} \right)^{12} - \left(\frac{\sigma}{r_{ij}} \right)^6 \right], \quad (2.14)$$

where ϵ_{ij} is the size of the well depth, and σ is the distance between the particles when the potential energy is zero. This functional form is chosen as it is repulsive at short distances and attractive at long distances. When only repulsions need to be represented, the Weeks-Chandler-Anderson (WCA) potential is a popular choice. The WCA potential is the Lennard-Jones potential shifted upwards and truncated at the LJ potential minimum [99].

Electrostatics cause more computational problems as they are long-range in character and when implemented naively, their computation scales quadratically with the number of charged particles. One could impose a radial cut-off at which we throw away all contributions from outside this radius, however, this then introduces significant inaccuracies. A method for circumventing this problem was devised by Ewald [100]. His suggestion was to partition the interaction potential into two terms: a short-range contribution and a long-range contribution, i.e. $\varphi(\mathbf{r}) = \varphi_{\text{short}}(\mathbf{r}) + \varphi_{\text{long}}(\mathbf{r})$. If the total potential is

$$E = \sum_{ij} \varphi(\mathbf{r}_i - \mathbf{r}_j) \quad (2.15)$$

then if we replace the long-range potential with its Fourier representation, then it follows that

$$E = \sum_{ij} \varphi_{\text{short}}(\mathbf{r}_i - \mathbf{r}_j) + \sum_{\mathbf{k}} \tilde{\Phi}_{\text{long}}(\mathbf{k}) |\tilde{\rho}(\mathbf{k})|^2, \quad (2.16)$$

where $\tilde{\Phi}_{\text{long}}$ and $\tilde{\rho}$ are the Fourier transform of the long-ranged potential and charge density respectively. The motivation behind this partitioning is that the short-range and long-range contributions now converge quickly in their respective spaces. The use of the Fourier transform requires the assumption that our system be periodic but this will be a common assumption with the use of PBCs as previously mentioned. The speed of the Fast Fourier Transform (FFT) renders this method much faster than persisting with real space calculations, with the method scaling as $N \log N$. The use of this method in MD is called Particle Mesh Ewald Molecular Dynamics (PMEMD).

2.1.4 Solvent Models

Finally, we must consider how we will model the water in our system. We have already mentioned that we may model our solvent implicitly via a Langevin thermostat but this alone neglects the electrostatic effects of water, hence, it is common to model water as a high dielectric region and include additional apolar solvation terms. Although explicit solvent is a more physically rigorous representation, implicit solvent models have the advantage of dramatically reducing

the degrees of freedom that must be sampled by eliminating those associated with the solvent.

The Generalised Born (GB) model is a computational method used in molecular dynamics simulations to approximate the electrostatic interactions between charged atoms in a system. The GB model is based on the Born solvation energy equation which relates the solvation energy of a molecule to the surface area of its solvent-accessible surface and the dielectric constant of the solvent. In the GB model, this relationship is generalised to account for the complex, non-linear dielectric response of water. This is achieved by dividing the solvent into regions of different dielectric constants, with each region corresponding to a different level of hydration.

In a GB simulation, the electrostatic interactions between the charged atoms in the solute are calculated using a combination of the Coulombic equation and a solvation energy term that accounts for the dielectric properties of the water. The solvation energy term is calculated using a set of empirical parameters that are fitted to experimental data. Though significantly less computationally expensive, the GB solvent model is far less accurate for energy calculations.

If we do wish to model the solvent explicitly then this will involve including all the solvent atoms in our force fields calculations. However, there are several choices as to how many interaction sites to include. Three-site models have three interaction points corresponding to the three atoms of the water molecule. Each site has a point charge, and the site corresponding to the oxygen atom also has a Lennard-Jones interaction. Since 3-site models achieve a high computational efficiency, these are widely used for many applications of molecular dynamics simulations, the most commonly used of which is TIP3P [101]. Most of the models use a rigid geometry matching that of actual water molecules. An exception is the SPC model [102], which assumes an ideal tetrahedral shape (HOH angle of 109.47°) instead of the observed angle of 104.5° . The four-point TIP4P rigid water model extends the TIP3P model by adding an additional massless site where the charge associated with the oxygen atom is placed. Models with 5 [103] and 6 [104] sites have also been developed.

The computational cost of a water simulation increases with the number of interaction sites in the water model, the run time being approximately proportional to the number of inter-atomic distances that need to be computed.

2.2 Amber All-atom Simulations

In this thesis we use the Amber Molecular Dynamics program when simulating systems in the all-atom representation. This program is contained within AmberTools, a set of auxiliary programs designed to work with the Amber engine. This is not to be confused with the Amber force field. Essentially Amber is contained within AmberTools with Amber being the core MD package and AmberTools also containing the packages for configuration and topology creation as well as trajectory processing. The standard file format for all-atom simulations is the Protein Data Bank (PDB) format, however, this file format is not storage efficient and so Amber style formats exist which reduce the size of these files through the inclusion of a topology file. In atomistic simulations specific clusters of atoms can be referred to as *residues*. The standard amino acids and nucleotides are common examples of residues. For example, the four DNA nucleotides are called DA, DT, DG and DC in PDB format, where DN is the residue associated with the base N.

Algorithmically, Amber’s molecular dynamics programs effectively use a leapfrog like integrator and has two primary MD programs, **sander** and **pmemd**. **sander** can perform minimizations and regular MD. **pmemd** is a version of **sander** that utilises the Particle Mesh Ewald method and is optimised for speed and for parallel scaling.

In this thesis we use the parmbsc1 [91] force field - a force field specifically designed to model nucleic acids. Parmbsc1 is parametrised using over 100 systems and approximately 140 μ s of total simulation time. The force field model builds upon its predecessor parmbsc0 [89] and uses quantum mechanical (QM) calculations for its fitting. Parmbsc0 itself is an update of the AMBER-parm99 force field. Parmbsc1 focuses on parametrising backbone degrees of freedom such as sugar puckering, glycosidic torsion and other torsional parameters. These parametrisations were

done from high-level QM calculations and optimised with respect to its previous parmbse0 value where required.

We opt for the TIP3P water model for explicit solvent simulations as TIP4P is too expensive in order to access the time-scales we wish to probe. When performing implicit solvent simulations we use the GB model, specifically we use a modification of the GB model which further improves agreement between Poisson-Boltzmann and explicit solvent data compared to the original formulation (GB-Neck2 [105]). We also note that the GPU versions of `sander` and `pmemd` do not support an electrostatic cut-off for non-periodic systems.

All atomistic simulations were visualised with Visual Molecular Dynamics (VMD) [106]. All renders were performed with the VMD Tachyon ray-tracer [107], such as Fig. 3.1 or in Blender after the conversion to a wave-front object such as Fig. 1.7.

2.3 OxDNA

As mentioned in Section 1.5.2, *oxDNA* is a nucleotide-level coarse grained model of DNA. *OxDNA* has been used to model a vast array of systems, proving ideal for modelling large structures related to nanotechnology [108–112], as well as biologically relevant systems [45, 46].

Each nucleotide is modelled as a rigid body with three interaction sites: the backbone site, stacking site and hydrogen bonding site. All interactions are pairwise with the pairs depending on the interaction type, see Fig. 2.2.

The electrostatics are modelled via a Debye-Hückel interaction. The interaction potential takes the following form:

$$V_{\text{oxDNA2}} = \sum_{\text{nn}} (V_{\text{backbone}} + V_{\text{stack}} + V_{\text{exc}}) \quad (2.17)$$

$$+ \sum_{\text{other}} (V_{\text{hb}} + V_{\text{cross stack}} + V_{\text{exc}} + V_{\text{coax stack}} + V_{\text{DH}}), \quad (2.18)$$

where nn denotes a sum over nearest neighbours, hb denotes hydrogen bonding, exc is excluded volume, stack is short for stacking and coax is short for coaxial. The functional forms of these interactions include: finitely extensible non-linear elastic (FENE) springs for backbone connectivity, Morse potentials for hydrogen

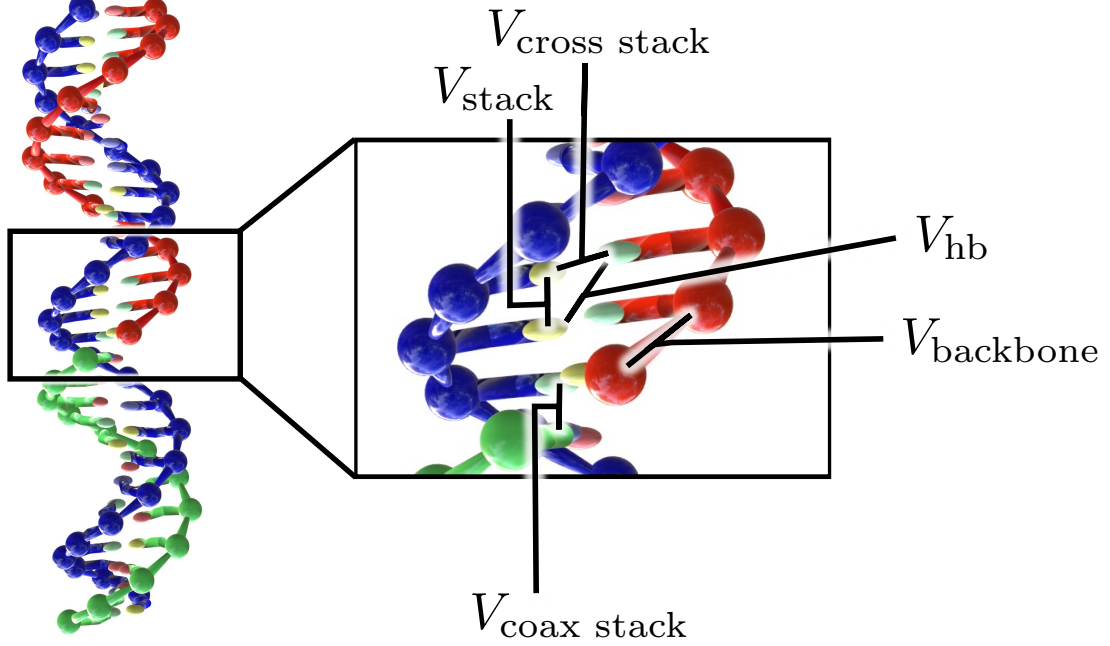


Figure 2.2: Outline of the oxDNA model and interaction sites.

bonding and stacking, harmonic springs for coaxial and cross stacking and a Lennard-Jones potential for soft repulsion. Attractive terms are modulated by angular factors to capture the dependence of these interactions on the relative orientations of the nucleotides.

This top-down approach was fitted to reproduce the geometry of B-DNA and thermodynamics of several systems including 14 base-oligomers [113], duplex hybridisation and hairpin formation [74], with stiffnesses tuned to reproduce DNA persistence lengths. The earliest version of oxDNA was sequence independent and did not display major and minor grooving, however, the latest oxDNA model, sometimes called oxDNA2, includes sequence dependence, solvent salt-concentration and groove asymmetry. Only oxDNA2 is used in this thesis and the term oxDNA will implicitly refer to oxDNA2.

OxDNA contains an MD option which utilises a version of the Davidchack quaternion integrator [114] and has a GPU implementation. Monte Carlo (MC) and Virtual Move Monte Carlo (VMMC) methods are also available with oxDNA as well as standard minimization algorithms such as steepest descent. For molecular

1 Simulation Unit	Physical Unit
Length	0.8518 nm
Temperature	3000 K
Mass	5.24×10^{-25} kg
Force	48.63 pN
Time	3.03 ps

Table 2.1: Unit conversions for oxDNA simulation units.

dynamics the solvent is modelled implicitly and an array of thermostats are available to maintain temperature including an Andersen-like, Berendsen-like and Langevin thermostat. The implementation of the Langevin thermostat uses a simple linear Stokes' drag term applied to each nucleotide assuming they are spherical in shape. It is worth noting that choosing a physically accurate linear drag term leads to prohibitively slowed dynamics. Hence, a relatively small drag term is chosen that accelerates the dynamics of the system while remaining in the over-damped regime [115].

All linear and circular oxDNA configurations and topologies were created via oxDNA utils, a series of Python packages which come with the oxDNA package. More complicated structures can be produced via an in-house Python package, and oxDNA files can also be created via conversion from other formats such as PDB via tacoxDNA [116].

Finally, all visualisations of oxDNA were performed with either Colgi2, oxView [117] or Blender. A custom Python script that utilises the Blender python API allows for high quality rendering of duplex DNA with bent nucleotides (see Fig. 2.2).

2.4 Mathematical Methods

In this section we give an overview of the relevant mathematical objects and techniques we will need in this thesis.

2.4.1 Elliptic Integrals and Functions

We briefly summarise the elliptic integrals and functions present in this thesis and the properties they have. We define the incomplete elliptic integrals of the

first, second and third kind to be

$$F(\varphi, k) = \int_0^\varphi \frac{d\theta}{\sqrt{1 - k^2 \sin^2 \theta}}, \quad (2.19)$$

$$E(\varphi, k) = \int_0^\varphi d\theta \sqrt{1 - k^2 \sin^2 \theta}, \quad (2.20)$$

$$\Pi(n, \varphi, k) = \int_0^\varphi \frac{1}{1 - n \sin^2 \theta} \frac{d\theta}{\sqrt{1 - k^2 \sin^2 \theta}} \quad (2.21)$$

respectively, where k is the elliptic modulus. Note that there exist equivalent formulations where all integrals are defined in regards to some elliptic parameter m , where $m = k^2$. For example, both SciPy and Mathematica adopt the m convention, however, most of the literature favours the use of k . These integrals are said to be *complete* when $\varphi = \pi/2$ and so we define the complete elliptic integrals of the first and second kind to be

$$K(k) = \int_0^{\pi/2} \frac{d\theta}{\sqrt{1 - k^2 \sin^2 \theta}}, \quad (2.22)$$

$$E(k) = \int_0^{\pi/2} d\theta \sqrt{1 - k^2 \sin^2 \theta} \quad (2.23)$$

$$\Pi(n, k) = \int_0^{\pi/2} \frac{1}{1 - n \sin^2 \theta} \frac{d\theta}{\sqrt{1 - k^2 \sin^2 \theta}} \quad (2.24)$$

Next we define the Jacobi amplitude as the function am that satisfies the equation

$$u = \int_0^{\text{am}(u, k)} \frac{d\theta}{\sqrt{1 - k^2 \sin^2 \theta}}. \quad (2.25)$$

I.e. the Jacobi amplitude is the inverse of the incomplete elliptic function of the first kind, $F(\text{am}(u, k), k) = u$. From here we may define an entire class of functions known as the Jacobi elliptic functions. The three most fundamental are

$$\text{sn}(u, k) = \sin(\text{am}(u, k)) \quad (2.26)$$

$$\text{cn}(u, k) = \cos(\text{am}(u, k)), \quad (2.27)$$

$$\text{dn}(u, k) = \frac{d}{du} \text{am}(u, k). \quad (2.28)$$

These three functions are related via identities $\text{sn}^2 + \text{cn}^2 = 1$ and $\text{dn}^2 + k^2 \text{sn}^2 = 1$, where we have suppressed the arguments of the elliptic functions for brevity. We can then define many other elliptic functions as quotients of the three primary functions

Periods	z -zeros			
	0	K	$K + iK'$	iK'
$4K, 2iK'$	sn	cd	dc	ns
$4K, 2K + 2iK'$	sd	cn	ds	nc
$4K, 4iK'$	sc	cs	dn	nd

Table 2.2: Periods and zeros for Jacobi elliptic functions.

where the new name is constructed by taking to the first letter of the numerator followed by the first letter of the denominator. For example we may define

$$\text{cd}(u, k) = \frac{\text{cn}(u, k)}{\text{dn}(u, k)}. \quad (2.29)$$

All the Jacobi elliptic functions are periodic in $\mathbb{R}$ and doubly-periodic in $\mathbb{C}$. A table summarising the zeros and periods of the elliptic functions are in Table 2.2. The following reciprocal modulus identities will prove useful:

$$kF(\phi, k) = F\left(\arcsin(k \sin \phi), \frac{1}{k}\right) \quad (2.30)$$

$$E(\phi, k) = kE\left(\arcsin(k \sin \phi), \frac{1}{k}\right) - \left(1 - \frac{1}{k^2}\right)F(\phi, k). \quad (2.31)$$

2.4.2 Differential Operators and Spectral Theory

In this thesis we will require some results pertaining to differential operators and their spectra. Hence, it will be useful to establish several definitions and known results. We note that spectral theory is a broad and rich field in its own right and we will not always define things with the same rigour in which mathematicians do. We define a differential operator to be an operator comprised of either derivatives or partial derivatives. For example a differential operator of order m which acts on function space of n variables $\{x_i\}_{i=1, \dots, n}$ is of the form

$$\hat{T} = \sum_{|\alpha| \leq m} a_\alpha(x_1, \dots, x_n) D^\alpha, \quad (2.32)$$

where $\alpha = (\alpha_1, \dots, \alpha_n)$ is a multi-index of non-negative integers, $|\alpha| = \alpha_1 + \dots + \alpha_n$ and a_α is a function whose domain is n -dimensional. The operator D^α is

$$D^\alpha = \frac{\partial^{|\alpha|}}{\partial x_1^{\alpha_1} \partial x_2^{\alpha_2} \dots \partial x_n^{\alpha_n}}. \quad (2.33)$$

For example the three-dimensional scalar Laplacian defined by

$$\Delta = \frac{\partial^2}{\partial x^2} + \frac{\partial^2}{\partial y^2} + \frac{\partial^2}{\partial z^2}, \quad (2.34)$$

is a differential operator that acts on functions $f : \mathbb{R}^3 \mapsto \mathbb{R}$ which are at least-twice differentiable. We further define a matrix-differential operator to be a matrix whose elements are scalar differential operators, e.g.

$$\hat{\mathbf{T}} = \begin{pmatrix} -\partial_x^2 & xy \\ xy & -\partial_y^2 \end{pmatrix} \quad (2.35)$$

is a 2×2 matrix differential operator which acts on 2-vectors whose components are functions $f : \mathbb{R}^2 \mapsto \mathbb{R}$. If we apply this operator to the vector $(\sin x \ x^2 y)^\top$, then

$$\begin{pmatrix} -\partial_x^2 & xy \\ xy & -\partial_y^2 \end{pmatrix} \begin{pmatrix} \sin x \\ x^2 y \end{pmatrix} = \begin{pmatrix} -\sin x + x^3 y^2 \\ xy \sin x \end{pmatrix}. \quad (2.36)$$

Equating the action of a differential operator on an object with some other object will trivially yield a differential equation. If we take the operator from Eq. 2.35, then the equation $\hat{\mathbf{T}}\mathbf{y} = 0$ will yield a 2×2 coupled system of second order partial differential equations (PDEs). As with most problems involving differential equations, in this thesis we will often have a differential equation that is subject to boundary conditions (BCs).

Given how we may cast differential operators into ordinary differential equations (ODEs) and PDEs via the action on some function, we may then define the eigenvalue problem for a differential operator. Consider a differential operator $\hat{T}$, then if $\exists y$ such that

$$\hat{T}y = \lambda y, \quad (2.37)$$

then y is an eigenfunction of $\hat{T}$ and λ the associated eigenvalue. For a scalar second order ODE, we can expect 2 independent solutions. For a 2×2 system of first order ODEs, we also expect 2 linearly independent solutions. The number of independent solutions (up to degeneracy) goes as the product of the spatial dimension of the operator and the order of the elements of the operator. E.g. for a 2×2 system of second order ODEs, we expect 4 independent solutions.

Loosely speaking the collection of eigenvalues $\{\lambda_k\}$, with bounded eigenfunctions of a differential operator is known as its *point spectrum* and does not have a dense domain. However, there also exist unbounded solutions where $(\hat{T} - \lambda\mathbb{I})$ is singular. Essentially, if a sequence of functions $\{y_n\}_{n \in \mathbb{N}}$ is unbounded for large n , then if the operator $(\hat{T} - \lambda\mathbb{I})$ takes the normalised vector $y_n / \|y_n\|$ arbitrarily close to 0 for large n , then λ is a member of the *continuous spectrum* of $\hat{T}$. Together with a third set called the *residual spectrum*, we obtain the spectrum of a differential operator as a union of these three sets.

If we equip our operator with some inner-product $\langle \cdot, \cdot \rangle$, then we may also define a *self-adjoint* or Hermitian differential operator as we can for regular matrices, i.e. $\langle \hat{T}x, y \rangle = \langle x, \hat{T}y \rangle$. For example, we may use the L^2 inner-product defined as

$$\langle f, g \rangle_{L^2} = \int_X f \bar{g} d\mu, \quad (2.38)$$

where f and g are functions on some space X with measure μ , and $\bar{g}$ indicates the conjugate of g . If f and g are complex-valued vectors, then we would define our inner-product as

$$\langle f, g \rangle = \int_X f g^\dagger d\mu, \quad (2.39)$$

where $\dagger$ denotes the Hermitian conjugate. Similarly to matrices, the self-adjointness of a differential operator ensures that its eigenvalues are real and can be ordered such that $\lambda_1 < \lambda_2 < \dots \rightarrow \infty$. As it turns out for self-adjoint operators, there is no residual spectrum.

Finally we must briefly describe how we perform operator algebra. As with regular operators, differential operators act right-wards. This is not important in cases where the operators commute e.g. ∂_x and ∂_y trivially commute on functions that are sufficiently smooth in both variable through Clairaut's theorem. There are, however, many examples where this is not the case. The most famous example is that of the position and momentum operators in QM. $\hat{x} = x$, $\hat{p} = -i\hbar\partial_x$ and we can compute their commutator via their action on some wavefunction ψ . From

this we may determine that $[x, -i\hbar\partial_x] = i\hbar$. The commutators we will need in this thesis include those involving ∂_s , ∂_s^2 and $f = f(s)$.

$$[\partial_s, f] = \frac{\partial f}{\partial s} \quad (2.40)$$

$$[\partial_s^2, f] = 2\frac{\partial f}{\partial s}\partial_s + \frac{\partial^2 f}{\partial s^2}. \quad (2.41)$$

We note that if f is a constant then both commutators vanish.

2.5 Topology and Geometry

In this thesis we will utilise results from differential geometry and topology pertaining to three key quantities: Twist (Tw), Writhe (Wr) and Linking Number (Lk). We will define each quantity and comment on what objects they are well-defined for.

Rigorously, we shall refer to 1D lines in 2D space as *plane-curves*, 1D lines in 3D space as *space-curves*. A *ribbon* may be defined as (X, U) where X is a space-curve which defines the centre-line of the ribbon, and U is the unit normal, hence, describing a 2D surface embedded in 3D space. A simple geometric property of space-curves that we utilise in this thesis is curvature. The curvature is defined as the magnitude of the rate of change of the unit tangent with respect to arc-length, i.e.

$$\kappa = \left\| \partial_s \hat{\mathbf{t}} \right\|. \quad (2.42)$$

2.5.1 Twist

Twist is a measure of the winding of a ribbon's normal around its centre line. From this we may define the twist as

$$\text{Tw} = \frac{1}{2\pi} \int ds \left(\frac{dU}{ds} \times U \right) \cdot \frac{dX}{ds}, \quad (2.43)$$

where dX/ds is the unit tangent to X . We note that this definition is local, i.e. the twist is simply a sum of contributions of a local twist density.

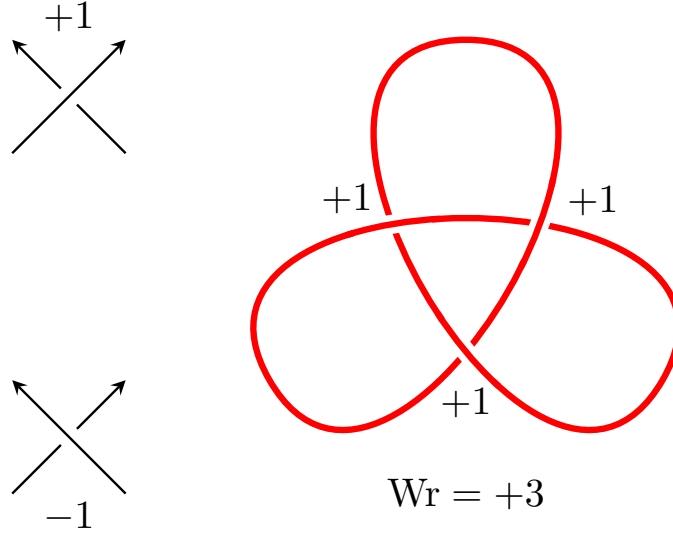


Figure 2.3: Convention for signed crossings for the calculation of the writhe of a link diagram. An example of the right-handed trefoil knot with $Wr = 3$ is given.

2.5.2 Writhe

Writhe is inherently defined for one-dimensional objects, although these curves can be embedded in different spaces which will affect the subsequent definitions. The most basic form of writhe is the writhe of an oriented link diagram. A link diagram is simply a collection of planar knots. The writhe is then defined as the total number of positive crossings minus the total number of negative crossings. The sign convention for which is given in Fig. 2.3.

Extending this notion to that of a space-curve, by taking a projection of said curve into the plane one can calculate the writhe of the resulting link diagram. However, this is just the writhe with respect to one vantage point. Following this procedure and averaging over all perspectives allows us to rigorously define the writhe of a closed space curve:

$$Wr = \frac{1}{4\pi} \int_C \int_C d\mathbf{r}_1 \times d\mathbf{r}_2 \cdot \frac{\mathbf{r}_1 - \mathbf{r}_2}{\|\mathbf{r}_1 - \mathbf{r}_2\|^3}, \quad (2.44)$$

where C is a closed-curve. This formula was first devised by Călugăreanu [118]. If we then parametrise the curve via its arc-length s , then

$$Wr = \frac{1}{4\pi} \int_0^L ds \int_0^L ds' \hat{\mathbf{t}}(s) \times \hat{\mathbf{t}}(s') \cdot \frac{\mathbf{r}(s) - \mathbf{r}(s')}{\|\mathbf{r}(s) - \mathbf{r}(s')\|^3}, \quad (2.45)$$

where L is the length of the curve and $\hat{\mathbf{t}}$ is unit tangent to C .

Unlike twist, this formula is clearly non-local and we note that Eq. 2.45 will diverge for curves which self-intersect. This can make it a non-trivial quantity to compute. Fuller, however, devised a local variant of writhe which relates the writhe of a space-curve to the writhe of another curve from which it can be continuously deformed from [119]. Fuller's formula for the writhe is

$$\text{Wr} = \text{Wr}_r + \frac{1}{2\pi} \int_0^L ds \frac{\hat{\mathbf{t}}_r \times \hat{\mathbf{t}}}{1 + \hat{\mathbf{t}}_r \cdot \hat{\mathbf{t}}} \cdot \frac{d(\hat{\mathbf{t}}_r + \hat{\mathbf{t}})}{ds}, \quad (2.46)$$

where $\hat{\mathbf{t}}$ is the unit tangent to the curve, $\hat{\mathbf{t}}_r$ is the unit tangent to the reference curve and Wr_r is the writhe of the reference curve. Another method of determining writhe is to compute the surface area enclosed by the tangent-indicatrix mapped onto the unit sphere [119], however, these formulae are only correct modulo an integer and we do not make use of them in this thesis.

2.5.3 Linking Number

The Linking number is a measure of the number of times two space-curves wrap around each other. Given this counting-like definition, $\text{Lk} \in \mathbb{Z}$ for closed curves. It can also be defined via a double integral, if we let the two space curves be γ_1 and γ_2 , then the linking number is

$$\text{Lk} = \frac{1}{4\pi} \int_{\gamma_1} \int_{\gamma_2} d\mathbf{r}_1 \times d\mathbf{r}_2 \cdot \frac{\mathbf{r}_1 - \mathbf{r}_2}{\|\mathbf{r}_1 - \mathbf{r}_2\|^3}. \quad (2.47)$$

Like writhe, the linking number is also a non-local quantity and in fact proves an even harder quantity to calculate. The quantities described thus far can be split into two groups: twist and writhe which are geometric objects and Lk which is a topological object. That is to say that Tw and Wr may change upon some deformation which preserves the topology of the system, whereas Lk is invariant under any such change. We also note that the formulae for linking number and writhe are very similar, however writhe is fundamentally a double integral over the same curve, whereas linking number is a double integral over two distinct curves.

Remarkably it was shown by White, Călugăreanu and Fuller [120] that the linking number of two closed curves can be decomposed into the sum of the twist and writhe of the associated ribbon defined by the two curves. I.e.

$$\text{Lk} = \text{Tw} + \text{Wr}, \quad (2.48)$$

where the writhe of the ribbon (X, U) is simply the writhe of the space-curve that defines its centre-line. These definitions apply very well to dsDNA where we can define vector U as simply pointing between complementary bases.

We will refer to how a ribbon distributes its topological invariant Lk into the two geometric quantities Tw and Wr as *twist-writhe partitioning*. Eq. 2.48 provides us with the advantage that, if the linking number of the system is known, then knowledge of either twist or writhe allows us to determine the other. Due to the relative difficulty in calculating Wr , it can prove easier to calculate the twist of a system and deduce the writhe in this way.

So far, all definitions of writhe and linking number apply only to closed curves, however, it will prove useful to be able to define them to open curves such as those in MT experiments. Initial methods to get around this topological caveat involved artificially closing the curves by adding a straight line segment or planar curve to connect the endpoints of the open configurations. Once this was done the closed formulae would be applied [121, 122]. More formal attempts to extend these ideas to open curves were made by Berger [123] and more recently by Prior and Neukirch [121] via the introduction of a new object called the *polar writhe*. The tangent-indicatrix method for open curves was also addressed by Starostin [124]. Ultimately, application of the closed form formulae to open systems remains the most common approach to define writhe and link in open systems.

2.6 Supercoiled DNA

Given the double-helical nature of dsDNA, even a relaxed straight duplex is coiled. Hence, we shall refer to the phenomenon of how this already coiled configuration further coils as *supercoiling*. As mentioned B-DNA topologically can be thought

of as a ribbon, this means we can apply some of the ideas and results we have discussed in Section 2.5 to DNA. See Fig. 2.4 for an example of how even a simple DNA minicircle is itself knotted.

2.6.1 Definitions

It is common to partition the linking number in the following way:

$$\text{Lk} = \text{Lk}_0 + \Delta\text{Lk}, \quad (2.49)$$

where Lk_0 is the linking number of the unstressed configuration and ΔLk is the linking number excess - a measure of how over/under supercoiled a configuration is. The natural linking number of a regular right-handed duplex is simply its twist, i.e. the length of the DNA divided by its distance per helical turn. Hence,

$$\text{Lk}_0 = \frac{\text{bp}}{h}, \quad (2.50)$$

where bp is the number of base-pairs and h is the helical pitch of the helix. h is approximately 10.5 bp for B-DNA. This quantity is positive for B-DNA as it is a right-handed double helix. It is common to then introduce σ , defined as

$$\sigma = \frac{\Delta\text{Lk}}{\text{Lk}_0}. \quad (2.51)$$

This quantity is called the *superhelical density* and it the preferred metric for quantifying supercoiling. In both eukaryotes and bacteria, the average superhelical density of DNA - including both constrained and unconstrained regions is approximately -0.06 to -0.07 .

2.6.2 Supercoiling Dynamics

Without defining the explicit Hamiltonian of a twistable worm-like chain, we may qualitatively explain many supercoiled phenomena via consideration of a simple inextensible wire under tension. All we must consider is that there are three main energy contributions to such a system: the mechanical energy from pulling the wire, the bending energy and the twisting energy. The mechanical energy

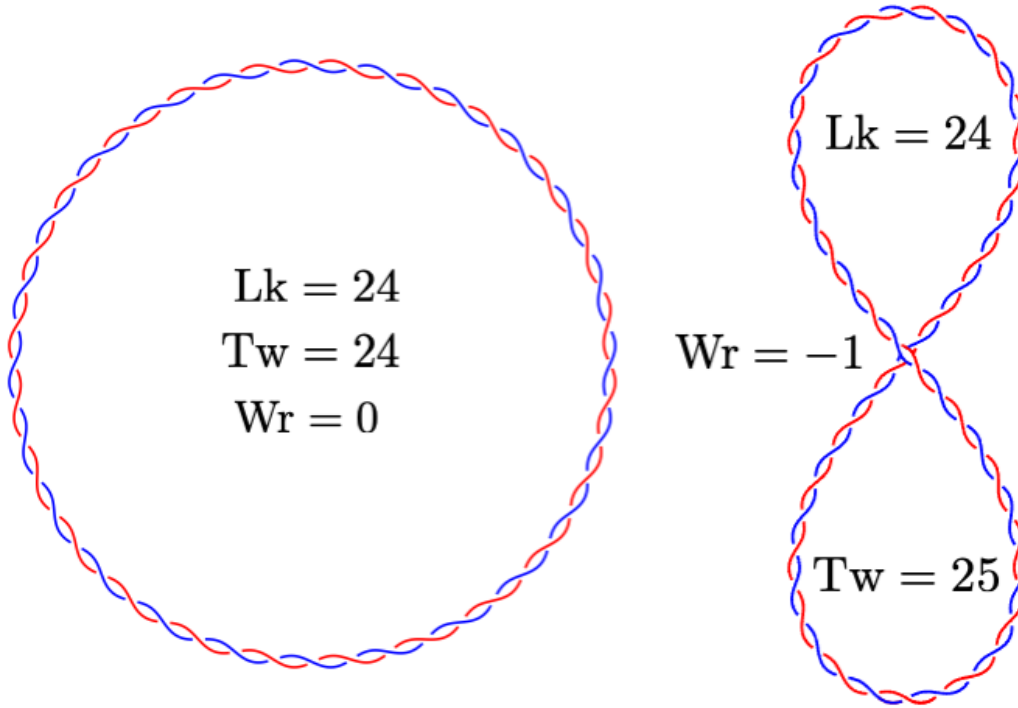


Figure 2.4: Knot diagrams of a minicircle with $Lk = Tw = 24$ and zero writhe (left) and a figure of eight configuration with the same linking number but with $Wr = -1$ and $Tw = 24$ (right). These two objects are topologically identical with different twist-writhe partitioning.

follows a simple relation $E = -f\Delta z$ where Δz is the end-to-end separation in the direction of a force with magnitude f .

Consider a twistable wire which is initially untwisted. At first the wire has a linking number of zero. By twisting the wire we have increased the magnitude of the linking number to some value. Twisting in the right-handed/left-handed direction will lead to positive/negative link. The wire must partition this link into twist and writhe. If it chooses to keep straight, then this carries zero writhe and so all the link must be stored as twist, but the wire remains fully extended and so has the minimum mechanical energy. Further increasing the magnitude of the link via twisting the ends, the wire will continue to store this link as twist until the benefit of remaining extended no longer out-ways the added cost of twisting more. At this point, which we shall call the buckling transition, the wire buckles and forms a loop, which we shall call a *curl*. This curl has a writhe of approximately unit magnitude,

with the sign matching the sign of the link. This allows the twist of the wire to be relieved by 1, decreasing the twisting energy of the wire. However, writhing will always increase the bending energy and decrease the end-to-end distance and so the energetic gain of reducing the twist must compensate for these increases.

If we further add to the magnitude of link by twisting the ends of the wire, the twist energy will again grow as the global twist increases. Again, the wire would like to relieve this excess twist by partitioning some of the link into extra writhe. The wire can do this in two ways, either by adding another curl if the wire is sufficiently long, or the curl can writhe further forming a *plectoneme*. The tip of the plectoneme, which we shall call the *loop*, remains with approximately unit magnitude writhe, with the extra writhe coming from what we shall call the *braid* of the plectoneme. Plectonemic braids are observed to be approximately solenoidal, where the handedness of the braid is such that the associated writhe has the correct sign. E.g. under-twisting a wire will lead to a negative link and so plectonemes with left-handed solenoidal braids will form as they carry the negative writhe required to reduce the twist of the system. We can assign a handedness of a plectoneme to be the same as the handedness of its braid. Twisting beyond this will simply increase the number of solenoidal repeats in the braid with each braid reducing the twist by writhing in the direction of the link.

This interplay between twisting, bending and pulling differs depending on several factors. For example, the strength of the external force will affect the critical superhelical density required to form plectonemes. The larger the force, the greater the gain from having a larger end-to-end distance, and so more link will be required for buckling and the subsequent writhing to prove beneficial. This explains why when you twist a wire into a plectoneme, simply pulling the wire sufficiently will return it to a straight twisted configuration. All of these structures and transitions can easily be seen by taking a twistable wire such as a laptop charger and applying a torque to the end and adjusting the force exerted.

These effects can also be seen for wires that are closed. The main difference is that the un-writhe configuration is a circle instead of a line and that the twisted

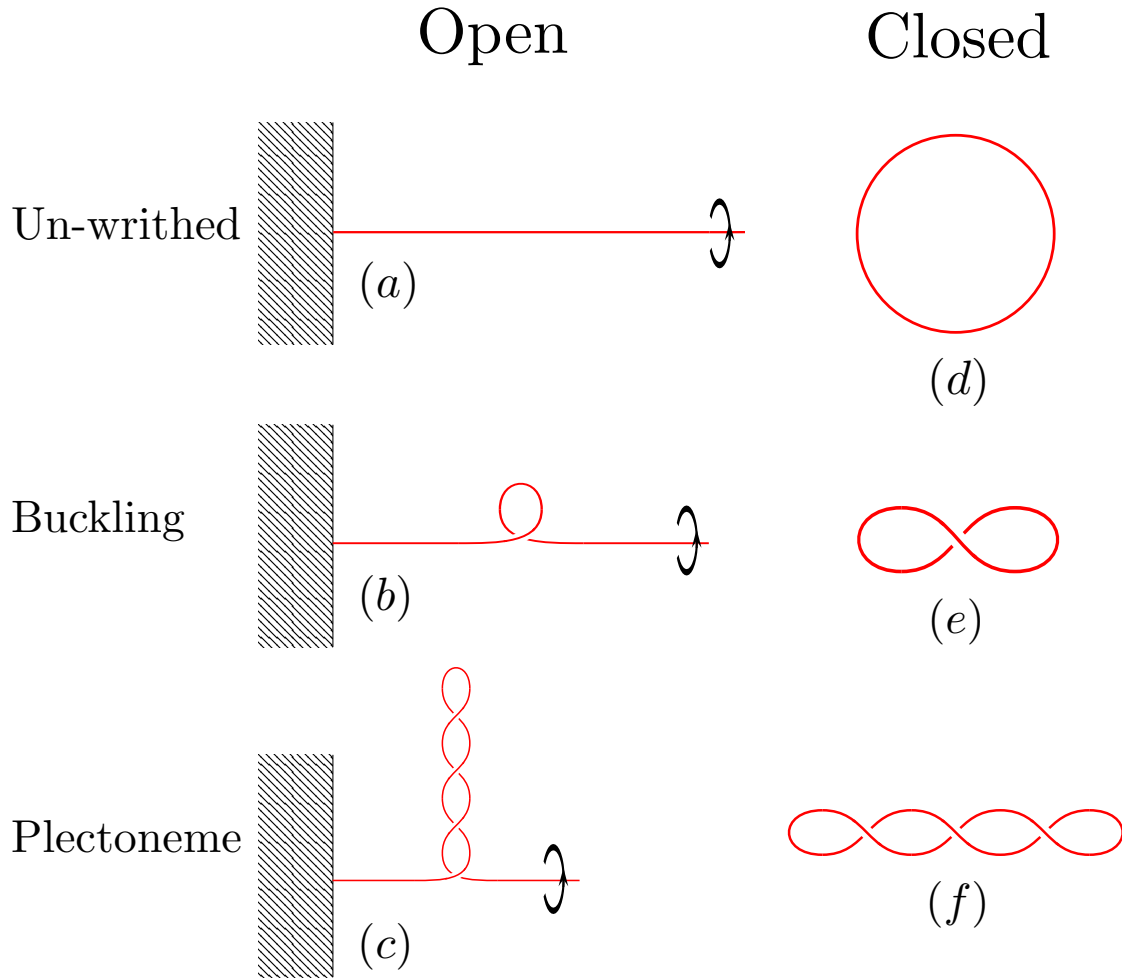


Figure 2.5: Schematic of supercoiling phenomena for open and closed systems. (a) straight line, (b) left-handed curl, (c) left-handed plectoneme, (d) planar circle, (e) left-handed figure of eight, (f) left-handed plectonemic minicircle.

circle will buckle to form a figure of eight shape beyond a critical amount of twist.

Examples of un-writhed, buckled and plectonemic structures are shown in Fig.

2.5 for both open and closed systems.

2.7 Data Analysis and Implementation

In this section we briefly describe the methods and issues we encounter when implementing some of the ideas established in this chapter.

2.7.1 Removing Intrinsic Helicity

Many quantities we wish to calculate in this thesis such as curvature and bending angles of duplexes rely on DNA being described as a single space-curve. For instance, if we wish to calculate the curvature of a duplex, what we really want is the curvature of the centre-line of the two-strands which comprise the duplex. Crudely, this can be obtained via simply finding the midpoint of each base-pair and constructing the centre-line from these points. However, due to the groove asymmetry of DNA, this centre-line will also have inherent helicity. The magnitude of this inherent helicity is often of the same magnitude or higher than the curvatures we are trying to probe. Hence, we need a more sophisticated method of calculating a more representative centre-line. There have been many such algorithms proposed to determine this so called *molecular contour* including 3DNA [125] but the one we have found to be the best is that of WrLine [126]. The Python package available with WrLine proved unsuitable for handling long trajectories due to its memory handling, and so we chose to implement the algorithm ourselves using an in-house Python script. The algorithm filters out local (i.e. sub-helical turn) irregularities using a sliding-window averaged over a single helical repeat.

We found that this method works excellently for closed-configurations but not as well for open ones. An example of the molecular contour produced using this method is shown in Fig. 2.6.

2.7.2 Calculating Twist and Writhe Numerically

In order to calculate twist and writhe for DNA, we must make a sensible choice of vectors that define the ribbon. If we define X_1 and X_2 , which are space curves that pass through the backbone of strand 1 and strand 2 respectively, we can define the centre-line X as simply the average of these two curves, i.e. $X = (X_1 + X_2) / 2$. The tangent can be trivially calculated as the unit vector which points between successive centre-line points. The unit normal U is then constructed via taking the vector that points between X_1 and X_2 and removing the component of this vector in the direction

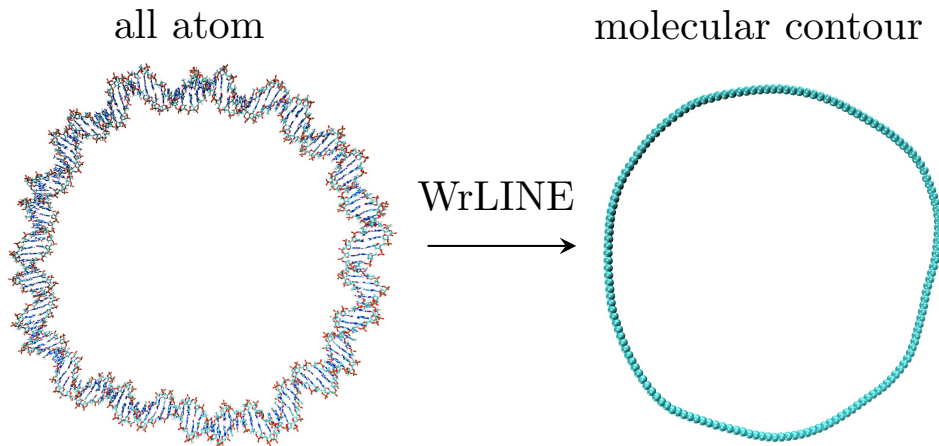


Figure 2.6: Example of the WrLINE algorithm for calculating the molecular contour applied to a 160-bp minicircle.

of the tangent to ensure orthogonality. The twist and writhe of a DNA configuration can then be calculated via numerical integration of Eq. 2.43 and Eq. 2.45.

This method was numerically implemented using both Python and Fortran where a spline-fitting method is used to describe the space-curves, however, these splines are constrained to pass through every point of the backbone. The definition of the backbone positions are model dependent. In this thesis these positions are chosen to be: the phosphate positions for all-atom simulations and the centre of mass site for oxDNA simulations.

This method was initially applied to seemingly great effect, with values of twist and writhe summing to the expected integer values of Lk to several decimal places. However, application of this method to some other systems revealed some algorithmic artefacts. For example, it was found that the writhe of a relatively flat minicircle was not as close to zero as expected. So despite the misleading evidence that this method can calculate the twist and writhe such that $Lk = Tw + Wr$, we had to change our approach to calculating these quantities.

To alleviate these issues, we elected to first calculate the molecular contour of the system using the WrLine algorithm, and subsequently determined the writhe via

simple discrete Riemannian integration of Eq. 2.45. The twist was then determined from the equation $Lk = Tw + Wr$. This method was found to be much more reliable. This is likely due to the fact that the inherent helicity of the centre-line of dsDNA causes the writhe integral to become highly oscillatory, leading to numerical errors when computing its integral.

The Riemannian double sum approximation of the the integral Eq. 2.45 scales quadratically with the number of points and so larger systems that require more data points to model will be computationally punished accordingly. This is of little concern when calculating the writhe of a single structure, however, it can become problematic when trying to compute the writhe every snapshot of a simulation. Luckily, the tangent field of a system will only marginally vary in a simulation if snapshots are taken sufficiently close together in time, making it an excellent candidate for applying the Fuller formula Eq. 2.46 with the previous snapshot and writhe providing the reference quantities required. To avoid small drifts from the correct writhe, one can choose to perform the full double integral writhe computation periodically to correct for this.

3

All-atom Simulations

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3.1 Introduction

All-atom simulations represent some of the most detailed methods available for molecular simulations, albeit at the cost of computational time. Hence, in this

chapter we will focus on studying systems that are suitable for all-atom investigation. With the current version of Amber and GPU resources available, this means we may not realistically simulate systems with more than 400-bp for longer than 100 ns in explicit solvent without dedicating weeks of resources.

The all-atom simulations in this thesis were performed using the Amber Molecular Dynamics package. All minimizations were performed with the AmberTools packages `pmemd` and `sander`, with all MD runs performed with `pmemd.cuda` or `pmemd.mpi`, the GPU and parallel CPU versions of `pmemd` respectively. All GPU runs performed were carried out on the Oxford Advanced Research Computing (ARC) or the Cambridge Service for Data Driven Discovery (CSD3) compute clusters. In this work we solely use the `parmbsc1` force field which is specifically parametrised for nucleic acids and is discussed in Section 2.2.

3.2 The All-atom Thymine Dimer

An immediate advantage of the all-atom representation is that it gives us the most transparent way to capture the effect of a thymine dimer. This is achieved via the creation of a new bespoke residue TT, with a TD consisting of two adjacent TT residues. Once these residues have been specified, we covalently bond the C5 and C6 carbon atoms on the residue pair. In addition to these extra bonds, both TT residues are subjected to a set of modified force field parameters that are specifically calculated for a thymine dimer. Note that we make no force field modifications to the two Adenine bases that lie complementary to the TD.

These force field modifications were calculated via the restrained electrostatic potential method (RESP) [127] by our collaborator Agnes Noy. Since the Amber force fields are built around accurate pairwise charges, and nucleic residues are constrained to have unit charge, we may derive a new residue via consideration of its modified charge. The RESP method calculates pairwise charges from gas-phase QM calculations that are designed to reproduce solution phase charge interactions and ensure that the rotationally degenerate atoms, such as methyl and methylene hydrogens, have equivalent charges.

3.3 MD Protocol

We now outline the basic protocol utilised in this work for creating and simulating atomistic systems. The procedure begins equivalently for explicit and implicit solvent simulations before diverging depending on the solvent model.

Given an input sequence, we generate an all-atom PDB file which can be either circular or linear. Linear configurations are generated with the AmberTools module `nab`, and circular configurations are made from linear strands with a custom script. For minicircles a value for Lk must also be specified. Given that a minicircle has zero writhe, the twist per base-pair can easily be calculated and adjusted such that the system has the correct value of Lk or σ .

If we wish to add a thymine dimer to the system, we simply change a pair of DT residues into a pair of TT residues in the PDB file. We then use the AmberTools program `tleap` to load the `parmbsc1` force field. A second `tleap` script is then called that bonds the end residues P and O3' atoms if the system is circular, and then bonds the C5 and C6 atoms of any pairs of TT residues if they are present. We must also remember at this point to load in any force field modifications if required. This is where the protocol for implicit and explicit solvent methods branch off.

3.3.1 Explicit Solvent

For explicit solvent simulations, a third `tleap` script solvates the system by adding water molecules and salt atoms. Na^+ ions are added until the net charge is zero, with the positive salt ions cancelling with the negatively charged backbone of the DNA. We then impose a truncated octahedral box with a 12 Å minimum distance between solute and box boundary and declare the choice of the TIP3P water model. This script produces a `.crd` Amber-style configuration file as well as a `.top` Amber topology file.

The system is initially minimized with the nucleic atoms held in place with harmonic restraints of strength 50 kCal/mol/Å². Then the whole system is minimized further using a combination of steepest descent and gradient descent. Typically we do not minimize for more than 10,000 cycles. Minimization is important as it

Run	k (kCal/mol/Å ²)	T (K)	Duration
Minimization 1	50.0	100	1,000 - 10,000 cycles
Minimization 2	None	100	1,000 - 10,000 cycles
Equilibration 1	50.0	100	10 ps
Equilibration 2	50.0	100-300	10 ps
Equilibration 3	50.0	300	10 ps
Equilibration 4	25.0	300	10 ps
Equilibration 5	10.0	300	10 ps
Equilibration 6	5.0	300	10 ps
Equilibration 7	2.5	300	10 ps
Equilibration 8	1.0	300	10 ps
Productive Run	None	300	10-100 ns

Table 3.1: Values of harmonic restraint strength k , temperature and run duration across all minimization, equilibration and productive phases of MD simulations.

provides an energetically reasonable starting point for the dynamics and reduces the likelihood of system instabilities or unrealistic behavior during the production run.

An electrostatic cut-off of 12 Å is chosen for both minimizations and these can be performed with either `pmemd` or `sander`. Unfortunately the GPU implementations of these programs are unsuitable for minimizations, leading to large artefacts in the system. We found the best method was to use `pmemd.MPI` with 8–16 CPU cores. Interestingly, we did not always see a speed-up if 32–48 cores were used.

We then perform 8 MD runs as part of the preparation phase. Unlike the minimizations these can utilise `pmemd.cuda` which leads to a significant speed up. The molecular dynamics then begins with the system heated to 300 K and the positional harmonic restraints relaxed over the course of 8 rounds of MD. Each run is 10 ps long and the details of these runs can be found in Table 3.1.

For minicircles with non-zero Lk, to prevent bubble formation we additionally constrain the system with Watson-Crick restraints via the `makeDISTRST` command in AmberTools. These preserve the Watson-Crick bonding across base-pairs. This additional step is especially important for minicircles with a TD as the system will often try and alleviate any torsional stress by simply forming a bubble at the TD site. We apply these restraints for the first 8 MD runs.

For the 9th and final MD run, which we will call the *production run*, we turn off all restraints and perform a long MD simulation (10–100 ns). From these longer runs we may observe dynamics, extract relevant quantities and compute their time-averages. Throughout all these MD phases we impose periodic boundary conditions and ensure constant pressure through a Berendsen barostat. We control the temperature with a Berendsen thermostat as well as using an electrostatic cut-off of 12 Å.

3.3.2 Implicit Solvent

For the implicit solvent protocol, we began by minimizing the system for 1000–10,000 cycles, which initially uses the steepest decent algorithm before switching to conjugate gradient descent around halfway through. Then, similarly to the explicit solvent method, we heated the system while slowly removing the harmonic restraints, but this time across 4 MD runs each 10 ps long. The first of these runs heated the system from 100 K to 300 K, with a restraint of 50 kCal/mol/Å². The next two runs then slowly reduced these constraints to 10 kCal/mol/Å² and then 1 kCal/mol/Å². Then the fourth run we applied the Watson-Crick type restraints if we wished to utilise them. Finally, we began a production run with no restraints.

A Langevin thermostat was used to control the temperature, with a collision frequency of 0.1 ps⁻¹ selected. We also imposed an electrostatic cut-off of 1000 Å as this is required for any GPU simulation of a non-periodic system in Amber. We used the Generalised Born (GBNeck2) implicit solvent model while ensuring there was no surface-area based non-polar free energy of solvation correction. A salt concentration was specified as there are no explicit ions for implicit solvent simulations. Finally, the SHAKE algorithm was utilised for all simulations.

This solvation protocol was implemented for undamaged minicircles, with a similar protocol already being used to great effect by Noy *et al.* [128]. This seemed to work well, quickly relaxing undamaged negatively supercoiled minicircles towards their preferred writhed configurations. Unfortunately, the implicit solvent methods did not yield sensible results when TT residues were present. Bubble formation was much more prevalent and attempts to restrain the system were

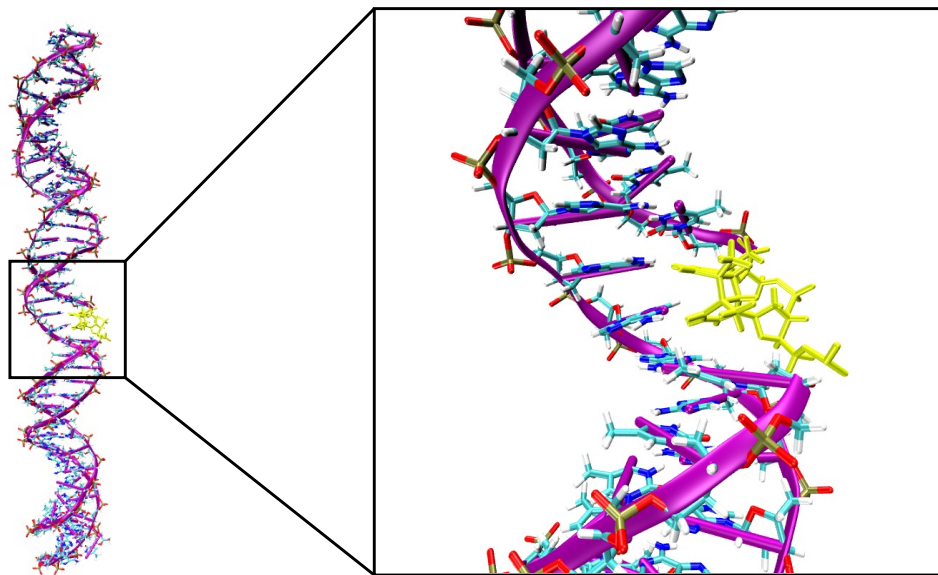


Figure 3.1: Snapshot of 40-bp poly(dA:dT) with centralised thymine dimer (yellow). Note the additional covalent bonds which join the C5 and C6 atoms of the two mutagenic bases. Visualisation and visual output performed in VMD.

less successful. This could be due to the artificially accelerated dynamics of the implicit solvent - the time-scale at which the DNA can alleviate torsional stress is much faster (approximately 10 times).

3.4 Linear DNA

We began our investigation into the structural effects of a TD in duplex DNA by studying a small poly(dA:dT) (a sequence of just thymines on one strand and adenines on the complementary strand). A 40-bp poly(dA:dT), with a thymine dimer placed in the centre between the 20th and 21st bases, was the first system probed in the all-atom picture. The system was prepared and simulated using the explicit solvent protocol detailed in Section 3.3.1 for 100 ns. See Fig. 3.1 for a snapshot of the damaged system.

3.4.1 Duplex flexibility

A common way to measure the flexibility of DNA is through its persistence length. This can be done in several ways, the most common of which is to compute

the tangent-autocorrelation function and compare to Eq. 1.1. However, given the relatively short length of our system compared to its persistence length ($L/\ell_p \approx 0.26$), it is difficult to obtain a good fit to the desired exponential decay. Another issue is that ℓ_p is a global quantity, it treats the whole polymer as a homogeneous WLC. Clearly this is inappropriate for damaged DNA where we see an area of relatively high increased local bending.

Instead we simply fitted a line of best fit either side of the duplex (neglecting end effects) and calculated the angle between them via the formula

$$\theta = \arccos(\hat{\mathbf{r}}_1 \cdot \hat{\mathbf{r}}_2), \quad (3.1)$$

where $\hat{\mathbf{r}}_{1,2}$ are the two directions of the lines of best fit, see Fig. 3.2. This angle was averaged over the entire trajectory of the 40-bp poly(dA:dT) and the spread measured via the standard deviation. We found that $\langle\theta\rangle = 22.86^\circ \pm 11.68^\circ$ for the damaged duplex compared with $\langle\theta\rangle = 20.26^\circ \pm 11.55^\circ$ for the control. It is clear that the magnitude of bending has increased.

One issue with this formulation is that it does not address the direction of bending and so we may not assert whether the thymine dimer causes bending in a specific direction. Furthermore, applying the definitions in Fig. 3.2 doesn't give a sufficiently local measure of bending because the two sections being used to define the vectors aren't sufficiently stiff. Thermal fluctuations will cause significant deviations from a straight configuration leading to length and temperature dependencies. These issues are addressed in the next section where we quantify bending using a different method.

Lines of best fit were simply taken to be the midpoint of the base-pairs of the duplexes. We opted not to utilise the WrLine algorithm to obtain a molecular contour for this system as the algorithm is not well suited for short duplexes and requires us to neglect too much of the DNA. The effect of inherent curvature is also less important when finding bending angles compared with calculating local properties such as curvature.

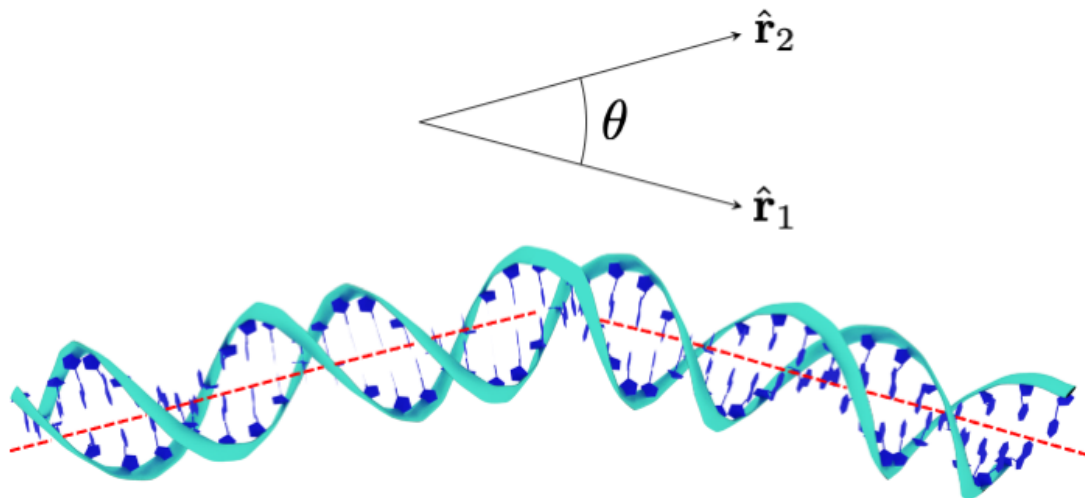


Figure 3.2: Schematic of bend angle calculation for kinked duplex with centralised damage including lines of best fits (red, dashed) through each half of the duplex and the angle formed between them.

3.4.2 Base-pair Step Parameters

The base-pair step (BPS) and base step parameters are used to describe the structural features of DNA. Specifically these parameters provide information about the local structure of DNA and how the parameters influence the interactions between DNA and other molecules. Both sets of parameters are comprised of three translational and three rotational parameters.

The base-pair step refers to the interaction between two adjacent base-pairs in a DNA double helix. It is defined by the relative position and orientation of the two base-pairs. The base-pair step parameters include the helical twist angle, the tilt angle, the roll angle, the slide displacement, the rise displacement and the shift displacement. All 6 BPS parameters are shown in Fig. 3.3.

Similarly there are the base step parameters that refer to the structural features of an individual base-pair. They include the propeller twist angle, the opening angle, the shear displacement, and the stretch displacement. Both of these sets of parameters require the definition of a base-pair step and base-step triad.

Using the program Curves+ [129], which adopts the Tsukuba convention [130] for defining the base-pair step triad, we calculated the time-averaged BPS parameters

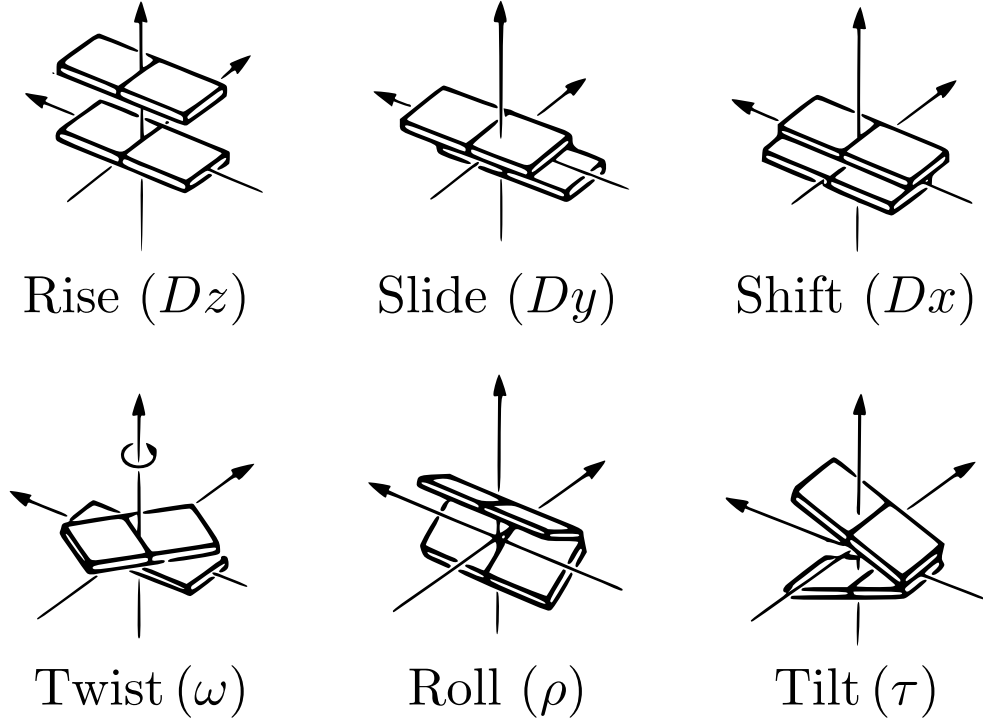


Figure 3.3: Schematic of all 6 base-pair step parameters: rise, slide, shift, twist, roll and tilt.

Linear 40 bp poly(dA:dT)			
BPS Parameter	Undamaged	Damaged	Change
slide / Å	-0.66 ± 0.58	-0.41 ± 0.34	0.25
shift / Å	0.17 ± 0.56	1.15 ± 0.46	0.98
rise / Å	3.37 ± 0.32	3.77 ± 0.20	0.40
twist °	34.60 ± 5.30	20.40 ± 4.80	-14.20
tilt °	0.90 ± 4.30	-16.70 ± 3.50	-17.60
roll °	-0.10 ± 5.40	22.30 ± 4.50	22.40

Table 3.2: Time averages, standard deviations and changes in BPS parameters for 20th BPS for damaged and undamaged linear 40-bp poly(dA:dT). The 20th BPS corresponds to the TD in the damaged system.

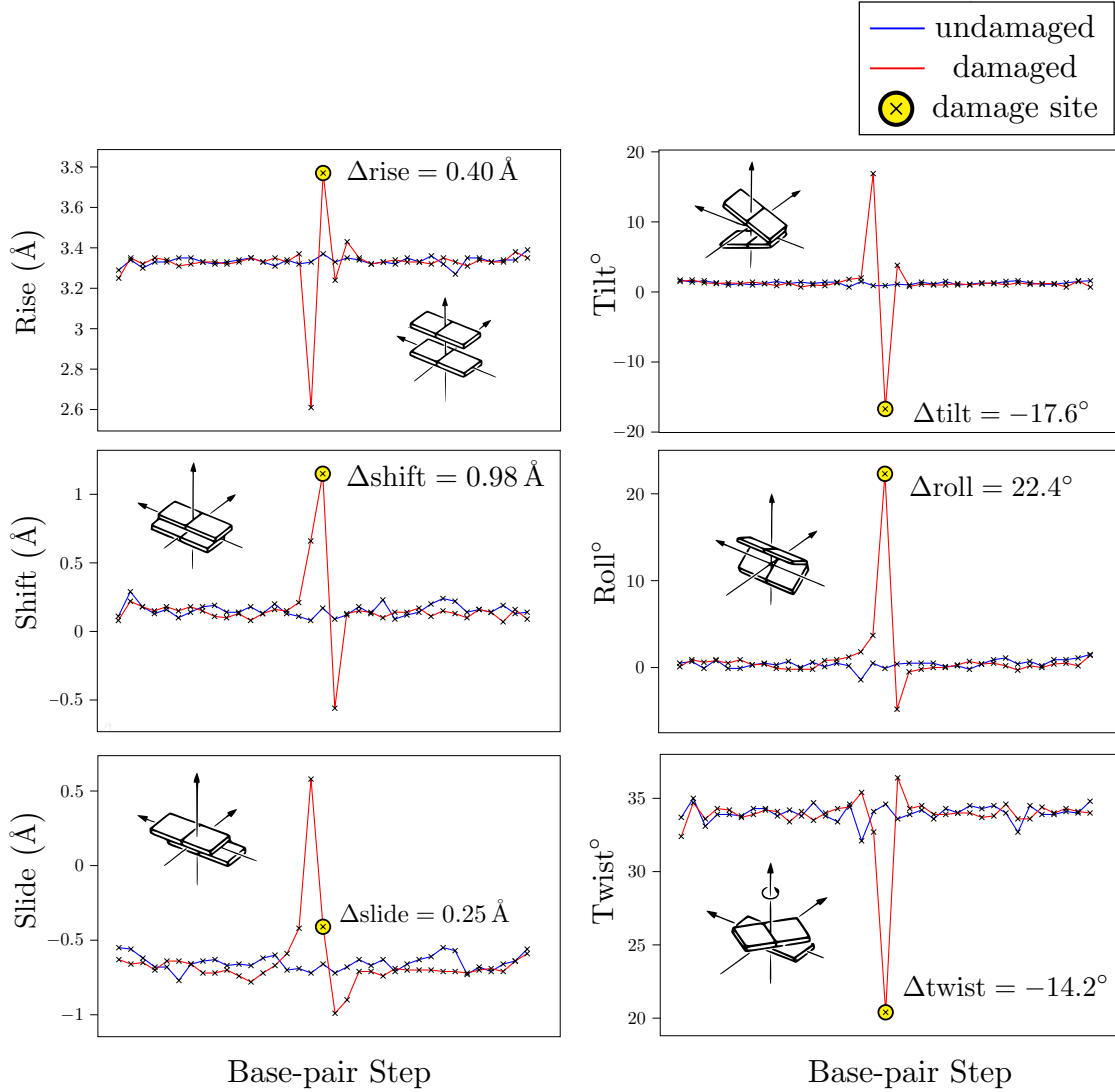


Figure 3.4: Plots of all 6 base-pair step parameters as a function of base-pair step for both damaged and undamaged linear poly(dA:dT) (TD is at the 20th BPS).

for every BPS in the configuration for both the damaged and undamaged 40-bp poly(dA:dT). The average BPS parameters for the 20th BPS (the TD for the damaged system) are detailed in Table 3.2 and each parameter plotted as a function of BPS in Fig. 3.4.

The difference in twist between the damaged and undamaged system -14.2° matches well with experiments which put this value between -9° [53] and -15° [56]. The predicted increase in roll of 22.4° and decrease in tilt of 17.6° are also in striking accordance with Ref. [53] that reports values of 22° and 17.8° , respectively.

Interestingly, all BPS parameters display a decrease in standard deviation despite the mean values of some quantities deviating from the control system significantly. This suggests that a TD is a more rigid structure than its undamaged counterpart. This is not unexpected given the extra bonds would be expected to further constrain the relative motion of the two thymine bases.

Fig. 3.3 indicates the effects that we see in the BPS parameters seems localized to the damage site and only a few base-pair steps either side, implying the TD has a relatively local effect on the duplex. In some degrees of freedom, compensatory changes in adjacent base-pair steps reduce the net effect of the TD. E.g. although the rise is larger for the TD base pair step, the previous BP step has a smaller rise than normal. But for others (roll, twist, slide) the change at the TD BPS dominates.

It must be noted that these values will not all have used the same base-pair triad definitions but this should only cause a subtle change in the experimental values. Another caveat is that we must note that the BPS triad definitions outlined in Ref. [125] were constructed to characterise the structure of undamaged B-DNA. It is unclear if applying these definitions to a mutated base-pair step will always lead to a sensible triad.

3.4.3 Induced Bending

We have already calculated the average bending angle formed at the centre of the damaged and undamaged poly(dA:dT) in Section 3.4.1 using a line of best fit approach. This suggested that the damaged duplex is on average more bent with marginally larger fluctuations. This method, as discussed, is not necessarily a good measure of local bending and so we propose another method. Given we have now calculated the BPS parameters, we may now deduce a bending angle from these results.

From the definitions given, we can define the bending angle between base-pairs in the base-pair step via $\theta = \sqrt{\rho^2 + \tau^2}$, where ρ is the roll and τ the tilt. Using this formula we estimate a biased bending angle of $\theta_0 = 26.79^\circ$, putting it in the same vicinity as Husain *et al.* [47] and Park *et al.* [53]. This definition however,

only focuses on the TD itself and not the two duplexes either side of the damage, hence providing a very local definition of the angle. As a sense check this method yields a bend angle in the undamaged duplex as being approximately zero due to the symmetric fluctuations of roll and tilt about zero.

These base-pair definitions do, however, provide us with a method of assigning a direction to the axial bending calculated in Section 3.4.1. Given that the mean values of tilt and roll of the damaged BPS are at least 4.7 standard deviations from zero, we may safely say that the duplex is largely bent in one specific direction. Hence, it is safe to argue that the value of 22° previously calculated is a reasonable estimate.

As noted, some of the disruptions induced by the TD to the BPS parameters are partially compensated by changes in the adjacent base-pair steps. Given that tilt is one of the parameters where this is true (see Fig. 3.3), we may deduce that the increase in roll is the main contribution to the induced bending. Hence, we may argue that the bending could be simply estimated as the increase in roll, making the bending angle around 22.4° putting it close to the estimation of 22° from the line of best fit method. We note however, that the line of best fit method provides an unsigned definition of bending, and so a like-for-like comparison may not be appropriate.

The method of Lim *et al.* outlined in Ref. [46] allows us to predict a signed bending angle using the data acquired in Section 3.4.1 by constructing a sign condition which determines if a bend is negative or positive. This method was applied to our system and yielded an angle around 19° . We, however, found this definition to be unsatisfactory as it fails to predict approximately zero bending in the undamaged duplex.

3.4.4 Hydrogen-Bonding Analysis

Another interesting question is how the hydrogen bonding is affected when the TD is present. We intuitively expect a reduction in the ability for the TDs to form hydrogen bonds with their complementary adenines, but to what extent? This is a useful question as the hydrogen bonding interaction strength is an input

parameter in the oxDNA TD model and so could help us infer if the choices made for that model are sensible.

Using an AmberTools program, we calculated whether or not two bases were hydrogen bonded for every frame of the MD production run. We then computed the hydrogen-bonding fraction, the fraction of the time there is deemed to be a hydrogen bond, across the trajectory. By default, the program defines that two atoms are hydrogen bonded based on a simple distance metric. The hydrogen-bonding fraction for both hydrogen bonds in an AT base-pair were computed as a function of base-pair index along the 40-bp poly(dA:dT). The results are shown in Fig. 3.5. Similarly to the the base-pair step parameters, we see a significant localized disruption in the hydrogen-bonding fraction for both hydrogen bonds. For the adenine N1 acceptor thymine N3 donor hydrogen bond, we see a decrease in the hydrogen-bonding fraction from approximately 0.7 to 0.39 for the 20th base-pair and a smaller reduction to 0.65 for the 21st. The following base-pair remains at around 0.65 until returning to a more standard value.

For the thymine O4 acceptor adenine N6 donor hydrogen bond, we also see a decrease in the hydrogen-bonding fraction for both mutated bases, this time from just above 0.65 to around 0.54, representing a comparatively smaller change. We do then see an increase in hydrogen-bonding fraction to the following base-pair to above 0.7 indicating that these atoms are on average closer to each other than they were in the system with no TD.

3.5 Minicircles

We now investigate how the presence of a TD affects topologically closed structures such as DNA minicircles.

3.5.1 Husain Minicircles

We have already discussed the famous circularization experiments of Husain *et al.* [47] in the context of the structural effects of TDs. In these experiments, Husain

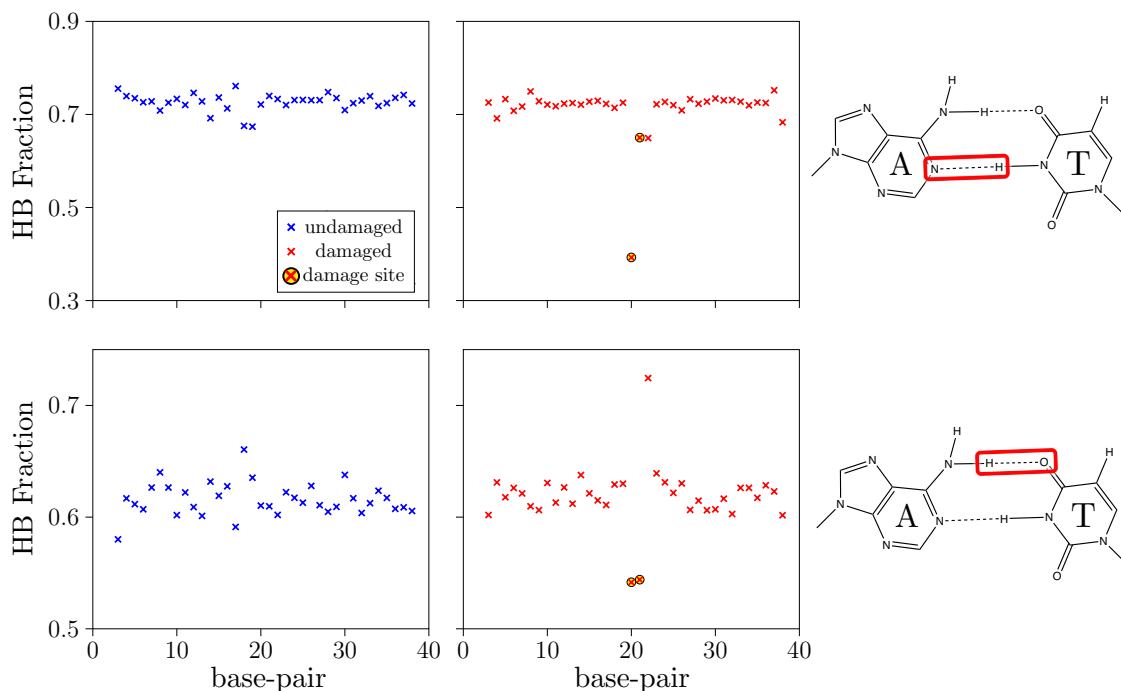


Figure 3.5: Plot of hydrogen-bonding fractions as a function of base-pair along a 40-bp poly(dA:dT). Left plots are for the undamaged system with the right plots for the damaged system with AT base-pairs 20 and 21 forming a TD. The top two plots represent the adenine N1 acceptor thymine N3 donor, with the bottom two representing a thymine O4 acceptor adenine N6 donor.

began with a self-complementary sequence of length 32 base-pairs given below:

$$\text{TTGGAGGAATTTCGTAGATCCGGTGCAGGCAAG.} \quad (3.2)$$

Thymine dimers were formed with the two adjacent thymines at the start of the sequence (indicated in red), this forms a base unit 32-mer. Many of these 32-mers were mixed together and ligated head to tail to form a range of open and closed configurations whose lengths are multiples of 32 base-pairs. Given the relatively short strands involved, we can simulate these in Amber on the 100 ns time-scale. We choose to simulate the most common structure formed in the experiments, which was the 160-bp minicircle containing 5 TDs.

Both undamaged and damaged minicircles of length 160-bp were generated and simulated using molecular dynamics with the methodology described in Section 3.3, see Fig. 3.6 for snapshot of the initial state. Watson-Crick restraints were not used as the system has only a small amount of torsional stress ($\sigma = -0.011$). A

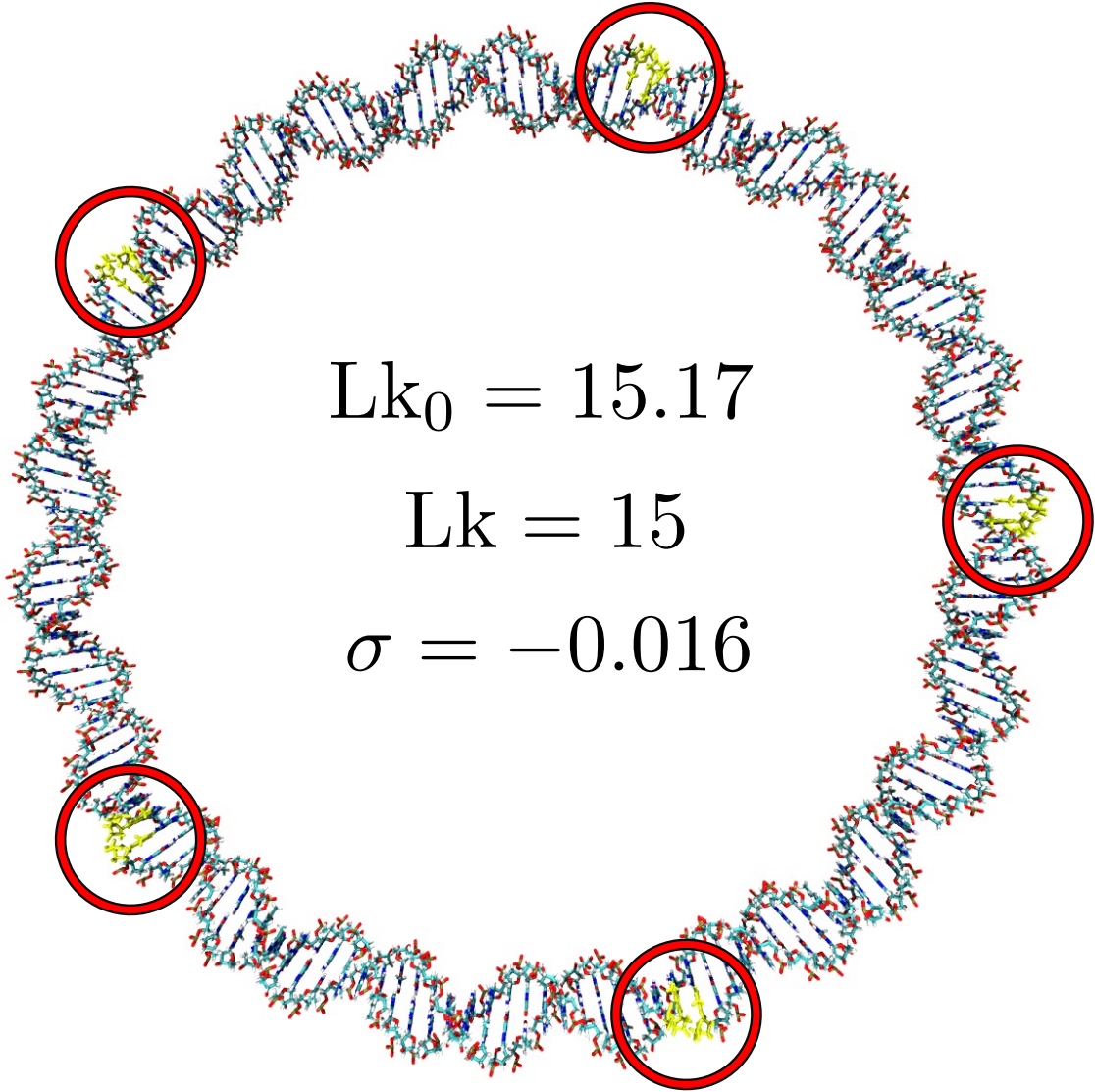


Figure 3.6: Image of initial 160-bp minicircle with 5 TDs (yellow and circled in red). This minicircle has a linking number of 15, corresponding to $Lk_0 = 15.28$ and a superhelical density of $\sigma = -0.011$.

snapshot of the the MD trajectory is shown in Fig. 3.7. It would appear from the simulation that the TDs are initialized very close to their preferred positions. The TDs are oriented such that positive roll and negative tilt will increase the bending angle into the major groove at each damage site. This is most easily seen in Fig. 3.8 which shows a snapshot of the damaged minicircle from a perspective aligned with the plane of the minicircle. If we look at the square bond formed in Fig. 3.8 by the dimerization, the more aligned the normal vector of this square is with the normal of the plane that defines the minicircle, the larger the contribution

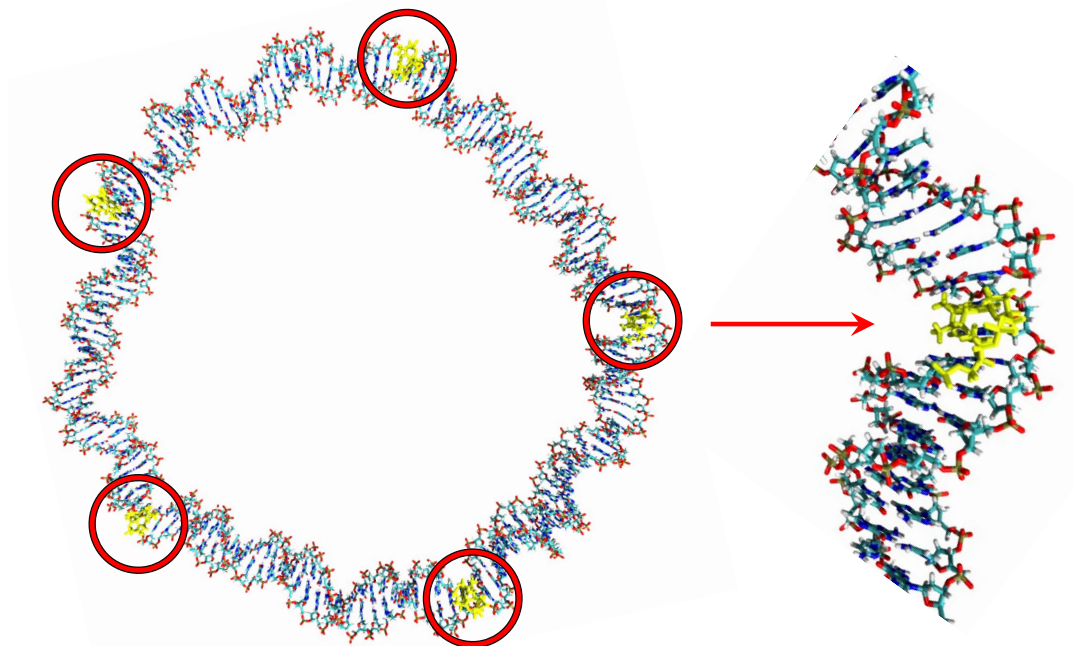


Figure 3.7: Snapshot of molecular dynamics simulation of 160-bp minicircle with 5 TDs (yellow and circled in red). A close-up a TD is also shown that displays a representative position for these mutated bases observed this simulation.

of bending is from the roll (vice-versa for tilt).

One may expect variation in a quantity called the “poloidal angle”, essentially a rotation of the duplex about its helical axis. Aksimentiev *et al.* refer to this mode as the “inside-out” mode [131]. They define the poloidal angle of a base-pair rigorously to be the angle formed between the vectors $\mathbf{a}$ and $\mathbf{b}$, where $\mathbf{a}$ is the vector that points from the duplex centre to the P1 phosphate, and $\mathbf{b}$ to be the vector that points from the duplex centre to the centre of mass of the minicircle. Since we observe very little variation in this inside-out mode, we conclude that the TDs in our minicircle are most likely initialized very close to their ideal positions. It would be interesting to see the consequences of performing the same simulation with the TDs far from their energy minima and whether transitions in this inside-out mode occur on time-scales accessibly by simulation. Kim *et al.* reported a characteristic time-scale of 61 ns in the poloidal angle auto-correlation for a 90-bp poly(dA:dT) minicircle [132].

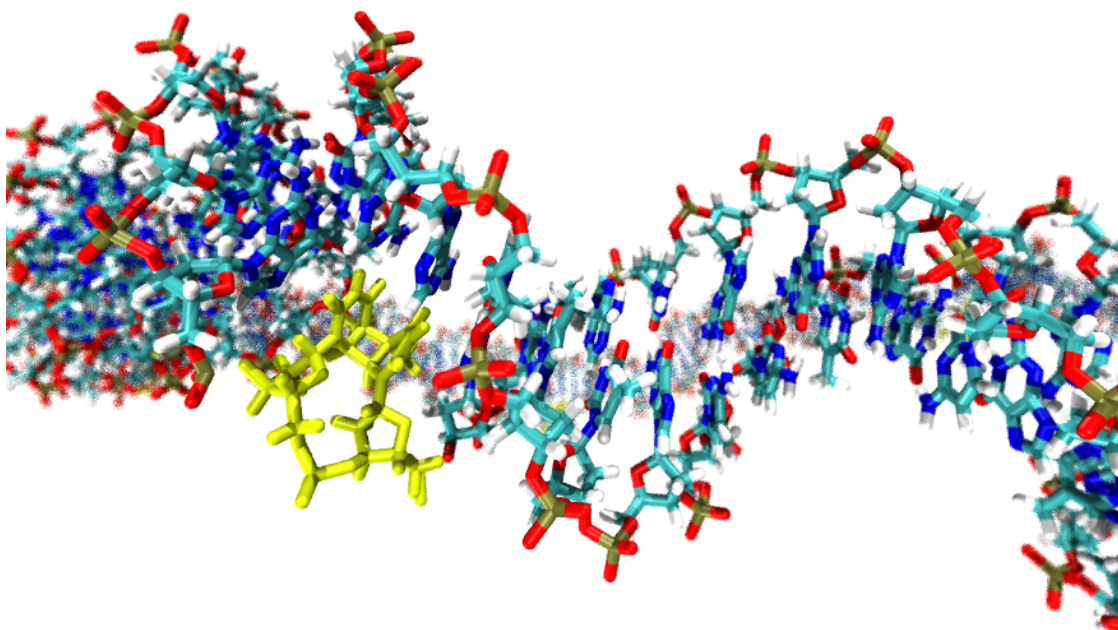


Figure 3.8: Snapshot of molecular dynamics simulation of specific TD (yellow) in 160-bp minicircle taken from perspective aligned with the plane of the minicircle.

3.5.2 Bending and Flexibility

It is clear from simple observation of the trajectories that we see strong preferential bending at the TD sites, just as observed by Husain *et al.* We attempt to quantify this effect via measurements of the both the curvature and the angle through the base-pairs either side of the damage. First, we apply the WrLine algorithm as described in Section 2.7.1 to create the molecular contour of the configuration. We then calculate the curvature via Eq. 2.42 along the chain for both the damaged and undamaged systems. The results are shown in Fig. 3.9.

As expected we see essentially noise for the undamaged configuration and five distinct peaks at the locations of the five thymine dimers. The average curvature

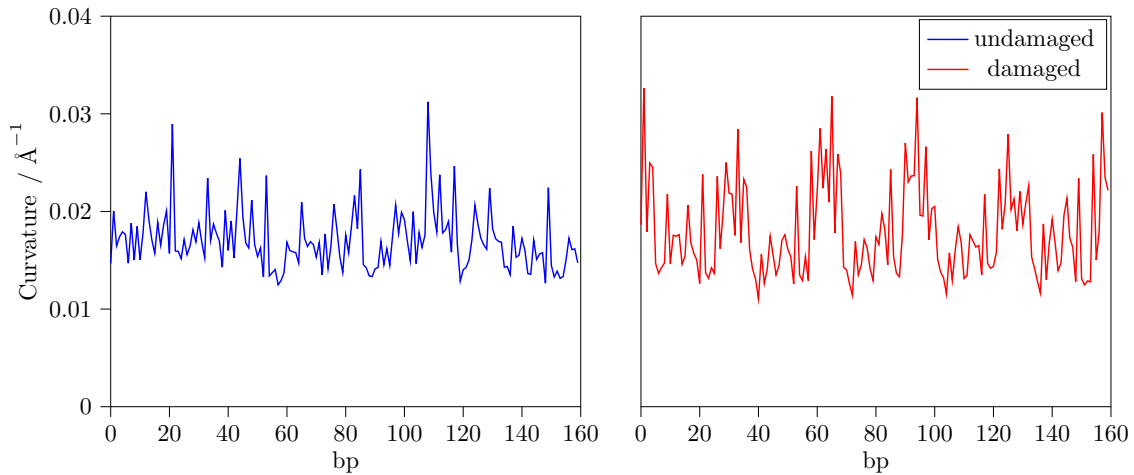


Figure 3.9: Plot of curvature as a function of base-pair index for both the undamaged (left) and damaged (right) 160-bp minicircles comprised of 5 32-mers.

for the undamaged circle was $0.017 \text{ \AA}^{-1}$, roughly the expected value of $2\pi/L \approx 2\pi/(3.4 \text{ \AA} \times 160) = 0.012 \text{ \AA}^{-1}$.

3.5.3 BPS parameters

The BPS parameters at the 5 TD sites were calculated every 5000 time-steps of each 100 ns trajectory and averaged over 10,000 frames using Curves+ [129]. The mean value of twist was 34.90° for the undamaged system when averaged across all 5 TDs and 20.28° for the damaged system when the same bases were considered. This equates to an average reduction of twist of 14.62° . This is a larger but relatively similar value to what we saw for the 40-bp poly(dA:dT) which showed a reduction in twist of 14.2° . We also observe a decrease in the standard deviation in BPS twist from 5.9° to 4.2° . This implies that the magnitude of the twist fluctuations at the damage sites are suppressed for the mutagenic bases. In fact we see a reduction in the fluctuations in all the BPS parameters for the damaged base-pair steps relative to control. This indicates that the TD as a whole is a more rigid structure than a regular AT base-pair, something we observed for the linear 40-bp poly(dA:dT) system. The difference in BPS values between the damaged and undamaged systems are shown in Table 3.4 which shows little variation between the 5 TDs.

Husain Minicircles			
BPS Parameter	Undamaged	Damaged	Change
slide / Å	-0.21 ± 0.62	-0.65 ± 0.33	-0.44
shift / Å	0.50 ± 0.76	1.17 ± 0.38	0.67
rise / Å	3.24 ± 0.27	3.76 ± 0.20	0.52
twist °	34.90 ± 5.90	20.28 ± 4.20	-14.62
tilt °	3.10 ± 3.88	-15.14 ± 3.44	-18.24
roll °	0.84 ± 5.52	23.90 ± 4.48	23.06

Table 3.3: Time averages, standard deviations and changes of BPS parameters for all mutated bases, averaged across all TDs (1st, 33rd, 65th, 97th and 129th base-pair steps) for damaged and undamaged Husain 160-bp minicircles.

	TD1	TD2	TD3	TD4	TD5
Δtwist°	-15.30	-15.60	-15.70	-13.10	-14.40
Δroll°	24.20	22.10	24.20	22.70	22.10
Δtilt°	-18.60	-18.40	-18.40	-17.40	-18.40
$\Delta\text{rise} / \text{Å}$	0.48	0.55	0.49	0.54	0.53
$\Delta\text{slide} / \text{Å}$	-0.37	-0.50	-0.42	-0.27	-0.61
$\Delta\text{shift} / \text{Å}$	0.67	0.58	0.67	0.88	0.55

Table 3.4: Difference in average BPS parameters for all 5 TDs (1st, 33rd, 65th, 97th and 129th base-pair steps) between damaged and undamaged 160-bp minicircles.

3.5.4 Twist-writhe Partitioning

Next we investigated how the damaged and undamaged minicircles distributed twist and writhe. A perfectly planar circle has null writhe and so partitions its linking number solely into twist. Our 160-bp minicircle has a linking number 15 and hence a twist of 15. $\text{Lk}_0 = 160/10.5 = 15.23$, $\Delta\text{Lk} = -0.23$ and so $\sigma = -0.016$. The value for the all-atom pitch of 10.5 bp/turn is the value used in Ref.[133].

The writhe of the molecular contour was then calculated for every frame of the 100 ns trajectory using the definitions given in Section 2.5. The twist values can then be determined from $\text{Lk} = \text{Tw} + \text{Wr}$. The averages of twist, writhe and excess twist across the trajectory were:

$$\begin{aligned}
\langle \text{Tw} \rangle &= 15.009 \pm 0.022, \quad \langle \text{Wr} \rangle = -0.009 \pm 0.022, \quad \langle \Delta\text{Tw} \rangle = -0.221 \pm 0.022, \\
\langle \text{Tw} \rangle_{\text{TD}} &= 14.991 \pm 0.021, \quad \langle \text{Wr} \rangle_{\text{TD}} = 0.009 \pm 0.021, \quad \langle \Delta\text{Tw} \rangle_{\text{TD}} = -0.239 \pm 0.022.
\end{aligned}$$

First we note that for the undamaged system, most of the ΔLk goes into ΔTw ,

with only a small negative writhe. Given that we have shown a TD will locally reduce twist, we might expect the twist of the entire system to decrease and this is in fact what we see. We can make an estimate of how much twist one might expect to lose by looking at the results from the 40-bp poly(dA:dT). The sum of BPS twists, when end effects were neglected, reduced by 9.3° . Hence, for the Husain 160-bp minicircle with 5 TDs, we estimate a reduction of approximately 45° in the sum of BPS twist. Tw, however, is measured in number of turns and so this equates to a change in twist of $\Delta\text{Tw} \approx -0.125$.

This predicted reduction is roughly seven times as large as difference between the twist for the undamaged system relative to the damaged that was computed at around 0.016. Of course, it should be noted these definitions of twist are not equivalent and that the sum of the locally defined BPS twist does not necessarily equate to Tw, although we do expect their values to be similar. However, as noted, reducing the local twist is not the only effect that a TD might have on twist-writhe partitioning. Given the increased axial flexibility and increased curvature of the duplex at the damage site, we may also expect that the writhe could increase in magnitude. For this under-twisted minicircle ($\sigma < 0$), this would lead to an increase in negative writhe.

The observed decrease in twist would then seem to clearly indicate that the ability for a TD to locally reduce twist is the dominant effect when considering twist-writhe partitioning. The fact that the reduction in twist is less than we might expect if we independently considered the local reduction in twist of each TD could be due to these competing effects. In addition, as the magnitude of the writhe in the undamaged system is so small, any substantive decrease in twist for the damaged system will lead to a positive writhe, as is observed. Larger decreases in twist would then lead to larger positive writhe, which are disfavoured due to their energy cost. Furthermore, the polygonal shape favoured by this specific placement of TDs further suppresses out-of-plane writhe fluctuations. These two effects are probably responsible for the relatively small change in Tw between the two systems.

The take home from this is that while the structural and energetic properties of a TD will perhaps have an intuitive effect on the twist-writhe partitioning of a damaged minicircle relative to the same system with no damage, the effect of writhe is far less trivial to predict.

3.5.5 Writhe Minicircles

We have seen that for linear systems or systems with small amounts of over/under twist that a TD remains within the duplex and causes relatively localized effects to structural parameters such as axial bending and BPS parameters. The next step is to probe systems with larger amount of torsional stress. Since bubble formation becomes more prevalent for $\sigma < -0.05$, our aim was to study a 210-bp minicircle with $Lk = -1$ which would yield a superhelical density of exactly $\sigma = -0.05$.

When using explicit solvent, it can take a long time for minicircles to evolve from their initial planar configuration to their preferred writhed state. Noy *et al.* [128] established an effective method for simulating undamaged circular supercoiled systems using an implicit solvent model in Amber. This method is much faster than using explicit solvent and so we attempted to utilise this protocol. It was found, however, that this method lead to severe artefacts and often bubble formation was difficult to avoid when TD(s) were present. This was the case even if the solvation processes involved long minimizations and relaxations.

If one were to perform an investigation into writhed minicircles with explicit water then this process of conversion from implicit to explicit solvent will require refinement. Alternatively, the generation of suitable initial structures from oxDNA or via construction from some pre-known elastic minima may also prove useful.

3.6 T-motifs for DNA Nanotechnology

The progress of DNA nanotechnology has been driven by the discovery of novel structural motifs with a wide range of shapes and uses. As part of a collaboration

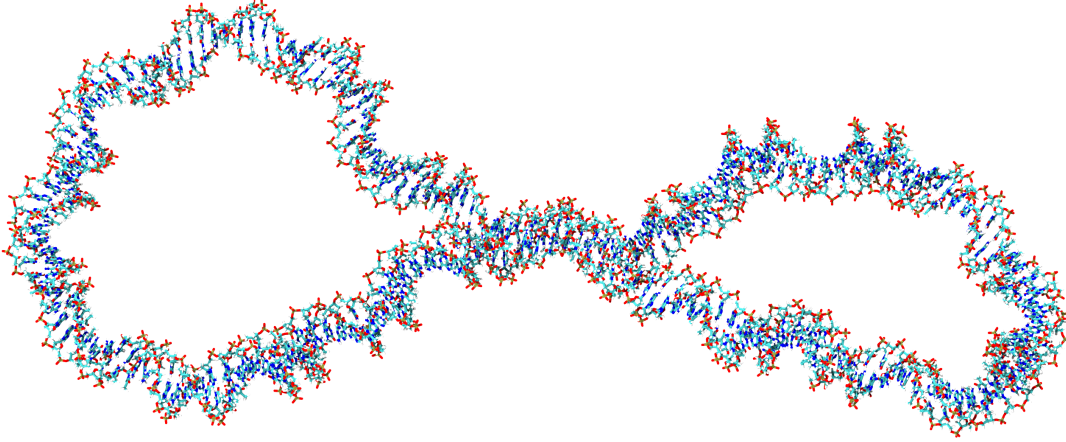


Figure 3.10: Snapshot of 210-bp minicircle with $\Delta Lk = -1$ in implicit solvent. The structure relaxes towards its preferred writhed state from an initially planar circle many orders of magnitude faster than for the explicit solvent.

with Behnam Najafi, a system of 3-armed, planar T-shaped motifs called T-motifs were studied using atomistic simulations to complement his investigation using oxDNA [134].

As shown in Fig. 3.11, a T-motif is a three-armed structure formed by two duplexes. Via the hybridization of a bulge loop in one duplex with a terminal sticky end of the other, two of the arms form an approximately continuous duplex with the third arm roughly perpendicular to this quasi-duplex. The geometry of the B-DNA double-helix implies that the junction will span a different groove depending on the polarity, $\mathcal{P} \in \{3', 5'\}$, of the sticky end. We use the notation $\mathbf{T}_{\mathcal{P}}^L$ to specify a T-motif with polarity $\mathcal{P}$ and bulge length L .

The three arms of the T-motif form right-angled junctions that can be utilized in the self assembly of larger DNA nanostructures. The motif is formed through the interaction of a bulge loop in one duplex and a sticky end of another. The polarity of the sticky end has significant consequences for the thermodynamic and geometrical properties of the T-motif: different polarities create junctions spanning different grooves of the duplex.

Najafi considered bulge sizes ranging from 3 to 9 nucleotides in length for both possible polarities leading to 14 total motifs. A T-motif with bulge length L nucleotides and polarity $\mathcal{P}$ will be denoted as $\mathbf{T}_{\mathcal{P}}^L$. He then simulated all systems using the virtual move Monte Carlo (VMMC) method. Ensemble averages of the three angles α , β and ϕ , defined in Fig. 3.11, indicated fully-formed 5' T-motifs have a stable, planar, and right-angled geometry ($\alpha \simeq \beta \simeq 90^\circ$, $\phi \simeq 0$) for bulge sizes $4 \leq L \leq 8$; for 3' T- motifs the range is more limited, $4 \leq L \leq 6$. Outside of these windows large fluctuations in these angles are observed due to the loss of a well-defined structure, albeit with the average values of the angles deviating not too far from the ideal values. The loss of rigidity is associated with breaking of stacking interactions at the junctions because of the unfavourable geometry.

All 14 T-motifs were simulated using the explicit all-atom protocol described in Section 3.3. The initial structures were obtained from oxDNA and converted to PDB format using tacoxDNA [116]. An example of a converted structure is shown in Fig. 3.12. Production runs of molecular dynamics were run for 200 ns of simulation time.

Angles α, β, ϕ , as defined in Fig. 3.11, were calculated every 5000 time-steps and averaged over the entire trajectory. The results are shown in Fig. 3.13. In order to find the best definitions of the arm-defining vectors **a**, **b** and **c**, we utilised the WrLine algorithm [126] that defines the molecular contour of duplex DNA (see Section 2.7). A line of best fit was then applied to the resulting molecular contour to define the vectors.

From Fig. 3.13 we can see that for motifs $\mathbf{T}_{5'}^{4-9}$ and $\mathbf{T}_{3'}^{3-6}$ we observe stable, planar, and right-angled geometries ($\alpha \simeq \beta \simeq 90^\circ$, $\phi \simeq 0$). For the $\mathbf{T}_{5'}^3$ and $\mathbf{T}_{3'}^9$ motifs we instead see large fluctuations in these angles albeit with the average values of the angles deviating not too far from the ideal values. These large fluctuations indicate the geometric incompatibility with the T-structure may be large.

In $\mathbf{T}_{3'}^7$ and $\mathbf{T}_{3'}^8$, the average ϕ angle deviates significantly from ideal value but with the fluctuations still relatively small. Indicating a relatively stable but undesirable structure. These two motifs form more of a right-handed triad of vectors, with all arms approximately orthogonal to each other. Fig. 3.14 shows a snapshot of a

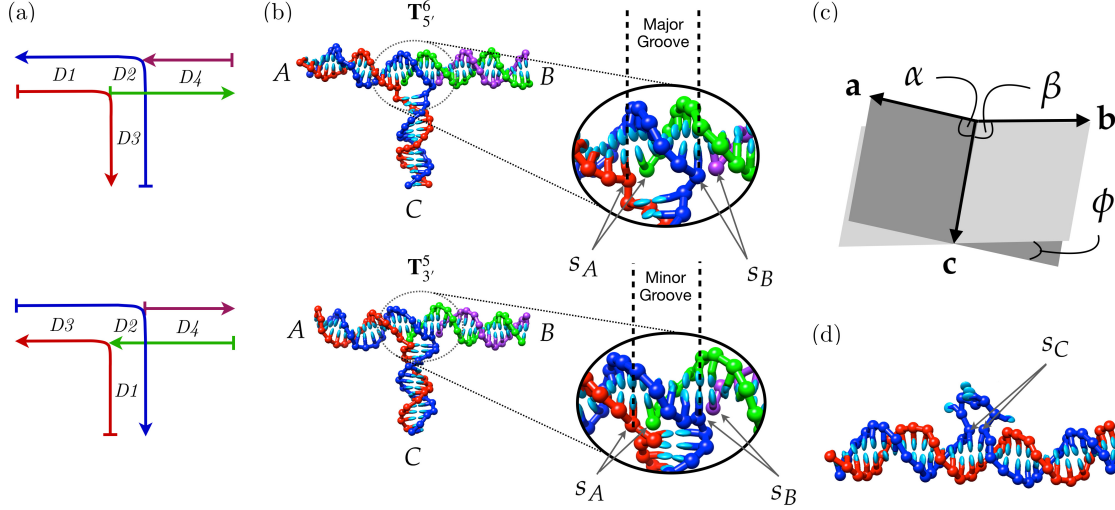


Figure 3.11: (a) Schematic representations of T-motifs, highlighting the four domains of interaction ($D1$ - $D4$) between the four component strands. The coloured arrows indicate the polarities of the strands from 5' to 3'. The junction is formed between two duplexes, one ($D4$) having a sticky end and one ($D1D3$) incorporating a bulge loop. The T-motif is formed through the hybridization of the sticky end and bulge loop in the junction domain $D2$. The geometry of the junction depends on the polarity of the sticky end. In the case of a 5' sticky end, the side branch of the junction is duplex $D3$, which straddles the major groove of the “nicked” duplex formed by coaxially stacked domains $D1D2D4$; in the case of a 3' sticky end, side branch $D1$ straddles the minor groove of the “nicked” duplex $D3D2D4$. We use the notation $\mathbf{T}_{\mathcal{P}}^L$ to describe a T-motif with a $D2$ domain of size L base-pairs and sticky end polarity $\mathcal{P} \in \{3', 5'\}$. (b) A T-motif comprises a side-branch duplex C straddling a doubly nicked duplex AB ; the positions of the two nicks delimit the junction domain $D2$. Snapshots from oxDNA simulations of fully formed T-motifs are shown, highlighting coaxial stacking sites s_A and s_B on either side of $D2$. (c) Scheme used to characterise the geometry of T-motifs. Vectors $\mathbf{a}$, $\mathbf{b}$ and $\mathbf{c}$, which correspond to the orientations of the three arms of the junction, are calculated by summing over base-normal vectors of the nucleotides in the 11 middle base pairs of the corresponding domain. We define angles α and β as positive for $(\mathbf{a} \times \mathbf{c}) \cdot (\mathbf{b} \times \mathbf{c}) < 0$ and ϕ as positive for $[(\mathbf{a} \times \mathbf{c}) \times (\mathbf{b} \times \mathbf{c})] \cdot \mathbf{c} < 0$. All three angles have positive values in the configuration shown here. (d) A third coaxial stacking interaction, at site s_C , can occur between the base pairs of $D1$ and $D3$ that flank the bulge; this configuration competes with the formation of the T-motif, especially for shorter bulge loops.

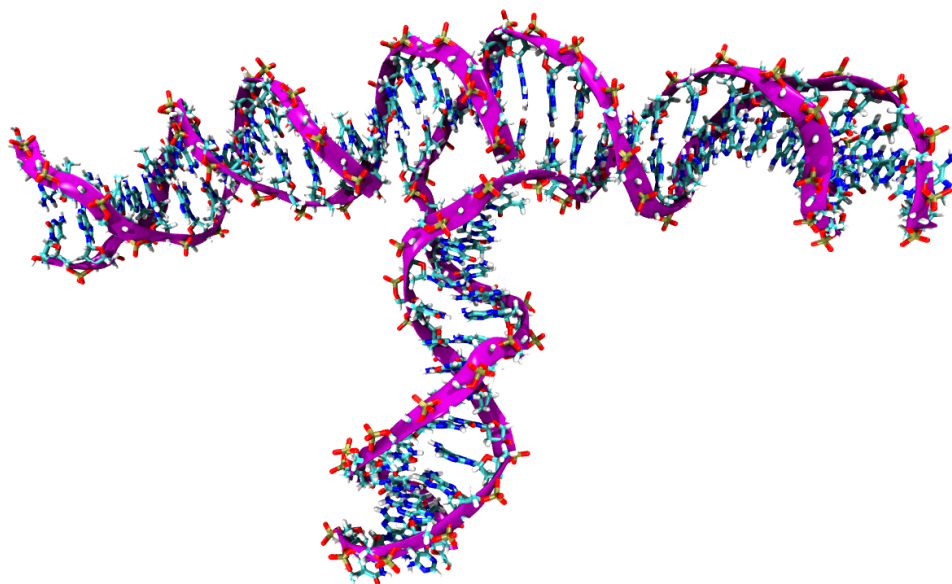


Figure 3.12: Snapshot at the end of a 200 ns all-atom molecular dynamics simulation of the $\mathbf{T}_{5'}^6$ motif.

representative configuration of the $\mathbf{T}_{3'}^7$ motif. This loss of a well-defined T structure for $\mathbf{T}_{3'}^{7-9}$ agrees with the oxDNA calculations.

3.7 Conclusion

To summarise we have taken a TD force field modification and applied it to a range of systems of varying length and topology to investigate the effects this mutagen would have compared to the undamaged systems in the all-atom representation.

It was found that a TD almost certainly bends the duplex, with a 40-bp poly(dA:dT) simulation indicating that this bending angle could be around $20 - 26^\circ$ depending on the definition of the angle, putting it well within many experimental estimates. The BPS parameters further provided good agreement with experiment,

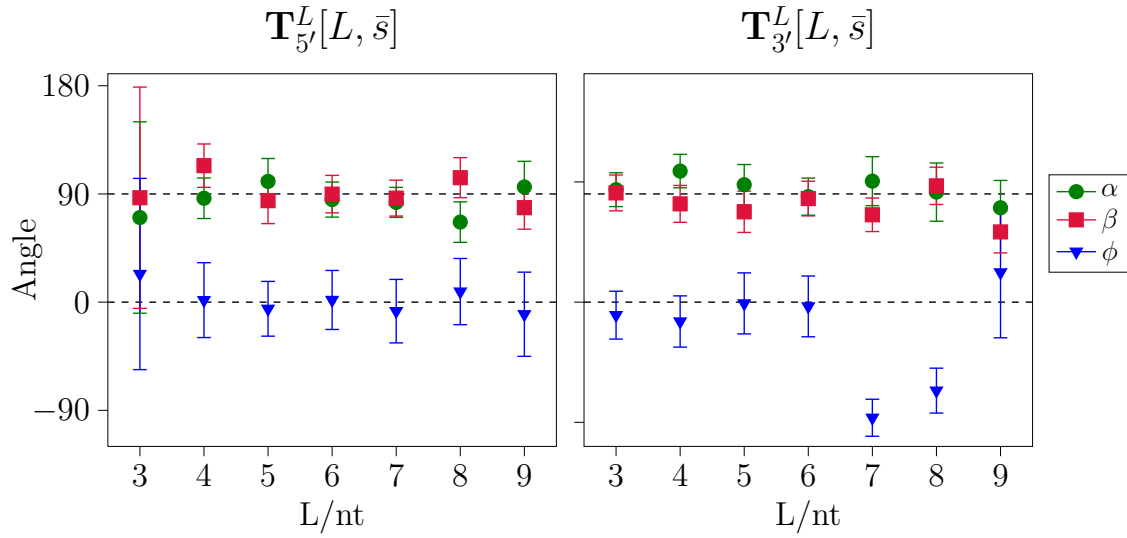


Figure 3.13: Mean values and standard deviations for angles between arms (see Fig. 3.11 for definitions) for the all-atom molecular dynamics simulations. The bulge size L is given in terms of nucleotides (nt). $5'$ motifs all remain approximately T-shaped whereas $3'$ motifs are only T-shaped for $3 \leq L \leq 6$.

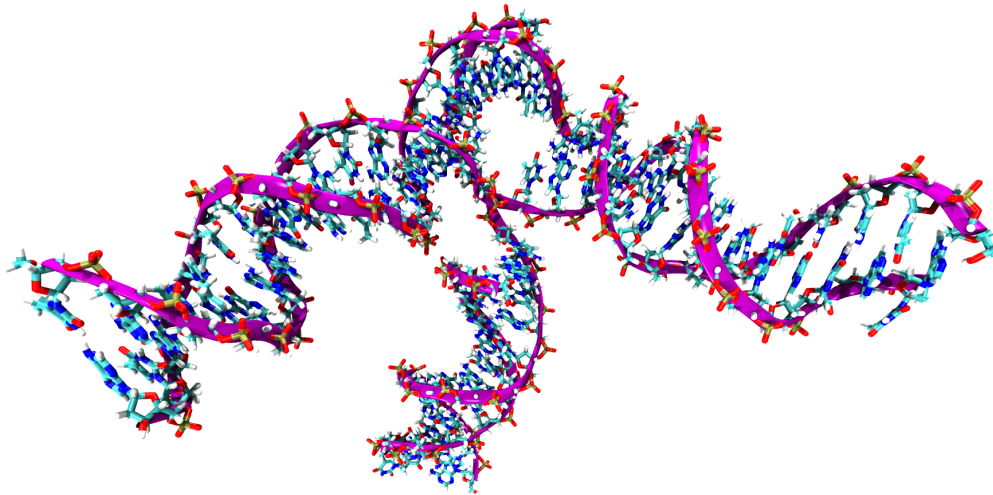


Figure 3.14: Snapshot at the end of the all-atom simulation of the $\mathbf{T}_3^7$ motif. Note that this motif deviates from the ideal T-shaped geometry.

especially for the rotational parameters, the caveat being that these values are not always agreed upon within the literature. The reduction in the fluctuations in these parameters, as quantified by their standard deviations, implied that this atomistic TD model is less flexible than a regular TT BPS. Simulations of minicircles allowed for the computation of curvature along the sequence that strongly demonstrated that the damaged locations are highly bent relative to the rest of the chain.

Twist-writhe partitioning was investigated for a 160-bp minicircle revealing that the presence of a TD causes a reduction in twist and hence, an increase in writhe when compared with the undamaged system. The physical properties of the TD were then used to argue why the minicircle may choose to partition twist and writhe in this way.

Despite the successes of the TD model on systems with low torsional stress, our methods could not be extended to under-twisted systems where the equilibrium structure is writhed. A combination of time-scales in explicit solvent simulations and the tendency for implicit solvent simulations to break the TD base-pairing lead to many issues. More work must be done to elucidate whether this is an issue of the MD protocol or an inherent shortcoming of the model.

Finally, atomistic simulations of a series of T-motifs provided more evidence to suggest the design of these motifs are suitable for use in self-assembling larger nanostructures. Complementing the coarse-grained simulations of Najafi, bar a couple of motifs, our all-atom simulations suggest that the structures remain largely in their idealised T shapes.

4

OxDNA Simulations

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4.1 Introduction

OxDNA provides us with a detailed yet pragmatic description of DNA. The nucleotide level coarse-graining simplifies the problem of modelling a complex structure such as DNA, while still capturing many of the structural, mechanical and thermodynamic properties of double- and single-stranded DNA. The computational savings of this coarse-graining along with its use of implicit solvent allow us to explore larger systems and longer time-scales than is feasible with the all-atom representation.

We first discuss the oxDNA Thymine dimer model(s) and then investigate several supercoiled systems and their topoisomers to probe the effect of this mutation on

dynamics and damage localization. Lim *et al.* [46] already explored the effect of TDs in plectonemic DNA using oxDNA, demonstrating that damaged systems form plectonemes for lower levels of under-twist [46] and preferentially pin damage and damage-induced bubbles towards the tip of plectonemic structures. This work complements the the study of Matek *et al.* [45] who established that bubbles will preferentially diffuse towards and become pinned at plectoneme tips.

4.1.1 OxDNA Thymine Dimer

In an attempt to model biologically motivated DNA inhomogeneities, Randisi developed a model of a TD in oxDNA [81]. The way to best capture this effect is perhaps less transparent than in the all-atom picture picture as we cannot just rely on the altered chemistry of the TD to motivate our modification (we may not bond the carbon atoms specifically if they do not exist!).

Instead, Randisi devised three models that all alter the oxDNA force field parameters in the vicinity of the TD. Intent on capturing the reported induced bending in straight duplexes along with oxDNA’s accurate thermodynamics, Randisi subsequently tuned the three TD models such that they resulted in the correct melting temperature drop given a desired bending angle. Due to the lack of certainty in the literature of this angle as discussed in Chapter 3, Randisi used two different angles, 15° and 30° across his three TD models. Initially a 40 bp poly(dA:dT) was minimized for various values of induced roll, to produced the desired bending angle for each model, then force field parameters pertaining to stacking and hydrogen bonding were adjusted to reproduce the desired melting temperature drop. An overview of these three models are given in Table 4.1.

A fundamental limitation of the current implementation of the oxDNA TD model is that it only supports a maximum of 2 TDs. The manner in which the TD is modelled in oxDNA means that adding more TDs is not necessarily trivial like in Amber when one simply needed to create a new residue.

Comparing these three models to our all-atom results of Chapter 3, we find our bending angle of approximately 23° sits roughly halfway between the two values

Features of TD Model			
	TD1	TD2	TD3
roll angle ϕ	37°	18.3°	27.1°
duplex angle θ_0	30°	15°	15°
stacking multiplier	1	1	10
# base-steps with modified stacking	2	2	1
hb weakening	10%	55%	60%
melting temperature drop [°C]	12.7	14.4	13.2

Table 4.1: Overview of TD Models developed by Randisi, all three models do not include a modification in tilt. The strength of the stacking potential is multiplied by the stacking multiplier. Reproduced from Ref. [81].

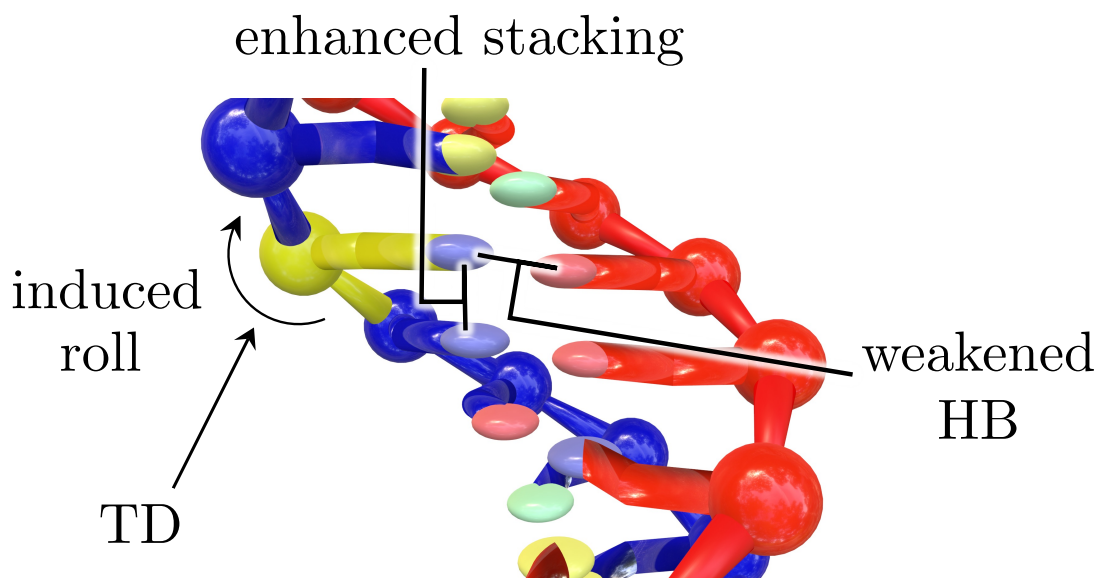


Figure 4.1: Close up of linear duplex containing a thymine dimer modelled using TD3 (yellow) after an energy minimization. Note that the TD3 model only explicitly induces roll in one nucleotide step and hence, only one nucleotide of the thymine dimer is explicitly coloured yellow.

used in the parametrisation of the oxDNA TDs. The oxDNA TDs largely obtain their bending angle through inducing roll, whereas the all-atom simulations also see significant variations in tilt (see Fig. 3.4). The relatively large magnitude in the reduction of hydrogen bonding seen in the all-atom simulations is more in keeping with TD2 and TD3. However, these values are not suitable for a like-for-like comparison. The all-atom metric of HB was defined as the proportion of the time hydrogen atoms were deemed close enough to be considered bonded, whereas the oxDNA HB weakening represents a reduction in the HB interaction

potential strength.

In this thesis we choose to implement the TD3 model as we believe all its modifications lie within sensible bounds compared with experiment and the all-atom results. It is also the model of choice for the investigation into plectoneme tip damage localization performed by Lim *et al.* [46]. That being said, it would be relatively easy to implement a new oxDNA TD model if one were to draw a more certain consensus over the effect of damage on the structural parameters. It was found that the effect of TD3 was a reduction in base-pair step twist of only around 2° in two base-pairs steps, a net effect of about 4° . This is significantly lower than the approximately 15° reduction in twist calculated for the all-atom TD.

4.1.2 Simulation Protocol

The comparatively simple protocol utilised in this thesis for oxDNA simulations contains the following steps: minimization of the initial structure for 10^4 steps, followed by a relaxation phase for 10^4 steps. Once this has been achieved, a MD production run can begin. For the systems in which we are interested, equilibrium is reached relatively quickly, with the slowest time-scale being the transition from planar minicircle into its preferred writhed structure.

The minimizations were performed on a CPU at 0 K, with the relaxation and MD performed with a GPU back-end at a temperature of 300 K. All simulations were run at a salt concentration of 0.1 M using the sequence dependent oxDNA2 model. A time-step of 0.002 simulation units was chosen with a Langevin thermostat with $\gamma = 0.04$ and $\gamma_{\text{rot}} = 0.0133$. As an example, 10^7 steps with a time-step of 0.002 yields a simulation time of 606 ns (see Table 2.1 for unit conversions). We note that in coarse-grained simulations, the time scales are often determined by the dynamics of the coarse-grained entities rather than individual particles. The behavior of these entities depends on the chosen level of coarse-graining and the specific interactions and forces included in the model. As a result, the time scales in coarse-grained simulations can be significantly different from the time scales observed in the fully atomistic system.

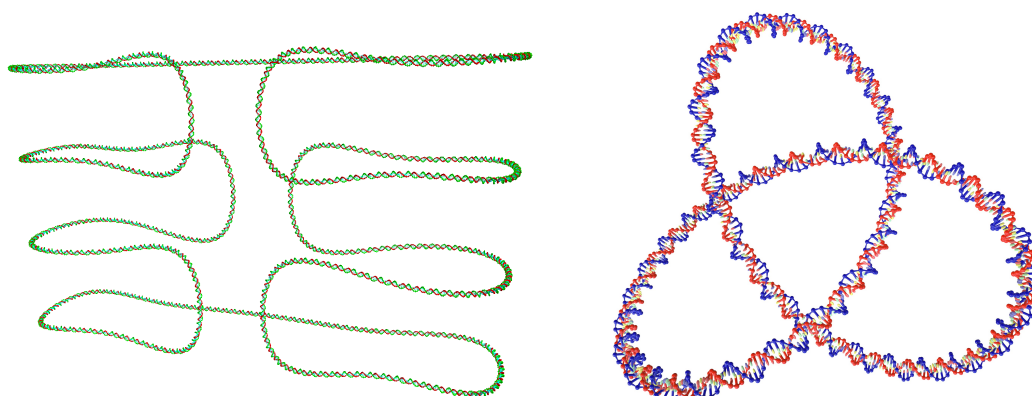


Figure 4.2: Examples of structures created using custom Python script. 4798 bp Hilbert space filling curve with $\sigma = -0.05$ (left) and 500 bp left-handed trefoil knot with $\sigma = 0$ (right).

Initial structures were created using the `oxDNA` utils scripts `generate.py` and `generate-sa.py` that create linear duplexes and planar minicircles with a desired linking number by controlling the twist. Another version of this tool was developed in Python to create more complex structures with desired linking numbers, however, structures generated in this way are not studied in this thesis. Once the centre line of the structure is constructed, a duplex with an ideal pitch is created that follows the centre-line. The twist and writhe are then calculated using the routine described in Section 2.7.2. The twist per base-pair is then varied such that the structure has the desired linking number. Examples created using this script include a 4798 bp Hilbert space filling curve with $\sigma = -0.05$ and a 500 bp left-handed trefoil knot with $\sigma = 0$, see Fig. 4.2.

Used in conjunction with the `tacoxDNA` `oxDNA` to PDB converter [116], it is trivial to also create all-atom configurations and topologies given a desired superhelical density with an arbitrary centre-line. This could prove very useful when the system in question is either hard to construct using pre-existing methods, or the time-scale to form the structure from a simpler initial structure requires too much computer time.

4.2 Linear DNA

The TD3 oxDNA model has been parametrised to have a resting bend angle of 15° , but what effect does this model have on the tangent autocorrelation of linear DNA? In order to compare directly to the Amber all-atom simulations. We simulated a 128 bp linear duplex which is a combination of four of the 32-mers studied by Husain *et al.* [47]. We then placed a TD halfway along the chain at bp 65. Both the damaged and undamaged systems were prepared, minimized and run for approximately $6 \mu\text{s}$ using the protocol in Section 4.1.2. The molecular contour was then calculated using the WrLine algorithm and from this the curvature along the chain was calculated every 1000 time-steps and averaged across the trajectory. The results for the curvature are shown in Fig. 4.3.

The curvature as expected has a peak centred around the damage site (bp 65). However, less expectedly, we see that this peak is noticeably wide with a small sub-trough in the centre of the peak. Hence, we may deduce that the oxDNA TD increases curvature substantially for a region of approximately 10 bp around the damage, but with a local minimum in curvature at the exact damage site. It is our belief that this dip in the curvature peak around the damage site is simply an algorithmic artefact from finding the molecular contour of damaged DNA. We note that the WrLine algorithm requires the positions of the 5 base-pairs either side of each step. The algorithm uses these base-pairs to form approximately one-helical turn. Hence, the reduction in twist caused by a TD could affect this method as it disrupts the ideal helical pitch of B-DNA.

4.3 Minicircles

4.3.1 Husain Minicircles

We replicate the 160-bp minicircles with $Lk = 15$ simulated in Section 3.5 using the oxDNA model. This time the pitch of oxDNA leads to $Lk_0 = 15.165$ and hence $\Delta Lk = -0.165$ and $\sigma = -0.011$. However, there is a fundamental limitation in the way the oxDNA TD model is implemented that means we may only simulate a

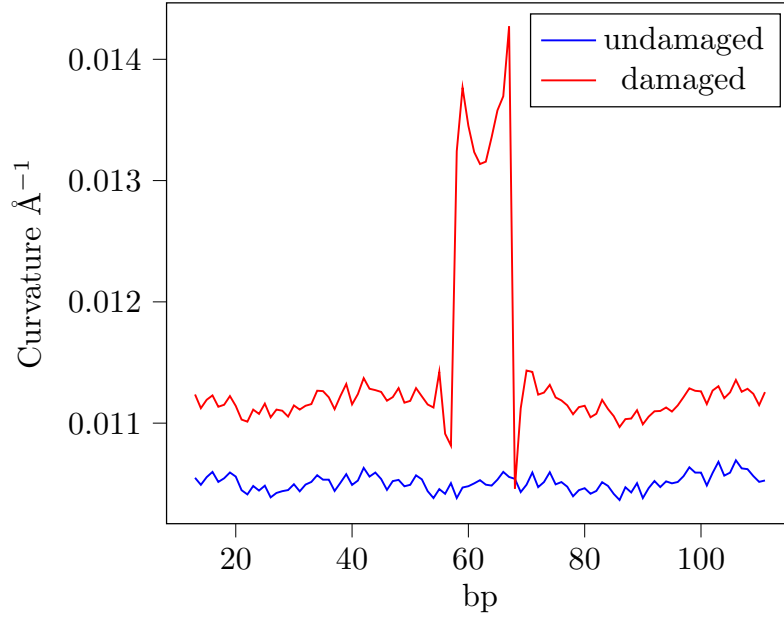


Figure 4.3: Plot of curvature as a function of base-pair index for both the undamaged (blue) and damaged (red) 128 bp linear strand.

system with at most 2 TDs. Hence, we shall replicate the 160-bp Sancar minicircle but only have TDs on the first two 32-mers (at BPS 1 and 33). Following the protocol outlined in Section. 4.1.2, we simulated the undamaged minicircle and the minicircle with 2 TDs present for 10^8 time-steps for a simulation time of $6 \mu\text{s}$. These simulations were considerably longer than achievable in Amber for a similar computer run-time ($\sim 100 \text{ ns}$).

Writhe was then calculated for every 1000 time-steps and time-averages computed across the trajectory, with corresponding twist values obtained from $\text{Lk} = \text{Tw} + \text{Wr}$. The average values were

$$\begin{aligned} \langle \text{Tw} \rangle &= 15.011 \pm 0.003, \quad \langle \text{Wr} \rangle = -0.011 \pm 0.003, \quad \langle \Delta \text{Tw} \rangle = -0.154 \pm 0.003 \\ \langle \text{Tw} \rangle_{\text{TD}} &= 15.015 \pm 0.004, \quad \langle \text{Wr} \rangle_{\text{TD}} = -0.015 \pm 0.004, \quad \langle \Delta \text{Tw} \rangle_{\text{TD}} = -0.150 \pm 0.0031. \end{aligned}$$

First, we observe that the mean twist of the undamaged minicircle in oxDNA is very similar to the value calculated for the minicircle when simulated in the all-atom representation. The average twist for the all-atom undamaged minicircle was 15.009 ± 0.022 , marginally lower in value. This is likely due to the fact that the ideal pitches of B-DNA will differ slightly between all-atom and oxDNA simulations.

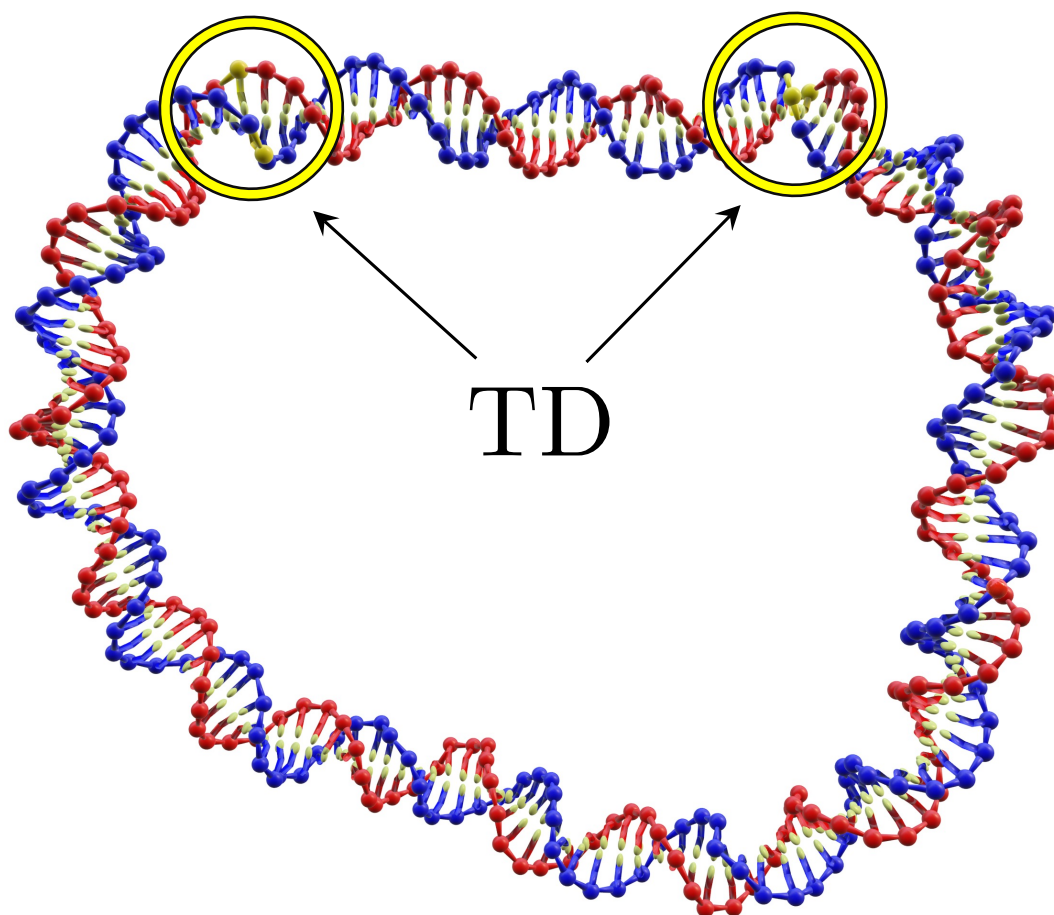


Figure 4.4: Snapshot at the end of the relaxation of 160 bp minicircle with $Lk = 15$ with 2 thymine dimers (yellow) at BPS 1 and 33. We note the straightening of the duplex between the TDs and the increased curvature on the rest of the minicircle as a result.

For oxDNA the pitch is 10.55 bp [80], whereas for all-atom it is 10.5 bp. This means that despite having the same linking number of 15, they have different values of Lk_0 and hence σ . For example for the all-atom system $\sigma = -0.016$ and for oxDNA $\sigma = -0.011$. Hence, the undamaged oxDNA minicircle is about 2/3 as under-twisted as the all-atom minicircle (albeit the magnitudes of these numbers are very small).

Interestingly for the damaged minicircle, we see an increase in twist and a decrease in writhe of about 0.004. This is in contrast to the all-atom simulations where the twist decreased by 0.018 from 15.009 for the undamaged minicircle to 14.991 for the minicircle with a TD present. To understand why this might be we will recap the competing effects contributing to twist-writhe partitioning. Since the TD decreases local twist, this could lead to a global increase in writhe. For our system, $\sigma < 0$ and so this equates to a decrease in negative writhe. However, due to the increased flexibility and curvature of the duplex at the TD site, we may also see an increase in the magnitude of writhe. For our minicircle, this would be an increase in negative writhe.

For the oxDNA minicircles, since we see an increase in negative writhe, then we must conclude that the increased flexibility and curvature of the duplex at the TD site is the dominant effect for twist-writhe partitioning. Whereas for the all-atom simulations, the dominant effect was the TDs ability to reduce twist. This is perhaps not surprising when we consider that there were 5 TDs in the all-atom simulations, compared with only 2 for the oxDNA simulations, and that the all-atom TD has been shown to absorb approximately 15° of BPS twist, compared with only 4° for the oxDNA TD.

We also note that the relative orientations of the 5 TDs in the all-atom simulations of the same minicircle were such that the minicircle favoured in plane configurations. However, with only 2 TDs in the oxDNA simulation, we do not see such a strong preference for TD orientation and hence, no additional penalty for out-of-plane fluctuations.

Curvature was also calculated as a function of base-pair step for both the damaged and undamaged systems. The results are shown in Fig. 4.5. Two very

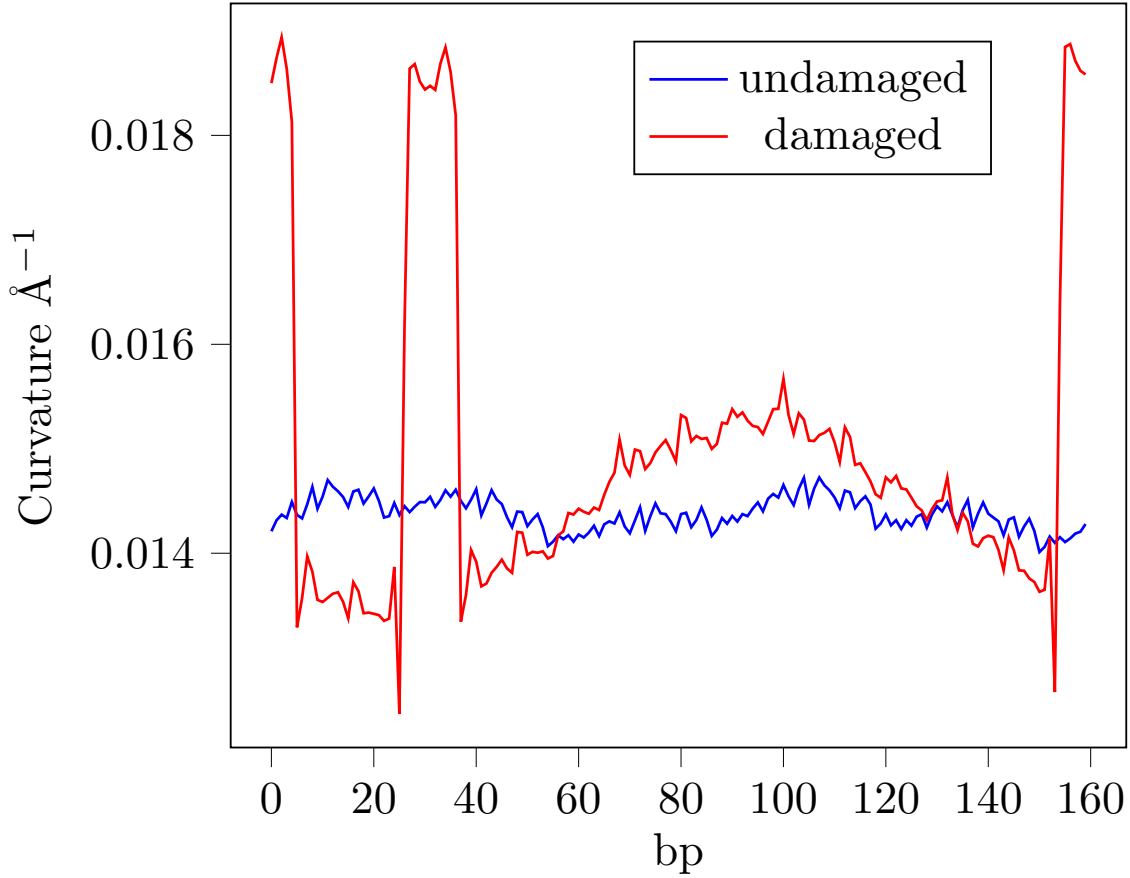


Figure 4.5: Plot of curvature as a function of base-pair index for both the undamaged (left) and damaged (right) 160 bp minicircles. Note the two distinct peaks (modulo 160 bp) for the damaged system indicating increased curvature at the TD sites.

prominent peaks are present at the two TD sites. We notice that the peaks show qualitatively the same characteristics as seen in Fig. 4.3 for the linear duplex with a TD, however, the increased curvature at the peaks relative to the background is larger. I.e. the curvature of the circle is more localized at the TD site for the minicircle than one might expect comparing with linear DNA. We also observe a peak in curvature centred halfway between the two TDs (halfway between the largest distance), indicative of a “horseshoe” like structure (see Fig. 4.4).

4.3.2 Twisted Minicircles

Next we investigated a series of larger minicircles with larger degrees of under-twist. We simulated a 336-bp minicircle with $\Delta Lk = 0.15$ and its negatively supercoiled topoisomers $\Delta Lk = -0.85, -1.85$ and -2.85 using the aforementioned protocol

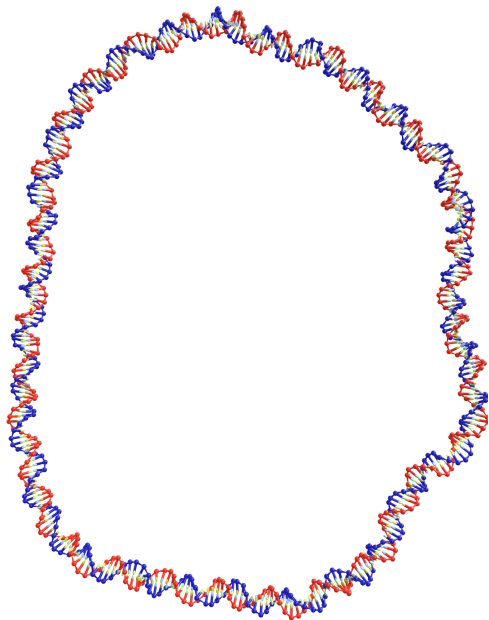
from Section 4.1.2 with one TD placed at the 60th base-pair step (indicated in red) for 10^8 time-steps equating to a simulation time of approximately $6 \mu\text{s}$. This sequence was studied by Noy *et al.* [135] and chosen as $336/10.5$ leads to an integer Lk_0 of 32, such that topoisomers would have integer ΔLk . However, as noted the pitch of B-DNA in oxDNA is closer to 10.55 and so in reality we have values slightly differing from the values $\Delta Lk = 0, -1, -2, -3$ one would expect if studying these systems in the all-atom picture. These topoisomers correspond to superhelical densities of $\sigma = 0.0047, -0.02667, -0.05808, -0.08948$. The sequence reads:

```
AAACGCGCGAGGCAGCTGTATGGCATGAAAGAGTTCTTCCCGGAAAACGCGGTGGAATATTTCGTTTC
CTACTACGACTACTATCAGCCGGAAGCCTATGTACCGAGTTCCGACACTTTCATTGAGAAAGATGCCT
CAGCTCTGTTACAGGTCACCTAATACCATCTAAGTAGTTGATTCATAGTGAAGTGCATATGTTGTGTTTT
ACAGTATTATGTAGTCTGTTTTTATGCAAAATCTAATTTAATATATTGATATTTATATCATTTTACG
TTTCTCGTTCAGCTTTTTTATACTAAGTTGAGCGAAACGGGAAGGGTTTTACCGATATCACCG.
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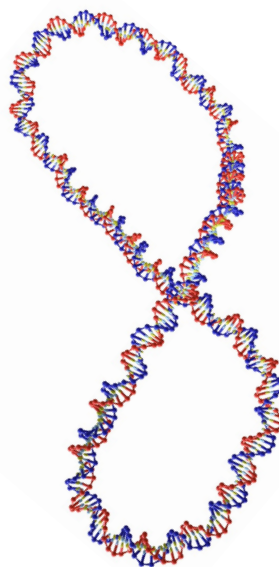
As expected the topoisomers with larger negative superhelical densities begin to writhe around themselves to form closed plectonemic structures, snapshots of each topoisomer are shown in Fig. 4.6. These simulations are not long enough to properly probe the ideas regarding damage diffusion but certain trajectories did qualitatively demonstrate periods where the TD situated itself at the tip of the plectonemic structure (see Fig. 4.7). In Fig. 4.7 we in fact see that one plectoneme tip is occupied by the TD and the other by a bubble. Given that bubbles will also have lower bending stiffnesses than un-melted DNA, they also receive an energetic reward for being situated at the point of highest curvature. In this instance there is a configuration where both the bubble and TD are in their preferred positions but there could arise scenarios where the TD and bubble compete to be located at a plectoneme tip.

The molecular contour of each simulation was calculated using the WrLine algorithm every 1000 time-steps for both the damaged and undamaged minicircles. The writhe of each topoisomers molecular contour was then calculated across the whole trajectory. Bubble formation was tracked across the simulations via the calculation of the angle between complementary bases. If this angle exceeded 60° then we deemed there to be a denaturation bubble at that base-pair. We note

$$\Delta Lk = 0.15$$



$$\Delta Lk = -0.85$$



$$\Delta Lk = -1.85$$



$$\Delta Lk = -2.85$$

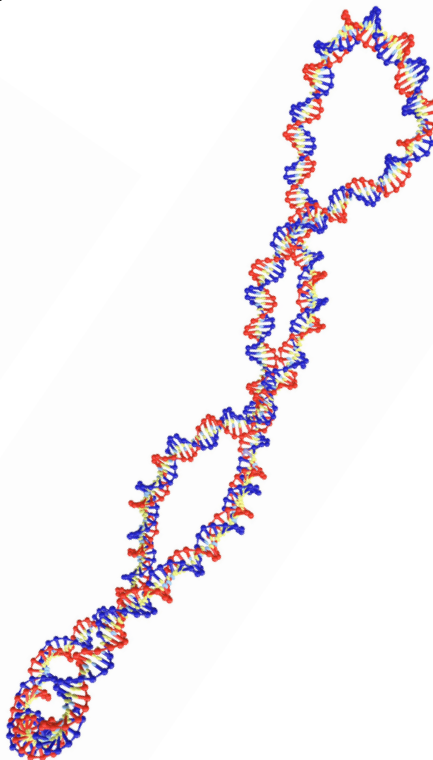


Figure 4.6: Snapshots of undamaged 336-bp minicircles for the four topoisomers $\Delta Lk = 0.15, -0.85, -1.85, -2.85$.

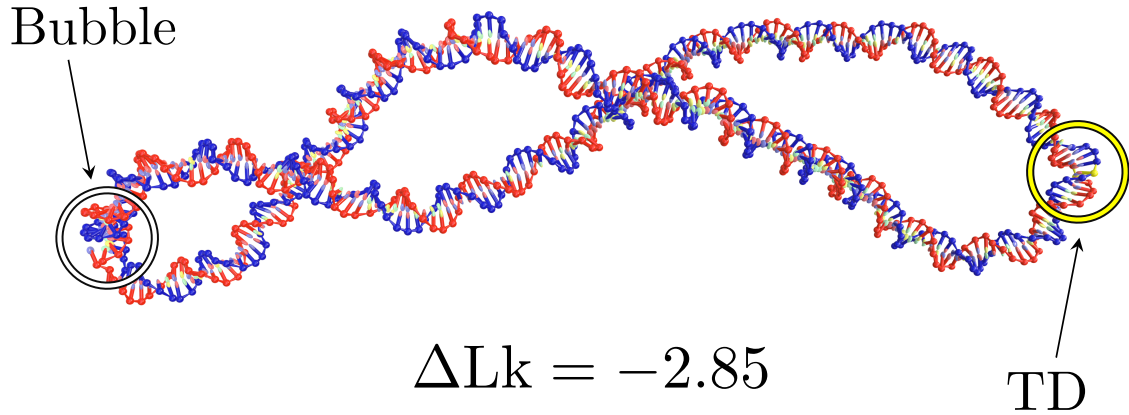


Figure 4.7: Snapshot of damaged 336-bp minicircles for the $\Delta Lk = -2.85$ topoisomer. In this configuration we see the TD at one tip of the plectonemic structure and a bubble at the opposite end.

that this choice is rather arbitrary. A snapshot of the undamaged $\Delta Lk = -2.85$ minicircle during a period of bubble formation is shown in Fig. 4.8.

The writhe results as a function of time are shown in Fig. 4.9. Given the large number of points (over 100,000), we calculated the average writhe every 200 snapshots and calculated the average of that period. Average writhe points are encompassed with a yellow circle if there was at least one bubble for the majority of the frames within the period. E.g. the first blue scatter point of the top left plot in Fig. 4.9 represents the average writhe of the first 200 frames of the simulation. This point is not encompassed by a yellow circle as there were fewer than 100 frames in this period where at least one bubble was detected.

From Fig. 4.9 we can see that the presence of a TD has little effect on writhe for small amounts of excess twist ($\Delta Lk = 0.15, -0.85$). For larger amounts of under-twist ($\Delta Lk = -1.85, -2.85$) we observe a more noticeable increase in negative writhe for the damaged system relative to the undamaged. We also see that bubbles are relatively uncommon for undamaged DNA with $\Delta Lk \geq -1.85$, with the amount of bubble formation increasing dramatically for $\Delta Lk = -2.85$.

We then plotted the writhe and number of base-pairs in bubbles for the $\Delta Lk = -2.85$ topoisomer on the same axis, see Fig. 4.10. From this figure we observe that

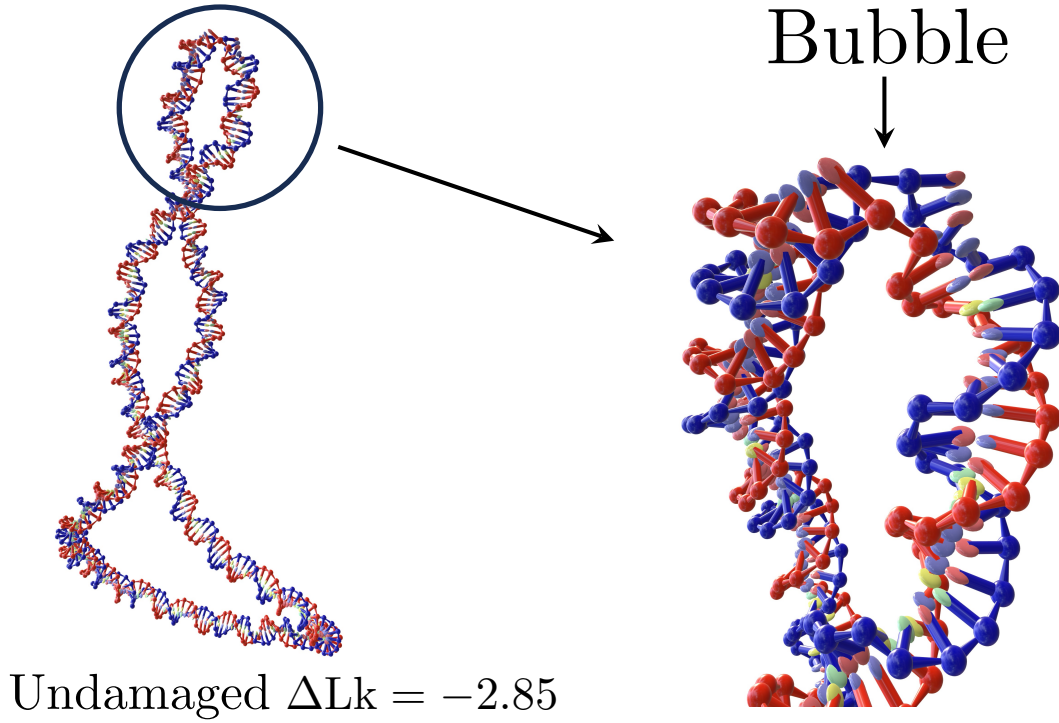


Figure 4.8: Snapshot of undamaged $\Delta Lk = -2.85$ 336-bp minicircle with bubble region indicated.

bubble formation is strongly correlated with writhe. There are two main reasons why bubbles could have an effect on the twist-writhe partitioning of DNA, with both reasons related to the increased flexibility of a bubble compared with regular DNA. Firstly, the rotational flexibility can allow twisted structures to relax torsional stress. In the case of under-twisted minicircles, this is achieved by increasing negative excess twist as it is easier to concentrate that twist into the bubble. This then has the effect of making the writhe of the structure less negative. Secondly, the increased axial flexibility could make structures easier to writhe given the lower cost associated with bending, whether that be positive or negative writhing. The idea that we can infer knowledge of bubble formation from the writhe, a quantity that can be calculated from the centre-line of the DNA, could prove a useful method for bubble detection if the resolution of the DNA is only to the duplex level.

These reasons are similar but subtly different to the reasons a TD might affect twist-writhe partitioning we discussed in Section 4.3.1 and Section 3.5. The difference is that a TD reduces local twist, whereas a bubble absorbs local ΔTw , i.e. it increases

the magnitude of ΔTw . The second is that a bubble is more flexible than a TD.

For the $\Delta Lk \leq -0.85$ topoisomers, the writhe in the absence of bubbles becomes more negative as the superhelical density becomes more negative, as expected. The writhe of the damaged systems relative to the undamaged also become increasingly more negative as ΔLk becomes more negative. For $\Delta Lk = -2.85$, the more negative writhe for the damaged system relative to the undamaged system is due to the larger amounts of bubble formation which can relieve excess under-twist as mentioned before. For $\Delta Lk = -1.85$ however, we see that during long periods of no bubbles, that the writhe of the minicircle with the TD is on average more negative than the undamaged minicircle.

Averages of writhe were then computed across the trajectories. To investigate the sole effect of the TD, samples were taken for periods of the trajectory without bubbles. The results for which are shown in Fig. 4.11. For the $\Delta Lk = 0.15$ minicircle, the undamaged minicircle has a writhe of approximately zero, but positive. Since the a TD will induce a local amount of negative twist, we might expect the writhe of the minicircle to become slightly more positive. However, we see that the writhe is largely unchanged and in fact, marginally decreases.

For the more negatively supercoiled minicircles, we see that the average writhe in the absence of bubbles decreases when a TD is present relative to the undamaged minicircle, with a larger effect for larger amount of negative excess link. This indicates that the dominant effect of the TD on twist-writhe partitioning is that the axial flexibility will act to increase the magnitude of the writhe. For our minicircles, this will mean an increase in negative writhe. This is perhaps not surprising given that the oxDNA TD absorbs only about 4° of twist.

The fact that the presence of a TD can be seen to increase negative writhe in under-twisted systems provides a possible explanation as to why we see less bubble formation in the damaged minicircles. Given that for a minicircle the excess link is distributed as

$$\Delta Lk = \Delta Tw + Wr, \quad (4.1)$$

an increase in negative writhe will decrease negative ΔTw as both quantities will be negative when $\Delta Lk < 0$. A reduction in ΔTw means that the minicircle is experiencing less under-twist, and under-twist is a major cause for denaturation bubbles.

Since it has been observed that bubbles preferentially diffuse towards the tips of plectonemic structures [45], any theoretical damage search method could potentially be aided by the fact that TDs may suppress bubble formation. Essentially, if a TD wishes to be located by some mechanism that we shall assume requires it to occupy the tip of a plectoneme, then its main competitor in the remaining undamaged section of the DNA for a place at the tip is the bubble - another twist absorbing and easily bent structure. The ability for a TD to inhibit another tip occupying object would enhance its own ability of reaching the tip. Coupled with the fact that bubble formation is more likely at a TD given the reduced melting temperature, this will again reduce the likelihood of the TD not being the most energetically favourable object to be positioned at the plectoneme tip.

4.4 Discussion and Future Work

In this section we have utilised the oxDNA model to investigate linear and circular DNA with and without a TD. We have shown that the oxDNA TD3 model causes regions of sharply increased curvature. Calculation of the writhes of various supercoiled minicircles revealed that the oxDNA TD has the effect of increasing negative writhe for under-twisted systems.

We observed that for the twisted minicircles, fewer bubbles were observed for the systems that had a TD. A possible explanation for this potential phenomenon was proposed based on the fact that an increase in negative writhe will decrease negative ΔTw . A reduction in ΔTw means the system experience less under-twist and hence, bubbles are less likely to form. Despite this, a significantly more extensive investigation will need to be undertaken. Given the relative ease of set-up and computational efficiency of the oxDNA model, there is large scope to probe many circular systems and their topoisomers to see whether or not this trend is universal.

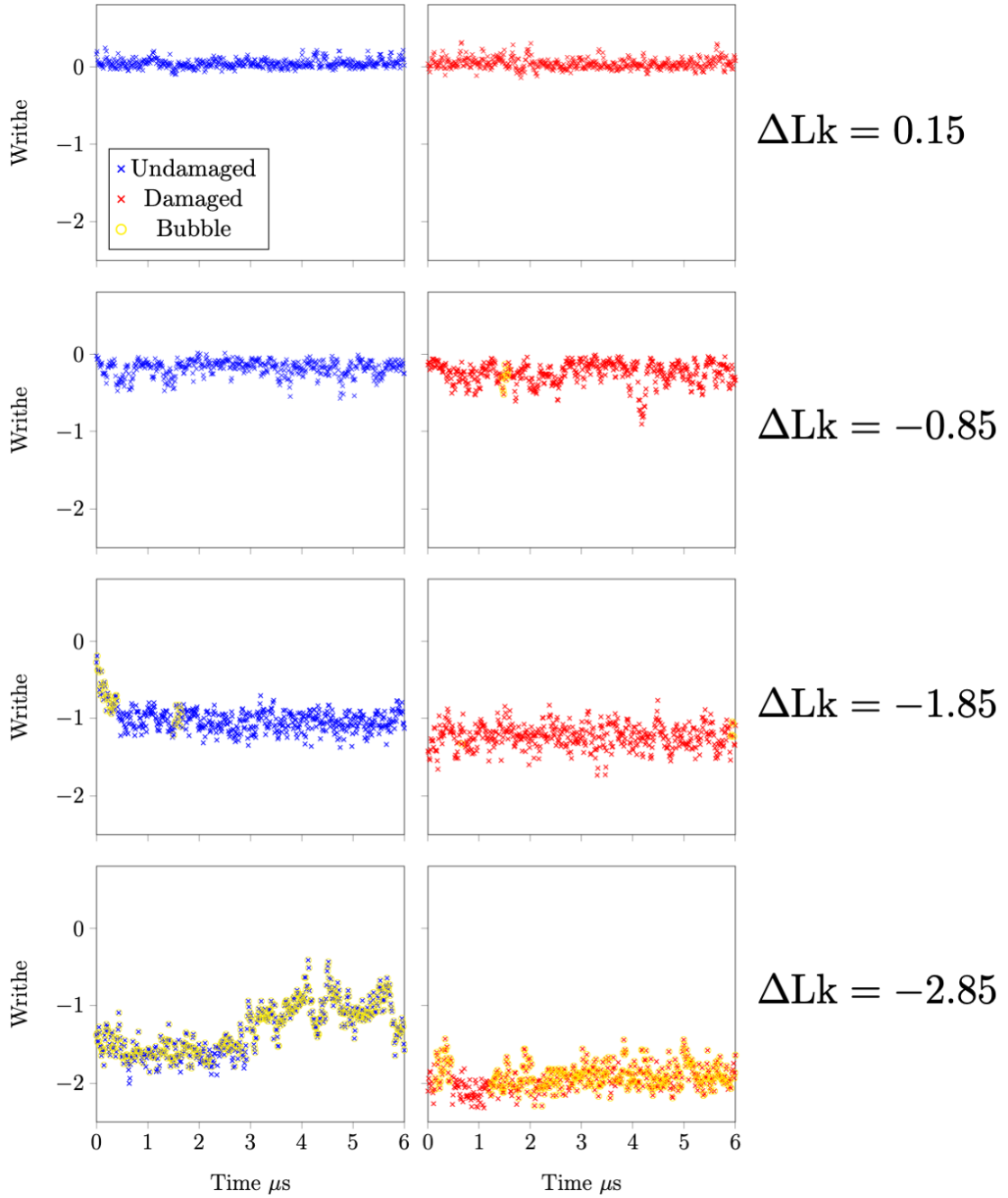


Figure 4.9: Writhe plots for four 336 bp topoisomers $\Delta Lk \in \{0.15, -0.85, -1.85, -2.85\}$ as a function of simulation time. Points correspond to the average writhe in a period of 200 frames of the simulation (100,000 time-steps). Points are encompassed with a yellow circle if there was at least one bubble for the majority of the frames within the period.

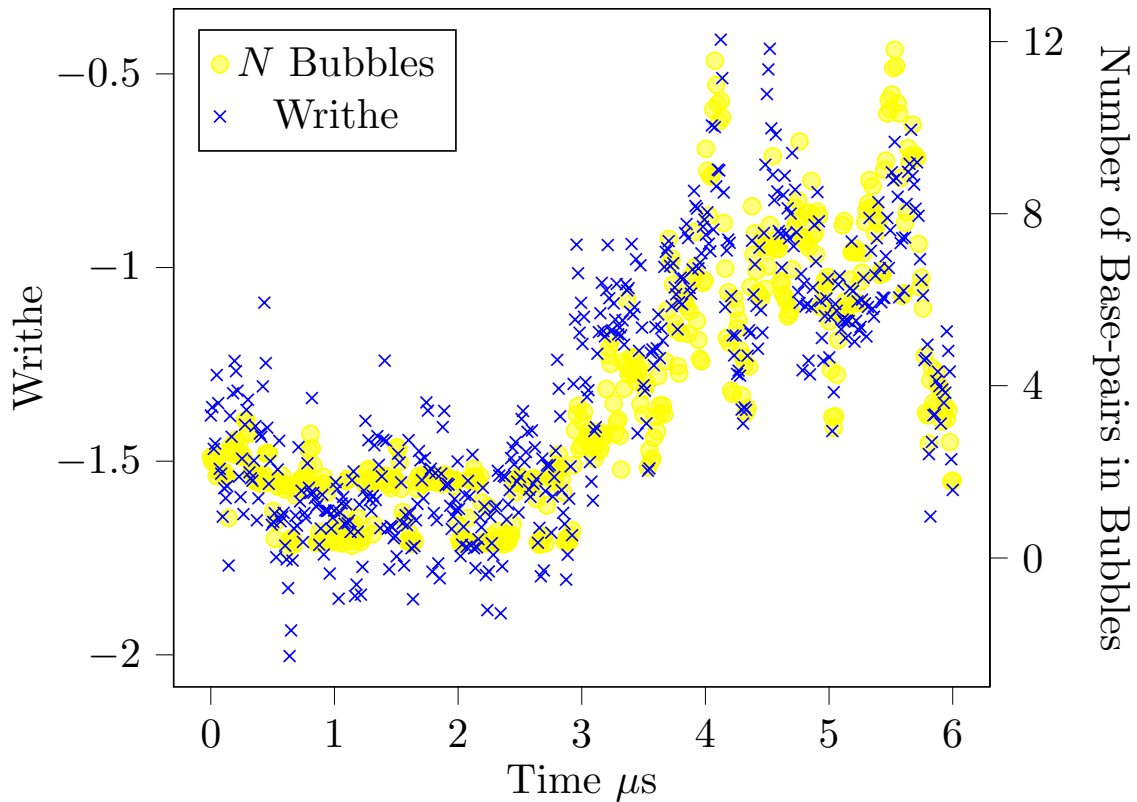


Figure 4.10: Plot of writhe and number of base-pairs that are denatured as a function of time for the undamaged $\Delta Lk = -2.85$ minicircle.

Another interesting avenue of future research is damage localization diffusion - the idea that the damage site will preferentially move towards the tip of plectonemic structures. This proposed mechanism has already been investigated for open MT-like systems by Lim *et al.* [46] but it would be interesting to perform the same analysis on closed systems. Once this has been done it would be interesting to look deeper into the interplay between bubbles and TDs and test some of the hypotheses about damage potentially inhibiting bubble formation.

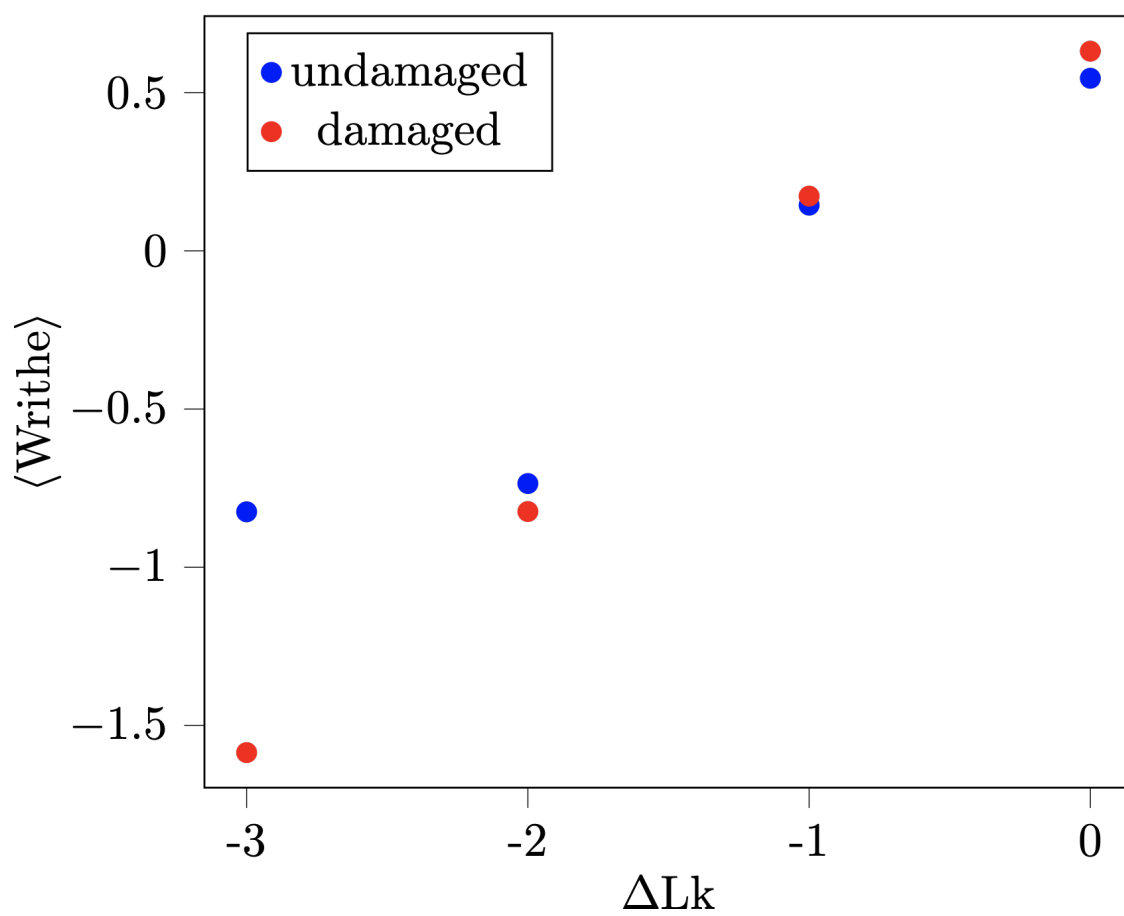


Figure 4.11: Average writhe plots for the four 336-bp topoisomers during periods with no bubbles.

5

Elastic Models of DNA

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5.1 Introduction

In this chapter we employ an energy-functional to find the elastic minima of curves and surfaces before adjusting them to incorporate damage. The first assumption we shall make is that undamaged dsDNA may be described as a homogeneous chain. This is a necessary assumption for this model and is well justified by

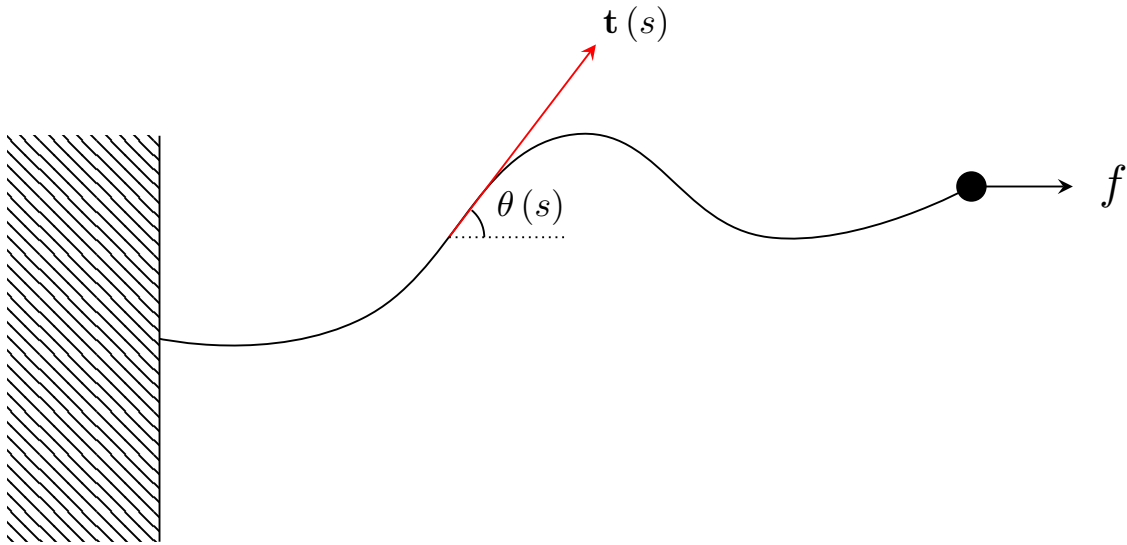


Figure 5.1: Diagram of WLC model of a MT experiment.

the approximately even distribution of the four canonical bases in real sequences. We begin with homogeneous planar elastica before defining the different types of damage that we will model. These ideas are then extended to three-dimensional twistable surfaces. We construct a piecewise model of a plectoneme for both planar and non-planar configurations and probe the effects of these damage types in the respective systems.

5.2 Planar Elastica

5.2.1 Homogeneous Elastica

Consider a plane-curve of length L with bending modulus A and forces constraining the ends of total magnitude f . If we define θ to be the angle formed between the curves tangent and the direction of the force (see Fig. 5.1), then we may define the Hamiltonian of the system to be

$$E = \int_0^L \left[\frac{A}{2} \dot{\theta}^2 - f \cos \theta \right] ds, \quad (5.1)$$

where s is the arc-length parameter. The first term of Eq. 5.1 is the bending contribution and the second term is the mechanical energy of the external force. The Euler-Lagrange (EL) equation will be $A\ddot{\theta} = f \sin \theta$. A trivial solution of the EL equation is the “straight chain” defined by $\theta = 0$ with energy $E = -fL$. For

non-trivial solutions we multiply both sides by $\dot{\theta}$ and integrate with respect to s . We get $\lambda\dot{\theta} = \sqrt{2(\mu - \cos\theta)}$, where $\lambda = \sqrt{A/f}$ is the so-called *deflection length* and μ is a constant of integration. The deflection length (sometimes referred to as the tension length or correlation length) shows the scale on which the polymer is deflected from ideal worm-like paths in order to conform to the constraint of having ends under tension [136]. This solution can then be re-expressed using that $\mu - \cos\theta = \mu + 1 - 2\cos^2(\theta/2)$. Rearranging our equation in $\dot{\theta}$:

$$\begin{aligned}\dot{\theta} &= \frac{2}{\lambda} \sqrt{\frac{\mu+1}{2} - \cos^2(\theta/2)} \\ &= \frac{2}{\lambda k} \sqrt{1 - k^2 \cos^2(\theta/2)},\end{aligned}\tag{5.2}$$

where $k^2 = 2/(\mu+1)$. An important thing to note is that we have taken the positive square root, this means we have chosen the branch where θ is necessarily increasing. Separating variables and integrating between the appropriate limits

$$\begin{aligned}\frac{1}{\lambda k} \int_0^s ds' &= \frac{1}{2} \int_{\theta_0}^{\theta} \frac{d\theta}{\sqrt{1 - k^2 \cos^2(\theta/2)}} \\ &= \frac{1}{2} \int_{\theta_0 - \pi}^{\theta - \pi} \frac{d\theta}{\sqrt{1 - k^2 \sin^2(\theta/2)}} \\ &= \int_{\theta_0/2 - \pi/2}^{\theta/2 - \pi/2} \frac{d\theta}{\sqrt{1 - k^2 \sin^2(\theta)}}\end{aligned}\tag{5.3}$$

Via the definition of the incomplete elliptic integral of the first kind (Eq. 2.19 from Section 2.4.1), Eq. 5.3 translates to

$$\frac{s - s_0}{\lambda k} = F\left(\frac{\theta(s) - \pi}{2}, k\right) - F\left(\frac{\theta_0 - \pi}{2}, k\right).\tag{5.4}$$

If we impose boundary conditions $\theta(0) = \theta_0$ and $\theta(L) = \theta_L$ then we obtain

$$\frac{L}{\lambda k} = F\left(\frac{\theta_L - \pi}{2}, k\right) - F\left(\frac{\theta_0 - \pi}{2}, k\right).\tag{5.5}$$

Solving Eq. 5.4 for θ ,

$$\theta(s) = \pi + 2\text{am}\left(\frac{s - s_0}{\lambda k} + F\left(\frac{\theta_0 - \pi}{2}, k\right), k\right),\tag{5.6}$$

where $\text{am}(u, k)$ is the Jacobi amplitude of u with elliptic modulus k . These solutions are the famous Euler elastica which include several notable examples such as the homoclinic loop and the Bernoulli lemniscate.

It will prove convenient to introduce the “loop energy” $E_{\text{loop}} = E + fL$ which is simply the difference between the energy of the elastica and the energy of the straight chain. The loop energy of this configuration can be found by inserting the solution Eq. 5.6 into the energy Eq. 5.1.

$$E = f \int_0^L ds \left[\mu - 2 \cos \theta \right] \quad (5.7)$$

$$= f \int_0^L ds \left[\frac{2}{k^2} + 1 - 4k^2 \text{sn}^2 \left(\frac{s}{\lambda k} + F \left(\frac{\theta_0 - \pi}{2}, k \right), k \right) \right] \quad (5.8)$$

$$= fL \left(1 - \frac{2}{k^2} \right) + \frac{4\sqrt{Af}}{k} \left[E \left(\text{am} \left(\frac{L}{\lambda k} + F \left(\frac{\theta_0 - \pi}{2}, k \right), k \right), k \right) - E \left(\frac{\theta_0 - \pi}{2}, k \right) \right], \quad (5.9)$$

where E is the elliptic integral of the second kind, sn is the Jacobi sine elliptic function and we have used an integral identity to evaluate the integral of sn^2 . Using Eq. 5.5, we may simplify this expression to

$$E = fL \left(1 - \frac{2}{k^2} \right) + \frac{4\sqrt{Af}}{k} \left[E \left(\frac{\theta_L - \pi}{2}, k \right) - E \left(\frac{\theta_0 - \pi}{2}, k \right) \right]. \quad (5.10)$$

In order to model a curl (see Section 2.6.2), we wish to consider a vertically symmetric loop. To achieve this we choose boundary conditions $\theta_0 = \pi$ and $\theta_L = 2\pi$. This only describes half the loop and so we must then make the transformation $E \mapsto 2E$ before finally ensuring $L \mapsto L/2$ in order for the full loop to have length L . Doing so yields a length constraint and loop energy of

$$kK(k) = \frac{L}{2\lambda}, \quad E = fL \left(1 - \frac{2}{k^2} \right) + \frac{8\sqrt{Af}}{k} E(k), \quad (5.11)$$

respectively. We can form parametric equations for the elastica via integration

$$x(s) = \int_0^s ds' \cos \theta \quad (5.12)$$

$$y(s) = \int_0^s ds' \sin \theta. \quad (5.13)$$

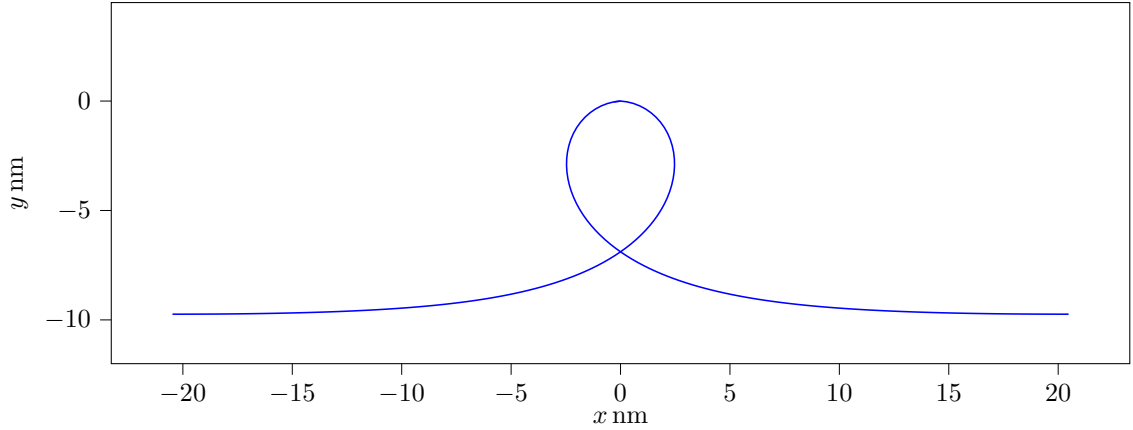


Figure 5.2: Example configuration of elastica of length 60 nm with $\theta_0 = 0$ and $\theta_L = 2\pi$. Elastica has a persistence length of 50 nm and the temperature is 300 K.

Hence, making use of the double angle formulae for sine and cosine and some integral identities,

$$x(s) = \left(\frac{2}{k^2} - 1\right)s - \frac{2\lambda}{k} \left[E\left(\frac{\theta(s) - \pi}{2}, k\right) - E\left(\frac{\theta_0 - \pi}{2}, k\right) \right] \quad (5.14)$$

$$y(s) = \frac{2\lambda}{k} \left[\sqrt{1 - k^2 \cos^2\left(\frac{\theta(s)}{2}\right)} - \sqrt{1 - k^2 \cos^2\left(\frac{\theta_0}{2}\right)} \right], \quad (5.15)$$

where $\theta(s)$ is defined by Eq. 5.6. Similar formulae were initially derived by Love [137] and are re-derived here for completeness. An example of a curl-like elastica with “flat” boundary conditions is shown in Fig. 5.2.

A case of note is when we dictate that $L \rightarrow \infty$. The diverging length constraint means we must have $k \rightarrow 1$. Unfortunately, this causes the energy itself to diverge trivially due to the $-fL$ term. Luckily, E_{loop} will remain finite in the limit $k \rightarrow 1$ and so using Eq. 5.11 we get

$$E_{\Delta}^{k=1} = 8\sqrt{Af}. \quad (5.16)$$

We shall refer to this specific case of elastica with unit elliptic modulus as the *homoclinic loop*.

5.2.2 Constraints

We have already defined the condition which ensures the correct length of a given elastica. Now we consider an elastica that is forced to be closed in the direction

of the force, in our case the x -direction. If a segment of length L is to be closed in x then via Eq. 5.14 we may deduce that

$$0 = \left(\frac{2}{k^2} - 1\right) L - \frac{2\lambda}{k} \left[E\left(\frac{\theta_L - \pi}{2}, k\right) - E\left(\frac{\theta_0 - \pi}{2}, k\right) \right]. \quad (5.17)$$

Another constraint we would like to impose is that of vanishing boundary curvature. This allows us to mimic saddle-point like regions where $\dot{\theta}$ changes sign without causing a discontinuity in the curvature. The curvature of our planar curve can be found by taking the arc-length derivative of Eq. 5.6 which yields

$$\dot{\theta} = \frac{2}{\lambda k} \operatorname{dn} \left(\frac{s}{\lambda k} + F\left(\frac{\theta_0 - \pi}{2}, k\right), k \right). \quad (5.18)$$

Equating this to zero at $s = L$, we find that

$$\frac{L}{\lambda k} = F\left[\arcsin\left(\frac{1}{k}\right), k\right] - F\left[\frac{\theta_0 - \pi}{2}, k\right]. \quad (5.19)$$

Combined with the closure condition, this means

$$(2 - k^2) \left[F\left(\arcsin\left(\frac{1}{k}\right), k\right) - F\left(\frac{\theta_0 - \pi}{2}, k\right) \right] = 2 \left[E\left(\arcsin\left(\frac{1}{k}\right), k\right) - E\left(\frac{\theta_0 - \pi}{2}, k\right) \right]. \quad (5.20)$$

For example, if we set $\theta_0 = \pi$ and we utilise Eq. 2.30, then we derive the condition $K(1/k) = 2E(1/k)$ which has a solution $k = 1.1002$. This is the Jacobi lemniscate. Ideally we would impose vanishing curvature at every boundary of our piecewise elastica so that our curvature profile is discontinuity free. Unfortunately, this is not possible. We may enforce $\dot{\theta}_L = 0$ as well as $x(L) = 0$ for the loop, but this does not allow us to find the equivalent solution for the first braid. Hence, we abandon this condition as it is evidently too strong.

Instead we shall allow discontinuities in the curvature at the endpoints of each segment. This means the tangent itself will remain continuous. As the energy is defined in a piecewise fashion, these discontinuities in the curvature won't cause the total energy to blow up if we only calculate the energies of each section and find their sum.

5.2.3 Twist-storing Elastica

If we consider a twistable object whose centre-line is confined to the plane with bending stiffness A and total twist Tw , parametrised by bending angle θ and twisting angle ψ , then the Hamiltonian of the system will be

$$E = \int_0^L \left[\frac{A}{2} \dot{\theta}^2 + \frac{C}{2} \dot{\psi}^2 - f \cos \theta \right] ds. \quad (5.21)$$

The solution to the EL equation in θ is the same as given in Section 5.2.1. With the solution for the twist variable following a simple linear form $\psi = p_\psi s + \psi_0$. The constant p_ψ is related to the twist via $p_\psi = 2\pi\text{Tw}/L$.

5.2.4 Planar Plectonemes

Now that we have the formulae to describe elastica with arbitrary boundary conditions with the ability to impose constraints such as closure in a particular direction we may introduce a new model for planar plectonemes. We propose a piecewise model to describe plectonemic structures via the choice of several boundary conditions (see Fig. 5.3). This is already a common method for modelling plectonemes, an example being the Clauvelin model [138]. The main limitation of considering a piecewise model is that the resulting structure is not necessarily an elastic minimum in its own right, simply a combination of other minima.

Our model is as follows. Consider a plectoneme of total length L and bending stiffness A and external force of magnitude f aligned in the x -direction. First we assume vertical symmetry. We begin by forming the plectoneme tip, which we shall call the loop. This is done by determining the angle $\theta_c = \theta(L_{\text{loop}}/2)$, where L_{loop} is the length of the loop, such that the loop is closed in the x -direction (if $\theta_0 = \pi$). This is done by implementing the closure condition Eq. 5.17. We model the braid as a series of n circular arcs of constant curvature, where their initial angle is set by θ_c . We impose a plectonemic radius r_0 , some value to which the circular arc must extend to, this determines the length of each braid segment and hence the total length of the braid.

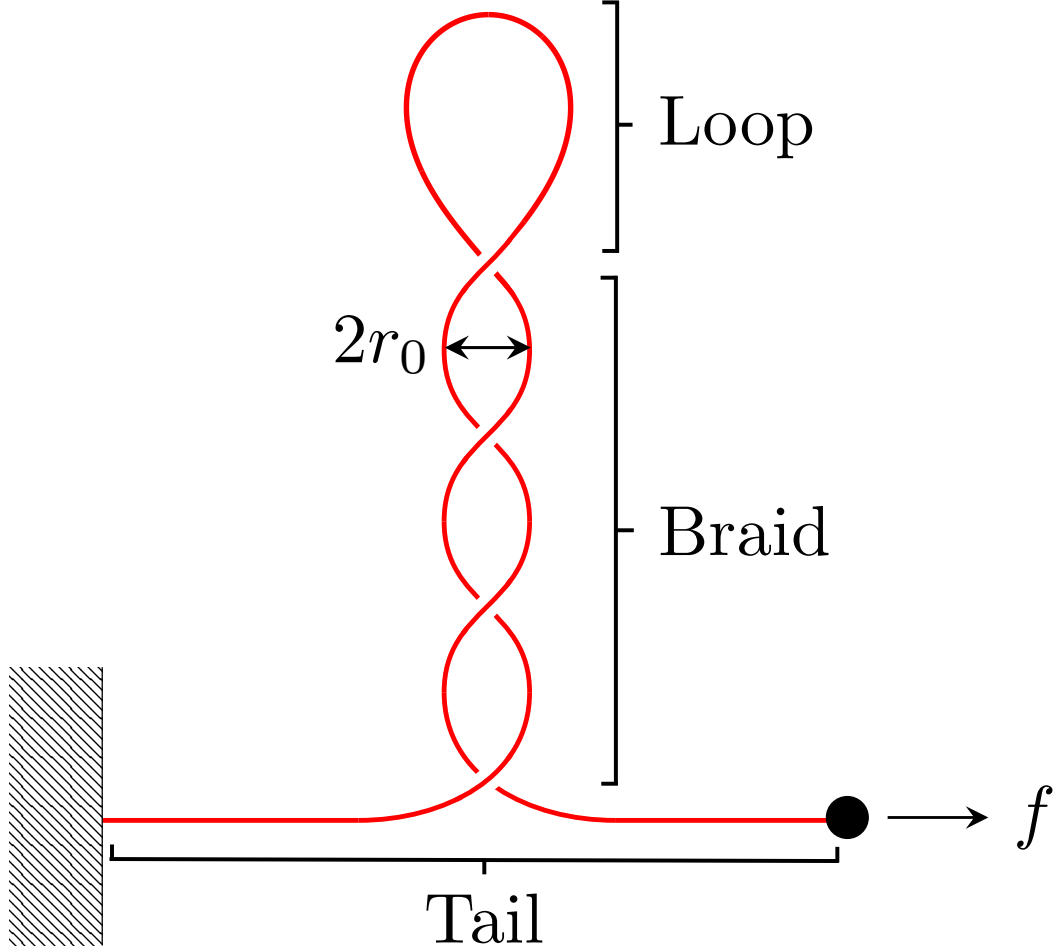


Figure 5.3: Schematic of planar plectoneme construction from loop, tail and braided regions.

Finally, the tail is constructed via ensuring $\theta(L_{\text{tail}}/2) = 2\pi$, where $L_{\text{tail}}/2$ is determined by how much length is left, i.e. $L_{\text{tail}}/2 = L/2 - L_{\text{loop}}/2 - L_{\text{braid}}/2$. The energy of the loop is

$$E_{\text{loop}} = \left(\frac{2}{k^2} - 1 \right) f L_{\text{loop}}, \quad (5.22)$$

where

$$\frac{L_{\text{loop}}}{2\lambda k} = F\left(\frac{\pi - \theta_c}{2}, k\right) \quad (5.23)$$

and θ_c satisfies the closure condition

$$F\left(\frac{\pi - \theta_c}{2}, k\right) (2 - k^2) - 2E\left(\frac{\pi - \theta_c}{2}, k\right) = 0. \quad (5.24)$$

If we let the length of the braid be L_{braid} , then the energy of the braid is

$$E_{\text{braid}} = \frac{A}{2L_{\text{braid}}} (\theta_L - \theta_0)^2. \quad (5.25)$$

We then choose L_{braid} such that the braid forms a plectoneme with the desired plectonemic radius r_0 . It is easy to show that this demands that

$$L_{\text{braid}} = \frac{r_0 (\theta_L - \theta_0)}{1 - \sin \theta_0}. \quad (5.26)$$

For a plectoneme of n braids, this amounts to a total bending energy of

$$E_{\text{braid}} = \frac{nA}{2r_0} (1 - \sin \theta_0) (\theta_L - \theta_0). \quad (5.27)$$

Finally the tail will have a length constraint of

$$\frac{L_{\text{tail}}}{2\lambda k} = K(k) - F\left(\frac{\pi - \theta_0}{2}, k\right) \quad (5.28)$$

and energy

$$E_{\text{tail}} = -fL_{\text{tail}} + 4\sqrt{Af} (1 - \cos \theta_0). \quad (5.29)$$

The total energy is obtained from summing the piecewise energy contributions. The determination of k_{loop} and k_{tail} in reality must be done numerically. For a plectoneme of a given length, radius and number of braids, we have only one free parameter - we choose this parameter to be k_{loop} . We implement our model numerically in Python by looping through all possible values of k_{loop} and determining the subsequent plectonemes which fulfil our constraints. We then simply choose the plectoneme with the lowest energy. An example of some plectonemes produced using this method are shown in Fig. 5.4.

5.3 Inhomogeneous Elastica

We now investigate the consequences of modifying the elastic parameters of our chain to see what configurations they might adopt. Following the work of Randisi [81], we introduce damage to our system in two ways:

- Kinked: an angle of θ_0 pays no bending energy.

$$L = 300 \quad \ell_p = 50$$

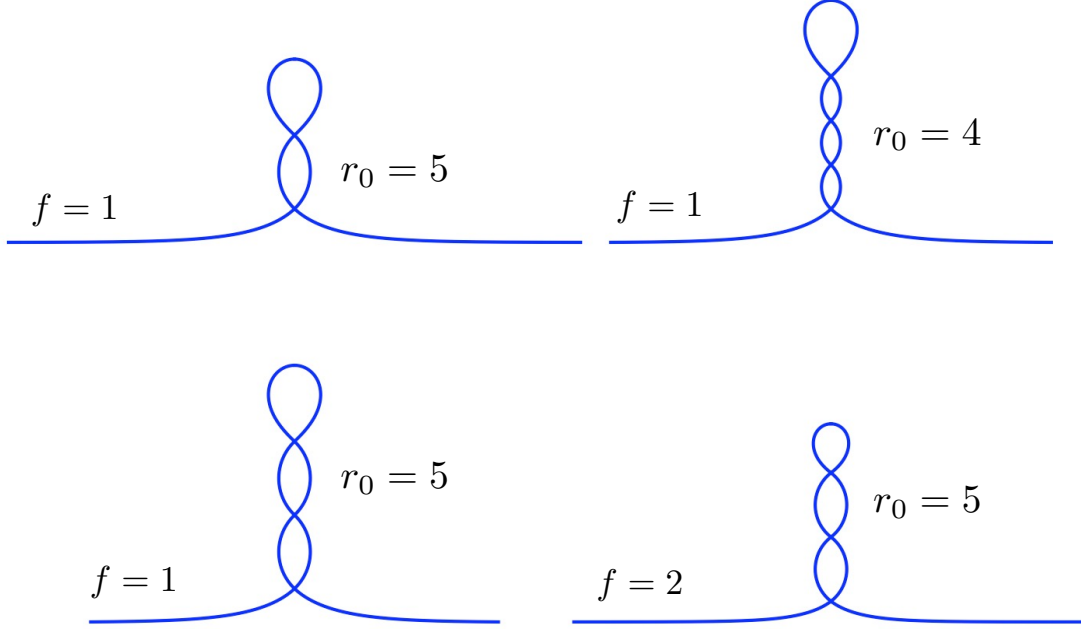


Figure 5.4: Four example plectonemes generated with the aforementioned methodology for several values of the plectonemic radius, number of braids and external force. Units of distance are nm and units of force are pN.

- Floppy: a segment of length L_1 has a reduced bending coefficient A_1 .

Randisi [81] employed a circular piecewise model to describe damaged curls. His argument is that, although not a solution to the EL equations, the loop energy of a circular configuration is similar to that of a the non-trivial “teardrop” shape of an elastica, and crucially has the same $\sqrt{Af}$ scaling, just with different prefactors. Randisi showed that for a kinked chain with natural rest angle θ_0 ,

$$\Delta E_{\text{loop}}^{\text{bent}} = \frac{\theta_0}{2\pi} E_{\text{loop}}. \quad (5.30)$$

For a floppy chain,

$$\Delta E_{\text{loop}}^{\text{flop}} = 2L_1 \left(\frac{A}{A_1} - 1 \right) f. \quad (5.31)$$

We now derive expressions which quantify the energy saving for having a damaged segment pinned to the tip of a looped/kinked structure, with no prior assumption of the geometry of said loops.

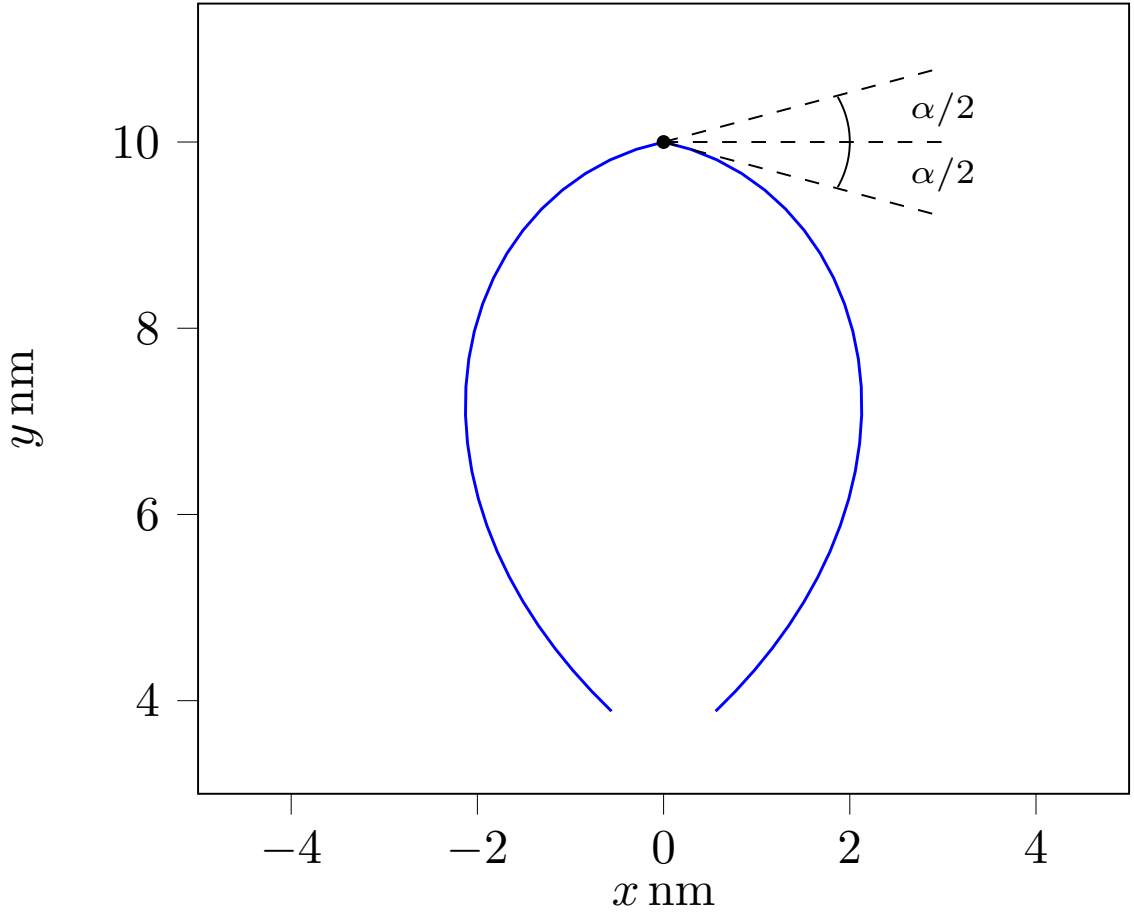


Figure 5.5: Geometry of a kinked elastica with rest angle α .

5.3.1 Kinked Elastica

For a kinked sequence, there is some angle α which pays no bending cost. Considering a symmetric elastica and setting $\theta_0 = \pi + \alpha/2$, from Eq. 5.10 we obtain

$$E_{\text{loop}} = \frac{2fL(k^2 - 1)}{k^2} + \frac{8\sqrt{Af}}{k} \left[E\left(\frac{\theta_L - \pi}{2}, k\right) - E\left(\frac{\alpha}{4}, k\right) \right]. \quad (5.32)$$

The length constraint is

$$\frac{L}{\lambda k} = F\left(\frac{\theta_L - \pi}{2}, k\right) - F\left(\frac{\alpha}{4}, k\right). \quad (5.33)$$

We note that there are no free parameters since the boundary conditions enforce a specific value of k .

5.3.2 Floppy Elastica

Consider a chain of length L , which has a segment of length L_1 with bending modulus A_1 , with the remaining chain of length $L - L_1$ having a bending modulus of A . If we have boundary conditions of $\theta(0) = \theta_0$ and $\theta(L) = \theta_L$, then the two media will join at some unknown angle $\theta(L_1) = \theta_1$.

The most general form of the piecewise energy is

$$\begin{aligned} E_{\text{loop}}^{\text{flop}} = & 2\sqrt{A_1}f \left[\frac{2}{k_1}E(\varphi_1, k_1) + \frac{k_1^2 - 1}{k_1}F(\varphi_1, k_1) \right] \\ & - 2\sqrt{A_1}f \left[\frac{2}{k_1}E(\varphi_0, k_1) + \frac{k_1^2 - 1}{k_1}F(\varphi_0, k_1) \right] \\ & + 2\sqrt{A}f \left[\frac{2}{k}E(\varphi_L, k) + \frac{k^2 - 1}{k}F(\varphi_L, k) \right] \\ & - 2\sqrt{A}f \left[\frac{2}{k}E(\varphi_1, k) + \frac{k^2 - 1}{k}F(\varphi_1, k) \right], \end{aligned} \quad (5.34)$$

where we define $\varphi_i = \theta_i/2 - \pi/2$ for $i = 0, 1, L$. This equation is obtained via Eq. 5.10 and using Eq. 5.5 to rewrite the linear terms of f as elliptic integrals using the length constraints given below:

$$\frac{L - L_1}{\lambda k} = F\left(\frac{\theta_L}{2} - \frac{\pi}{2}, k\right) - F\left(\frac{\theta_1}{2}, k\right), \quad (5.35)$$

$$\frac{L_1}{\lambda_1 k_1} = F\left(\frac{\theta_1}{2}, k_1\right) - F\left(\frac{\theta_0}{2}, k_1\right). \quad (5.36)$$

Eq. 5.36 can be rearranged to give

$$\frac{\theta_1}{2} = \text{am}\left(\frac{L_1}{\lambda_1 k_1} + F\left(\frac{\theta_0}{2}, k_1\right), k_1\right). \quad (5.37)$$

Hence, we may write both length constraints as a single equation

$$\frac{L - L_1}{\lambda k} = F\left(\frac{\theta_L}{2} - \frac{\pi}{2}, k\right) - F\left(\text{am}\left(\frac{L_1}{\lambda_1 k_1}, k_1\right) + F\left(\frac{\theta_0}{2}, k_1\right), k\right). \quad (5.38)$$

And so rearranging further

$$F\left(\frac{\theta_L}{2} - \frac{\pi}{2}\right) = \frac{L - L_1}{2k\lambda} + F\left(\text{am}\left(\frac{L_1}{2\lambda_1 k_1}, k_1\right) + F\left(\frac{\theta_0}{2}, k_1\right), k\right). \quad (5.39)$$

Using Eq. 5.35 and Eq. 5.36, the energy Eq. 5.34 simplifies to

$$E_{\text{loop}}^{\text{flop}} = 2\sqrt{A_1 f} \left[\frac{2}{k_1} (E(\varphi_1, k_1) - E(\varphi_0, k_1)) + \frac{k_1^2 - 1}{k_1^2} \frac{L_1}{\lambda_1} \right] \\ + 2\sqrt{A f} \left[\frac{2}{k} (E(\varphi_L, k) - E(\varphi_1, k)) + \frac{k^2 - 1}{k^2} \frac{L - L_1}{\lambda} \right]. \quad (5.40)$$

This energy expression appears to have 3 free parameters k_1 , k and φ_1 . However, Eq. 5.35 and Eq. 5.36 eliminate two of these and so we effectively have only one free parameter. This means we may minimize this energy via a simple one-dimensional derivative. Choosing to minimise with respect to k_1 , where we must take care as k and φ_1 are functions of k_1 ,

$$\frac{dE_{\text{loop}}^{\text{flop}}}{dk_1} = 4\sqrt{A_1 f} \left[\frac{1}{k_1} \frac{d}{dk_1} [E(\varphi_1, k_1) - E(\varphi_0, k_1)] - \frac{E(\varphi_1, k_1) - E(\varphi_0, k_1)}{k_1^2} + \frac{L_1}{\lambda_1 k_1^3} \right] \\ + 4\sqrt{A f} \left[\frac{dk}{dk_1} \left(\frac{1}{k} \frac{d}{dk} [E(\varphi_L, k) - E(\varphi_1, k)] + \frac{E(\varphi_L, k) - E(\varphi_1, k)}{k^2} \right) \right. \\ \left. + \frac{L - L_1}{\lambda k^3} \frac{dk}{dk_1} \right]. \quad (5.41)$$

Using that

$$\frac{d}{dk} E(\phi, k) = \frac{d\phi}{dk} \sqrt{1 - k^2 \sin^2 \phi} + \frac{E(\phi, k) - F(\phi, k)}{k}, \quad (5.42)$$

and equating the derivative to zero:

$$0 = \frac{k_1 \left(\frac{d\varphi}{dk_1} \sqrt{1 - k_1^2 \sin^2 \varphi} \right) - F(\varphi_1, k_1) + F(\varphi_0, k_1)}{k_1^2} + \frac{L_1}{\lambda_1 k_1^3} \\ + \sqrt{\frac{A}{A_1}} \frac{dk}{dk_1} \left(\frac{L - L_1}{\lambda k^3} + \frac{F(\varphi_1, k) - F(\varphi_L, k) - k \frac{d\varphi}{dk} \sqrt{1 - k^2 \sin^2 \varphi_1}}{k^2} \right) \quad (5.43)$$

Utilising the length constraints Eq. 5.35 and Eq. 5.36 and rearranging

$$0 = \frac{d\varphi}{dk_1} \frac{\sqrt{1 - k_1^2 \sin^2 \varphi}}{k_1} - \sqrt{\frac{A}{A_1}} \frac{dk}{dk_1} \frac{d\varphi}{dk} \frac{\sqrt{1 - k^2 \sin^2 \varphi}}{k}. \quad (5.44)$$

By the chain rule $\frac{d\varphi}{dk_1} = \frac{dk}{dk_1} \frac{d\varphi}{dk}$ and so we may cancel this term as a common factor (it can be shown to be non-zero) and rearrange to show that

$$\frac{\sqrt{1 - k_1^2 \sin^2 \varphi_1}}{k_1} = \sqrt{\frac{A}{A_1}} \frac{\sqrt{1 - k^2 \sin^2 \varphi_1}}{k}. \quad (5.45)$$

This can be reformulated as

$$\frac{1}{k_1} \operatorname{dn} \left(\frac{L_1}{\lambda_1 k_1} + F \left(\frac{\theta_0 - \pi}{2}, k_1 \right), k_1 \right) = \sqrt{\frac{A}{A_1}} \frac{1}{k} \operatorname{dn} \left(F \left(\frac{\theta_L - \pi}{2}, k \right) - \frac{L - L_1}{\lambda k}, k \right). \quad (5.46)$$

We will refer to this equation as the minimization condition. It simply means given boundary conditions θ_0 and θ_L , along with the length constraint, one may deduce k_1 and k such that the elastic energy of the piecewise elastica is minimized. In reality Eq. 5.46 must be solved numerically. It has been seen empirically that this stationary point is in fact a minimum, though this can only be proven rigorously in certain cases.

5.3.3 Damaged Curls

In an attempt to model curls with a centralised damage site, we make use of the formulae derived previously, imposing suitable boundary conditions. If we assume vertical symmetry, then it is easy to deduce that a suitable choice for boundary conditions is $\theta_0 = \pi + \alpha/2$ and $\theta_L = 2\pi$. We also must then set $L \mapsto L/2$. Inserting these into Eq. 5.47 and Eq. 5.33

$$E_{\text{loop}}^{\text{kinked}} = \frac{8\sqrt{A}f}{k} \left[E(k) - E\left(\frac{\alpha}{4}, k\right) \right] - \frac{2Lf}{k^2} + 2Lf, \quad (5.47)$$

and

$$\frac{L}{2\lambda k} = K(k) - F\left(\frac{\alpha}{4}, k\right). \quad (5.48)$$

Recall that for infinitely long chains $L \rightarrow \infty$, we require $k \rightarrow 1$. In the case where $k = 1$, for a kinked segment of natural rest angle α , it is easy to show that the exact energy of this bent elastica is

$$E_{\text{loop}}^{\text{kinked}} = 8\sqrt{A}f \left(1 - \sin\left(\frac{\alpha}{4}\right) \right). \quad (5.49)$$

This means a cost of

$$\Delta E_{\text{loop}}^{\text{bent}} = E_{\text{loop}} \sin\left(\frac{\alpha}{4}\right). \quad (5.50)$$

A similar result was derived initially by Sankararaman and Marko [70].

For a small rest angle, a simple Taylor expansion of sine yields $\Delta E_{\text{loop}}^{\text{bent}} \approx \theta_0 E_{\text{loop}}/4$. This is a larger deficit than the Randisi model which predicts $\Delta E_{\text{loop}}^{\text{bent}} = \theta_0 E_{\text{loop}}/2\pi$. This is to be expected as for elastica with $k \neq 0$ (i.e. non circular) you pay more energy for bending at the tip of the loop and so a bent region here will save more energy.

For floppy curls, the correct boundary conditions are $\theta_0 = \pi$ and $\theta_L = 2\pi$. Inserting these values into Eq. 5.46 the minimization condition becomes

$$\frac{\text{dn}\left(\frac{L_1}{\lambda_1 k_1}, k_1\right)}{k_1} = \sqrt{\frac{A}{A_1}} \frac{\text{dn}\left(K(k) - \frac{L-L_1}{\lambda k}, k\right)}{k}. \quad (5.51)$$

If we further set $L \rightarrow \infty$, this still demands $k \rightarrow 1$ and so this condition becomes

$$\sqrt{\frac{A_1}{A}} = k_1 \text{cd}\left(\frac{L_1}{\lambda_1 k_1}, k_1\right). \quad (5.52)$$

This does not yield an analytically tractable result for the energy reduction. However, this minimization condition for curls can be investigated in the limit of a small damage region. If we assume that $\theta_1/4 \ll 1$, then $E(\theta_1/4, k_1) = F(\theta_1/4, k_1) \approx \theta_1/4 + \mathcal{O}(\theta_1/4)^3$ and so from the boundary condition:

$$\frac{\theta_1}{4} \approx \frac{L_1}{2k_1\lambda_1}. \quad (5.53)$$

Plugging this into the energy, and using $\sin x \approx x$ for $x \ll 1$,

$$\begin{aligned} E_{\text{loop}}^{\text{flop}} &\approx 4\sqrt{A_1}f \frac{1}{k_1} \left[\frac{L_1}{2k_1\lambda_1} \right] + 8\sqrt{A}f \left[1 - \frac{L_1}{2k_1\lambda_1} \right] + 2L_1f \\ &= \frac{2L_1f}{k_1^2} + 8\sqrt{A}f - \frac{4L_1f}{k_1} \sqrt{\frac{A}{A_1}} + 2L_1f. \end{aligned} \quad (5.54)$$

Minimizing with respect to k_1 ,

$$\frac{dE_{\text{loop}}^{\text{flop}}}{dk_1} = \frac{4L_1f}{k_1^3} \left(k_1 \sqrt{\frac{A}{A_1}} - 1 \right) = 0, \quad (5.55)$$

which gives us that $k_1 = \sqrt{A_1/A}$. Computing the double derivative

$$\frac{d^2 E_{\text{loop}}^{\text{flop}}}{dk_1^2} = \frac{4L_1f}{k_1^4} \left(3 - 2k_1 \sqrt{\frac{A}{A_1}} \right). \quad (5.56)$$

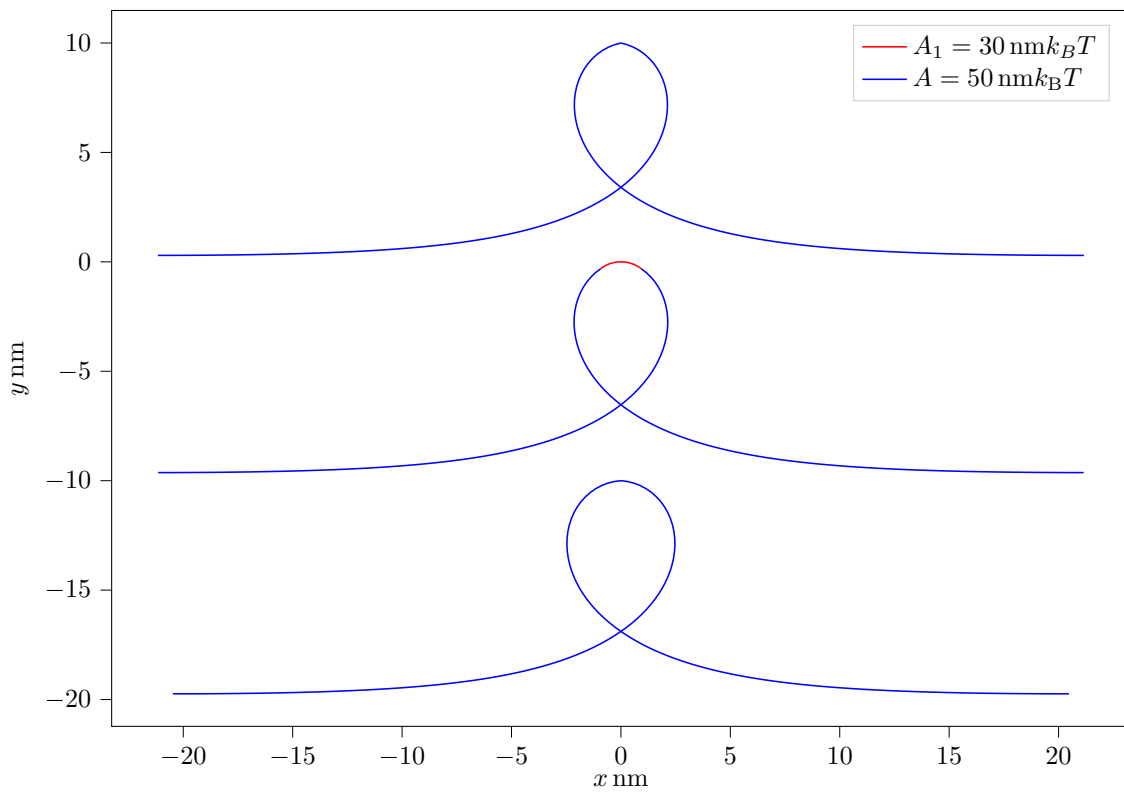


Figure 5.6: Plot of damaged and undamaged curl configurations of length 60 nm with external force 2 pN. Top: kinked curl with $\theta_0 = 30^\circ$, middle: floppy curl with $L_1 = 2$ nm and bottom: homogeneous curl.

If we evaluate this at $k_1 = \sqrt{A_1/A}$,

$$\left. \frac{d^2 E_{\text{loop}}^{\text{flop}}}{dk_1^2} \right|_{k_1=\sqrt{A_1/A}} = \frac{4L_1 f A^2}{A_1^2} > 0. \quad (5.57)$$

Therefore, we can deduce this is in fact a minimum. If we now plug this critical value for k_1 back into the loop energy, we get

$$E_{\text{loop}}^{\text{flop}} \approx 8\sqrt{Af} + 2L_1 \left(1 - \frac{A}{A_1}\right) f. \quad (5.58)$$

Hence, the change in energy of the loop, which is defined to be $\Delta E_{\text{loop}} = E_{\text{loop}} - E_{\text{loop}}^{\text{flop}}$, is

$$\Delta E_{\text{loop}}^{\text{flop}} = 2L_1 \left(\frac{A}{A_1} - 1 \right) f. \quad (5.59)$$

This is in agreement with the Randisi model [81]. This is interesting as this shows that in the limit of a small damage region, we recover an identical expression to that which used a circular approximation but that made no assumption on the size of the defect. It is rather trivial, though laborious, to include higher order corrections to this formula. For example, it can be shown that to quadratic order

$$\Delta E_{\text{loop}}^{\text{flop}} = 2L_1 f \left(1 - \frac{A}{A_1}\right) - \frac{L_1^3 f^2 A}{6A_1^2} \left(1 - \frac{A}{A_1}\right). \quad (5.60)$$

5.3.4 Damaged Circles

Using inhomogeneous elastica, we may construct a circular configuration with n evenly spaced damaged sites. This allows us to model the minicircle experiments of Huasin *et al.* [47] in Chapter 3. To do this we use the floppy damage model and initially describe only a portion of the circle. We may then construct the rest via symmetry. For example, for the 5TD minicircle, we choose $\theta_0 = \pi$ and $\theta_L = 4\pi/5$. Hence, the only free parameters are A_1 and L_1 . The analysis of Chapter 3 indicated a relatively local effect on the structure of a TD and so we choose L_1 to be 3 bp. We then calculated the minimal energy configuration for several values of A_1 ; these are shown in Fig. 5.7.

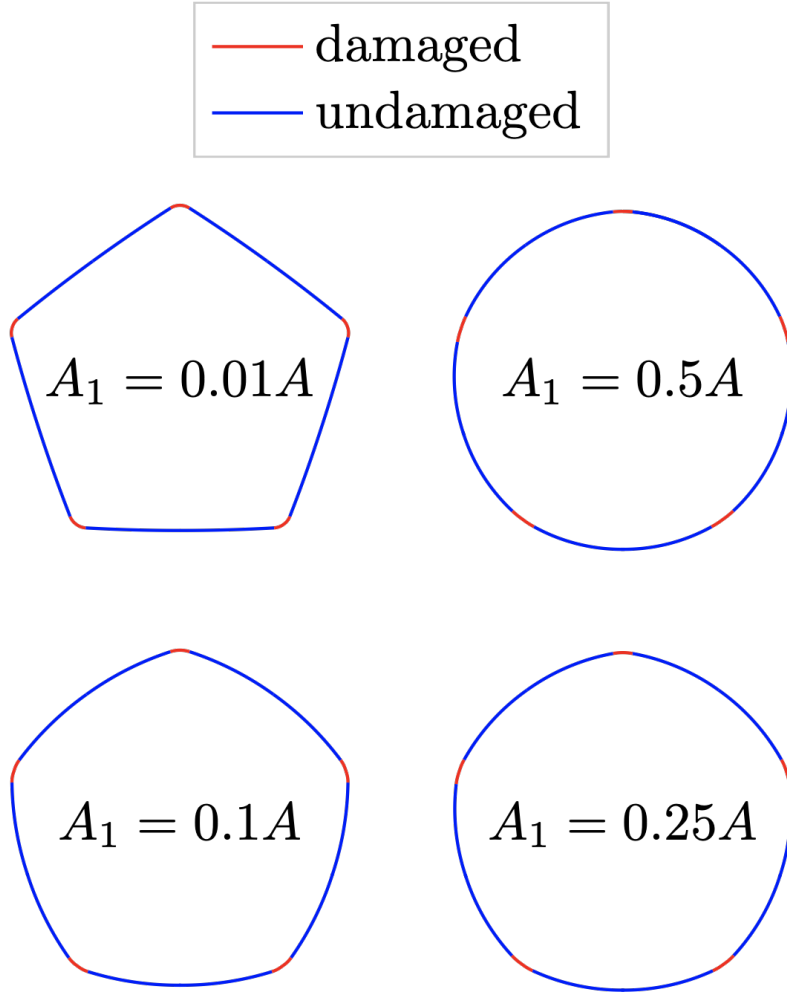


Figure 5.7: Elastic minima of 160-bp circular configurations with 5 TDs of length 3 bp. Blue regions have a bending stiffness of $50 \text{ nm} k_B T$, with red regions having a reduced bending stiffness A_1 . Four different degrees of bending reduction shown $A_1/A = 0.01, 0.1, 0.25, 0.5$.

A relatively large reduction in the bending stiffness is required to significantly deviate the structure from a circle. Potentially indicating that the floppy model of damage alone is unsuitable for modelling TDs given that Husain *et al.* saw relatively strong bending. This is likely to be an effect of the relatively small sizes of the sections (each section is only approximately 20% of the persistence length).

5.3.5 Damaged Plectonemes

We now apply our kinked and floppy models of damage to the piecewise plectoneme model established in Section 5.2.4. The way this is achieved is similar to that of

adding the damage to a curl: we place the damage at the tip of the “loop” and this then determines the subsequent boundary conditions such that we obtain a plectoneme. The difference from the homogeneous plectoneme will be that the damage will affect these boundary conditions. For floppy damage this involves the inclusion of a new free parameter, the elliptic modulus of the damaged region. This requires variation in an additional parameter before the most energetically favourable plectoneme can be selected. Fig. 5.8 shows examples of a homogeneous plectoneme and its two damaged variants.

We could expect to see a reduction in the plectoneme energy when damage is included for three reasons. The first is that the plectoneme will pay less bending energy at the tip. Secondly, it could choose to increase its end-to-end separation if the tail size is increased. Finally, the damage could allow for a steeper inclination of the braid thus reducing its bending energy further. By steeper we mean the tangent of the braided region forms a larger angle with the axis aligned with the force.

In order to investigate the energetic savings for the various damage models, we calculated the energy of homogeneous, kinked and floppy plectonemes for various forces. A plectonemic radius of 3 nm was chosen. The results are shown in Fig. 5.9.

As expected the floppy elastica saves bending energy in both the loop and the tail as well as having a longer end-to-end distance. Unexpectedly, however, is the increased bending energy of the kinked elastica. Despite saving energy by kinking the loop, it leads to a braid that forms a smaller angle with the axis aligned with the force, which increases the braids bending energy - negating the saving at the tip. In fact, the kinked model is less favourable at low force than the homogeneous plectoneme, eventually becoming more favourable for some characteristic force. This is clearly a shortcoming of the model, in reality a kinked plectoneme should be able to form braids with larger angles to the force axis and see the analogous saving of the floppy model.

There are several other obvious deficiencies with the planar models. The need to set a plectonemic radius means that we do not see the natural tightening of plectonemic coils with increased force as we intuitively expect. Solving boundary

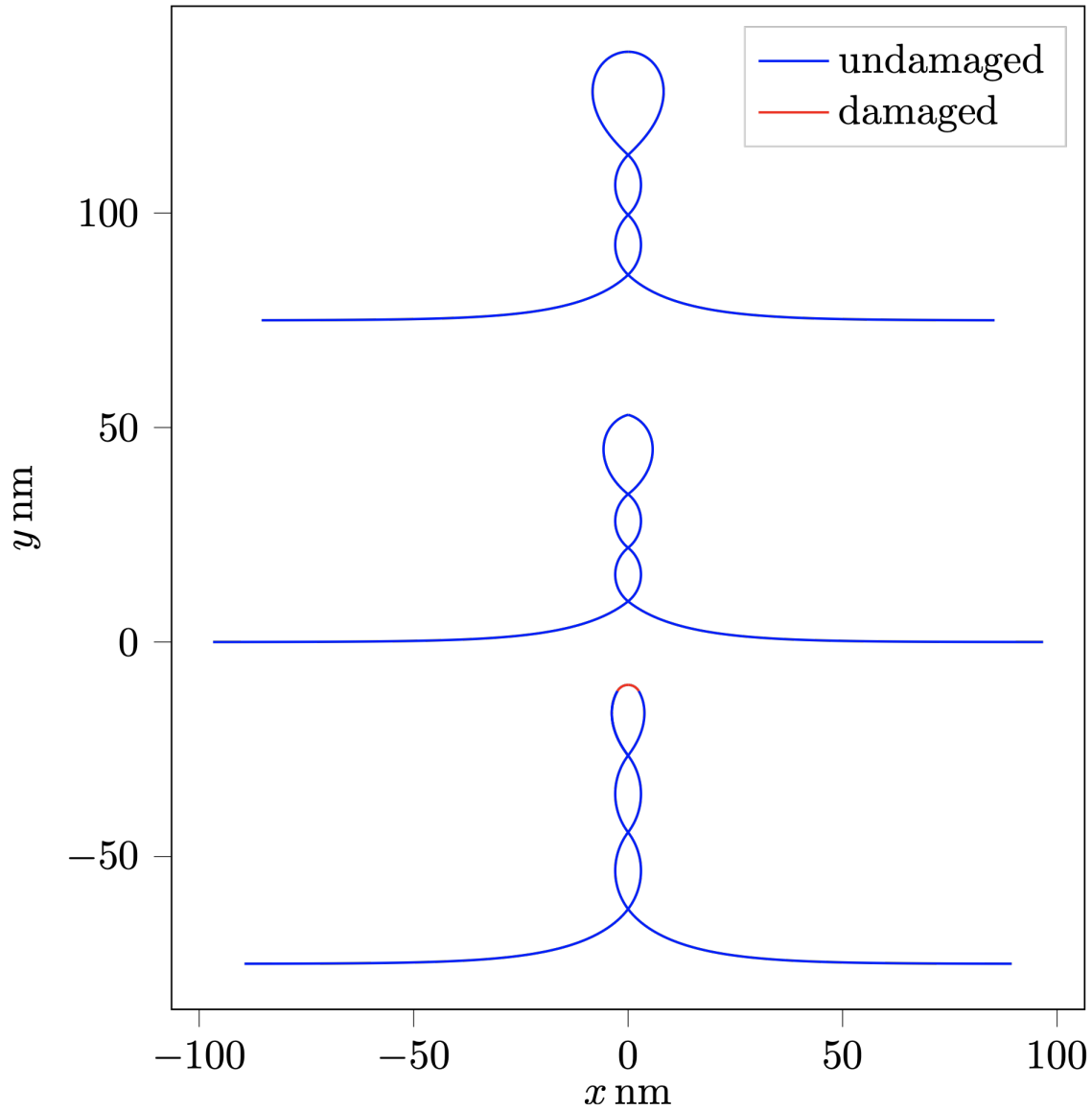


Figure 5.8: 300 nm plectonemes formed by a homogeneous chain, a kinked chain and a floppy chain. The kinked chain has a rest angle of 30° and the floppy segment is 6 nm in length and has a reduced bending stiffness of $31 k_B T \text{ nm}$. The homogeneous chain has a bending stiffness of $50 k_B T \text{ nm}$ and is under an applied force of 1 pN.

conditions is also a difficult task that adds numerical complexity and reduces the robustness of the algorithm as the equations often do not find appropriate solutions.

5.4 3D Elastica

The major deficiency in the space-curve approximation of DNA is that it inherently describes a one-dimensional object and, hence, cannot be used to describe something

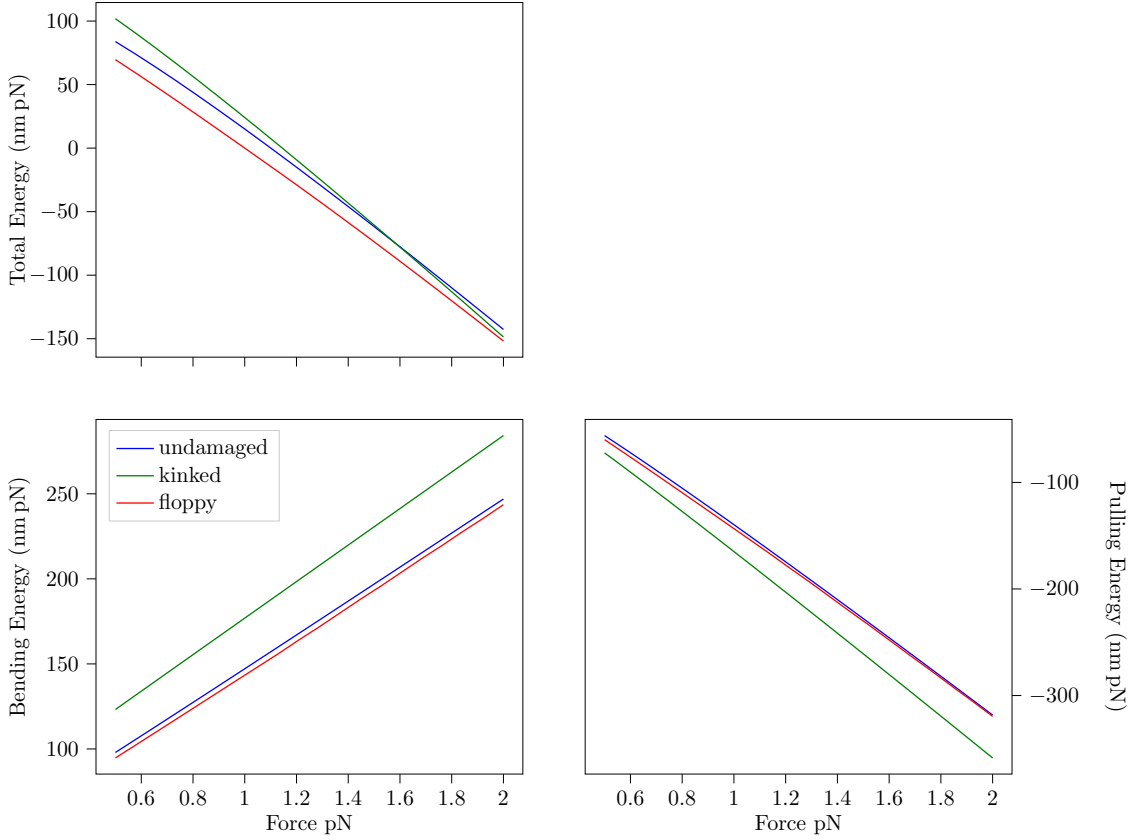


Figure 5.9: Plots of total energy, bending energy and mechanical energy of damaged and undamaged plectonemes of length 300 nm with a plectonemic radius of 3 nm as a function of external force. The kinked plectonemes had a rest angle of 30° , the floppy plectonemes have floppy regions of length 3 nm and have a reduced bending coefficient of $31 k_B T \text{ nm}$ compared to the homogeneous chain which has a bending coefficient of $50 k_B T \text{ nm}$.

that can be twisted. This is rectified if we instead consider an orthonormal triad of vectors $\{\hat{\mathbf{e}}_i\}_{i=1,2,3}$ that describes a moving frame. This now describes a two-dimensional surface embedded in three-dimensional space. This type of object is called a *ribbon* and can now be twisted. We shall refer to this model as the twistable worm-like chain (TWLC). Strictly speaking, this describes a *Darboux frame*. It is the analogue of the Frenet–Serret frame as applied to surface geometry.

The following framework was established by Benham [139, 140] with further work done by Starostin [141] and then by Fain *et al.* [142, 143]. Consider three Euler angles θ, ϕ, ψ that describe the evolution of a triad of vectors via a composition of three rotations. The individual vectors $\hat{\mathbf{e}}_1$, $\hat{\mathbf{e}}_2$ and $\hat{\mathbf{e}}_3$ evolve according to the equation

$$\hat{\mathbf{e}}_i(s) = \mathbf{R}_3(\psi) \mathbf{R}_1(\theta) \mathbf{R}_3(\phi) \hat{\mathbf{e}}_i(0). \quad (5.61)$$

If we let $\mathbf{R} = \mathbf{R}_3(\psi) \mathbf{R}_1(\theta) \mathbf{R}_3(\phi)$, then the rate of change of our triad is $\partial_s \hat{\mathbf{e}}_i(s) = \dot{\mathbf{R}} \hat{\mathbf{e}}_i(0)$. But we may invert this rotation $\hat{\mathbf{e}}_i(0) = \mathbf{R}^\top \hat{\mathbf{e}}_i(s)$ and hence obtain that

$$\partial_s \hat{\mathbf{e}}_i(s) = \dot{\mathbf{R}} \mathbf{R}^\top \hat{\mathbf{e}}_i(s). \quad (5.62)$$

$\dot{\mathbf{R}} \mathbf{R}^\top$ is skew-symmetric and its action on a vector can hence be represented as a cross-product. It can then be shown that $\exists \boldsymbol{\Omega}$ such that $\dot{\mathbf{e}}_i = \boldsymbol{\Omega} \times \hat{\mathbf{e}}_i$, where

$$\boldsymbol{\Omega} = \begin{pmatrix} \dot{\phi} \sin \theta \sin \psi + \dot{\theta} \cos \psi & -\dot{\phi} \sin \theta \cos \psi + \dot{\theta} \sin \psi & \dot{\phi} \cos \theta + \dot{\psi} \end{pmatrix}. \quad (5.63)$$

One observation that can be made is that upon the rotation $\psi \rightarrow \psi - \pi/2$, $\Omega_1 \rightarrow \Omega_2 \rightarrow -\Omega_1$. The curvature is

$$\kappa^2 = \|\partial_s \hat{\mathbf{t}}\|^2 \quad (5.64)$$

$$= \Omega_1^2 + \Omega_2^2 \quad (5.65)$$

$$= \dot{\phi}^2 \sin^2 \theta + \dot{\theta}^2. \quad (5.66)$$

Consider the elastic Hamiltonian parametrised by the three Euler angles θ, ϕ, ψ

$$E[\theta, \phi, \psi] = \int_{-L/2}^{L/2} ds \frac{A}{2} (\dot{\phi}^2 \sin^2 \theta + \dot{\theta}^2) + \frac{C}{2} (\dot{\phi} \cos \theta + \dot{\psi})^2 - f \cos \theta, \quad (5.67)$$

where we have contributions from bending twisting and pulling respectively. The Euler-Lagrange equation for a variable ϕ is

$$\frac{\partial \mathcal{L}}{\partial \phi} - \frac{d}{ds} \left(\frac{\partial \mathcal{L}}{\partial \dot{\phi}} \right) = 0. \quad (5.68)$$

Hence, the Euler-Lagrange equations for ϕ and ψ give that

$$\dot{\psi} = \frac{p_\psi}{C} - \dot{\phi} \cos \theta \quad (5.69)$$

$$\dot{\phi} = \frac{p_\phi - p_\psi \cos \theta}{A \sin^2 \theta}, \quad (5.70)$$

where $p_\phi = \partial E / \partial \dot{\phi}$ and $p_\psi = \partial E / \partial \dot{\psi}$ are conserved momenta. In fact, $p_\psi = C (\dot{\phi} \cos \theta + \dot{\psi})$ is related to the twist as

$$\text{Tw} = \frac{1}{2\pi} \int_{-L/2}^{L/2} ds (\dot{\phi} \cos \theta + \dot{\psi}) \quad (5.71)$$

$$= \frac{L p_\psi}{2\pi C}. \quad (5.72)$$

The EL equation in θ gives us that

$$A\ddot{\theta} = A\dot{\phi}^2 \cos \theta \sin \theta - C\dot{\phi} \sin \theta (\dot{\phi} \cos \theta + \dot{\psi}) + f \sin \theta. \quad (5.73)$$

There are two families of trivial solutions that satisfy these equations. The first is the twistable straight chain, defined by $\theta = \pi/2$, $\phi = 0$ and $\psi = p_\psi s/C$. It has an energy

$$E_{\text{line}} = -fL + \frac{2\pi^2 C}{L} \text{Tw}^2. \quad (5.74)$$

The other trivial solution is that of a solenoid. If we seek a solenoid with n complete turns and a total length L , then $\theta = \delta$ and it follows from Eq. 5.73 and Eq. 5.70 that

$$\theta = \delta \quad (5.75)$$

$$\phi = \frac{2\pi n s}{L} + \phi_0 \quad (5.76)$$

$$\psi = \left[\frac{2\pi n \cos \delta}{L} \left(\frac{A}{C} - 1 \right) + \frac{fL}{2\pi n C} \right] s + \psi_0. \quad (5.77)$$

From these equations we may deduce the twist of the solenoid:

$$\text{Tw}_{\text{helix}} = \frac{A}{C} n \cos \delta + \frac{fL^2}{4\pi^2 n C}. \quad (5.78)$$

The energy of such a solenoid is then simply

$$E_{\text{solenoid}} = \frac{2\pi^2 n^2 A}{L} \sin^2 \delta + \frac{2\pi^2 C}{L} \text{Tw}^2 - fL \cos \delta. \quad (5.79)$$

There also exists a family of non-trivial saddle point solutions. These solutions are very general and can be used to form a variety of periodic structures such as 3D curls, figures of eight and trefoil knots. These solutions depend on the boundary conditions imposed on the shapes. The full solutions to the EL equations and associated quantities of such configurations are derived in Appendix A for completeness and were originally derived by Benham [139]. A summary of the results is given below. Defining $u = \cos \theta$, if $c \leq u \leq b$ and a is a constant that can be determined by the system constraints then

$$u = (b - c) \text{sn}^2 \left(\frac{s}{\lambda k} \sqrt{\frac{b - c}{2}}, k \right) + c. \quad (5.80)$$

$$\phi(s) = \frac{1}{\sqrt{a-c}} \left[\sqrt{\frac{(a+1)(b+1)}{1+c}} \Pi \left(\frac{c-b}{c+1}, \operatorname{am} \left(\frac{s}{\lambda} \sqrt{\frac{a-c}{2}}, k \right), k \right) \right. \quad (5.81)$$

$$\left. + \sqrt{\frac{(a-1)(1-b)}{1-c}} \Pi \left(\frac{b-c}{1-c}, \operatorname{am} \left(\frac{s}{\lambda} \sqrt{\frac{a-c}{2}}, k \right), k \right) \right]. \quad (5.82)$$

$$\begin{aligned} \psi(s) = & p_\psi \left(\frac{1}{C} - \frac{1}{A} \right) s + \frac{1}{\sqrt{a-c}} \left[\sqrt{\frac{(a+1)(b+1)}{1+c}} \Pi \left(\frac{c-b}{c+1}, \operatorname{am} \left(\frac{s}{\lambda} \sqrt{\frac{a-c}{2}}, k \right), k \right) \right. \\ & \left. - \sqrt{\frac{(a-1)(1-b)}{1-c}} \Pi \left(\frac{b-c}{1-c}, \operatorname{am} \left(\frac{s}{\lambda} \sqrt{\frac{a-c}{2}}, k \right), k \right) \right]. \end{aligned} \quad (5.83)$$

This describes an elastica whose value of u goes $c \rightarrow b$.

5.4.1 3D Plectonemes

We now attempt to extend the plectoneme model from the previous section to include twist. We again model a plectoneme as a piecewise model comprised of the braid, the loop and the tail. We construct each and then form the entire plectoneme via appropriate reflections. Several 3D plectoneme models already exist, the most notable being the models of Clauvelin *et al.* [138], Purohit [144] and Marko and Neukirch [65].

We model the loop as a 3D elastica and choose $c = -1$; this leaves two unknowns, a and b . We then impose closure in y and closure up to a constant in x , this allows us to set a plectonemic radius.

$$0 = \int_0^{L_{\text{loop}}/2} ds \, z_{\text{loop}} \quad (5.84)$$

$$r_0 = \int_0^{L_{\text{loop}}/2} ds \, x_{\text{loop}}. \quad (5.85)$$

If we were to allow the loop elastica to be “complete”, then we would have two unknown roots and two conditions that results in zero free parameters for the loop, i.e. the loop is uniquely determined by R alone. However, it is found that often the loop wishes to adopt configurations that are not “complete”. Hence, we choose L_{loop} to be the one free parameter for our loop. Since the loop is closed in the direction of the force, the force enters our Hamiltonian simply as a Lagrange multiplier and

hence its choice is not necessarily equal to the external force. One more danger to be avoided is configurations that self-intersect but not at the ends of the loop. This is avoided by performing a check on the loop to see if the $x > r_0 \forall s \in [0, L_{\text{loop}}]$.

We model the braid as a solenoid whose inclination is determined by the loops end angle. This ensures the z -component of the tangents match up between loop and braid. The parameters that determine a solenoid are the pitch p , radius r and the number of turns n . The inclination of a solenoid is determined by the pitch and radius and so, given an input radius, the pitch can be determined, leaving us with r and n as free parameters.

Finally, we model the tail as a planar elastica that can store twist energy via the addition of the ψ parameter. Technically this is only an elastic minimum in 2D, however, it will only deviate marginally from the full 3D elastica and has the benefit of changes in p_ψ leaving the writhe unchanged.

To summarise we can generate a plectoneme with the following free parameters: L_{loop} , R , n and p_ψ . We have two global constraints to fulfil, the total length L and linking number Lk . Hence, there are 2 truly free parameters. To find the optimal plectoneme we simply vary the remaining parameters to find which is the most energetically favourable. There are clearly many ways to do this. Below we describe what we have found to be the optimum procedure.

Given L_{loop} , R and n construct the loop. Once b_{loop} is known, we can deduce the value δ that defines the inclination angle of the solenoidal braid. The pitch of the braid is then calculable given we know its radius and inclination. This leaves us with some length that we construct the tail from. We choose initially for the tail to store zero twist. This method ensures that the plectoneme fulfils the length criteria. Solving the length constraint for the tail, when the tail is long, can be numerically challenging due to the divergence of the elliptic integral of the first kind as the elliptic modulus tends to unity. Hence, we make use of the approximate expression

$$\theta(s) = 4 \arctan \left[\tan \left(\frac{\theta_L}{4} \right) e^{(s-L)/\lambda} \right]. \quad (5.86)$$

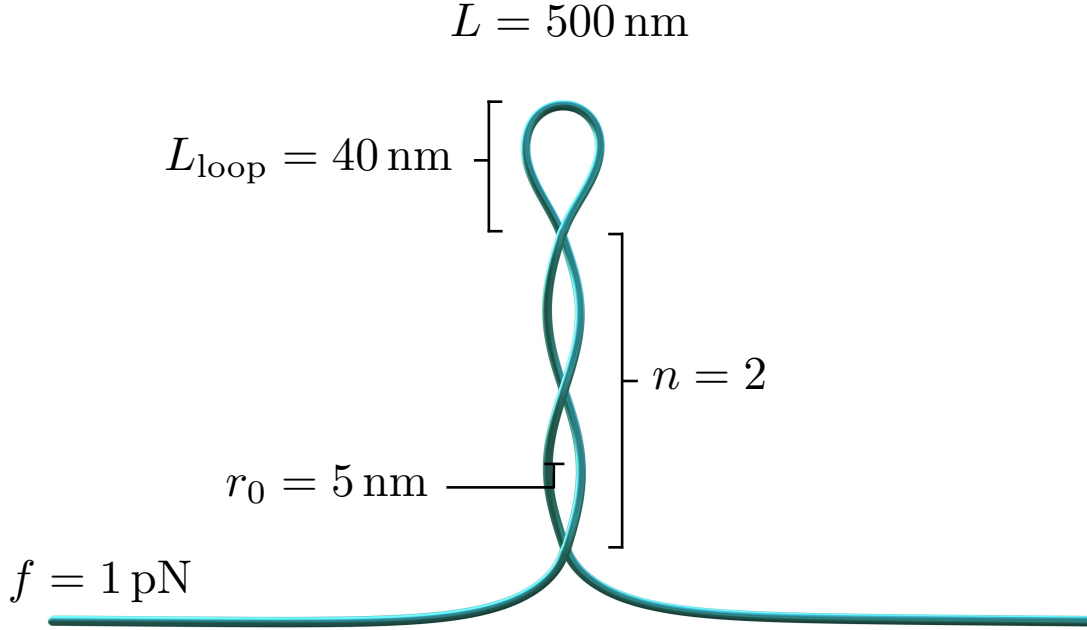


Figure 5.10: Left-handed plectoneme with 2 braids formed for a system of length 500 nm and a plectonemic radius of $r_0 = 5 \text{ nm}$.

For sufficiently long tails, this approximation leads to an exponentially decaying error compared with solving the tail exactly.

We then calculate the twist and writhe of the entire plectoneme to obtain the linking number. We compare this to the desired linking number which allows us to deduce the amount of excess twist we must put into the tail. As the twist controlling parameter p_ψ does not affect the writhe, we need not calculate it a second time.

Writhe, when calculated with the discretised double integral described in Section 2.7.2, is by far the most computationally expensive part of the algorithm. Hence we opt to only perform one full writhe calculation for every n , and then calculate all writhes with the same number of braids using Fuller's formula described by Eq. 2.46 with the previous plectoneme providing the reference curve and writhe. This leads to significantly increased efficiency while maintaining a high level of accuracy if our varied parameters are sufficiently finely spaced. An example plectoneme of length 500 nm is given in Fig. 5.10.

As for the choice of plectonemic radius we have two options. One method is

to add an electrostatic term into our energy that captures the cost of forming a more tightly wound plectoneme. This approach has been used most notably in Refs. [145] and [146]. Both utilise Debye-Hückel theory, but with the latter including a self-interaction contribution. Hence, we choose to adopt the potential proposed by Brahmachari and Marko [146] which is as follows:

$$\beta U_{\text{elec}}(R, \delta) = \zeta K_0 \left(\frac{2R}{\lambda_D} \right) Z(a) + \frac{\zeta c_1 \lambda_D^2}{R^2 a^2 (1 + c_2 a^2)}, \quad (5.87)$$

where λ_D is the *Debye length*, $a = \cot \delta$, $Z(a) = 1 + m_1 a^2 + m_2 a^4$, $\zeta = 2A\ell_B \nu^2$ and ℓ_B is the *Bjerrum length*. The disadvantage of this method as it requires variation in another variable, and so adds to the computational cost of the procedure.

A more ad-hoc method is to use experimental data to choose our value of r_0 . Boles *et al.* [147] describes the relationship of plectonemic radius vs superhelical density, however, this is limited to a specific salt concentration. Another limitation is that the experimental data is for closed DNA, and so will be force independent. Given the relatively low computational cost of our plectonemic model so far, we opt for the use of the more general electrostatic method of Brahmachari and Marko.

Of course the plectoneme is not always the most favourable configuration for a supercoiled system. As discussed in Section 5.4, the twisted line and curl are also possible configurations depending on the superhelical density of the system. Hence, we may calculate the most favourable of these for an array of forces and σ . Due to the non-trivial electrostatics of the curl, we only consider the plectoneme and straight line configurations as possibilities. The results are shown in Fig. 5.11 where the blue dots represent B-DNA and the red dots are plectonemes.

5.4.2 3D Damage and Future Work

The aim of this section was to propose a new plectonemic model of DNA, that utilised the elastic minima solutions to construct a piecewise plectoneme. The aim was to create a more sophisticated model than its predecessors via more careful consideration of boundaries and a more detailed writhe calculation. The hope

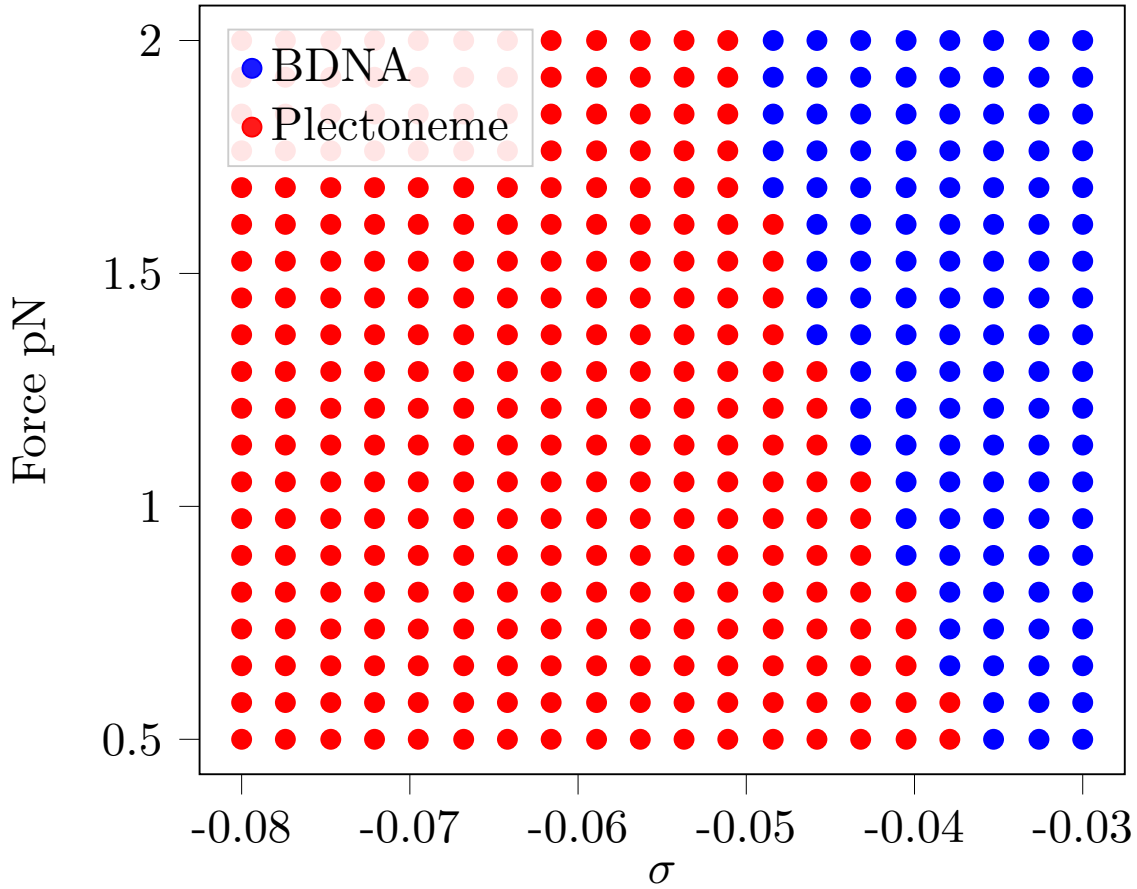


Figure 5.11: Phase diagram of 600 bp DNA with $A = 50 k_B T \text{ nm}$, $C = 100 k_B T \text{ nm}$ for a range of forces and superhelical densities.

was that we could generate spatially reasonable plectonemes with sensible twist distributions while keeping the computational cost low.

Combining the work done on plectonemes and inhomogeneous elastica, it is possible to insert the floppy or kinked defect models into the plectoneme generating a model to investigate the effects of tip-damaged localisation. A 3D version of the kinked model would entail a twist rest angle as well as a bending rest angle, for example approximately 15° for a TD. For floppy 3D damage we would simply need to construct the damaged region where we see a reduction in the twist stiffness C as well as in the bending stiffness A .

With such damages at the tip of the plectoneme, the lower energy required to bend the loop as well as the reduction in twist the system needs to accommodate should cause the plectonemic state to become favourable at lower forces and smaller

superhelical densities. Hence, we expect to see a plectonemic buckling transition at smaller values of σ compared with the homogeneous system.

Unfortunately, there was not enough time to implement 3D damage to the plectoneme model established in this section and it will be left as future work for now. The main challenge would be finding robust methods for solving the increasingly difficult boundary conditions for the interface of the damaged and undamaged sections. A more realistic option might be to do a numerical minimization using gradient descent where the starting configuration is an undamaged plectoneme generated using the current method.

5.5 Conclusion

In this chapter we introduced the planar worm-like chain, characterising DNA by its bending modulus. We then utilised the resulting elastica to demonstrate how several supercoiled configurations could be replicated in the plane. This involved developing a planar plectoneme model which was comprised of piecewise curves, although our 2D plectonemes are not strictly elastic minima, simply comprised of piecewise elastic minima.

We then investigated the effect of placing a damaged region in our chain, following on the work of Randisi [81] who defined the kinked and floppy damaged types. We derived a general expression for the elastic minimum of two chains with differing bending moduli and applied this expression to curls and circles. We then inserted this damage model into the tips of our planar plectonemes and probed how the energies of these damaged plectonemes compared with the undamaged ones. It was seen that beyond some force both damaged models were energetically favoured, however we note the limitations of the planar model that ensure we must keep a fixed plectonemic radius.

Finally, we considered a twistable worm-like chain that was not restricted to the plane and re-derived the common elastic minima of the straight chain, solenoid and 3D elastica. Extending the ideas utilised in the planar plectoneme model, we developed a piecewise 3D plectoneme model which included an electrostatic

contribution to the energy, and an accurate writhe calculation. Using this model we generated a plectoneme/line phase diagram which agreed with the expected trend.

6

Thermal Fluctuations and Statistical Mechanics

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6.1 Introduction

Up until now we have dealt with a WLC theory of a string or ribbon which simply parametrizes our object according to some quantities such as A , C , L , ℓ_P and f .

For example, we could set $\ell_P = 50 \text{ nm}$ for B-DNA and $\ell_P = 2 \text{ m}$ for a stiff wire. The elastic minima of both objects would depend on the same formulae, i.e. this is a scale-independent description. This means there is no physics to discern a macroscopic WLC from a microscopic one. This should be of some concern so we must ask ourselves, what is the most important physics we have neglected in our microscopic model? The short answer is temperature, or more specifically, the consequences of temperature. Both a steel wire and in-vivo DNA are immersed in fluids which have a non-zero temperature. If the thermal kick from the fluid is sufficient, it can cause the configuration to explore a new region of phase-space. The difference in order of magnitude of the energy of a macroscopic WLC and a microscopic one is the reason we only see meaningful fluctuations in the latter.

In this thesis we shall assume our DNA is in equilibrium and so we expect it to fluctuate about some *mean-field* configuration. But how do we capture this effect in our current theory? Incorporating thermal fluctuations is really a question of incorporating entropy, hence we must devise a way to take into account the number of accessible states to our WLC.

This idea was first used by Marko and Siggia [62] who determined that for a straight chain, we see a reduction in the bending modulus A . This phenomenon can be referred to as a *softening* in the elastic parameters. Moroz and Nelson [68] then derived the revised bending constants for a TWLC in the fixed torque ensemble. Other notable applications of this technique include the TWLC with twist-bend coupling [34] and the WLC homoclinic loop [148]. In this chapter we consider the effects of thermal fluctuations on a range of configurations beyond what has previously been studied.

The development of a more rigorous theory of thermal fluctuations for twistable ribbons and the application of this theory to new configurations will allow us to gain more insight into the DNA energy landscape. The scope of what structures have currently been investigated in this manner are currently limited to only a few configurations. The hope is that the quantification of the effect of thermal

fluctuations on novel configurations will aid the understanding of all potential phases of DNA.

6.2 Polymeric Path Integrals

Though mostly associated with quantum mechanics, the origins of the path integral come from Wiener [149] who developed a framework to consider the contributions of all possible trajectories of a Brownian particle. Expectation values can then be computed via weighted sums over these paths. This formalism was then adapted to quantum mechanics by Feynman [150] with the interpretation that all possible paths are realised.

This method was then adapted to capture the effects of thermal fluctuations in molecular systems. If one can model a system of particles through an energy functional of some path property, a tangent for example, then we can construct a partition function which is a weighted sum over that field. I.e.

$$Z = \int \mathcal{D}\hat{\mathbf{t}} e^{-\beta E[\hat{\mathbf{t}}]}, \quad (6.1)$$

where $\mathcal{D}\hat{\mathbf{t}}$ is the integration *measure* over tangent space and $\beta = 1/k_B T$. There are two main ways we can study the path integral in Eq. 6.1: direct integration or an approximation using a mean-field approach. In the case of the former, we simply require an appropriate parametrization of the integration space and the resulting integral to be computable.

6.2.1 Discrete Path Integrals

We begin with the discrete WLC, i.e. a Kratky-Porod model of a polymer [61]. Consider the energy of an N -link polymer of sub-length b with total length $L = Nb$, see Fig. 6.1. The bending energy is

$$E_{\text{bend}} = -\frac{1}{2b} \sum_{n=1}^{N-1} \kappa_n \|\hat{\mathbf{u}}_{n+1} - \hat{\mathbf{u}}_n\|^2, \quad (6.2)$$

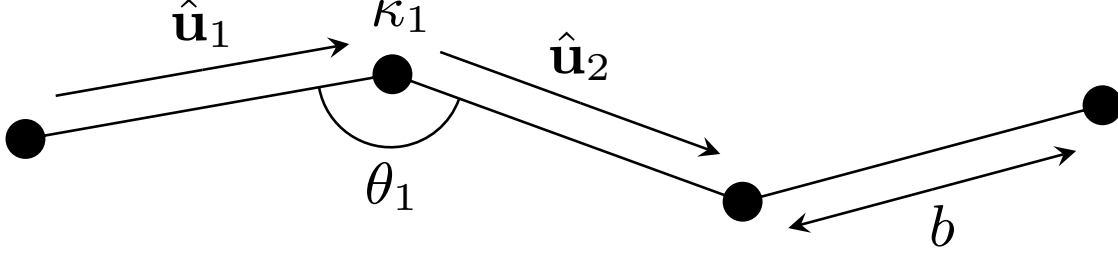


Figure 6.1: Schematic of discrete WLC for 4 particles with 3 links of bond length b . In the Kratky-Porod model, bonds are inextensible and the energy of each site is a function of the stiffness of that site and the angle between the bonds.

where $\hat{\mathbf{u}}_n = (\mathbf{r}_{n+1} - \mathbf{r}_n) / \|\mathbf{r}_{n+1} - \mathbf{r}_n\|$ is a discrete unit tangent. We can in fact compute the partition function of this exactly:

$$Z_N = \prod_{n=1}^{N-1} \left(1 - e^{-2\beta\kappa_n/b}\right). \quad (6.3)$$

From this we can determine the average tangent-tangent correlation

$$\langle \hat{\mathbf{u}}_j \cdot \hat{\mathbf{u}}_k \rangle = \prod_{i=j}^k \left[\coth \left(\frac{\beta\kappa_i}{b} \right) - \frac{b}{\beta\kappa_i} \right]. \quad (6.4)$$

The derivations for these formulae are given in Appendix B and are based on the derivation for the homogeneous chain that is outlined throughout Chapter 15 of Ref. [151]. Essentially these formulae render the homogeneous formulae site dependent. But what effect does this have on the decay of the tangent autocorrelation? As we can see from Fig. 6.2 a single site with modified bending stiffness only exhibits a small change in the autocorrelation. This could make detection of inhomogeneities via this method challenging and implies for small damage regions we don't see a large change in the average flexibility of the whole chain.

6.2.2 Continuous Path Integrals

We now introduce the second method of computing polymeric path integrals detailed in this thesis through an illustrative example of the homogeneous straight-chain. Recall that the energy functional for the WLC is

$$E[\hat{\mathbf{t}}] = \int_0^L \left[\frac{A}{2} \|\partial_s \hat{\mathbf{t}}\|^2 - \mathbf{f} \cdot \hat{\mathbf{t}} \right] ds, \quad (6.5)$$

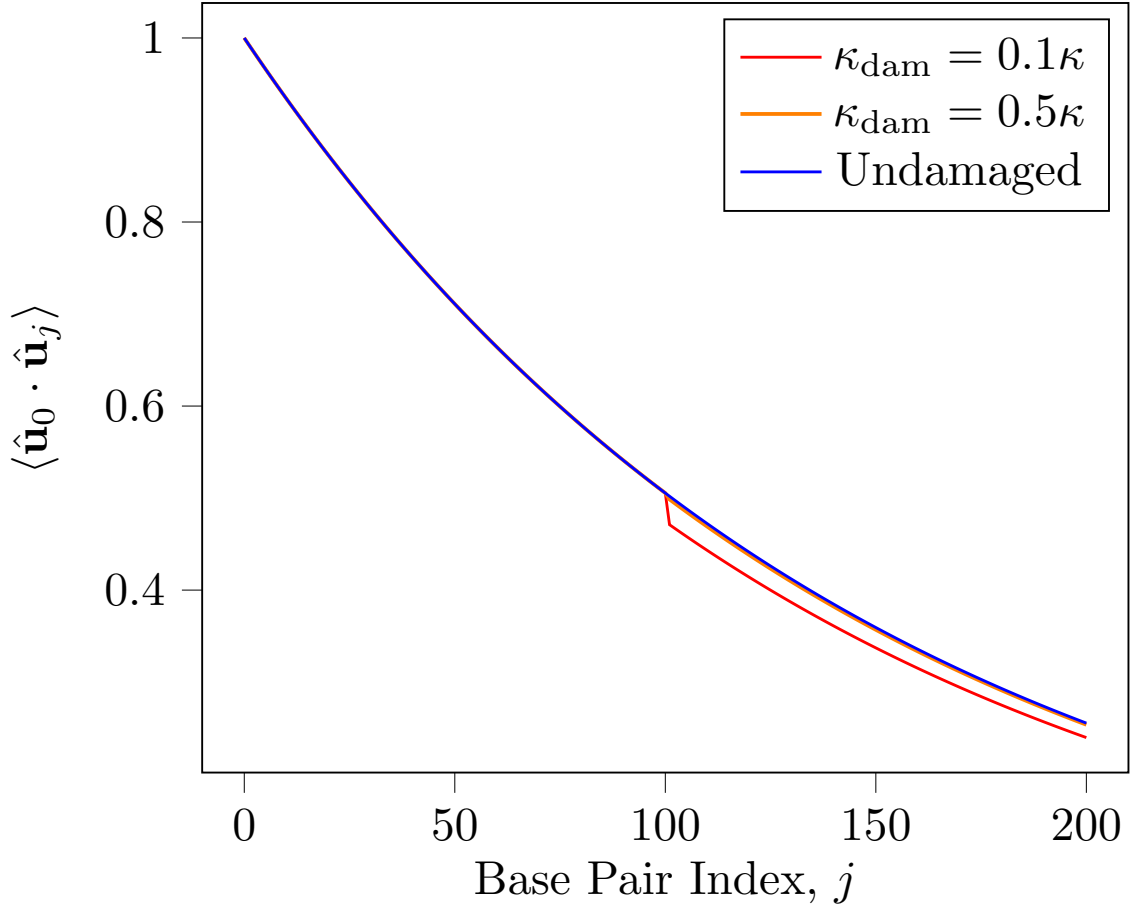


Figure 6.2: Plot of tangent autocorrelation as a function of base-pair index for a 200-bp chain for both a homogeneous chain and an inhomogeneous chain. The homogeneous bending stiffness is $\kappa = 50 k_B T \text{ nm}$ and the inhomogeneous chains have reduced bending stiffnesses of $\kappa_{\text{dam}} = 0.5\kappa$ and $\kappa_{\text{dam}} = 0.1\kappa$ at a single site located at the 100th bp.

where $\hat{\mathbf{t}}$ is the unit tangent and the force $\mathbf{f}$ is oriented in the z -direction. For the straight chain aligned with the z -axis, $\kappa = 0$ and so $E_c = -fL$, where c denotes the mean-field or *classical* solution. We model the thermal fluctuations explicitly through a fluctuation in the tangent field. For an arbitrary fluctuation, the modified tangent $\tilde{\mathbf{t}}$ will simply be

$$\tilde{\mathbf{t}} = t_1 \hat{\mathbf{x}} + t_2 \hat{\mathbf{y}} + t_3 \hat{\mathbf{z}}. \quad (6.6)$$

If classically $\mathbf{t}_c = \hat{\mathbf{z}}$, then we expect $\hat{\mathbf{t}}$ will still largely point in this direction for small fluctuations. Hence, we let the modified tangent $\tilde{\mathbf{t}}$, take the form

$$\tilde{\mathbf{t}} = t_1 \hat{\mathbf{x}} + t_2 \hat{\mathbf{y}} + \sqrt{1 - t_1^2 - t_2^2} \hat{\mathbf{z}}, \quad (6.7)$$

where $t_1, t_2 \ll 1$. If we perform a Taylor expansion on $\hat{\mathbf{z}}$ coefficient, we arrive at

$$\tilde{\mathbf{t}} \approx t_1 \hat{\mathbf{x}} + t_2 \hat{\mathbf{y}} + \left(1 - \frac{t_1^2 + t_2^2}{2}\right) \hat{\mathbf{z}}. \quad (6.8)$$

Hence, now $\tilde{\mathbf{t}}$ is of the form $\tilde{\mathbf{t}} = \mathbf{t}_c + \delta\mathbf{t}$, where $\delta\mathbf{t}$ is a “fluctuation tangent” that is small in magnitude. We are now in the position to construct a partition function. Since we assumed that our system is fluctuating around some mean-field configuration, then the path integral will be an integral over all the possible fluctuations of the tangent, not an integral over all tangents as was the case for the path integral in Section 6.2.1. Hence,

$$Z = \iint \mathcal{D}\delta\mathbf{t} e^{-\beta E[\tilde{\mathbf{t}}]}, \quad (6.9)$$

where $\mathcal{D}\delta\mathbf{t}$ is the integration measure over fluctuation-tangent space. If we enter the tangent of the form in Eq. 6.8 into Eq. 6.5 then

$$E[\tilde{\mathbf{t}}] = -fL + \int_0^L ds \frac{A}{2} [(\partial_s t_1)^2 + (\partial_s t_2)^2] + \frac{f}{2} (t_1^2 + t_2^2). \quad (6.10)$$

We then recognise $-fL$ as our mean field energy E_c and we use integration by parts to show

$$E[\tilde{\mathbf{t}}] = E_c + \frac{A}{2} \int_0^L ds \left[-t_1 \partial_s^2 t_1 - t_2 \partial_s^2 t_2 + \frac{1}{\lambda^2} (t_1^2 + t_2^2) \right]. \quad (6.11)$$

This requires the boundary conditions to evaluate to zero, via either vanishing boundary conditions or periodic boundary conditions. The second term in Eq. 6.11 is called the *second order variation* in the energy and represents the energy contribution of the fluctuations. The procedure of expanding about some classical path to obtain the classical energy plus some fluctuation contribution is called the *semi-classical approximation*. Inserting Eq. 6.11 into our partition function:

$$Z = e^{-\beta E_c} \iint \mathcal{D}\delta\mathbf{t} \exp \left[-\frac{\beta A}{2} \int_0^L ds \left[-t_1 \partial_s^2 t_1 - t_2 \partial_s^2 t_2 + \frac{1}{\lambda^2} (t_1^2 + t_2^2) \right] \right]. \quad (6.12)$$

The next step is to write the measure as $dt_1 dt_2$. Despite $t_1, t_2 \ll 1$, we argue that we may integrate each component between $-\infty$ and ∞ as the main contribution will come from smaller values. This is called the *saddle-point approximation*. Its

purpose is that it now renders the tangent space domain infinite and hence, the integral becomes an analytically tractable Gaussian.

$$Z = e^{-\beta E_c} \iint_{\mathbb{R}^2} \exp \left[-\frac{\beta A}{2} \int_0^L ds - t_1 \partial_s^2 t_1 - t_2 \partial_s^2 t_2 + \frac{1}{\lambda^2} (t_1^2 + t_2^2) \right]. \quad (6.13)$$

The result of this Gaussian integral is

$$Z = \frac{2\pi e^{-\beta E_c}}{\beta A \text{Det} \left(-\partial_s^2 + \frac{1}{\lambda^2} \right)}, \quad (6.14)$$

where both integration variables contribute equally an inverse square-root term. We define $\text{Det } \hat{O}$ to be the *functional determinant* of the differential operator $\hat{O}$, see Section 2.4.2 for an overview of differential operators. Since it is only the logarithm of the partition functional that corresponds to physical observables, we are permitted to shed multiplicative constants. Finally, we let $e^{-\beta E_c}$ be the classical partition function Z_c and subsequently define Z_{fluc} such that $Z = Z_c Z_{\text{fluc}}$ which gives us

$$Z_{\text{fluc}} = \frac{1}{\text{Det} (-\partial_s^2 + 1/\lambda^2)}. \quad (6.15)$$

This functional determinant can be calculated in several ways, the most common of which is using Fourier methods. It is common in the literature to change variables to the Fourier components of the fluctuation tangent and proceed from there without introducing the notion of the functional determinant. This is beneficial when working with systems where Fourier methods are applicable and can hide a lot of the dirty tricks developed by physicists to make sense of these path integrals. In this thesis we shall endeavour to work with the functional determinant and choose to evaluate such objects using Fourier techniques if the situation demands it.

Finally, observables we shall determine include the free energy

$$\mathcal{F} = -k_B T \ln Z, \quad (6.16)$$

as well as the relative end-to-end distance

$$\frac{\langle \Delta z \rangle}{L} = -\frac{1}{L} \frac{\partial \mathcal{F}}{\partial f}. \quad (6.17)$$

Similarly to defining a fluctuation partition function, we may define the fluctuation free energy as $\beta \mathcal{F}_{\text{fluc}} = -\ln Z_{\text{fluc}}$.

6.3 Functional Determinants

In order to progress any further we must make sense of what a functional determinant is. Consider a differential operator $\hat{O}$. The easiest way to extend the notion of the determinant would be to write

$$\text{Det}\hat{O} = \prod_n \Lambda_n, \quad (6.18)$$

where $\hat{O}\psi_n = \Lambda_n\psi_n$, Λ_n and ψ_n are the eigenvalues and associated eigenfunctions of $\hat{O}$ respectively. The theory of differential operators is established more thoroughly in Section 2.4.2. From the theory of ordinary differential equations we know that we can only solve an eigenfunction problem with regards to particular boundary conditions. Hence, a functional determinant must also depend on the imposed boundary conditions.

This formulation can be problematic for several reasons. The first is that the presence of a zero mode will cause the functional determinant to vanish. The second is that given the number of eigenvalues isn't necessarily bounded, the determinant can and often will diverge.

We once again return to our differential operator for the straight WLC, $\hat{O} = -\partial_s^2 + 1/\lambda^2$. Solving the ODE $\hat{O}\psi = \Lambda\psi$ in the domain $s \in [0, L]$, subject to Dirichlet boundary conditions we find that

$$\psi \propto \sin\left(L\sqrt{\Lambda_n + \frac{1}{\lambda^2}}\right), \quad (6.19)$$

where $\Lambda_n = n^2\pi^2/L - 1/\lambda^2$, $n \in \mathbb{N}_+$. Inserting this into our formula

$$\text{Det}\hat{O} = \prod_{n=1}^{\infty} \left(\frac{n^2\pi^2}{L^2} + \frac{1}{\lambda^2}\right). \quad (6.20)$$

Clearly this infinite product diverges. This was something quantum physicists discovered long ago when computing the path integral of a harmonic oscillator. Their solution was to renormalize the determinant by dividing by the determinant of another operator such that the infinities “cancel out”. This rather ad-hoc method of dealing with unwanted infinities is a necessary evil when dealing with path

integrals and is part and parcel of the field. The method of renormalization is made more rigorous via the Riemann zeta definition of the determinant which we will cover later. If we define $\hat{O}_{\text{free}} = -\partial_s^2$, then the determinant of the free operator is just $n^2\pi^2/L^2$. Therefore

$$\frac{\text{Det}\hat{O}}{\text{Det}\hat{O}_{\text{free}}} = \prod_{n=1}^{\infty} \left(1 + \frac{L^2}{n^2\pi\lambda^2}\right). \quad (6.21)$$

The infinite product representation of sin can then be used to show that

$$\frac{\text{Det}\hat{O}}{\text{Det}\hat{O}_{\text{free}}} = \frac{\sinh(L/\lambda)}{L/\lambda}. \quad (6.22)$$

Hence,

$$\beta\mathcal{F}_{\text{fluc}} = \ln\left(\frac{L}{\lambda}\right) - \ln \sinh(L/\lambda). \quad (6.23)$$

In the strong force limit, i.e. $f \gg 1$,

$$\mathcal{F} \approx -fL \left(1 - \frac{1}{2\beta\sqrt{Af}}\right) + \mathcal{O}(f^{-1}). \quad (6.24)$$

We can therefore calculate the relative end-to-end extension:

$$\frac{\langle\Delta z\rangle}{L} = 1 - \frac{1}{2\beta\sqrt{Af}}. \quad (6.25)$$

This indicates a shortening of the mean end-to-end distance that reflects the reduced stiffness of our DNA. Despite the successes in the WLC in describing dsDNA, something that structurally is far more complex than a homogeneous one-dimensional string, it is possible to extend this method to a two-dimensional surface embedded in three-dimensional space which allows for the storing of twist energy. Finally, it is worth noting that we are justified in assuming the strong force limit as this indicates that the DNA is held tightly and the transverse fluctuations are small.

6.3.1 Methods for Functional Determinants

We now outline two possible methods of calculating the functional determinant of a differential operator which will be required later. Consider the fourth-order differential operator of the form

$$\hat{\Lambda}^{(4)} = \partial_s^4 - \xi\partial_s^2 + \chi, \quad (6.26)$$

where $\xi, \chi \in \mathbb{R}$ are constants. It can be shown that

$$\ln \frac{\text{Det} \hat{\Lambda}^{(4)}}{\text{Det} \hat{\Lambda}_{\text{free}}^{(4)}} \approx \frac{1}{2} \sqrt{\xi + \sqrt{4\chi}} \quad (6.27)$$

for Dirichlet boundary conditions if we assume that $\chi > \xi^2/4$, a justification we shall make along the way. This can be done in two ways. The first is via Fourier methods, the second via the factorization of the operator. Beginning with the latter, factorizing the differential operator into two second order operators:

$$\hat{\Lambda}^{(4)} = \left(-\partial_s^2 + \frac{\xi}{2} + \sqrt{\frac{\xi^2}{4} - \chi} \right) \left(-\partial_s^2 + \frac{\xi}{2} - \sqrt{\frac{\xi^2}{4} - \chi} \right). \quad (6.28)$$

If we assume $\xi^2/4 - \chi < 0$, then

$$\hat{\Lambda}^{(4)} = \left(-\partial_s^2 + \frac{\xi}{2} + i\sqrt{\chi - \frac{\xi^2}{4}} \right) \left(-\partial_s^2 + \frac{\xi}{2} - i\sqrt{\chi - \frac{\xi^2}{4}} \right). \quad (6.29)$$

Renormalising by the free operator $\hat{\Lambda}_{\text{free}}^{(4)} = \partial_s^4$, which we shall also factorise,

$$\frac{\text{Det} \hat{\Lambda}^{(4)}}{\text{Det} \hat{\Lambda}_{\text{free}}^{(4)}} = \frac{\text{Det} \left(-\partial_s^2 + \frac{\xi}{2} + i\sqrt{\chi - \frac{\xi^2}{4}} \right) \text{Det} \left(-\partial_s^2 + \frac{\xi}{2} - i\sqrt{\chi - \frac{\xi^2}{4}} \right)}{\text{Det} (-\partial_s^2) \text{Det} (-\partial_s^2)}. \quad (6.30)$$

We can now calculate these two quotients of functional determinants:

$$\frac{\text{Det} \hat{\Lambda}^{(4)}}{\text{Det} \hat{\Lambda}_{\text{free}}^{(4)}} = \frac{\sinh(L\sqrt{E_+})}{L\sqrt{E_+}} \frac{\sinh(L\sqrt{E_-})}{L\sqrt{E_-}}, \quad (6.31)$$

where $E_{\pm} = \frac{\xi}{2} \pm i\sqrt{\chi - \frac{\xi^2}{4}}$. We recognise these two values as the two roots of the associated characteristic equation of $\hat{\Lambda}^{(4)}$ in the upper $\mathbb{C}$ -plane. Let us now find the value of this square root. Let the root $\sqrt{E_+}$ be $\gamma + i\eta$. Then

$$\gamma + i\eta = \sqrt{\frac{\xi}{2} + i\sqrt{\chi - \frac{\xi^2}{4}}}. \quad (6.32)$$

ξ and $\chi - \xi^2/4$ are real and positive and so we expect $\xi/2 + i\sqrt{\chi - \xi^2/4}$ to be in the first quadrant and hence, also its square root. However, $\xi/2 - i\sqrt{\chi - \xi^2/4}$ will be in the fourth quadrant and so its square root will be in the second quadrant. This means $\sqrt{E_{\pm}} = \gamma \pm i\eta$. Squaring and equating real and imaginary parts

$$\begin{aligned} \gamma^2 - \eta^2 &= \frac{\xi}{2} \\ 4\gamma^2\eta^2 &= \chi - \frac{\xi^2}{4}. \end{aligned}$$

These can then be combined to give a quartic equation in η ,

$$\eta^4 + \frac{\xi}{2}\eta^2 - \frac{\chi}{4} + \frac{\xi^2}{16} = 0. \quad (6.33)$$

Solving for $\gamma, \eta \in \mathbb{R}_{\geq 0}$

$$\gamma = \frac{1}{2}\sqrt{\xi + \sqrt{4\chi}} \quad (6.34)$$

$$\eta = \frac{1}{2}\sqrt{-\xi + \sqrt{4\chi}}. \quad (6.35)$$

Finally given that $\sinh(x) \sim e^x$ for $x \gg 1$,

$$\frac{1}{L} \ln \left(\frac{\text{Det} \hat{\Lambda}^{(4)}}{\text{Det} \hat{\Lambda}_{\text{free}}^{(4)}} \right) \approx \sqrt{E_+} + \sqrt{E_-} + \mathcal{O} \left(\frac{\ln L}{L} \right). \quad (6.36)$$

Hence,

$$\frac{1}{2L} \ln \left(\frac{\text{Det} \hat{\Lambda}^{(4)}}{\text{Det} \hat{\Lambda}_{\text{free}}^{(4)}} \right) \approx \gamma + \mathcal{O} \left(\frac{\ln L}{L} \right). \quad (6.37)$$

We have therefore shown that the logarithm of the renormalised functional determinant for a fourth operator of the class described above will simply be proportional to the real part of the solution to the quartic characteristic equation of the associated ODE.

Using the Fourier method, consider the eigenvalue problem $\hat{\Lambda}^{(4)} y_n = \lambda_n y_n$. If we switch to the Fourier basis $y_n = \sum_q e^{iqs} (\tilde{y}_n)_q$, then our eigenvalue equation becomes

$$\sum_q \left[(q^4 + \xi q^2 + \chi) - \lambda_n \right] e^{iqs} (\tilde{y}_n)_q = 0. \quad (6.38)$$

Hence for each q , the eigenvalue is just $\lambda_n = q^4 + \xi q^2 + \chi$. Given that

$$\ln \frac{\text{Det} \hat{\Lambda}^{(4)}}{\text{Det} \hat{\Lambda}_{\text{free}}^{(4)}} = \sum_n \ln \left(\frac{\lambda_n}{\lambda_n^{\text{free}}} \right), \quad (6.39)$$

exchanging sums for integrals via $\sum_n \rightarrow \frac{L}{2\pi} \int dq$ and using $\beta \mathcal{F}_{\text{fluc}} = \ln [\text{Det} \hat{\Lambda}^{(4)} / \text{Det} \hat{\Lambda}_{\text{free}}^{(4)}]$ we arrive at

$$\frac{\beta \mathcal{F}_{\text{fluc}}}{L} = \frac{1}{2\pi} \int_0^\infty dq \ln \left(\frac{q^4 + \xi q^2 + \chi}{q^4} \right) \quad (6.40)$$

$$= \frac{1}{4\pi} \int_{-\infty}^\infty dq \ln \left(1 + \xi/q^2 + \chi/q^4 \right) \quad (6.41)$$

$$= \frac{1}{2\pi} \int_{-\infty}^\infty dq \frac{\xi q^2 + 2\chi}{q^4 + \xi q^2 + \chi}. \quad (6.42)$$

This integral can be computed via complex analysis, which requires finding the roots of the denominator. These roots will be the same as the for the characteristic equation, but with $\xi \mapsto -\xi$. Using the residue theorem:

$$\frac{\beta \mathcal{F}_{\text{fluc}}}{L} = i \sum_p \text{Res}(f, q_p). \quad (6.43)$$

If we integrate along the real line, we encompass the two poles in the upper $\mathbb{C}$ -plane. Recall the residue for a simple pole is

$$\text{Res}(f, z_0) = \lim_{z \rightarrow z_0} (z - z_0) f(z). \quad (6.44)$$

Hence,

$$\frac{\beta \mathcal{F}_{\text{fluc}}}{L} = i [\text{Res}(f, q_1) + \text{Res}(f, q_1)] \quad (6.45)$$

$$= \frac{i(\xi q_1^2 + 2\chi)}{(q_1 - q_2)(q_1 - q_3)(q_1 - q_4)} + \frac{i(\xi q_2^2 + 2\chi)}{(q_2 - q_1)(q_2 - q_3)(q_2 - q_4)}, \quad (6.46)$$

where $q_1 = \eta + i\gamma$, $q_2 = -\eta + i\gamma$, $q_3 = \eta - i\gamma$ and $q_4 = -\eta - i\gamma$. Therefore,

$$\frac{\beta \mathcal{F}_{\text{fluc}}}{L} = i \left[\frac{\xi(\eta + i\gamma)^2 + 2\chi}{(2\eta)(2i\gamma)(2\eta + 2i\gamma)} + \frac{\xi(\eta - i\gamma)^2 + 2\chi}{(-2\eta)(-2\eta + 2i\gamma)(2i\gamma)} \right] \quad (6.47)$$

$$= \frac{\xi}{4\gamma} + \frac{\beta}{2\gamma(\gamma^2 + \eta^2)} \quad (6.48)$$

$$= \gamma. \quad (6.49)$$

This agrees with the solution obtained using the operator method. The success of the Fourier technique should be of little surprise given that an ODE with constant coefficients will be rendered trivially algebraic in the Fourier basis.

6.3.2 Gel'fand-Yaglom Theorem

The methods proposed thus far have all been applied to the relatively simple case of differential operators with constant coefficients. However, with the growing demand from physicists to improve techniques available to solve functional determinants, Gel'fand and Yaglom [152] devised a powerful tool that yields a beautifully elegant form. Consider a one-dimensional self-adjoint differential operator $\hat{L}_1$ of the form

$$\hat{L}_1 = \hat{L}_0 + V, \quad (6.50)$$

with general boundary conditions

$$\mathbf{M} \begin{pmatrix} y(0) \\ y'(0) \end{pmatrix} + \mathbf{N} \begin{pmatrix} y(L) \\ y'(L) \end{pmatrix} = 0, \quad (6.51)$$

where $\mathbf{M}$ and $\mathbf{N}$ are 2×2 matrices which fully encode said boundary conditions. E.g. for Dirichlet boundary conditions

$$\mathbf{M} = \begin{pmatrix} 1 & 0 \\ 0 & 0 \end{pmatrix}, \quad \mathbf{N} = \begin{pmatrix} 0 & 0 \\ 1 & 0 \end{pmatrix}. \quad (6.52)$$

We suppose $\hat{L}_1$ and $\hat{L}_0$ have non-degenerate point spectra which are bounded from below and non-zero. Suppose that $\{y_j^{(1)}(x), y_j^{(2)}(x)\}$ are the two linearly independent solutions to the homogeneous problem $\hat{L}_j y_j = 0$ (with any BC), then if we define

$$\mathbf{Y}_j(x) = \begin{pmatrix} y_j^{(1)}(x) & y_j^{(2)}(x) \\ y_j^{(1)'}(x) & y_j^{(2)'}(x) \end{pmatrix}, \quad (6.53)$$

the ratio of the functional determinants of the two differential operators are given by the formula:

$$\frac{\text{Det} \hat{L}_1}{\text{Det} \hat{L}_0} = \frac{\det(\mathbf{M} + \mathbf{N} \mathbf{Y}_1(L))}{\det(\mathbf{M} + \mathbf{N} \mathbf{Y}_0(L))}, \quad (6.54)$$

with the condition that $\mathbf{Y}_j(0) = \mathbb{I}_2$.

The proof of this theorem revolves around complex analysis through the construction of a complex valued function whose poles are the discrete energies of our operator. After taking the logarithm of the infinite product of our eigenvalues to produce an infinite sum, one can relate this expression to the Riemann zeta function, analytically continued to the origin.

The power of this theorem is that all we require to compute this expression is simply the solution to the homogeneous ODEs $\hat{L}_j y_j = 0$. As an example we again consider the simple operator of the form $\hat{L}_1 = -\partial_s^2 + A$ on the interval $s \in [0, L]$ with Dirichlet boundary conditions. We must hence solve the following ODEs

$$(-\partial_s^2 + A) y_1 = 0, \quad y_1(0) = 0, \quad y_1'(0) = 1 \quad (6.55)$$

$$-\partial_s^2 y_0 = 0, \quad y_0(0) = 0, \quad y_0'(0) = 1. \quad (6.56)$$

These have the solutions

$$y_1(s) = \frac{1}{\sqrt{A}} \sinh(s\sqrt{A}) \quad (6.57)$$

$$y_0(s) = s. \quad (6.58)$$

The Gel'fand-Yaglom theorem for Dirichlet boundary conditions is simply $\text{Det}\hat{L}_1/\text{Det}\hat{L}_0 = y_1(L)/y_0(L)$ and so

$$\frac{\text{Det}\hat{L}_1}{\text{Det}\hat{L}_0} = \frac{\sinh(L\sqrt{A})}{L\sqrt{A}}. \quad (6.59)$$

This agrees with the expression given by Eq. 6.22 with $A = 1/\lambda^2$.

Generalising the Gel'fand-Yaglom theory to the class of second order matrix differential operators was achieved by Forman [153]. For a 2×2 matrix differential operator $\hat{\mathbf{L}}$ of the form

$$\hat{\mathbf{L}} = \mathbf{P}_0(s) \partial_s^2 + \mathbf{P}_1(s) \partial_s + \mathbf{P}_2(s), \quad (6.60)$$

the functional determinant of said operator is

$$\text{Det}\hat{\mathbf{L}} = \exp \left[\frac{1}{2} \int_a^b dt \text{Tr} \mathbf{P}_1(t) \mathbf{P}_0^{-1}(t) \right] \det(\mathbf{M} + \mathbf{N}\mathbf{Y}(b)). \quad (6.61)$$

Where now $\mathbf{Y}$ will be a 4×4 matrix as a 2×2 system of second order ODEs will have 4 linearly independent solutions. Obtaining $\mathbf{Y}$ in this case with the condition $\mathbf{Y}(a) = \mathbb{I}_4$ can prove challenging and so McKane and Tarlie further define $\mathbf{H}$ as

$$\mathbf{H}(s) = \begin{pmatrix} \mathbf{y}_1 & \mathbf{y}_2 & \mathbf{y}_3 & \mathbf{y}_4 \\ \dot{\mathbf{y}}_1 & \dot{\mathbf{y}}_2 & \dot{\mathbf{y}}_3 & \dot{\mathbf{y}}_4 \end{pmatrix}, \quad (6.62)$$

where the columns of $\mathbf{H}$ are the 4 linearly independent solutions to $\hat{\mathbf{L}}\mathbf{y} = 0$ and their derivatives. This homogeneous ODE is called the *Jacobi accessory equation*. Non-trivial solutions to the Jacobi accessory equation are often called *conjugate points*. This is convenient as now if we let

$$\mathbf{Y}(s) = \mathbf{H}(s) \mathbf{H}^{-1}(a), \quad (6.63)$$

we can see that the initial condition $\mathbf{Y}(a) = \mathbb{I}_4$ is immediately fulfilled.

There are instances where this determinant will still vanish, this occurs when the solutions to the Jacobi accessory equation vanish at the boundaries. These eigenfunctions are known as *zero modes*. In order to make sense of a the functional determinant of a singular matrix differential operator, one needs to separate the contribution of the zero mode(s). This can occur when evaluating a Gaussian integral obtained by expanding a functional integral about a non-trivial classical solution (an instanton).

In the case of zero modes, several procedures have been developed to remove said singularities. McKane and Tarlie devised a method which involves perturbing the boundary conditions [154]. Kirsten and McKane then developed another technique using contour integration [155]. Falco [156] more recently developed a method of removing multiple zero modes from matrix differential operators, generalizing the methods of McKane and Tarlie. We shall simply list the results here.

Consider an n -dimensional matrix differential operator $\hat{\Omega}$ of the form Eq. 6.60 with Dirichlet boundary conditions on the domain $[a, b]$. $\hat{\Omega}$ is called a non-Schrödinger operator if $\mathbf{P}_1 \neq 0$. We may convert this differential operator into a Schrödinger form via the transformation

$$\hat{\mathbf{O}} = \mathbf{p}^{-1} \hat{\Omega} \mathbf{p}. \quad (6.64)$$

This operator is *iso-spectral* to $\hat{\Omega}$. Performing this transformation, we find that

$$\hat{\mathbf{O}} = \partial_s^2 + \mathbf{p}^{-1} \left(2\dot{\mathbf{p}} + \mathbf{P}_0^{-1} \mathbf{P}_1 \mathbf{p} \right) \partial_s + \mathbf{p}^{-1} \left(\ddot{\mathbf{p}} + \mathbf{P}_0^{-1} \mathbf{P}_1 \dot{\mathbf{p}} + \mathbf{P}_0^{-1} \mathbf{P}_2 \mathbf{p} \right). \quad (6.65)$$

We may recover a Schrödinger operator if $\dot{p} = -\frac{1}{2} \mathbf{P}_0^{-1} \mathbf{P}_1 p$. Solving this matrix ODE, with the initial condition $p(a) = \mathbb{I}$:

$$\mathbf{p} = \exp \left[-\frac{1}{2} \int_a^s dy \mathbf{P}_0^{-1} \mathbf{P}_1 \right]. \quad (6.66)$$

Following the notation of Falco [156], suppose that the solutions to the homogeneous problem satisfy $\hat{\Omega} \psi_i = 0$ for $i = 1, \dots, 2r$. The zero modes are the

null-eigenfunctions that also vanish at the boundary, i.e. $\psi(a) = 0$. We now construct the matrix Ψ , such that

$$\Psi_{\hat{\Omega},n} = \begin{pmatrix} \psi_{1,1} & \cdots & \psi_{n,1} \\ \vdots & \ddots & \vdots \\ \psi_{1,n} & \cdots & \psi_{n,n} \end{pmatrix}. \quad (6.67)$$

For Dirichlet boundary conditions, the maximum number of zero modes is $n = r$. The functional determinant with the zero modes extracted, which we shall denote as $\text{Det}'\hat{\Omega}$ is

$$\text{Det}'\hat{\Omega} = \frac{(-1)^r}{\gamma^r} \frac{\det p(a)}{\det p(b)} \frac{\det \dot{\Psi}_{\hat{\Omega}r}(b)}{\det \dot{\Psi}_{\hat{\Omega}r}(a)} \frac{\det \langle \psi_{\hat{O}}, \psi_{\hat{O}} \rangle}{\left| \det \dot{\Psi}_{\hat{O}r}(b) \right|^2}, \quad (6.68)$$

where $\Psi_{\hat{L}r}$ is the block matrix of zero modes of $\hat{L}$ and $\psi_{\hat{O}}$ are the null eigenfunctions of $\hat{O}$. When the transformation p is unitary, $\det p = 1$, $\det \langle \psi_{\hat{O}}, \psi_{\hat{O}} \rangle = \det \langle \psi_{\hat{\Omega}}, \psi_{\hat{\Omega}} \rangle$ and $\Psi_{\hat{O}r} = p \Psi_{\hat{\Omega}r}$. Eq. 6.68 reduces to

$$\text{Det}'\hat{\Omega} = \frac{(-1)^r}{\gamma^r} \frac{\det \dot{\Psi}_{\hat{\Omega}r}(b)}{\det \dot{\Psi}_{\hat{\Omega}r}(a)} \frac{\det \langle \psi_{\hat{\Omega}}, \psi_{\hat{\Omega}} \rangle}{\left| \det \left(p(b) \dot{\Psi}_{\hat{\Omega}r}(b) + \dot{p}(b) \Psi_{\hat{\Omega}r}(b) \right) \right|^2} \quad (6.69)$$

This means that we may deduce the functional determinant of our operator if all-zero modes are found. For example, if we had a 2×2 matrix differential operator with Dirichlet BC, then we only need find 2 zero modes.

It is common that in the calculations of fluctuation determinants for non-trivial saddle-point solutions, zero modes arise. Consider the classical solution Φ_{cl} which has degeneracy in some variables. These degenerate solutions can be parameterized by points z of some manifold $\mathcal{M}$. Coordinates z_i on the manifold typically express broken symmetries of the classical action. Due to the degeneracy, the variation in these parameters will cause no change in energy and hence,

$$\psi_i = \frac{\partial \Phi_{\text{cl}}(x; z)}{\partial z} \quad (6.70)$$

will be the null eigenfunctions of the fluctuation operator. Such eigenfunctions that vanish at the boundary we shall call “physical” zero modes.

The contribution of these physical zero modes must be calculated explicitly beyond the Gaussian approximation. The additional contribution to the partition function is of the form $\tilde{Z} \approx \Lambda_0 Z$. The contribution of Λ_0 can be shown to be [156]

$$\Lambda_0 = \frac{1}{\pi^{n/s}} \int dz_1 \dots dz_n \sqrt{\det \langle \psi, \psi \rangle}. \quad (6.71)$$

The astonishing fact about this result is that the Jacobian contribution $\sqrt{\det \langle \psi, \psi \rangle}$ will cancel with many of the results for differential operators that have had zero modes extracted. Note how this factor will cancel with the numerators of several formulae previously given.

6.4 Continuous Path Integral of an Inhomogeneous Chain

If we consider a straight chain with a small region of reduced bending stiffness, as we did in Section 5.3.2 when considering elastic minima, we can then perform a path integral over the fluctuation field to quantify the effects of thermal agitations on a floppy sequence.

The derivation of the resultant formulae are given in Appendix C, with only the results stated here. The partition function is given by

$$Z = \frac{e^{\beta f L} \sqrt{\beta f L}}{\sinh^2(L/2\lambda)} \left[1 - \frac{L\epsilon}{2\lambda} \coth\left(\frac{L}{2\lambda}\right) \right] + \mathcal{O}(\epsilon^2), \quad (6.72)$$

where $\epsilon = (1 - A_1/A) L_1/L$. The relative end-to-end extension is given by

$$\frac{\langle \Delta x \rangle}{L} = 1 - \frac{1}{2\beta\sqrt{A}f} - \frac{1}{4\beta\sqrt{A}f} \left(1 - \frac{A_1}{A} \right) \frac{L_1}{L}. \quad (6.73)$$

Hence, on top of the reduction in end-to-end distance predicted for homogeneous chains due to entropic elasticity, we predict a further shortening due to the floppy segment. This formula sensibly limits to the homogeneous value upon setting $A_1 \rightarrow A$ or $L_1 \rightarrow 0$. To our knowledge this is a new result. It would be interesting to try and validate this formula via MD or VMMC simulations of a floppy Kratky-Porod polymer model.

6.5 Fluctuation Theory of Ribbons

We now try and extend our fluctuation theory to the twistable worm like chain model as described in Section 5.4. The deformation of this triad is described by the equation of motion

$$\frac{d\hat{\mathbf{e}}_i}{ds} = \boldsymbol{\Omega} \times \hat{\mathbf{e}}_i. \quad (6.74)$$

where $\boldsymbol{\Omega} = (\Omega_1 \ \Omega_2 \ \Omega_3)$. Since any cross product can be written as a multiplication by a skew-symmetric matrix, we may write $\partial_s \hat{\mathbf{e}}_i = \mathbf{M} \hat{\mathbf{e}}_i$, where

$$\mathbf{M} = \begin{pmatrix} 0 & \Omega_3 & -\Omega_2 \\ -\Omega_3 & 0 & \Omega_1 \\ \Omega_2 & -\Omega_1 & 0 \end{pmatrix}. \quad (6.75)$$

For example,

$$\frac{d\hat{\mathbf{e}}_1}{ds} = \Omega_3 \hat{\mathbf{e}}_2 - \Omega_2 \hat{\mathbf{e}}_3. \quad (6.76)$$

The triad is chosen such that $\hat{\mathbf{e}}_3$ is the tangent vector, $\hat{\mathbf{e}}_1$ is normal to the surface and $\hat{\mathbf{e}}_2 = \hat{\mathbf{e}}_3 \times \hat{\mathbf{e}}_1$. It can be shown that $\Omega_1^2 + \Omega_2^2 = \kappa^2$ and Ω_3 is the twist density. The elastic Hamiltonian for a ribbon of length L is

$$\mathcal{H} = \frac{1}{2} \int_0^L ds \ \Omega_\mu M_{\mu\nu} \Omega_\nu, \quad (6.77)$$

where

$$M_{\mu\nu} = \begin{pmatrix} A_1 & 0 & 0 \\ 0 & A_2 & G \\ 0 & G & C \end{pmatrix}, \quad (6.78)$$

is the stiffness matrix. Its elements are: the bending stiffnesses A_1 and A_2 , the torsional stiffness C and the twist-bend coupling G . In this thesis we will be utilising the isotropic-bending version of this model ($A_1 = A_2 = A$), as well as no twist-bend coupling $G = 0$. Expanding out Eq. 6.78 with these assumptions, we get

$$\mathcal{H} = \frac{1}{2} \int_0^L ds \ A (\Omega_1^2 + \Omega_2^2) + C \Omega_3^2. \quad (6.79)$$

Converting our Hamiltonian into a functional of triad vectors

$$E[\{\hat{\mathbf{e}}_i\}] = \frac{1}{2} \int_0^L ds \ A (\partial_s \hat{\mathbf{e}}_3)^2 + C (\hat{\mathbf{e}}_1 \times \partial_s \hat{\mathbf{e}}_1 \cdot \hat{\mathbf{e}}_3)^2 - 2\mathbf{f} \cdot \hat{\mathbf{e}}_3, \quad (6.80)$$

where we have now included an external force $\mathbf{f}$. Note that for closed curves the inclusion of $\mathbf{f}$ is equivalent to a Lagrange multiplier. If we consider a tangent of the form $\hat{\mathbf{t}} = \begin{pmatrix} q_1 & q_2 & \sqrt{1-q^2} \end{pmatrix}$ in the $\hat{\mathbf{e}}_i$ basis, assuming the fluctuations are mostly in the transverse components of the tangent, i.e. $q \ll 1$. Then to quadratic order $\hat{\mathbf{t}} = \begin{pmatrix} q_1 & q_2 & 1 - q^2/2 \end{pmatrix}$ where $q^2 = q_1^2 + q_2^2$. This means we can express $\hat{\mathbf{t}}$ as

$$\hat{\mathbf{t}} = \hat{\mathbf{t}}_0 + \delta\mathbf{t}, \quad (6.81)$$

where $\hat{\mathbf{t}}_0 = \hat{\mathbf{e}}_3$ is the mean-field tangent, and $\delta\mathbf{t} = \begin{pmatrix} q_1 & q_2 & -(q_1^2 + q_2^2)/2 \end{pmatrix}$ is the fluctuation tangent.

If we wish to calculate $\partial_s \hat{\mathbf{t}}$, we must be careful to remember that not only do the coefficients of the tangent vectors change, but also the direction of the basis vectors.

$$\partial_s \hat{\mathbf{t}} = \partial_s \mathbf{q} + \mathbf{M}\mathbf{q}. \quad (6.82)$$

The norm squared of this is

$$(\partial_s \hat{\mathbf{t}})^2 = (\partial_s \mathbf{q} + \mathbf{M}\mathbf{q})^\top (\partial_s \mathbf{q} + \mathbf{M}\mathbf{q}) \quad (6.83)$$

$$= (\partial_s \mathbf{q}^\top - \mathbf{q}^\top \mathbf{M}) (\partial_s \mathbf{q} + \mathbf{M}\mathbf{q}) \quad (6.84)$$

$$= \partial_s \mathbf{q}^\top \partial_s \mathbf{q} + \partial_s \mathbf{q}^\top \mathbf{M}\mathbf{q} - \mathbf{q}^\top \mathbf{M} \partial_s \mathbf{q} - \mathbf{q}^\top \mathbf{M}^2 \mathbf{q}. \quad (6.85)$$

Given that we integrate this quantity with respect to arc-length, using integration by parts, we may permute derivatives of a product if we simply pick up a minus sign. Coupled with the fact we will assume the boundary terms vanish, it can then be shown that Eq. 6.85 is identical to

$$(\partial_s \hat{\mathbf{t}})^2 = -\mathbf{q}^\top \partial_s^2 \mathbf{q} + \partial_s \mathbf{q}^\top \mathbf{M}\mathbf{q} - \mathbf{q}^\top \mathbf{M} \partial_s \mathbf{q} - \mathbf{q}^\top \mathbf{M}^2 \mathbf{q}. \quad (6.86)$$

Repeating the same trick again

$$(\partial_s \hat{\mathbf{t}})^2 = -\mathbf{q}^\top \partial_s^2 \mathbf{q} - 2\mathbf{q}^\top \mathbf{M} \partial_s \mathbf{q} - \mathbf{q}^\top \partial_s \mathbf{M} \mathbf{q} - \mathbf{q}^\top \mathbf{M}^2 \mathbf{q}. \quad (6.87)$$

Writing $\mathbf{q} = \hat{\mathbf{e}}_3 + \delta\mathbf{q}$,

$$(\partial_s \hat{\mathbf{t}})^2 = (\Omega_1^2 + \Omega_2^2) + 2(\Omega_2 \partial_s q_1 - \Omega_1 \partial_s q_2 - \Omega_1 \Omega_3 q_1 - \Omega_2 \Omega_3 q_2) \quad (6.88)$$

$$+ \delta\mathbf{q}^\top \partial_s^2 \delta\mathbf{q} - 2\delta\mathbf{q}^\top \mathbf{M} \partial_s \delta\mathbf{q} - \delta\mathbf{q}^\top \partial_s \mathbf{M} \delta\mathbf{q} - \delta\mathbf{q}^\top \mathbf{M}^2 \delta\mathbf{q}. \quad (6.89)$$

I.e. the “curvature operator” is $-\partial_s^2 - 2\mathbf{M}\partial_s - (\partial_s\mathbf{M}) - \mathbf{M}^2$. It actually turns out that for a fluctuation of the type described, the skew-symmetric parts of this operator yield zero when the matrix element is taken with the tangent vector. Hence, the operator may be simplified to $-\partial_s^2 - 2\mathbf{M}\partial_s - \mathbf{M}^2$. This operator acts on a tangent vector, however, we may split this up and integrate over the two degrees of freedom in the fluctuation. Doing so yields

$$\int_0^L ds \left(\partial_s \hat{\mathbf{t}} \right)^2 = \int_0^L ds \Omega_1^2 + \Omega_2^2 + \int_0^L ds \mathbf{q}^\top \hat{\mathbf{T}}_{\text{bend}} \mathbf{q} + \mathcal{O}(\delta \mathbf{q}^4), \quad (6.90)$$

where

$$\hat{\mathbf{T}}_{\text{bend}} = \frac{A}{2} \begin{pmatrix} -\partial_s^2 + \Omega_3^2 - \Omega_1^2 & -\Omega_1\Omega_2 - 2\Omega_3\partial_s \\ -\Omega_1\Omega_2 + 2\Omega_3\partial_s & -\partial_s^2 + \Omega_3^2 - \Omega_2^2 \end{pmatrix}. \quad (6.91)$$

This operator is self-adjoint with respect to the inner product

$$\langle \mathbf{u}, \mathbf{v} \rangle = \int_0^L ds \mathbf{u}^\top \mathbf{v}, \quad (6.92)$$

which is what we expect as we desire real eigenvalues and therefore, a real determinant. The stretching term is trivial to compute

$$-\mathbf{f} \cdot \hat{\mathbf{t}} = -fL + \delta \mathbf{q}^\top \begin{pmatrix} f/2 & 0 \\ 0 & f/2 \end{pmatrix} \delta \mathbf{q} + \mathcal{O}(\delta \mathbf{q}^3). \quad (6.93)$$

6.5.1 Twist Operator

We are not yet in the position to rewrite the fluctuation contribution to the twist energy as we currently only describe the change in the fluctuation’s tangent, and not its other two vectors $\hat{\mathbf{e}}_2$ and $\hat{\mathbf{e}}_1$ which are required to quantify the ribbon’s twist. Luckily there is a work-around for this. Let the linking numbers of the mean-field and perturbed ribbons be Lk and Lk' respectively. Then we partition twist and writhe using White’s theorem for both configurations, i.e. $\text{Lk} = \text{Tw} + \text{Wr}$ and $\text{Lk}' = \text{Tw}' + \text{Wr}'$. Subtracting these two equations yields

$$\text{Lk} - \text{Lk}' = \text{Tw} - \text{Tw}' + \text{Wr} - \text{Wr}'. \quad (6.94)$$

We demand that our fluctuation field leave the topology of the system unchanged. For a second order theory such as this, we may simply impose that $\text{Lk} - \text{Lk}' = \mathcal{O}(q^3)$.

This means that to second order, the RHS of Eq. 6.94 must vanish. Rearranging Eq. 6.94, we find

$$(\text{Tw}')^2 = \text{Tw}^2 + 2\text{Tw}(\text{Wr} - \text{Wr}') + (\text{Wr} - \text{Wr}')^2 + \mathcal{O}(q^3). \quad (6.95)$$

Applying Fuller's theorem with the choice that the reference curve is the mean-field configuration, we get $\hat{\mathbf{t}} = \hat{\mathbf{e}}_3$.

$$\text{Wr}' - \text{Wr} = \frac{1}{2\pi} \int_0^L \left[\frac{\hat{\mathbf{e}}_3 \times \hat{\mathbf{t}}}{1 + \hat{\mathbf{e}}_3 \cdot \hat{\mathbf{t}}} \cdot \frac{d(\hat{\mathbf{e}}_3 + \hat{\mathbf{t}})}{ds} \right] ds. \quad (6.96)$$

$$= \frac{1}{2\pi} \int_0^L \left[\frac{q_1 \hat{\mathbf{e}}_2 - q_2 \hat{\mathbf{e}}_1}{1 + \sqrt{1 - q^2}} \cdot (\Omega_2 \hat{\mathbf{e}}_1 - \Omega_1 \hat{\mathbf{e}}_2 + \partial_s \mathbf{q} + \mathbf{M}\mathbf{q}) \right] ds. \quad (6.97)$$

Performing a Taylor expansion gives

$$\text{Wr}' - \text{Wr} = \frac{1}{4\pi} \int_0^L ds \left[-2q_1 \Omega_1 - 2q_2 \Omega_2 + q_1 \partial_s q_2 - q_2 \partial_s q_1 + \Omega_3 (q_1^2 + q_2^2) + \mathcal{O}(q^3) \right]. \quad (6.98)$$

The above can be more compactly written as

$$\text{Wr}' - \text{Wr} = \frac{1}{4\pi} \int_0^L ds \mathbf{q}^\top \begin{pmatrix} \Omega_3 & \partial_s \\ -\partial_s & \Omega_3 \end{pmatrix} \mathbf{q}, \quad (6.99)$$

once linear terms have been neglected. The twist operator can then be obtained from Eq. 6.95. Hence we obtain

$$E_{\text{twist}}[\hat{\mathbf{t}}] = E_{\text{twist}} + \int_0^L ds \mathbf{q}^\top \hat{\mathbf{T}}_{\text{twist}} \mathbf{q} + \mathcal{O}(q^2), \quad (6.100)$$

where

$$\hat{\mathbf{T}}_{\text{twist}} = \frac{C}{2} \begin{pmatrix} -\Omega_3^2 & -\Omega_3 \partial_s \\ \Omega_3 \partial_s & -\Omega_3^2 \end{pmatrix}. \quad (6.101)$$

This is also a self-adjoint operator.

6.6 Applications of Fluctuation Theory

Combining the operators derived so far, we obtain

$$\hat{\mathbf{T}} = \frac{A}{2} \begin{pmatrix} -\partial_s^2 + \left(1 - \frac{C}{A}\right) \Omega_3^2 - \Omega_1^2 + \cos \delta / \lambda^2 & -\Omega_1 \Omega_2 - \left(2 - \frac{C}{A}\right) \Omega_3 \partial_s \\ -\Omega_1 \Omega_2 + \left(2 - \frac{C}{A}\right) \Omega_3 \partial_s & -\partial_s^2 + \left(1 - \frac{C}{A}\right) \Omega_3^2 - \Omega_2^2 + \cos \delta / \lambda^2 \end{pmatrix}. \quad (6.102)$$

We now apply this theory to several systems including the straight chain, solenoids and non-trivial 3D elastica. We shall see that we recover the results of previous theories as special cases of our more general operator.

6.6.1 Twistable Straight Chain

For the twistable straight chain, we have $\Omega_1, \Omega_2 \rightarrow 0$, $\Omega_3 = 2\pi/L$ and so

$$\hat{\Lambda}^{(2)} = \frac{A}{2} \begin{pmatrix} -\partial_s^2 + 4\pi^2 \left(1 - \frac{C}{A}\right) / L^2 + 1/\lambda^2 & -2\pi \left(2 - \frac{C}{A}\right) \partial_s / L \\ 2\pi \left(2 - \frac{C}{A}\right) \partial_s / L & -\partial_s^2 + 4\pi^2 \left(1 - \frac{C}{A}\right) / L^2 + 1/\lambda^2 \end{pmatrix}. \quad (6.103)$$

Expanding out into a fourth order differential operator

$$\hat{\Lambda}^{(4)} = \partial_s^4 - \left[\frac{4\pi^2}{L^2} \left(2 - \frac{2C}{A} - \left(2 - \frac{C}{A} \right)^2 \right) + \frac{2}{\lambda^2} \right] \partial_s^2 + \left[\frac{4\pi^2}{L^2} \left(1 - \frac{C}{A} \right) + \frac{1}{\lambda^2} \right]^2. \quad (6.104)$$

This is a fourth order scalar differential operator with constant coefficients, hence we know that the free energy is just related to the imaginary part of the quartic characteristic equation (see Section 6.3.1).

$$\frac{\beta \mathcal{F}_{\text{fluc}}}{L} = \frac{1}{2} \sqrt{\xi + 2\sqrt{\chi}} \quad (6.105)$$

$$= \frac{1}{2} \left[\frac{2}{\lambda^2} - \frac{4\pi^2}{L^2} \left(2 + \frac{C^2}{A^2} \right) + 2 \left(\frac{4\pi^2}{L^2} \left(1 - \frac{C}{A} \right) + \frac{1}{\lambda^2} \right) \right]^{1/2} \quad (6.106)$$

$$= \left[\frac{1}{\lambda^2} - \frac{\pi^2 C^2}{A^2 L^2} \right]^{1/2}. \quad (6.107)$$

Taylor expanding for $\lambda \ll 1$ which corresponds to the large force limit,

$$\frac{\beta \mathcal{F}_{\text{fluc}}}{L} \approx \frac{1}{\lambda} \left(1 - \frac{\pi^2 C^2 \lambda^2}{2A^2 L^2} \right). \quad (6.108)$$

Hence,

$$\mathcal{F} = -fL \left(1 - \frac{1}{\beta \sqrt{A} f} \right) + \frac{2\pi^2 C}{L} \left(1 - \frac{C}{4A\beta \sqrt{A} f} \right). \quad (6.109)$$

We have successfully recovered the Moroz-Nelson revised twist stiffness constants [63].

6.6.2 Solenoidal DNA

We consider a helical configuration formed by a TWLC with constant curvature and torsion. A helix of length L with n turns, radius R and pitch p can be

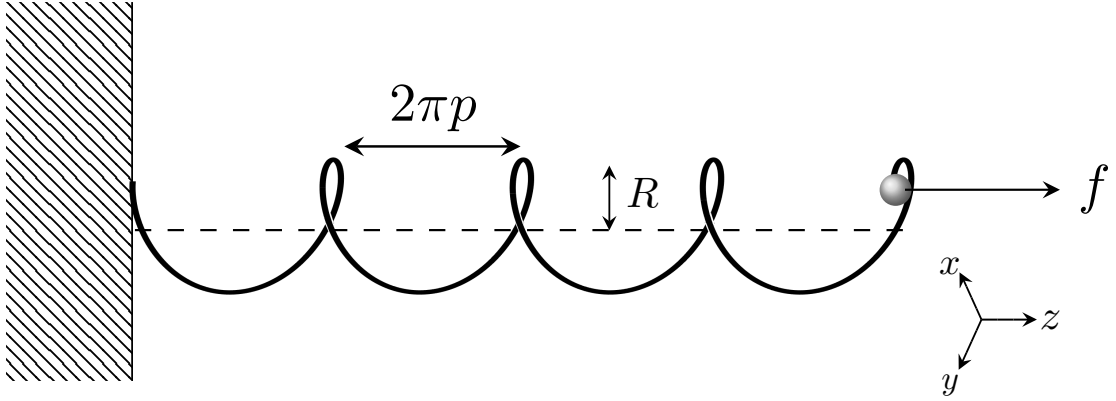


Figure 6.3: Plot of right-handed solenoidal configuration in a molecular-tweezer set-up with external force aligned with the helical axis.

described by the following equation

$$\mathbf{r}(s) = R \cos\left(\frac{2\pi ns}{L}\right) \hat{\mathbf{x}} + R \sin\left(\frac{2\pi ns}{L}\right) \hat{\mathbf{y}} \pm \frac{2\pi nps}{L} \hat{\mathbf{z}}, \quad (6.110)$$

where the $\pm$ refers to right- and left-handed helices, respectively. Fig. 6.3 shows an example of the helix geometry. Note that the definition of pitch here differs by a factor of 2π to how it is commonly defined for DNA, this is to simplify the formulae involved. The length of the helix is $L = 2\pi n \sqrt{R^2 + p^2}$ and so

$$\mathbf{r}(s) = R \cos\left(\frac{s}{\sqrt{R^2 + p^2}}\right) \hat{\mathbf{x}} + R \sin\left(\frac{s}{\sqrt{R^2 + p^2}}\right) \hat{\mathbf{y}} \pm \frac{ps}{\sqrt{R^2 + p^2}} \hat{\mathbf{z}}. \quad (6.111)$$

The unit tangent is obtained via taking an arc-length derivative and normalizing:

$$\hat{\mathbf{t}}(s) = \left[-R \sin\left(\frac{s}{\sqrt{R^2 + p^2}}\right) \hat{\mathbf{x}} + R \cos\left(\frac{s}{\sqrt{R^2 + p^2}}\right) \hat{\mathbf{y}} \pm p \hat{\mathbf{z}} \right] / \sqrt{R^2 + p^2}. \quad (6.112)$$

The solenoidal elastic minimum was characterised in Eq. 5.4 where we derived expressions for the Euler angles, energy and twist. This is everything we require in order to utilise the fluctuation operator we have derived in this section. The only additional thing we require is the writhe of the solenoid.

We may compute the writhe of our solenoid via Fuller's theorem Eq. 2.46. Taking

the reference configuration to be a straight chain aligned with the z -axis,

$$\Delta W_r = \frac{1}{2\pi} \int_0^L ds \frac{\hat{\mathbf{z}} \times \hat{\mathbf{t}}}{1 + \hat{\mathbf{z}} \cdot \hat{\mathbf{t}}} \cdot \frac{d}{ds} (\hat{\mathbf{z}} + \hat{\mathbf{t}}) \quad (6.113)$$

$$= \frac{1}{(R^2 + p^2)} \frac{R^2}{1 + p/\sqrt{R^2 + p^2}} \quad (6.114)$$

$$= \frac{\tau}{2\pi L} \left[\sqrt{1 + \left(\frac{\kappa}{\tau}\right)^2} + 1 \right], \quad (6.115)$$

where we have used that $\kappa = R/(R^2 + p^2)$ and $\tau = \pm p/(R^2 + p^2)$. This was shown by Starostin [124] to be equivalent to the double integral formulation of the writhe given by Eq. 2.45.

Incorporating the twist degree of freedom to our solenoid as described in Section 5.4, the twist is related to p_ψ via $p_\psi = 2\pi C \Delta \text{Tw}/L$. Hence, for a given ΔLk we can describe the solenoidal elastic minimum subject to certain boundary conditions, i.e. the opening angle. The matrix differential operator is

$$\hat{\mathbf{T}} = \frac{A}{2} \begin{pmatrix} -\partial_s^2 + \left(1 - \frac{C}{A}\right) \Omega_3^2 - \Omega_1^2 + \cos \delta / \lambda^2 & -\Omega_1 \Omega_2 - \left(2 - \frac{C}{A}\right) \Omega_3 \partial_s \\ -\Omega_1 \Omega_2 + \left(2 - \frac{C}{A}\right) \Omega_3 \partial_s & -\partial_s^2 + \left(1 - \frac{C}{A}\right) \Omega_3^2 - \Omega_2^2 + \cos \delta / \lambda^2 \end{pmatrix}. \quad (6.116)$$

Making use of the rotated version of $\hat{\mathbf{T}}$ derived in Appendix D, with $\dot{\theta} = 0$ and $\tau = \dot{\phi} \cos \theta$ we obtain

$$\hat{\mathbf{T}}_{\text{rot}} [\Omega_3, \delta] = \begin{pmatrix} -\partial_s^2 + \tau^2 - \frac{C}{A} \Omega_3 \tau + \cos \delta / \lambda^2 & \left(2\tau - \frac{C}{A} \Omega_3\right) \partial_s \\ -\left(2\tau - \frac{C}{A} \Omega_3\right) \partial_s & -\partial_s^2 - \kappa^2 + \tau^2 - \frac{C}{A} \Omega_3 \tau + \cos \delta / \lambda^2 \end{pmatrix} \quad (6.117)$$

This is the exact same form of fluctuation operator as derived by Rollin [157]. Interestingly, that derivation was performed in the Frenet-Serret basis, but as we have seen there exists a simple rotation which takes our Darboux frame to the Frenet frame which only carries a constant Jacobian and hence does not affect the physics. There are systems that are not suited to the less general Frenet approach, i.e. systems with non-linear twist density. This is elucidated in Appendix ?? that shows for a rotation to the Frenet frame, we only recover a Hermitian operator if $\ddot{\psi} = 0$.

Our problem is now a case of finding the functional determinant of this operator that is of the form

$$\text{Det} \begin{pmatrix} -\partial_s^2 + a & c\partial_s \\ -c\partial_s & -\partial_s^2 + b \end{pmatrix}. \quad (6.118)$$

If we expand the functional matrix determinant into a fourth-order scalar functional determinant, then said operator is

$$\partial_s^4 - (a + b - c^2) \partial_s^2 + ab. \quad (6.119)$$

Solving a functional determinant essentially comes down to solving the associated polynomial characteristic equation. This would be seen if one were to transform to Fourier space and perform the path integral over the Fourier components of the transverse tangent. Let's call the roots of this quartic equation w, x, y, z . There are several possibilities for the root structure: four real roots, two real roots and two imaginary roots or four imaginary roots (here the number of roots is up to algebraic multiplicity). Recall that for an operator of the form:

$$\partial_s^4 - \xi \partial_s^2 + \chi \quad (6.120)$$

we have shown that

$$\frac{\beta \mathcal{F}_{\text{fluc}}}{L} = \frac{1}{2} \sqrt{\xi + 2\sqrt{\chi}}. \quad (6.121)$$

This means for $\xi = a + b - c^2$ and $\chi = ab$, where $a = \tau^2 - C\Omega_3\tau/A + \cos\delta/\lambda^2$, $b = -\kappa^2 - \tau^2 - C\Omega_3\tau/A + \cos\delta/\lambda^2$ and $c = 2\tau - C\Omega_3/A$ Eq. 6.121 reduces to

$$\frac{\beta \mathcal{F}_{\text{fluc}}}{L} = \frac{\cos^{1/2}\delta}{\lambda} - \frac{\lambda}{4\cos^{1/2}\delta} \kappa^2 - \frac{\lambda}{4\cos^{1/2}\delta} \frac{C^2}{A^2} \Omega_3^2. \quad (6.122)$$

Given that the classical energy is $E_c = \frac{AL}{2} \kappa^2 - fL \cos\delta + \frac{CL}{2} \Omega_3^2$,

$$\begin{aligned} \mathcal{F} = & \frac{AL}{2} \left(1 - \frac{1}{2\beta\sqrt{Af}\cos^{1/2}\delta} \right) \kappa^2 - \left(1 - \frac{1}{\beta\sqrt{Af}\cos^{1/2}\delta} \right) fL \cos\delta \\ & + \frac{CL}{2} \left(1 - \frac{C}{4\beta A\sqrt{Af}\cos^{1/2}\delta} \right) \Omega_3^2. \end{aligned} \quad (6.123)$$

Hence, the effective bending constants and quantities can be found by comparing Eq. 6.123 and the classical energy. Doing so leaves us with

$$A_{\text{eff}} = A \left(1 - \frac{1}{2\beta\sqrt{Af}\cos^{1/2}\delta} \right) \quad (6.124)$$

$$C_{\text{eff}} = C \left(1 - \frac{C}{4\beta A\sqrt{Af}\cos^{1/2}\delta} \right) \quad (6.125)$$

$$\Delta z_{\text{eff}} = L \left(1 - \frac{1}{\beta\sqrt{Af}\cos^{1/2}\delta} \right). \quad (6.126)$$

This may seem initially worrying as the effective bending modulus A_{eff} does not seem to limit to its twistable straight chain counterpart when setting $\delta \rightarrow 0$, however, in this limit $\kappa \rightarrow 0$ and so the logic of reading off A_{eff} from the free energy is invalid.

An investigation by Dittmore and Neumann [158] made an empirical prediction on the softening of C as a function of force via the consideration of a solenoidal phase of DNA. They suggested that their expression compared favourably to the experimental data of Mosconi *et al.* [38]. This idea of a solenoidal phase of DNA was also investigated by Randisi [81] and Rollin [157]. They speculated that for large negative superhelical densities, we observe a state of fully melted DNA. This melted DNA then has drastically altered elastic properties ($A = 7 \text{ nm}$, $C = 19 \text{ nm}$) that make the free energy of the solenoidal phase more favourable when fluctuations are considered. This phase of DNA is called L-DNA. Rollin predicted that the solenoidal phase with fluctuations is the preferred state of L-DNA. Interestingly, the solenoidal configuration is not the most energetically favourable when no fluctuations are considered, implying that the solenoidal phase is stabilised by thermal fluctuations.

6.7 Fluctuations of Non-trivial Elastic Minima

We have now applied our fluctuation theory to both the twistable straight chain and a solenoidal configuration, both of which are trivial solutions to the TWLC Euler-Lagrange equations, but what happens if we try and apply our operator to non-trivial elastic minima? The only example of application of fluctuation theory to a non-trivial elastic minimum in the literature is to the class of un-twistable planar loops [148].

Next we turn our attention to the non-trivial solutions of the TWLC Hamiltonian. Unfortunately, the complexity of the solutions means we cannot easily obtain the solutions to the homogeneous problem $\hat{\mathbf{T}}\mathbf{y} = 0$ through traditional techniques. Luckily we may abuse another method utilised by those looking at conventionally intractable systems.

As discussed in Section 6.3.2, by Jacobi's theorem [159] we may obtain null eigenfunctions of the fluctuation operator by simply differentiating our classical

solution with respect to the constants of integration. Taking, for example, the s_0 derivative of the classical fluctuation tangent

$$\mathbf{y}_1 = \frac{\partial \delta \mathbf{t}_c}{\partial s_0} = \begin{pmatrix} \Omega_2 \\ -\Omega_1 \end{pmatrix}, \quad (6.127)$$

where we are in the triad basis. One does indeed find that $\hat{\mathbf{T}}\mathbf{y}_1 = 0$ through a lengthy calculation. The next constants to consider are the ψ_0 and ϕ_0 invariances. If the classical solution is governed by the frame evolution

$$\hat{\mathbf{e}}_i(s) = \mathbf{R}_3(\psi) \mathbf{R}_1(\theta) \mathbf{R}_3(\phi) \hat{\mathbf{e}}_i(0), \quad (6.128)$$

we may simply differentiate to obtain our solution. This predicts another solution when taking a ϕ_0 derivative

$$\mathbf{y}_2 = \sin \theta \begin{pmatrix} \cos \psi \\ \sin \psi \end{pmatrix}. \quad (6.129)$$

Unfortunately, taking the derivative with respect to ψ_0 yields the zero vector. In fact we may even deduce the generic derivative of $\mathbf{t}_c$ with respect to some arbitrary variable x using this method.

$$\frac{\partial \mathbf{t}_c}{\partial x} = \begin{pmatrix} -\sin \psi \partial_x \theta + \sin \theta \cos \psi \partial_x \phi \\ \cos \psi \partial_x \theta + \sin \theta \sin \psi \partial_x \phi \end{pmatrix}. \quad (6.130)$$

If we apply the fluctuation operator to this vector and equate it to zero such that it satisfies the null eigenvalue equation, we see that it requires

$$\frac{\partial}{\partial x} (\dot{\phi} \cos \theta) = 0, \quad (6.131)$$

i.e. our solution must obey this identity. But what is $\dot{\phi} \cos \theta$? Essentially it represents the torsion (up to a constant of proportionality) of the Darboux frame which isn't additionally twisted by the final rotation in ψ . We may view this frame as describing a Frenet ribbon. So we must ponder, with what quantities is the torsion of the Frenet ribbon constant? Intuitively s_0 , ψ_0 and ϕ_0 fulfil this, however with the ψ_0 derivative not yielding a non-zero result, we will have to keep searching.

As noted, we only required three of the four linearly independent solutions to the system of differential equations as the system has a constant Wronskian which should allow us to determine the last solution from the other three.

As discussed in Section 6.3.2, zero modes correspond to symmetries in the classical action. It is interesting to see whether or not the solutions $\mathbf{y}_1$ and $\mathbf{y}_2$ are zero modes. As we can see, Eq. 6.129 vanishes at the boundary and is hence a zero mode when $\sin \theta$ is initially zero, i.e. the ribbon's tangent is aligned with the external force. This corresponds to the rotational invariance of the ϕ_0 rotation about the force direction.

Less trivial is the s_0 derivative solution.

$$\mathbf{y}_1 = \begin{pmatrix} -\dot{\phi} \sin \theta \cos \psi + \dot{\theta} \sin \psi \\ -\dot{\phi} \sin \theta \sin \psi - \dot{\theta} \cos \psi \end{pmatrix}. \quad (6.132)$$

If we again assume $\sin \theta = 0$ at the boundary, then we additionally require $\dot{\theta} = 0$ for $\mathbf{y}_1$ to be a zero mode. From Appendix A, we see that this is fulfilled if Eq. A.4 is zero. $\sin \theta = 0$ requires $b = 1$ and $p_\psi = p_\phi = p$. Hence, Eq. 5.18 vanishing means

$$0 = \frac{E_0}{f} - 1 - \frac{p^2}{2Af} \lim_{\theta \rightarrow 0} \frac{1 - \cos \theta}{\sin^2 \theta}. \quad (6.133)$$

This limit trivially evaluates to $-\frac{1}{2}$ and inserting this into Eq. 6.133 we get

$$0 = \frac{E_0}{f} - 1 + \frac{p^2}{2Af}. \quad (6.134)$$

Eq. A.23 and Eq. A.28 can then be used to show that Eq. 6.134 is equivalent to

$$ac + 3a + 3c + 1 = 0. \quad (6.135)$$

6.7.1 Using the Rotated Operator

We note that the two solutions to our null-eigenvalue problem for our general operator, Eq. 6.127 and Eq. 6.129 both look like rotations of simpler vectors by a rotation in the Euler angle ψ , i.e.

$$\mathbf{y}_1 = \begin{pmatrix} \cos \psi & -\sin \psi \\ \sin \psi & \cos \psi \end{pmatrix} \begin{pmatrix} \sin \theta \\ 0 \end{pmatrix}, \quad \mathbf{y}_2 = \begin{pmatrix} \cos \psi & -\sin \psi \\ \sin \psi & \cos \psi \end{pmatrix} \begin{pmatrix} \dot{\phi} \sin \theta \\ \dot{\theta} \end{pmatrix}, \quad (6.136)$$

where we have inverted the sign of the second solution. Hence, we could consider investigating the equivalent problem but for the rotated operator derived in Appendix D:

$$\hat{\mathbf{T}}_{\text{rot}} = -\partial_s^2 + \begin{pmatrix} 0 & \ddot{\phi} \sin \theta / \dot{\theta} \\ -\ddot{\phi} \sin \theta / \dot{\theta} & 0 \end{pmatrix} \partial_s + \begin{pmatrix} \ddot{\theta} \cot \theta - \dot{\theta}^2 & -\ddot{\phi} \cos \theta + 2\dot{\theta} \dot{\phi} \sin \theta \\ \ddot{\phi} \cos \theta & \ddot{\theta} \cot \theta - \dot{\phi}^2 \sin^2 \theta \end{pmatrix}. \quad (6.137)$$

First, a lengthy calculation confirms that both $(\sin \theta \ 0)^\top$ and $(\dot{\phi} \sin \theta \ \dot{\theta})^\top$ are null eigenvectors of the differential operator $\hat{\mathbf{T}}_{\text{rot}}$. Next we utilise the fascinating method outlined in [160]. Given a homogeneous differential equation, if some of the solutions are known, then they can be "factored out" just as can be done for algebraic equations. Let's try the factorization on this operator:

$$\hat{\Omega} = I\partial_s - \begin{pmatrix} (\dot{\phi} \sin \theta)' & (\sin \theta)' \\ \ddot{\theta} & 0 \end{pmatrix} \begin{pmatrix} \dot{\phi} \sin \theta & \sin \theta \\ \dot{\theta} & 0 \end{pmatrix}^{-1} \quad (6.138)$$

$$= I\partial_s - \frac{1}{\dot{\theta} \sin \theta} \begin{pmatrix} (\dot{\phi} \sin \theta)' & (\sin \theta)' \\ \ddot{\theta} & 0 \end{pmatrix} \begin{pmatrix} 0 & -\sin \theta \\ -\dot{\theta} & \dot{\phi} \sin \theta \end{pmatrix} \quad (6.139)$$

$$= I\partial_s - \frac{1}{\dot{\theta} \sin \theta} \begin{pmatrix} -\dot{\theta}^2 \cos \theta & -\ddot{\phi} \sin^2 \theta \\ 0 & -\ddot{\theta} \sin \theta \end{pmatrix}. \quad (6.140)$$

Using some identities from the derivation of the EL equations, we can show that

$\hat{\Omega} = \partial_s + \mathbf{X}$, where

$$\mathbf{X} = - \begin{pmatrix} \dot{\theta} \cot \theta & \ddot{\phi} \sin \theta / \dot{\theta} \\ 0 & \ddot{\theta} / \dot{\theta} \end{pmatrix}. \quad (6.141)$$

Let us check this solution by solving

$$\dot{\mathbf{y}} - \begin{pmatrix} \dot{\theta} \cot \theta & \ddot{\phi} \sin \theta / \dot{\theta} \\ 0 & \ddot{\theta} / \dot{\theta} \end{pmatrix} \mathbf{y} = 0. \quad (6.142)$$

If we let $\mathbf{y} = (y_1 \ y_2)^\top$, then

$$\dot{y}_2 - \frac{\ddot{\theta}}{\dot{\theta}} y_2 = 0. \quad (6.143)$$

We may solve this via the integrating factor, $\int ds \ \ddot{\theta} / \dot{\theta} = \log \dot{\theta}$ and so $\dot{y}_2 / \dot{\theta} - \ddot{\theta} y_2 / \dot{\theta}^2 = 0$ and so $\partial_s (y_2 / \dot{\theta}) = 0$. Hence $y_2 = \dot{\theta}$. We note that $y_2 = 0$ is also a solution. The ODE in y_1 is

$$\dot{y}_1 - \dot{\theta} \cot \theta y_1 - \ddot{\phi} \sin \theta / \dot{\theta} y_2 = 0. \quad (6.144)$$

The integrating factor is $1/\sin \theta$ and so

$$\frac{\dot{y}_1}{\sin \theta} - \frac{\dot{\theta} \cos \theta}{\sin^2 \theta} = \ddot{\phi}. \quad (6.145)$$

Hence,

$$\partial_s \left(\frac{y_1}{\sin \theta} \right) = \ddot{\phi}. \quad (6.146)$$

Integrating

$$y_1 = \sin \theta \int ds \ddot{\phi} \quad (6.147)$$

$$= \dot{\phi} \sin \theta + C \sin \theta. \quad (6.148)$$

If $y_2 = 0$, then $y_1 = \sin \theta$. Hence we have recovered both independent solutions and have a differential operator that shares zeros with our full operator.

$$\hat{\mathbf{T}}_{\text{rot}} = - \left[\partial_s + \begin{pmatrix} \dot{\theta} \cot \theta & 0 \\ \ddot{\phi} \sin \theta / \dot{\theta} & \ddot{\theta} / \dot{\theta} \end{pmatrix} \right] \left[\partial_s - \begin{pmatrix} \dot{\theta} \cot \theta & \ddot{\phi} \sin \theta / \dot{\theta} \\ 0 & \ddot{\theta} / \dot{\theta} \end{pmatrix} \right] \quad (6.149)$$

We have shown that our full operator is iso-spectral to the “twistless” operator, which can then be factorized as

The issue is we require the solutions to the associated fully expanded ODE. Given we know the zeros of the left operator, then we can simply determine what functions must “pass through” the right operator in order to generate these zeros.

$$\begin{pmatrix} -\partial_s + \dot{\theta} \cot \theta & \ddot{\phi} \sin \theta / \dot{\theta} \\ 0 & -\partial_s + \ddot{\theta} / \dot{\theta} \end{pmatrix} \begin{pmatrix} z_1 \\ z_2 \end{pmatrix} = \begin{pmatrix} 0 \\ 1/\dot{\theta} \end{pmatrix}. \quad (6.150)$$

The expanded out ODES are $-\dot{z}_1 + \dot{\theta} \cot \theta z_1 + \ddot{\phi} \sin \theta z_2 / \dot{\theta} = 0$ and $-\dot{z}_2 + \ddot{\theta} z_2 / \dot{\theta} = 1/\dot{\theta}$. Solving the latter ODE with the integrating factor method

$$z_2 = -\dot{\theta} \left[\int \frac{1}{\dot{\theta}^2} ds + \text{const} \right]. \quad (6.151)$$

Plugging this into the first ODE and solving

$$z_1 = \sin \theta \left[-\text{const} \times \dot{\phi} + \int \ddot{\phi} \int \frac{1}{\dot{\theta}^2} ds \right]. \quad (6.152)$$

Given that $\begin{pmatrix} \dot{\phi} \sin \theta & \dot{\theta} \end{pmatrix}^\top$ is a zero of the right operator, then this will just result in a trivial zero of the left operator and so we will neglect it here. Hence

$$\mathbf{z} = \begin{pmatrix} -\sin \theta \int \ddot{\phi} \left(\int 1/\dot{\theta}^2 \right) \\ -\dot{\theta} \int 1/\dot{\theta}^2 \end{pmatrix}. \quad (6.153)$$

Integrating by parts and factoring out minus 1,

$$\mathbf{z} = \begin{pmatrix} \sin \theta \left(\dot{\phi} \int 1/\dot{\theta}^2 - \int \dot{\phi}/\dot{\theta}^2 \right) \\ \dot{\theta} \int 1/\dot{\theta}^2 \end{pmatrix} \quad (6.154)$$

The final vector solution $\mathbf{w}$ can be found by solving

$$\begin{pmatrix} -\partial_s + \dot{\theta} \cot \theta & \ddot{\phi} \sin \theta / \dot{\theta} \\ 0 & -\partial_s + \ddot{\theta} / \dot{\theta} \end{pmatrix} \begin{pmatrix} w_1 \\ w_2 \end{pmatrix} = \begin{pmatrix} 1/\sin \theta \\ -\dot{\phi}/\dot{\theta} \end{pmatrix}. \quad (6.155)$$

The ODEs are $-\dot{w}_1 + \dot{\theta} \cot \theta w_1 + \ddot{\phi} \sin \theta w_2 / \dot{\theta} = 1/\sin \theta$ and $-\dot{w}_2 + \ddot{\theta} w_2 / \dot{\theta} = -\dot{\phi}/\dot{\theta}$.

Again, solving the latter ODE first

$$w_2 = \dot{\theta} \left[\int \frac{\dot{\phi}}{\dot{\theta}^2} + \text{const} \right]. \quad (6.156)$$

Finally, subbing this in and solving

$$w_1 = \sin \theta \left[\int \left(\ddot{\phi} \int \frac{\dot{\phi}}{\dot{\theta}^2} \right) + \text{const} \times \dot{\phi} - \int \frac{1}{\sin^2 \theta} \right]. \quad (6.157)$$

Using some integration by parts and neglecting the zeros of the right operator,

$$\mathbf{w} = \begin{pmatrix} \sin \theta \left[\dot{\phi} \int \dot{\phi}/\dot{\theta}^2 - \int \dot{\phi}^2/\dot{\theta}^2 - \int \csc^2 \theta \right] \\ \dot{\theta} \int \dot{\phi}/\dot{\theta}^2 \end{pmatrix}. \quad (6.158)$$

In theory we now have all four linearly independent solutions and everything we need to calculate our functional determinant. In order to apply the formulae establish in this chapter, we must be able to evaluate the matrix $\mathbf{H}(s)$ at the boundary terms 0 and L . In the case of no zero modes, we find that if we let $[\mathbf{H}^{-1}(0)]_{ij} = a_{ij}$ and $[\mathbf{H}(L)]_{ij} = b_{ij}$, then

$$\mathbf{H}(0) = \begin{pmatrix} \frac{p_\phi - p_\psi c}{A\sqrt{1-c^2}} & \sqrt{1-c^2} & 0 & 0 \\ 0 & 0 & a_{32} & a_{42} \\ 0 & 0 & a_{33} & a_{43} \\ \left(\frac{p_\phi - p_\psi c}{A^2(1-c^2)^{3/2}} \right) + \frac{\sqrt{1-c^2}}{\lambda^2} & 0 & a_{34} & a_{44} \end{pmatrix}. \quad (6.159)$$

And

$$\mathbf{H}(L) = \begin{pmatrix} \frac{p_\phi - p_\psi b}{A\sqrt{1-b^2}} & \sqrt{1-b^2} & b_{31} & b_{41} \\ 0 & 0 & b_{32} & b_{42} \\ 0 & 0 & b_{33} & b_{43} \\ \frac{(p_\phi - p_\psi b)(p_\phi b - p_\psi)}{A^2(1-b^2)^{3/2}} + \frac{\sqrt{1-b^2}}{\lambda^2} & 0 & b_{34} & b_{44} \end{pmatrix}, \quad (6.160)$$

where we have computed all terms which vanish in both matrices and we elect not to calculate certain elements as they eventually get multiplied with zeros at some point and so do not contribute to the final result. All non-trivial elements are calculated in a very long winded calculation outlined in Appendix E.

Consider a_{33} ,

$$\partial_s \left[\sin \theta \left(\dot{\phi} \int 1/\dot{\theta}^2 - \int \dot{\phi}/\dot{\theta}^2 \right) \right] = \dot{\theta} \cos \theta \left(\dot{\phi} \int 1/\dot{\theta}^2 - \int \dot{\phi}/\dot{\theta}^2 \right) + \ddot{\phi} \sin \theta \int 1/\dot{\theta}^2. \quad (6.161)$$

When evaluated at $s = 0$,

$$a_{33} = \left(\frac{p_\psi}{A} - \frac{2c(p_\phi - p_\psi c)}{A(1-c^2)} \right) a_{32}. \quad (6.162)$$

Given that the quantity we must calculate is

$$\det \left(\mathbf{M} + \mathbf{N} \mathbf{H}(L) \mathbf{H}^{-1}(0) \right), \quad (6.163)$$

then we can simplify the task if we determine an expression for this first in terms of the two matrices $\mathbf{H}(L)$ and $\mathbf{H}^{-1}(0)$. Given the known zeros of the matrices involved, we arrived at the simplified formula

$$\det \left(\mathbf{M} + \mathbf{N} \mathbf{H}(L) \mathbf{H}^{-1}(0) \right) = \frac{(a_{21}b_{11} - a_{11}b_{21})(a_{42}b_{32} - a_{32}b_{42})}{a_{14}a_{21}(a_{33}a_{42} - a_{32}a_{43})}. \quad (6.164)$$

From Appendix E

$$a_{32} = \frac{\lambda^2 \sqrt{1-c^2}}{(c-a)(b-c)} \quad (6.165)$$

$$a_{42} = \frac{\lambda^2 (p_\phi - p_\psi c)}{A(b-c)(a-c)\sqrt{1-c^2}}. \quad (6.166)$$

Similarly,

$$b_{32} = \frac{\lambda^2 \sqrt{1-b^2}}{(b-c)(b-a)}. \quad (6.167)$$

$$b_{42} = \frac{\lambda^2 (p_\phi - p_\psi b)}{A(b-c)(b-a)\sqrt{1-b^2}} \quad (6.168)$$

Next we consider a_{43} . Expanding out

$$\partial_s \left[\sin \theta \left(\dot{\phi} \int \dot{\phi} / \dot{\theta}^2 - \int \dot{\phi}^2 / \dot{\theta}^2 - \int \csc^2 \theta \right) \right] = \ddot{\phi} \sin \theta \int \dot{\phi} / \dot{\theta}^2 ds - \csc \theta.$$

When evaluated at zero, the entire first term will vanish, leaving

$$a_{43} = \left(\frac{p_\psi}{A} - \frac{2c(p_\phi - p_\psi c)}{A(1-c^2)} \right) a_{42} - \frac{1}{\sqrt{1-c^2}}. \quad (6.169)$$

Therefore

$$a_{33}a_{42} - a_{32}a_{43} = a_{42}a_{32} - a_{32} \left(a_{42} - \frac{1}{\sqrt{1-c^2}} \right) = \frac{a_{32}}{\sqrt{1-c^2}}. \quad (6.170)$$

We now have all the elements of Eq. 6.164. We also note that

$$a_{21}b_{11} - a_{11}b_{21} = \sqrt{1-c^2} \frac{p_\phi - p_\psi b}{A\sqrt{1-b^2}} - \frac{p_\phi - p_\psi c}{A\sqrt{1-c^2}} \sqrt{1-b^2}. \quad (6.171)$$

Unfortunately there was not enough time to properly interpret this result or apply formulae to cases where $b = 1$ or $c = -1$ (which will lead to zero modes) and we shall leave this as future work. Despite this, it must be noted that the core calculation has been completed and relevant techniques to compute the resulting expressions have been elucidated. It would be interesting to see whether the factorisation of the fluctuation operator can be applied to different differential operators. This would be very powerful as theoretically for any 2×2 second order differential operator, as long as 2 of the solutions to the Jacobi accessory equation can be obtained via simple differentiation of the classical solution then we simply have to solve a 2×2 system of first order ODEs as we did in our example here.

6.8 Conclusion

In this section we have introduced the polymeric path integral and how its applications to WLC have already been used to renormalise the elastic parameters of DNA and explain how entropy effectively softens DNA.

Beginning with the discrete path integral formulation, we compute the path integral partition function of an inhomogeneous Kratky-Porod model. Using this we then derived the expected deviation to the standard exponential decay for tangent-tangent correlations.

We then investigated the path integral semi-classical approximation in polymer physics and applied it to a floppy WLC. In the limit of a small damage region, we quantified the reduction in end-to-end separation of the polymer in regards to the damage region length and degree of stiffness reduction, a new result and one that could be easily validated via simulation.

We then derived a new generalised fluctuation operator for a twistable worm-like chain in the fixed link ensemble. This operator is independent of the parametrization of the chain in the absence of an external force. We then applied this operator to the straight, solenoidal and non-trivial solutions of the TWLC EL equations, successfully recovering some well-known results as well as computing the relevant quantities required to calculate the fluctuation contribution of the non-trivial saddle point solutions, objectively the most sophisticated configurations ever considered using polymeric path integrals.

During this chapter we also outlined a systematic approach to tackling fluctuation problems that utilises some results from the generalised Gelfand-Yaglom theorem and outline how this approach may be used to tackle the unsolved problem of the 3D twistable elastica.

These techniques allow us to make predictions on the free energy landscape of various mean-field configurations of DNA and hence assert when particular configurations become more or less favourable. The power of these analytic models is the ability to vary the elastic constants and other system variables without needing to re-run new and expensive free-energy calculations.

7

Conclusion

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7.1 Conclusion

In this thesis, we have applied and developed several tools to aid the understanding of DNA's response to mechanical stress and thermal fluctuations. The range of detail of the models utilised in this work allows for a uniquely broad view into how DNA responds to these phenomena. The different length-scales that we may appropriately probe with each level of description give us a deeper overall perspective.

Through the atomistic TD model outlined in Chapter 3, we demonstrated how this model of DNA damage induces an axial bending of approximately 20° through the explicit solvent simulations of a 40-bp poly(dA:dT). Analysis of the time-averaged BPS parameters of this system showed good agreement with experiment and the fluctuations in the parameters indicated that this TD model forms a more rigid BPS than its undamaged counterpart. We then applied this TD model to the minicircles studied by Husain *et al.* [47]. Calculation of the curvatures of a 160-bp

minicircle with 5 TDs indicated strong preferential bending at the damage sites, as seen in the experiments. Given that the sum of twist and writhe is a constant for any topologically closed system, the twist writhe partitioning of these minicircles was also investigated. The effects of the TD on twist and writhe partitioning were discussed and it was found that the damaged minicircle preferred to remain more planar than expected if we were to assume that it would writhe out of the plane to compensate any reduction in twist induced by the 5 TDS.

The coarse-grained simulations of Chapter 4 allowed for the study of larger systems and longer time-scales through the well established oxDNA model. The nucleotide-level approximation significantly speeds up the dynamics compared with atomistic simulation while retaining the double-stranded complexity of B-DNA. A 160-bp minicircle was simulated with two TDs which displayed the same characteristic peaks in curvature as seen in the atomistic simulations. A series of negatively supercoiled 336-bp topoisomers were simulated which explored the effect of DNA damage on under-twisted minicircles. These results, while only one specific system of topoisomers, suggested an intriguing possible property of TDs. It was found that the writhe of these negatively supercoiled minicircles became more negative, which correspondingly lead to a reduction in under-twist. Given that excess under-twist leads to bubble formation, it is reasonable to propose that the presence of a TD could suppress bubble formation. This could have major implications on the dynamics of damage localization. Given that simulations have shown that both bubbles and TDs preferentially locate themselves at the tip of plectonemic structures, then the ability for a TD to impede a potential competitor for this spot would lend credence to proposed notions that tip localization is utilised by search enzymes.

Chapter 5 details how equilibrium configurations of DNA can be approximated through a WLC Hamiltonian that describes DNA through its elastic constants. Initially, planar elastica were studied, with the development of several planar models that include DNA damage through a local modification to the chain stiffness or chain geometry. Reductions in energy for these damaged models were calculated for curls, with exact formulae deducible in the limit of small damage sites. A piecewise

plectoneme model was developed and the damage models were introduced to the tips of these structures to investigate the energetics of localization of DNA damage at the plectoneme tip. A fully three-dimensional TWLC was then utilised to create a more realistic plectoneme model which could now include electrostatics and have a desired linking number. This plectoneme model was shown to follow the expected trends as force and superhelical density were varied.

Finally, extending the elastic theory of Chapter 5, Chapter 6 described how a semi-classical approximation and resulting second order variational in the Hamiltonian can be used to quantify the effect of thermal fluctuations on an equilibrium elastic minimum through a polymeric path integral. A discrete WLC was initially considered with free ends, with the effect of a floppy sequence on the tangent autocorrelation calculated. A second order fluctuation theory was then developed for a twistable ribbon with the associated differential operator derived. This theory was then applied to the trivial straight chain and solenoidal configurations. A generalised method of calculating the resulting functional determinant was then discussed before it was applied to the non-trivial saddle point solutions to the TWLC EL equations. All the elements required to compute the functional determinant of the non-trivial saddle point solutions fluctuation operator was successfully obtained. This is a new result and we believe that the more general framework developed and the ideas around operator factorisation could potentially lead to the application of polymer path integrals to even more sophisticated configurations.

7.2 Future Work

There are many things that require more work beyond what was achieved in this thesis, the most interesting of which we shall discuss here.

Despite the successes of modelling TDs in circular systems with little torsional stress, more work needs to be done in order to determine whether the all-atom TD model can provide an appropriate description of writhed structures. If so, then the effect these TDs have on twist-writhe partitioning could prove very interesting. It would also be fascinating to see if the idea that TDs suppress bubble formation

is seen in atomistic simulations. Refinement of conversion between implicit and explicit solvent simulations detailed in Section 3.5.5 would reduce the time spent equilibrating non-planar minicircles. This would then allow for the simulation of writhed minicircles containing a TD.

Chapter 4 outlines the oxDNA TD model already utilised by Lim *et al.* [46] to investigate damage localization in supercoiled structures. Specifically they looked into MT-like plectoneme experiments with external torque injected into the system. However, it would be interesting to see if we see similar results for closed minicircles with topological stress. Another interesting question that arises from Chapter 4 is whether TDs do in fact suppress bubble formation, the limited results supporting this assertion leave scope for a much more comprehensive investigation into this idea.

The 3D plectonemic model established in Chapter 5 provided some encouraging results indicating that this model can be used to generate reasonably behaved plectonemes. More work will have to be done to validate this model, with currently only qualitative observations of the resulting plectonemes and a reasonable B-DNA/plectoneme phase diagram as the supporting evidence. Extension of the damage models for planar plectonemes described in Section 5.2.4 are then the next main area to explore. Given the consensus in this thesis that TDs reduce local twist and naturally kink DNA, these traits can be incorporated into the 3D plectonemic model that, unlike the planar model, has a twist degree of freedom. Also unlike the planar model, the full 3D plectonemes will have the ability to modify the radius of their solenoidal braid. The relatively crude manner in which the ideal plectonemes are generated could also be improved with more sophisticated optimization techniques.

The main unsolved problem of Chapter 6 is the interpretation of the the results of the non-trivial saddle point solutions. Despite the four independent solutions to the null eigenvalue problem for the relevant matrix differential operator being computed, we did not have time to correctly interpret the consequences this has on the free energy and end-to-end separation of the configurations. Despite the overall success of the method, the fluctuation theory developed however, is far

from a comprehensive description of dsDNA. A more generalised version of the theory based on a Hamiltonian that includes twist-bend coupling as well as bending anisotropy could be developed. This would allow us to quantify the effect of these characteristics on the free energy of this now more realistic model of dsDNA.

List of Abbreviations

1D, 2D, 3D	. . .	One-, two- or three dimensional, referring in this thesis to spatial dimensions in an image.
A		Adenine
BC		Boundary Condition
BER		Base-excision repair
bp		Base-pair
BPS		Base-pair Step
DNA		Deoxyribonucleic Acid
dsDNA		Double-stranded DNA
C		Cytosine
CPD		Cis-syn Pyrimidine Dimer
EL		Euler-Lagrange
FENE		Finitely Extensible Non-linear Elastic
FF		Force Field
FFT		Fast Fourier Transform
G		Guanine
GB		Generalised Born
GPU		Graphics Processing Unit
LJ		Lennard-Jones
MC		Monte Carlo

MD		Molecular Dynamics
MMR		Mismatch Repair
MT		Magnetic Tweezer
NAP		Nucleoid Associated Protein
NER		Nucleotide Excision Repair
NMR		Nuclear Magnetic Resonance
ODE		Ordinary Differential Equation
OT		Optical Tweezer
PBC		Periodic Boundary Condition
PDB		Protein Data Bank
PDE		Partial Differential Equation
PMEMD	. . .	Particle Mesh Ewald Molecular Dynamics
QM		Quantum Mechanics
RNA		Ribonucleic Acid
T		Thymine
TD		Thymine Dimer
TWLC		Twistable Worm-like Chain
U		Uracil
UV		Ultraviolet
VdW		Van der Waals
VMMC		Virtual Move Monte Carlo
WLC		Worm-like chain

Appendices



Explicit formulae for 3D WLC

A.1 Solving the Euler-Lagrange Equations for TWLC

Using that $C(\dot{\phi} \cos \theta + \dot{\psi}) = p_\psi$ and the expression for $\dot{\phi}$ given by Eq. 5.70 to eliminate ϕ and ψ from Eq. 5.73, we get

$$A\ddot{\theta} = A \left(\frac{p_\phi - p_\psi \cos \theta}{A \sin^2 \theta} \right)^2 \cos \theta \sin \theta - p_\psi \dot{\phi} \sin \theta + f \sin \theta \quad (\text{A.1})$$

$$= \frac{\cos \theta}{A \sin^3 \theta} (p_\phi - p_\psi \cos \theta)^2 - p_\psi \frac{p_\phi - p_\psi \cos \theta}{A \sin \theta} + f \sin \theta \quad (\text{A.2})$$

$$= \frac{\cos \theta}{A \sin^3 \theta} [p_\phi^2 - p_\phi p_\psi (\cos \theta + \sec \theta) + p_\psi^2] + f \sin \theta \quad (\text{A.3})$$

If we multiply by $\dot{\theta}$ and integrate with respect to s , picking up an integration constant of E_0 we get

$$\frac{A}{2} \dot{\theta}^2 = E_0 - f \cos \theta - \frac{p_\phi^2 + p_\psi^2}{2A} \csc^2 \theta + \frac{p_\phi p_\psi \cos \theta}{A \sin^2 \theta}. \quad (\text{A.4})$$

If we then let $u = \cos \theta$,

$$\dot{u}^2 = \frac{2(1-u^2)}{A} (E_0 - fu) - \frac{1}{A^2} (p_\phi^2 + p_\psi^2 - 2p_\phi p_\psi u). \quad (\text{A.5})$$

We can rewrite this as

$$\dot{u}^2 = \frac{2f}{A} u^3 - \frac{2E_0}{A} u^2 + \left(\frac{2p_\phi p_\psi}{A^2} - \frac{2f}{A} \right) u + \frac{2E_0}{A} - \frac{1}{A^2} (p_\phi^2 + p_\psi^2) \quad (\text{A.6})$$

$$= \frac{2f}{A} \left[u^3 - \frac{E_0}{f} u^2 + \left(\frac{p_\phi p_\psi}{Af} - 1 \right) u + \frac{E_0}{f} - \frac{1}{2Af} (p_\phi^2 + p_\psi^2) \right]. \quad (\text{A.7})$$

From this expression it is clear that $\dot{u}^2$ is simply a cubic in u and can be solved via separation of variables. In order to integrate the reciprocal square root of a cubic it will be useful to factorize the cubic. Cubics have two types of solution, three real roots, or one real root and two complex roots which are conjugate to each other. We are only interested in the case of three real roots, however, we note that little mention is made in the literature on the possibility of complex roots. It can be shown, for example that the discriminant of the right hand side of Eq. A.7 in the special case that $p_\psi = \pm p_\phi = p$ leads to a discriminant of

$$\Delta = \frac{(p^2 - 2AE_0 \mp 2Af)^2 (A(E_0 \mp f)^2 \mp 4fp^2)}{A^3 f^4}. \quad (\text{A.8})$$

This quantity can only be negative and hence, we can only get complex roots, when

$$|p| \geq \sqrt{Af} \left(\frac{E_0}{f} + 1 \right) / 2. \quad (\text{A.9})$$

Let us now suppose that we may label the roots $a, b, c \in \mathbb{R}$ such that we may rearrange Eq. A.7 as

$$\frac{ds}{\lambda} = \frac{1}{\sqrt{2}} \frac{du}{\sqrt{(u-a)(u-b)(u-c)}}. \quad (\text{A.10})$$

We now make choices about the ordering of u in this hierarchy such that the square root in Eq. A.10 yields a real result. The consequence of this is that we may only have two orderings for the roots

$$a \geq b \geq u \geq c \quad \text{or} \quad u \geq a \geq b \geq c. \quad (\text{A.11})$$

This corresponds to a sign combination of $--+$ and $+++$ which give the desired net positive. For the first case u can oscillate between b and c - this is the case we are most interested in. For this case we may integrate Eq. A.10 using a result from Byrd [161]:

$$\frac{s}{\lambda} = \frac{1}{\sqrt{2}} \int_c^u \frac{du}{\sqrt{(a-u)(b-u)(u-c)}} \quad (\text{A.12})$$

$$= \sqrt{\frac{2}{a-c}} F \left(\arcsin \left(\sqrt{\frac{u-c}{b-c}} \right), k \right), \quad (\text{A.13})$$

where $F(u, k)$ is the incomplete elliptic integral of the first kind (see definition in Section 2.4.1) and $k^2 = (b - c) / (a - c)$. Inverting this we find

$$u = (b - c) \operatorname{sn}^2 \left(\frac{s}{\lambda k} \sqrt{\frac{b - c}{2}}, k \right) + c. \quad (\text{A.14})$$

If we impose that $u(0) = c$ and $u(L) = b$ then Eq. A.13 produces the length constraint

$$\frac{L}{\lambda k} = \sqrt{\frac{2}{b - c}} K(k). \quad (\text{A.15})$$

The formulae differ if we wish to integrate from u to b .

$$\frac{s}{\lambda} = \frac{1}{\sqrt{2}} \int_u^b \frac{du}{\sqrt{(a - u)(b - u)(u - c)}} \quad (\text{A.16})$$

$$= \sqrt{\frac{2}{a - c}} F \left(\arcsin \left(\frac{1}{k} \sqrt{\frac{b - u}{a - u}} \right), k \right), \quad (\text{A.17})$$

with the same elliptic modulus $k^2 = (b - c) / (a - c)$. If we again invert this for u ,

$$u = \frac{b - ak^2 \operatorname{sn}^2 \left(\frac{s}{\lambda k} \sqrt{\frac{b - c}{2}}, k \right)}{\operatorname{dn}^2 \left(\frac{s}{\lambda k} \sqrt{\frac{b - c}{2}}, k \right)}. \quad (\text{A.18})$$

$u(0) = b$ as expected, and setting $u(L) = c$ we see we get the same solution as before:

$$\frac{L}{\lambda k} \sqrt{\frac{b - c}{2}} = K(k). \quad (\text{A.19})$$

These two solutions are in fact identical, modulo a translation in s of L , this can be easily shown given $\operatorname{sn}(u + K, k) = \operatorname{cd}(u, k)$ and that $\operatorname{dn}(u + K, k) = \sqrt{1 - k^2} \operatorname{nd}(u, k)$ (see Section 2.4.1 for definitions of the Jacobi elliptic functions and elliptic integrals). This works both ways and so we may think about the elastica natural oscillating between b and c .

This choice corresponds to a cubic of the form

$$\dot{u}^2 = \frac{2f}{A} (u - a)(u - b)(u - c) \quad (\text{A.20})$$

$$= \frac{2f}{A} \left[u^3 - (a + b + c)u^2 + (ab + ac + bc)u - abc \right]. \quad (\text{A.21})$$

But recall

$$\dot{u}^2 = \frac{2f}{A} \left[u^3 - \frac{E_0}{f} u^2 + \left(\frac{p_\phi p_\psi}{Af} - 1 \right) u + \frac{E_0}{f} - \frac{1}{2Af} (p_\phi^2 + p_\psi^2) \right]. \quad (\text{A.22})$$

Hence, by comparing coefficients we may read off that

$$\frac{E_0}{f} = a + b + c \quad (\text{A.23})$$

$$\frac{p_\phi p_\psi}{Af} - 1 = ab + ac + bc \quad (\text{A.24})$$

$$\frac{E_0}{f} - \frac{1}{2Af} (p_\phi^2 + p_\psi^2) = -abc. \quad (\text{A.25})$$

We can combine some of these relations to get

$$\frac{p_\phi p_\psi}{Af} + \frac{E_0}{f} - 1 = a + b + c + ab + ac + bc. \quad (\text{A.26})$$

Hence,

$$\frac{1}{2Af} (p_\phi^2 + p_\psi^2 + 2p_\phi p_\psi) = a + b + c + ab + ac + bc + abc + 1. \quad (\text{A.27})$$

Therefore,

$$\frac{1}{2Af} (p_\phi + p_\psi)^2 = (1 + c)(1 + b)(1 + a). \quad (\text{A.28})$$

We can also subtract equations instead

$$\frac{1}{2Af} (p_\phi^2 + p_\psi^2) - \frac{p_\phi p_\psi}{Af} = a + b + c - ab - ac - bc + abc - 1. \quad (\text{A.29})$$

Therefore,

$$\frac{1}{2Af} (p_\phi - p_\psi)^2 = (a - 1)(1 - b)(1 - c). \quad (\text{A.30})$$

This can be solved as $p_\psi + p_\phi = \pm \sqrt{2Af} \sqrt{(1 + c)(1 + b)(1 + a)}$ and $p_\psi - p_\phi = \pm \sqrt{2Af} \sqrt{(1 - c)(1 - b)(a - 1)}$. This corresponds to 4 choices of sign combinations. The choice of branch is supposedly non-trivial according to [143]. If we choose two +’s, then

$$2p_\psi = \sqrt{2Af} \left[\sqrt{(1 + c)(1 + b)(1 + a)} + \sqrt{(1 - c)(1 - b)(a - 1)} \right]. \quad (\text{A.31})$$

Squaring this we find

$$p_\psi^2 = Af \left(a + b + c + abc \pm \sqrt{(1 - c^2)(1 - b^2)(a^2 - 1)} \right). \quad (\text{A.32})$$

Now

$$\dot{\phi} = \frac{p_\phi - p_\psi u}{A(1-u^2)} = \frac{p_\phi}{A(1-u^2)} - \frac{p_\psi}{A} \frac{u}{1-u^2}. \quad (\text{A.33})$$

Therefore $p_\phi = \sqrt{2Af} \sqrt{(1+c)(1+b)(1+a)} - p_\psi$.

$$\dot{\phi} = \frac{p_\phi - p_\psi u}{A(1-u^2)} \quad (\text{A.34})$$

$$= \frac{p_\phi}{A(1-u^2)} - \frac{p_\psi}{A} \frac{u}{1-u^2}. \quad (\text{A.35})$$

Integrating

$$\phi = \int_c^u \left(\frac{p_\phi}{A(1-u^2)} - \frac{p_\psi}{A} \frac{u}{1-u^2} \right) \frac{ds}{du} du. \quad (\text{A.36})$$

This can be decomposed into two integrals, both of the form

$$\int_c^u \frac{R(u) du}{\sqrt{(a-u)(b-u)(u-c)}} = \frac{2}{\sqrt{a-c}} \int_0^{u_1} R[c - (c-b) \text{sn}^2 u] du, \quad (\text{A.37})$$

where $\text{sn} u_1 = (u-c)/(b-c)$.

The two functions will be $R_1(u) = 1/(1-u^2)$ and the other $R_2(u) = u/(1-u^2)$.

We can decompose these using $1/(1-u^2) = 1/2(1+u) + 1/2(1-u)$.

$$I_1 = \frac{1}{2} \int_0^{u_1} \frac{du}{1-c-(b-c)\text{sn}^2 u} + \frac{du}{1+c+(b-c)\text{sn}^2 u} \quad (\text{A.38})$$

$$= \frac{1}{2(c+1)} \Pi\left(\frac{c-b}{1+c}, \text{am}, k\right) + \frac{1}{2(1-c)} \Pi\left(\frac{b-c}{1-c}, \text{am}, k\right). \quad (\text{A.39})$$

Similarly

$$I_2 = \frac{1}{2} \int_0^{u_1} du \frac{c+(b-c)\text{sn}^2 u}{1-c-(b-c)\text{sn}^2 u} + \frac{c+(b-c)\text{sn}^2 u}{1+c+(b-c)\text{sn}^2 u} \quad (\text{A.40})$$

$$= -\frac{1}{2(c+1)} \Pi\left(\frac{c-b}{1+c}, \text{am}, k\right) + \frac{1}{2(1-c)} \Pi\left(\frac{b-c}{1-c}, \text{am}, k\right). \quad (\text{A.41})$$

This means

$$\phi(s) = \frac{1}{\sqrt{2Af}\sqrt{a-c}} \left[\frac{p_\phi + p_\psi}{1+c} \Pi\left(\frac{c-b}{c+1}, \text{am}\left(\frac{s}{\lambda} \sqrt{\frac{a-c}{2}}, k\right), k\right) \right. \quad (\text{A.42})$$

$$\left. + \frac{p_\phi - p_\psi}{1-c} \Pi\left(\frac{b-c}{1-c}, \text{am}\left(\frac{s}{\lambda} \sqrt{\frac{a-c}{2}}, k\right), k\right) \right] + \phi_0. \quad (\text{A.43})$$

Finally

$$\dot{\psi} = \frac{p_\psi}{C} - \dot{\phi} \cos \theta \quad (\text{A.44})$$

$$= \frac{p_\psi}{C} - \frac{p_\phi u - p_\psi u^2}{A(1-u^2)} \quad (\text{A.45})$$

$$= p_\psi \left(\frac{1}{C} - \frac{1}{A} \right) + \frac{p_\psi - p_\phi u}{A(1-u^2)}. \quad (\text{A.46})$$

Integrating the first term is trivial, we then recognise the second term as Eq. [A.35](#) with p_ψ and p_ϕ permuted. Hence we may simply write that

$$\begin{aligned} \psi(s) &= p_\psi \left(\frac{1}{C} - \frac{1}{A} \right) s + \frac{1}{\sqrt{2Af}\sqrt{a-c}} \left[\frac{p_\phi + p_\psi}{1+c} \Pi \left(\frac{c-b}{c+1}, \text{am}(u_1, k), k \right) \right. \\ &\quad \left. - \frac{p_\phi - p_\psi}{1-c} \Pi \left(\frac{b-c}{1-c}, \text{am}(u_1, k), k \right) \right] + \psi_0. \end{aligned} \quad (\text{A.47})$$

Now that we have the solutions for the Euler angles we may calculate the energy of said configurations. Starting with the bending energy

$$\kappa^2 = \dot{\theta}^2 + \dot{\phi}^2 \sin^2 \theta \quad (\text{A.48})$$

$$= \frac{2}{A} \left[E_0 - f \cos \theta - \frac{1}{2A} \frac{p_\phi^2 + p_\psi^2 - 2p_\phi p_\psi u}{1-u^2} \right] + \frac{(p_\phi - p_\psi u)^2}{A^2(1-u^2)} \quad (\text{A.49})$$

$$= \frac{2}{\lambda^2} (E_0/f - \cos \theta) - p_\psi^2/A^2 \quad (\text{A.50})$$

$$= \frac{2}{\lambda^2} (\gamma - \cos \theta), \quad (\text{A.51})$$

where $\gamma = E_0/f - p_\psi^2/2Af$.

The energy is hence,

$$E = \int_0^L ds \frac{A}{2} \kappa^2 + \frac{C}{2} \Omega_3^2 - f \cos \theta \quad (\text{A.52})$$

$$= \frac{p_\psi^2}{2} \left(\frac{1}{C} - \frac{1}{A} \right) L + E_0 L - 2f \int_0^L ds \cos \theta. \quad (\text{A.53})$$

To compute the integral in Eq. [A.53](#) we need another result from Byrd [\[161\]](#)

$$\int_0^L ds \cos \theta = \frac{\lambda}{\sqrt{2}} \int_c^b \frac{u \, du}{\sqrt{(a-u)(b-u)(u-c)}} \quad (\text{A.54})$$

$$= \frac{\lambda\sqrt{2}}{\sqrt{a-c}} \int_0^{u_1} c - (c-b) \text{sn}^2 u \, du \quad (\text{A.55})$$

$$= \frac{\lambda\sqrt{2}}{\sqrt{a-c}} \left[\left(c - \frac{c-b}{k^2} \right) \frac{L}{\lambda k} \sqrt{\frac{b-c}{2}} + \frac{c-b}{k^2} E(k) \right] \quad (\text{A.56})$$

$$= aL - \lambda\sqrt{2}\sqrt{a-c}E(k). \quad (\text{A.57})$$

Hence, using that $E_0 = (a + b + c)f$, we arrive at an energy of

$$E = \frac{p_\psi^2}{2} \left(\frac{1}{C} - \frac{1}{A} \right) L + f(a + b + c)L - 2afL + 2\sqrt{2Af}\sqrt{a-c}E(k). \quad (\text{A.58})$$

A.2 Constraints

In general we have three unknowns in the system a, b, c and three equations determined by the boundary conditions in θ, ϕ, ψ . Hence in general, as in the planar case, the elastica is completely determined by the boundary conditions. Some of these initial conditions however will prove an arbitrary choice, such as initial angles ϕ_0 and ψ_0 for closed configurations. In these cases we will want to impose other constraints which will in-turn determine the elastica. One constraint we may wish to impose on our system is a desired Linking number. It has been showed via Fuller's theorem that [162] that

$$\text{Lk} = \mathcal{I} + \frac{1}{2\pi} \int_0^L ds \left(\dot{\phi} + \dot{\psi} \right) \quad (\text{A.59})$$

where $\mathcal{I}$ is an integer that encodes the knotting of the loop. It is equal to the number of signed crossings of the loop, assuming that it has been flattened out as much as possible.

Using this formula (for now we shall assume $\mathcal{I} = 0$)

$$\text{Lk} = \frac{1}{2\pi} (\phi(L) + \psi(L)) \quad (\text{A.60})$$

$$= \frac{p_\psi}{2\pi} \left(\frac{1}{C} - \frac{1}{A} \right) L + \frac{1}{\pi\sqrt{a-c}} \sqrt{\frac{(1+a)(1+b)}{1+c}} \Pi \left(\frac{c-b}{c+1}, k \right) \quad (\text{A.61})$$

$$= \text{Tw} \left(1 - \frac{C}{A} \right) + \frac{1}{\pi\sqrt{a-c}} \sqrt{\frac{(1+a)(1+b)}{1+c}} \Pi \left(\frac{c-b}{c+1}, k \right), \quad (\text{A.62})$$

where we have used the relation $p_\psi = 2\pi C \text{Tw} / L$. This allows us to read off the writhe

$$\text{Wr} = -\frac{C}{A} \text{Tw} + \frac{1}{\pi\sqrt{a-c}} \sqrt{\frac{(1+a)(1+b)}{1+c}} \Pi \left(\frac{c-b}{c+1}, k \right). \quad (\text{A.63})$$

For DNA closed in the z -direction, $\int_0^L ds \cos \theta = 0$ and so the energy has the rather simple form

$$E = \left[E_0 f + \frac{p_\psi^2}{2} \left(\frac{1}{C} - \frac{1}{A} \right) \right] L. \quad (\text{A.64})$$

This integral condition for closure has already been computed in Eq. A.57 which allows us to rewrite the condition as

$$\sqrt{2}aL/\lambda - 2\sqrt{a-c}E(k) = 0. \quad (\text{A.65})$$

Combining this with the length expression Eq. A.15 we get $aK = (a-c)E$.

B

Path Integral of Discrete WLC

Here we derive the partition function for a heterogeneous discrete worm-like chain using arguments found in [151]. Consider the energy of an N -link polymer of sub-length b with total length $L = Nb$. The bending energy is

$$E_{\text{bend}} = -\frac{1}{2b} \sum_{n=1}^{N-1} \kappa_n \|\hat{\mathbf{u}}_{n+1} - \hat{\mathbf{u}}_n\|^2. \quad (\text{B.1})$$

Where $\hat{\mathbf{u}}_n = (\mathbf{r}_{n+1} - \mathbf{r}_n) / \|\mathbf{r}_{n+1} - \mathbf{r}_n\|$ is a discrete unit tangent. Using the fact that $\|\hat{\mathbf{u}}_{n+1} - \hat{\mathbf{u}}_n\|^2 = 2(1 - \hat{\mathbf{u}}_{n+1} \cdot \hat{\mathbf{u}}_n) = 2(1 - \cos \Delta\vartheta_n)$, where $\Delta\vartheta_n$ is the angle between $\hat{\mathbf{u}}_{n+1}$ and $\hat{\mathbf{u}}_n$. We may rewrite our $e^{-\beta H}$ term as

$$\exp \left[-\frac{\beta \kappa_n}{b} (1 - \cos \Delta\vartheta_n) \right]. \quad (\text{B.2})$$

The three dimensional time-sliced measure near the surface of a sphere is

$$\mathcal{D}\hat{\mathbf{u}} = \prod_{n=2}^{N-2} \int \frac{d\hat{\mathbf{u}}_n}{2\pi b / \beta \kappa_n}. \quad (\text{B.3})$$

If parametrise $\hat{\mathbf{u}}$ in spherical coordinates, $\hat{\mathbf{u}} = (\cos \varphi \cos \theta \quad \sin \varphi \cos \theta \quad \sin \theta)$, then

$$\cos \Delta\vartheta_n = \cos \theta_{n+1} \cos \theta_n + \sin \theta_{n+1} \sin \theta_n \cos (\varphi_{n+1} - \varphi_n), \quad (\text{B.4})$$

and the measure becomes

$$\mathcal{D}\hat{\mathbf{u}} = \prod_{n=2}^{N-2} \int \frac{d \cos \theta_n d\varphi_n}{2\pi b / \beta \kappa_n}. \quad (\text{B.5})$$

The un-normalized probability distribution of the polymer given initial and final tangents $\hat{\mathbf{u}}_1$ and $\hat{\mathbf{u}}_N$ is a weighted sum over all paths $\{\hat{\mathbf{u}}_n\}_{2 \leq n \leq N-1}$ which connect particle 2 with particle $N + 1$:

$$P(\mathbf{u}_N | \mathbf{u}_1) = \prod_{n=2}^{N-1} \int \frac{d\hat{\mathbf{u}}_n}{2\pi b / \beta \kappa_n} \exp \left[-\frac{\beta}{b} \sum_{m=1}^{N-1} \kappa_m \|\hat{\mathbf{u}}_{m+1} - \hat{\mathbf{u}}_m\|^2 \right]. \quad (\text{B.6})$$

The partition function is then the integral over all initial and final vectors of this probability, averaged over 4π (surface area of a sphere),

$$Z_N = \frac{1}{4\pi} \int d\hat{\mathbf{u}}_1 \int d\hat{\mathbf{u}}_N P(\mathbf{u}_N | \mathbf{u}_1) \quad (\text{B.7})$$

$$= \frac{1}{4\pi} \int d\hat{\mathbf{u}}_1 \int d\hat{\mathbf{u}}_N \prod_{n=2}^{N-1} \int \frac{d\hat{\mathbf{u}}_n}{2\pi b / \beta \kappa_n} \exp \left[-\frac{\beta}{b} \sum_{m=1}^{N-1} \kappa_m \|\hat{\mathbf{u}}_{m+1} - \hat{\mathbf{u}}_m\|^2 \right] \quad (\text{B.8})$$

$$= \frac{b}{2\beta \kappa_1} \int d\hat{\mathbf{u}}_N \prod_{n=1}^{N-1} \int \frac{d\hat{\mathbf{u}}_n}{2\pi b / \beta \kappa_n} \exp \left[-\frac{\beta \kappa_n}{b} \|\hat{\mathbf{u}}_{n+1} - \hat{\mathbf{u}}_n\|^2 \right], \quad (\text{B.9})$$

where we have absorbed the $\hat{\mathbf{u}}_1$ integral into the product allowing us to combine the product index and exponent summation index. Replacing the measure and exponent with their spherical coordinate counterparts and neglecting the prefactor

$$Z_N = \int d\hat{\mathbf{u}}_N \prod_{n=2}^{N-1} \int \frac{d \cos \theta_n d\varphi_n}{2\pi b / \beta \kappa_n} \exp \left[-\frac{\beta}{b} \sum_{n=1}^{N-1} \kappa_n (1 - \cos \Delta \vartheta_n) \right]. \quad (\text{B.10})$$

We now use the identity

$$e^{h \cos \theta} = \sum_{\ell=0}^{\infty} a_{\ell}(h) \frac{2\ell+1}{4\pi} P_{\ell}(\cos \theta), \quad (\text{B.11})$$

where $a_{\ell}(h) = (2\pi)^{3/2} I_{\ell+1/2}(h) / h^{1/2}$. We then expand the Legendre polynomial in terms of spherical harmonics

$$P_{\ell}(\cos \Delta \vartheta_n) = \frac{4\pi}{2\ell+1} \sum_{m=-\ell}^{\ell} Y_{\ell m}(\theta_{n+1}, \varphi_{n+1}) Y_{\ell m}^*(\theta_n, \varphi_n). \quad (\text{B.12})$$

Hence

$$e^{h \cos \Delta \vartheta_n} = \sum_{\ell=0}^{\infty} a_{\ell}(h) \sum_{m=-\ell}^{\ell} Y_{\ell m}(\theta_{n+1}, \varphi_{n+1}) Y_{\ell m}^*(\theta_n, \varphi_n). \quad (\text{B.13})$$

Therefore,

$$\begin{aligned} Z_N &= \int d\hat{\mathbf{u}}_N \prod_{n=1}^{N-1} \int \frac{d\hat{\mathbf{u}}_n}{b / \beta \kappa_n} \exp \left[-\frac{\beta \kappa_n}{2b} (1 - \cos \Delta \vartheta_n) \right] \\ &= \int d\hat{\mathbf{u}}_N \prod_{n=1}^{N-1} \int \frac{d\hat{\mathbf{u}}_n}{\pi b / \beta \kappa_n} e^{-\beta \kappa_n / b} \sum_{\ell=0}^{\infty} a_{\ell}(h_n) \sum_{m=-\ell}^{\ell} Y_{\ell m}(\hat{\mathbf{u}}_{n+1}) Y_{\ell m}^*(\hat{\mathbf{u}}_n), \end{aligned} \quad (\text{B.14})$$

where $h_n = \beta\kappa_n/b$. We may integrate out intermediate pairs

$$Y_{\ell m}(\theta_{n+1}, \varphi_{n+1}) Y_{\ell m}^*(\theta_n, \varphi_n) Y_{\ell m}(\theta_n, \varphi_n) Y_{\ell m}^*(\theta_{n-1}, \varphi_{n-1}) \quad (\text{B.15})$$

via the use of the orthogonality relation

$$\int d\hat{\mathbf{x}} Y_{\ell m}(\hat{\mathbf{x}}) Y_{\ell' m'}^*(\hat{\mathbf{x}}) = \delta_{\ell\ell'} \delta_{mm'} \quad (\text{B.16})$$

leaving us with

$$Z_N = \int d\hat{\mathbf{u}}_1 \int d\hat{\mathbf{u}}_N Y_{\ell m}(\hat{\mathbf{u}}_1) Y_{\ell m}^*(\hat{\mathbf{u}}_N) \prod_{n=1}^{N-1} e^{-h_n} \sum_{\ell=0}^{\infty} a_{\ell}(h_n). \quad (\text{B.17})$$

The final integrations are easy since

$$\int d\hat{\mathbf{u}}_n Y_{\ell m}(\hat{\mathbf{u}}_n) = \sqrt{4\pi} \delta_{\ell 0} \delta_{m 0}, \quad (\text{B.18})$$

and so the partition function simplifies to

$$Z_N = 4\pi \prod_{n=1}^{N-1} \frac{e^{-h_n}}{\pi b / \beta\kappa_n} a_0(h_n) \quad (\text{B.19})$$

$$= 4\pi \prod_{n=1}^{N-1} \frac{e^{-h_n}}{\pi b / \beta\kappa_n} \frac{(2\pi)^{3/2}}{\sqrt{h_n}} I_{1/2}(h_n). \quad (\text{B.20})$$

$I_{1/2}(z) = \sqrt{2/\pi x} \sinh(z)$ and so neglecting constant multipliers which do not effect the physics,

$$Z_N = 4\pi \prod_{n=1}^{N-1} \frac{e^{-\beta\kappa_n/b}}{\pi b / \beta\kappa_n} \frac{(2\pi)^{3/2}}{\sqrt{\beta\kappa_n/b}} \sqrt{\frac{2}{\pi\beta\kappa_n/b}} \sinh(\beta\kappa_n/b) \quad (\text{B.21})$$

$$\sim \prod_{n=1}^{N-1} (1 - e^{-2\beta\kappa_n/b}). \quad (\text{B.22})$$

For the homogeneous chain, i.e. $\kappa_n = \kappa \forall n$ and so,

$$Z_N = [1 - e^{-2\beta\kappa/b}]^{N-1}. \quad (\text{B.23})$$

The partition function was previously defined as the integral

$$Z_N = \int \frac{d\hat{\mathbf{u}}_N}{4\pi} \prod_{n=1}^{N-1} \int \frac{d\hat{\mathbf{u}}_n}{A_n} \exp \left[-\frac{\beta\kappa_n}{b} (1 - \hat{\mathbf{u}}_{n+1} \cdot \hat{\mathbf{u}}_n) \right] \quad (\text{B.24})$$

$$= \int \frac{d\hat{\mathbf{u}}_N}{4\pi} \prod_{n=1}^{N-1} \int \frac{\beta\kappa_n d\hat{\mathbf{u}}_n}{2\pi b} \exp \left[-\frac{\beta\kappa_n}{b} (1 - \hat{\mathbf{u}}_{n+1} \cdot \hat{\mathbf{u}}_n) \right] \quad (\text{B.25})$$

Hence, we may obtain the average $\langle \hat{\mathbf{u}}_{n+1} \cdot \hat{\mathbf{u}}_n \rangle$ via differentiation with respect to κ_m

$$\frac{\partial Z_N}{\partial \kappa_m} = \frac{\partial}{\partial \kappa_m} \int \frac{d\hat{\mathbf{u}}_N}{4\pi} \prod_{n=1}^{N-1} \int \frac{\beta \kappa_n d\hat{\mathbf{u}}_n}{2\pi b} \exp \left[-\frac{\beta \kappa_n}{b} (1 - \hat{\mathbf{u}}_{n+1} \cdot \hat{\mathbf{u}}_n) \right] \quad (\text{B.26})$$

$$= \int \frac{d\hat{\mathbf{u}}_N}{4\pi} \left(\prod_{n=1, n \neq m}^{N-1} \int \frac{\beta \kappa_n d\hat{\mathbf{u}}_n}{2\pi b} \exp \left[-\frac{\beta \kappa_n}{b} (1 - \hat{\mathbf{u}}_{n+1} \cdot \hat{\mathbf{u}}_n) \right] \right) \quad (\text{B.27})$$

$$\times \int \frac{\beta}{2\pi b} d\hat{\mathbf{u}}_m \left(1 - \frac{\beta \kappa_m}{b} (1 - \hat{\mathbf{u}}_{m+1} \cdot \hat{\mathbf{u}}_m) \right) e^{-\beta E_m}. \quad (\text{B.28})$$

Hence,

$$\frac{\partial Z_N}{\partial \kappa_m} = \frac{\beta}{b} \langle \hat{\mathbf{u}}_{m+1} \cdot \hat{\mathbf{u}}_m - 1 \rangle Z_N \quad (\text{B.29})$$

$$+ \int \frac{d\hat{\mathbf{u}}_N}{4\pi} \left(\prod_{n=1, n \neq m}^{N-1} \int \frac{\beta \kappa_n d\hat{\mathbf{u}}_n}{2\pi b} \exp \left[-\frac{\beta \kappa_n}{b} (1 - \hat{\mathbf{u}}_{n+1} \cdot \hat{\mathbf{u}}_n) \right] \right) \quad (\text{B.30})$$

$$\times \int \frac{\beta}{2\pi b} d\hat{\mathbf{u}}_m e^{-\beta E_m} \quad (\text{B.31})$$

$$= \frac{\beta}{b} \langle \hat{\mathbf{u}}_{m+1} \cdot \hat{\mathbf{u}}_m - 1 \rangle Z_N + \frac{Z_N}{\kappa_m}. \quad (\text{B.32})$$

Rearranging

$$\langle \hat{\mathbf{u}}_{m+1} \cdot \hat{\mathbf{u}}_m \rangle = 1 + \frac{b}{\beta} \frac{1}{Z_N} \frac{\partial Z_N}{\partial \kappa_m} - \frac{b}{\beta \kappa_m}. \quad (\text{B.33})$$

It can be shown that $\langle \hat{\mathbf{u}}_{n+1} \cdot \hat{\mathbf{u}}_{n-1} \rangle = \langle \hat{\mathbf{u}}_{n+1} \cdot \hat{\mathbf{u}}_n \rangle \langle \hat{\mathbf{u}}_n \cdot \hat{\mathbf{u}}_{n-1} \rangle$ and so

$$\langle \mathbf{u}_{n+1} \cdot \mathbf{u}_{n-1} \rangle = \left(1 + \frac{1}{Z_N} \frac{\beta}{b} \frac{\partial Z_N}{\partial \kappa_{n+1}} \right) \left(1 + \frac{1}{Z_N} \frac{\beta}{b} \frac{\partial Z_N}{\partial \kappa_n} \right). \quad (\text{B.34})$$

Calculating this derivative

$$\frac{\partial Z_N}{\partial \kappa_i} = \frac{\partial}{\partial \kappa_i} \prod_{n=1}^{N-1} (1 - e^{-2\beta \kappa_n/b}) \quad (\text{B.35})$$

$$= \frac{2\beta}{b} e^{-2\beta \kappa_i/b} \prod_{n=1, n \neq i}^{N-1} \frac{\beta \kappa_n}{b} (1 - e^{-2\beta \kappa_n/b}). \quad (\text{B.36})$$

Dividing by the partition function:

$$\frac{1}{Z_N} \frac{\partial Z_N}{\partial \kappa_i} = \frac{2\beta}{b} \frac{e^{-2\beta \kappa_i/b}}{1 - e^{-2\beta \kappa_i/b}}. \quad (\text{B.37})$$

Hence

$$1 + \frac{1}{Z_N} \frac{b}{\beta} \frac{\partial Z_N}{\partial \kappa_i} = \coth(\beta \kappa_i/b). \quad (\text{B.38})$$

By induction it follows that

$$\langle \hat{\mathbf{u}}_j \cdot \hat{\mathbf{u}}_k \rangle = \prod_{i=j}^k \left[\coth \left(\frac{\beta \kappa_i}{b} \right) - \frac{b}{\beta \kappa_i} \right]. \quad (\text{B.39})$$

I.e. the correlations are simply products of Langevin functions. In the case of an infinitely long homogeneous chain where $\kappa \gg bk_B T$, $\coth(z) - 1/z \sim 1 - 1/z$ and so

$$\langle \hat{\mathbf{u}}_j \cdot \hat{\mathbf{u}}_k \rangle = \left[1 - \frac{L}{\beta N \kappa} \right]^{|j-k|} \approx e^{-|j-k|b/\beta \kappa}. \quad (\text{B.40})$$

C

Thermal Fluctuations of the Inhomogeneous Straight Chain

For our inhomogeneous chain of length L with segment of length L_1 with a weakened bending stiffness $A_1 < A$, the Hamiltonian reads

$$H = \int_{-L/2}^{L/2} \frac{A(s)}{2} \dot{\phi}^2 - f \cos \phi \, ds, \quad (\text{C.1})$$

where

$$A(s) = \begin{cases} A_1 & |s| \leq L_1/2 \\ A & \text{otherwise.} \end{cases} \quad (\text{C.2})$$

If we consider quadratic fluctuations away from the straight chain, the fluctuation Hamiltonian $\Delta H = H - H_{\phi=0}$

$$\Delta H = \frac{1}{2} \int_{-L/2}^{L/2} A(s) \dot{\phi}^2 + f \phi^2 ds. \quad (\text{C.3})$$

The partition function is

$$Z_1 = \int \mathcal{D}[\phi] e^{-\beta \Delta H}. \quad (\text{C.4})$$

If we let $\phi(s) = \sum_n \tilde{\phi}_n e^{2\pi i n s/L}$. Then $\dot{\phi}(s) = \sum_n \omega_n \tilde{\phi}_n e^{i \omega_n s}$, where $\omega_n = 2\pi n/L$.

The measures transforms as

$$\int \mathcal{D}[\phi] = \int d\tilde{\phi}_0 \prod_{n \in \mathbb{N}} \int d\tilde{\phi}_n d\tilde{\phi}_n^*. \quad (\text{C.5})$$

This transformation is the statistical mechanics version of the what Quantum Physicist would call a transformation to Matsubara frequency space, with ω_n denoting the Matsubara frequency. The fact that this is a product over the natural numbers is important. Our fluctuation Hamiltonian becomes

$$\begin{aligned}
\Delta H &= \frac{1}{2} \int_{-L/2}^{L/2} \sum_{n \in \mathbb{Z}} \sum_{m \in \mathbb{Z}} (f - A(s) \omega_n \omega_m) \tilde{\phi}_n \tilde{\phi}_m e^{i(\omega_n + \omega_m)s} ds \\
&= \frac{1}{2} \sum_n \sum_m \tilde{\phi}_n \tilde{\phi}_m \left[f \int_{-L/2}^{L/2} e^{i(\omega_n + \omega_m)s} ds - \omega_n \omega_m \int_{-L/2}^{L/2} A(s) e^{i(\omega_n + \omega_m)s} ds \right] \\
&= \frac{1}{2} \sum_n \sum_m \tilde{\phi}_n \tilde{\phi}_m \left[fL \delta_{n,-m} - \omega_n \omega_m \tilde{A}_{\omega_n + \omega_m} \right]. \tag{C.6}
\end{aligned}$$

It can be shown that

$$\tilde{A}(\omega_n) = AL \delta_{n,0} + (A_1 - A) L \frac{\sin[L_1 n \pi / L]}{n \pi}. \tag{C.7}$$

For $L_1/L \ll 1$, we may Taylor expand to show

$$\tilde{A}_{\omega_n + \omega_m} \simeq AL \left(\delta_{n,-m} + \left(\frac{A_1}{A} - 1 \right) \frac{L_1}{L} \right) + \mathcal{O}[(L_1/L)^2]. \tag{C.8}$$

This simplifies our Hamiltonian

$$\begin{aligned}
\Delta H &= \sum_{n \in \mathbb{Z}} \left(\frac{fL}{2} + \frac{2\pi^2 A}{L} n^2 \right) \tilde{\phi}_n \tilde{\phi}_{-n} - \frac{2\pi^2 A}{L} \left(\frac{A_1}{A} - 1 \right) \frac{L_1}{L} \sum_n \sum_m nm \tilde{\phi}_n \tilde{\phi}_m \\
&= \sum_{n \in \mathbb{Z}} \left(\frac{fL}{2} + \frac{2\pi^2 A}{L} n^2 \right) |\tilde{\phi}_n|^2 + \frac{2\pi^2 A}{L} \left(\frac{A_1}{A} - 1 \right) \frac{L_1}{L} \sum_{n \in \mathbb{Z}} \sum_{m \in \mathbb{Z}} nm \tilde{\phi}_n \tilde{\phi}_m^*,
\end{aligned}$$

where we have used the fact that $\tilde{\phi}_n^* = \tilde{\phi}_{-n}$. We can see that the double sum will contribute just single quadratic term in $\tilde{\phi}_n$ for every m , hence we can rewrite the above expression as

$$\Delta H = \sum_{n \in \mathbb{Z}} \left(\frac{fL}{2} + \frac{2\pi^2 A_{\text{eff}}}{L} n^2 \right) |\tilde{\phi}_n|^2 + \frac{2\pi^2 A}{L} \left(\frac{A_1}{A} - 1 \right) \frac{L_1}{L} \sum_{n \in \mathbb{Z}} nm \tilde{\phi}_n \tilde{\phi}_m^*, \tag{C.9}$$

where $A_{\text{eff}} = A \left(1 - \left(1 - \frac{A_1}{A} \right) \frac{L_1}{L} \right)$ and the primed sigma notation implies $m \neq n$. Our partition function now reads

$$Z_1 = \prod_{k \in \mathbb{N}} \int d\tilde{\phi}_k d\tilde{\phi}_k^* \exp \left[-\beta \left(\sum_{n \in \mathbb{Z}} \left(\frac{fL}{2} + \frac{2\pi^2 A_{\text{eff}}}{L} n^2 \right) |\tilde{\phi}_n|^2 + \frac{2\pi^2 A}{L} \left(\frac{A_1}{A} - 1 \right) \frac{L_1}{L} \sum_{n \in \mathbb{Z}} \sum_{m \in \mathbb{Z}} nm \tilde{\phi}_n \tilde{\phi}_m^* \right) \right]. \tag{C.10}$$

The issue preventing further progress here is that the measure is a product over $\mathbb{N}$ and not $\mathbb{Z}$. To rectify this we will convert the summation index of the argument of the exponent to match the measure. The first term is easy as it is symmetric upon the index transformation $n \rightarrow -n$, and so

$$\sum_{n \in \mathbb{Z}} \left(\frac{fL}{2} + \frac{2\pi^2 A_{\text{eff}}}{L} n^2 \right) \tilde{\phi}_n \tilde{\phi}_n^* = 2 \sum_{n \in \mathbb{N}} \left(\frac{fL}{2} + \frac{2\pi^2 A_{\text{eff}}}{L} n^2 \right) \tilde{\phi}_n \tilde{\phi}_n^* + fL \tilde{\phi}_0 \tilde{\phi}_0^*. \quad (\text{C.11})$$

The second term is can be re-expressed as

$$\sum_{n \in \mathbb{Z}} \sum_{m \in \mathbb{Z}}' nm \tilde{\phi}_n \tilde{\phi}_m^* = \sum_{n \in \mathbb{N}} n (\tilde{\phi}_n - \tilde{\phi}_n^*) \sum_{m \in \mathbb{N}}' m (\tilde{\phi}_m - \tilde{\phi}_m^*) \quad (\text{C.12})$$

$$= \sum_{n \in \mathbb{N}} n \tilde{\phi}_n c + \sum_{n \in \mathbb{N}} n \tilde{\phi}_n^* c^*, \quad (\text{C.13})$$

where $c = \sum_{m \in \mathbb{N}}' m \tilde{\phi}_m$. This is good news as now our fluctuation Humiliation is of the form

$$\sum_{n \in \mathbb{N}} (\tilde{\phi}_n \tilde{\phi}_n^* + c \tilde{\phi}_n + c^* \tilde{\phi}_n^*). \quad (\text{C.14})$$

Which firstly, means we can take out the summation in the exponent and merge it with the product defining the measure,

$$Z_1 = \prod_{n \in \mathbb{N}} \int d\tilde{\phi}_n d\tilde{\phi}_n^* \exp \left[-\beta \left(2 \left(\frac{fL}{2} + \frac{2\pi^2 A_{\text{eff}}}{L} n^2 \right) |\tilde{\phi}_n|^2 + b \tilde{\phi}_n + b^* \tilde{\phi}_n^* \right) \right] \quad (\text{C.15})$$

The integrals are complex Gaussians of the form

$$\int dz dz^* e^{-a z z^* - b z - b^* z^*} = \frac{\pi}{a} e^{b b^* / a}, \quad (\text{C.16})$$

This is a trivial consequence of completing the square $a z z^* + b z + b^* z^* = a \left(z + \frac{b}{a} \right) \left(z + \frac{b}{a} \right)^* - b b^* / a$ with $a \in \mathbb{R}$. But $b = \frac{2\pi^2 A}{L} \left(\frac{A_1}{A} - 1 \right) \frac{L_1}{L} \sum_{m \in \mathbb{N}}' m \tilde{\phi}_m \sim \epsilon$, where $\epsilon = L_1/L \ll 1$. This means $b b^* \sim \mathcal{O}(\epsilon^2)$ and so $e^{b b^* / a} \sim 1 + \mathcal{O}(\epsilon^2)$. In this factorization, $a = 2fL + 4\pi^2 A_{\text{eff}} n^2 / L$ and so

$$Z_1 = \prod_{n \in \mathbb{N}} \frac{\pi}{\beta} \frac{1}{fL + 4\pi^2 A_{\text{eff}} n^2 / L} + \mathcal{O}(\epsilon^2). \quad (\text{C.17})$$

We haven't forgotten that A_{eff} is a function of ϵ , but we will not Taylor expand it yet as it will be easier to compute the infinite product first and Taylor expand after. This product is certainly divergent. However we may borrow some tricks

from Quantum path integrals to “regularize” this divergent product via a rather illegal looking factorization of the multiplicand. This way we can assign a finite value to our divergent part of the partition function yielding a result. These finite numbers are derived from an analytic continuation of the Riemann zeta function outside of its domain of convergence. We factorize the product as

$$Z_1^{-1} = \prod_{n \in \mathbb{N}} \frac{4\beta\pi A_{\text{eff}}}{L} \prod_{n \in \mathbb{N}} n^2 \prod_{n \in \mathbb{N}} \left[1 + \left(\frac{fL^2}{4\pi^2 A_{\text{eff}} n^2} \right) \right] + \mathcal{O}(\epsilon^2). \quad (\text{C.18})$$

We now use the Riemann-zeta regularized products

$$\prod_{n \in \mathbb{N}} a = \frac{1}{\sqrt{a}}, \quad \prod_{n \in \mathbb{N}} n = \sqrt{2\pi}, \quad (\text{C.19})$$

along with the convergent infinite product

$$\prod_{n \in \mathbb{N}} \left[1 + \left(\frac{z}{\pi n} \right)^2 \right] = \frac{\sinh(z)}{z}. \quad (\text{C.20})$$

Putting this all together

$$Z_1^{-1} = \frac{\sqrt{L}}{\sqrt{4\beta\pi A_{\text{eff}}}} \cdot 2\pi \cdot \frac{\sinh(L\sqrt{f}/2\sqrt{A_{\text{eff}}})}{L\sqrt{f}/2\sqrt{A_{\text{eff}}}} \quad (\text{C.21})$$

$$= \frac{2\sqrt{\pi}}{\sqrt{\beta f L}} \sinh(L/2\lambda_{\text{eff}}), \quad (\text{C.22})$$

where $\lambda_{\text{eff}} = \sqrt{A_{\text{eff}}/L}$. Hence our full partition function in 3D is

$$Z = \frac{e^{\beta f L} \sqrt{\beta f L}}{\sinh^2(L/2\lambda_{\text{eff}})}. \quad (\text{C.23})$$

Where we have used that in three dimensions [148]:

$$Z = e^{\beta f L} Z_1^2. \quad (\text{C.24})$$

Taking the logarithm

$$\log Z = \beta f L + \log \beta f L - 2 \log \sinh\left(\frac{L}{2\lambda_{\text{eff}}}\right). \quad (\text{C.25})$$

Hence

$$\frac{\langle \Delta x \rangle}{L} = 1 - \frac{1}{\beta f L} - \frac{1}{2\beta \sqrt{A f}} \coth\left(\frac{L}{2\lambda_{\text{eff}}}\right). \quad (\text{C.26})$$

This yields the large force behaviour,

$$\frac{\langle \Delta x \rangle}{L} = 1 - \frac{1}{2\beta\sqrt{A_{\text{eff}}f}}. \quad (\text{C.27})$$

If we now replace A_{eff} with $A \left(1 - \left(1 - \frac{A_1}{A}\right) \frac{L_1}{L}\right)$, then

$$\frac{\langle \Delta x \rangle}{L} \simeq 1 - \frac{1}{2\beta\sqrt{Af}} \left[1 + \frac{1}{2} \left(1 - \frac{A_1}{A}\right) \frac{L_1}{L}\right] + \mathcal{O}(\epsilon^2) \quad (\text{C.28})$$

$$= 1 - \frac{1}{2\beta\sqrt{Af}} - \frac{1}{4\beta\sqrt{Af}} \left(1 - \frac{A_1}{A}\right) \frac{L_1}{L}. \quad (\text{C.29})$$

We can see from this expression that the relative end-to-end distance slightly decreases relative to the homogeneous case.

D

Calculation of Transformed Fluctuation Operator

Applying the transformation outlined in Section 6.3.2 to the fluctuation operator Eq. 6.102, we set

$$\mathbf{P}_0 = -\mathbb{I}_2 \quad (\text{D.1})$$

$$\mathbf{P}_1 = \left(2 - \frac{C}{A}\right) \Omega_3 \begin{pmatrix} 0 & -1 \\ 1 & 0 \end{pmatrix} \quad (\text{D.2})$$

$$\mathbf{P}_2 = \begin{pmatrix} \left(1 - \frac{C}{A}\right) \Omega_3^2 - \Omega_1^2 + \frac{\cos \theta}{\lambda^2} & -\Omega_1 \Omega_2 \\ -\Omega_1 \Omega_2 & \left(1 - \frac{C}{A}\right) \Omega_3^2 - \Omega_1^2 + \frac{\cos \theta}{\lambda^2} \end{pmatrix}. \quad (\text{D.3})$$

$\mathbf{P}_1$ is skew-symmetric and so p is unitary. Specifically from Eq. 6.66 we can determine that

$$\mathbf{p} = \begin{pmatrix} \cos \varphi & -\sin \varphi \\ \sin \varphi & \cos \varphi \end{pmatrix}, \quad (\text{D.4})$$

where $\varphi(s) = \left(2 - \frac{C}{A}\right) \Omega_3 s$. Let us now consider the action of a rotation of this form on the generic Hermitian operator

$$\hat{\mathbf{T}} = \begin{pmatrix} -\partial_s^2 + A & C + D\partial_s \\ C - D\partial_s & -\partial_s^2 + B. \end{pmatrix} \quad (\text{D.5})$$

We now compute the transformed operator from Eq. 6.65. Any rotation matrix will commute with any skew-symmetric matrix with equal diagonal elements. Therefore

$\mathbf{p}$ and its inverse will commute with any of its derivatives and $\mathbf{P}_1$ and so from Eq. 6.65 we can show that

$$\hat{\mathbf{O}} = \partial_s^2 + \mathbf{p}^{-1} (2\dot{\mathbf{p}} - \mathbf{P}_1 \mathbf{p}) \partial_s + \mathbf{p}^{-1} (\ddot{\mathbf{p}} - \mathbf{P}_1 \dot{\mathbf{p}} - \mathbf{P}_2 \mathbf{p}) \quad (\text{D.6})$$

$$= \partial_s^2 + (2\mathbf{p}^{-1} \dot{\mathbf{p}} - \mathbf{P}_1) \partial_s + (\mathbf{p}^{-1} \ddot{\mathbf{p}} - \mathbf{p}^{-1} \mathbf{P}_1 \dot{\mathbf{p}} - \mathbf{p}^{-1} \mathbf{P}_2 \mathbf{p}). \quad (\text{D.7})$$

Performing these matrix multiplications

$$\begin{aligned} \hat{\mathbf{O}} = & -\partial_s^2 + \begin{pmatrix} 0 & D + 2\dot{\varphi} \\ -(D + 2\dot{\varphi}) & 0 \end{pmatrix} \partial_s + \begin{pmatrix} (D + \dot{\varphi}) \dot{\varphi} & \ddot{\varphi} \\ -\ddot{\varphi} & (D + \dot{\varphi}) \dot{\varphi} \end{pmatrix} \\ & + \begin{pmatrix} A \cos^2 \varphi + B \sin^2 \varphi + C \sin 2\varphi & (B - A) \sin \varphi \cos \varphi + C \cos 2\varphi \\ (B - A) \sin \varphi \cos \varphi + C \cos 2\varphi & A \sin^2 \varphi + B \cos^2 \varphi + C \sin 2\varphi \end{pmatrix} \end{aligned} \quad (\text{D.8})$$

. If we let $2\dot{\varphi} + D = 0$, then we get a Schrödinger operator

$$\hat{\mathbf{O}} = -\partial_s^2 + \begin{pmatrix} A \cos^2 \varphi + B \sin^2 \varphi - \dot{\varphi}^2 & (B - A) \sin \varphi \cos \varphi \\ (B - A) \sin \varphi \cos \varphi & A \sin^2 \varphi + B \cos^2 \varphi - \dot{\varphi}^2 \end{pmatrix}. \quad (\text{D.9})$$

Using that $A = (1 - \frac{C}{A}) \Omega_3^2 + \cos \theta / \lambda^2 - \Omega_1^2$, $B = (1 - \frac{C}{A}) \Omega_3^2 + \cos \theta / \lambda^2 - \Omega_2^2$, $C = -\Omega_1 \Omega_2$ and $D = -(2 - \frac{C}{A}) \Omega_3$. This leads to the operator

$$\hat{\mathbf{O}} = -\partial_s^2 + \begin{pmatrix} -\frac{C^2}{4A^2} \Omega_3^2 + \cos \theta / \lambda^2 - \mu^2 & -\mu\nu \\ -\mu\nu & -\frac{C^2}{4A^2} \Omega_3^2 + \cos \theta / \lambda^2 - \nu^2 \end{pmatrix}. \quad (\text{D.10})$$

where $\mu = (\Omega_1 \cos \varphi + \Omega_2 \sin \varphi)$ and $\nu = (\Omega_1 \sin \varphi - \Omega_2 \cos \varphi)$. This can be factorised as

$$\hat{\mathbf{O}} = -\partial_s^2 + \left(-\frac{C^2}{4A^2} \Omega_3^2 + \cos \theta / \lambda^2 \right) \mathbf{I} - \mathbf{Q}^\top \mathbf{Q} \quad (\text{D.11})$$

If instead we choose $\varphi = -\psi$, this leads to the non-Schrödinger operator

$$\begin{aligned} \hat{\mathbf{O}} = & -\partial_s^2 + \begin{pmatrix} 0 & \frac{C}{A} \Omega_3 - 2\dot{\phi} \cos \theta \\ -\frac{C}{A} \Omega_3 + 2\dot{\phi} \cos \theta & 0 \end{pmatrix} \partial_s \\ & + \begin{pmatrix} \cos \theta / \lambda^2 - \dot{\theta}^2 + \dot{\phi}^2 \cos^2 \theta - \frac{C}{A} \Omega_3 \dot{\phi} \cos \theta & -\ddot{\psi} - \dot{\theta} \dot{\phi} \sin \theta \\ \ddot{\psi} - \dot{\theta} \dot{\phi} \sin \theta & \cos \theta / \lambda^2 - \dot{\phi}^2 \sin^2 \theta + \dot{\phi}^2 \cos^2 \theta - \frac{C}{A} \Omega_3 \dot{\phi} \cos \theta \end{pmatrix}. \end{aligned} \quad (\text{D.12})$$

Via use of the EL equations, we may even simplify this operator one final time:

$$\hat{\mathbf{O}} = -\partial_s^2 + \begin{pmatrix} 0 & \ddot{\phi} \sin \theta / \dot{\theta} \\ -\ddot{\phi} \sin \theta / \dot{\theta} & 0 \end{pmatrix} \partial_s + \begin{pmatrix} \ddot{\theta} \cot \theta - \dot{\theta}^2 & -\ddot{\phi} \cos \theta + 2\dot{\theta} \dot{\phi} \sin \theta \\ \ddot{\phi} \cos \theta & \ddot{\theta} \cot \theta - \dot{\phi}^2 \sin^2 \theta \end{pmatrix}. \quad (\text{D.13})$$

Interestingly the operator Eq. D.12 isn't necessarily Hermitian, we require $\ddot{\psi} = 0$ in order for it to be self-adjoint. Furthermore if $\dot{\theta} = 0$, $\kappa = \dot{\phi} \cos \theta$ and $\tau = \dot{\phi} \sin \theta$ and so

$$\hat{\mathbf{O}} = -\partial_s^2 + \begin{pmatrix} \cos \delta / \lambda^2 + \tau^2 - \frac{C}{A} \Omega_3 \tau & \left(\frac{C}{A} \Omega_3 - 2\tau \right) \partial_s \\ - \left(\frac{C}{A} \Omega_3 - 2\tau \right) \partial_s & -\frac{C}{A} \Omega_3^2 + \cos \delta / \lambda^2 + \tau^2 - \kappa^2 - \frac{C}{A} \Omega_3 \tau \end{pmatrix}. \quad (\text{D.14})$$

This is the fluctuation operator derived by Rollin [157] which was performed in the Frenet-Serret basis without the twist degree of freedom. I.e. the fluctuation operator of a twistable worm-like chain when the final twist rotation is undone in this case is equivalent to the fluctuation operator which is only comprised of two rotations (the Frenet ribbon). Therefore, we must ask, do we need to include the twist variable if the determinant will be the same if we don't include it? Well as pointed out, this transformation requires $\ddot{\psi} = 0$ so that the resulting operator is Hermitian. Hence, we are only effectively permitted to neglect the twist variable ψ in instances where the mean-field configuration has a uniform distribution of twist.

E

Calculation of matrix $\mathbf{H}(s)$ at the boundary

In order to calculate the functional determinant of the non-trivial solutions to the TWLC we are required to evaluate the matrix $\mathbf{H}(s)$ at the boundary points $s = 0, L$. The elements of $\mathbf{H}(s)$ that we are required to compute are

$$\dot{\theta} \int 1/\dot{\theta}^2 ds, \quad \dot{\theta} \int \dot{\phi}/\dot{\theta}^2 ds, \quad \dot{\theta} \int \dot{\phi}^2/\dot{\theta}^2 ds. \quad (\text{E.1})$$

We may either compute these integrals with respect to s or change variables to u . Both methods are outlined here. We note that despite evaluating indefinite integrals at the two boundary points, we are not computing definite integrals.

E.1 Arc Length Integration

To begin we must compute the indefinite integral

$$\int \frac{1}{\dot{\theta}^2} ds, \quad (\text{E.2})$$

where $\cos \theta = c + (b - c) \text{sn}^2 \left(s\lambda\sqrt{a - c}/2, m \right)$. If we let $x = qs$ where $q = \sqrt{a - c}/\lambda\sqrt{2}$, then $\cos \theta = c + (b - c) \text{sn}^2 (x, m)$ and $\int 1/\dot{\theta}^2 ds = q^{-3} \int 1/[\theta'(x)]^2 dx$. Implicitly we shall use prime notation to indicate a derivative with respect to x and

keep the dot notation for s derivatives. Differentiating, $-\theta' \sin \theta = 2(b-c) \operatorname{cnsndn}$ and hence,

$$\int \frac{1}{\dot{\theta}^2} ds = \frac{1}{4(b-c)^2 q^3} \int \frac{\sin^2 \theta}{\operatorname{sn}^2 \operatorname{cn}^2 \operatorname{dn}^2} dx. \quad (\text{E.3})$$

The domains transform from $0 \leq s \leq L$ to $0 \leq x \leq K(k)$. If we use our expression for $\cos \theta$ to evaluate $\sin^2 \theta$

$$\int \frac{1}{\dot{\theta}^2} ds = \frac{1}{4(b-c)^2 q^3} \int \frac{1 - c^2 - 2c(b-c) \operatorname{sn}^2 - (b-c)^2 \operatorname{sn}^4}{\operatorname{sn}^2 \operatorname{cn}^2 \operatorname{dn}^2} dx. \quad (\text{E.4})$$

The new integral can be split up into three terms

$$I = \int \frac{1 - c^2}{\operatorname{sn}^2 \operatorname{cn}^2 \operatorname{dn}^2} - \frac{2c(b-c)}{\operatorname{cn}^2 \operatorname{dn}^2} - \frac{(b-c)^2 \operatorname{sn}^2}{\operatorname{cn}^2 \operatorname{dn}^2} dx. \quad (\text{E.5})$$

We can also replace $\operatorname{sn}^2 = (1 - \operatorname{cn}^2)$ to give

$$I = \int \frac{1 - c^2}{\operatorname{sn}^2 \operatorname{cn}^2 \operatorname{dn}^2} - \frac{(b-c)(b+c)}{\operatorname{cn}^2 \operatorname{dn}^2} + \frac{(b-c)^2}{\operatorname{dn}^2} dx. \quad (\text{E.6})$$

We can now compute these integrals separately

$$\int \operatorname{nd}^2 = \frac{1}{m'} \left(E + m \frac{\operatorname{cnsn}}{\operatorname{dn}} \right) \quad (\text{E.7})$$

$$\int \operatorname{nc}^2 \operatorname{nd}^2 = \frac{1}{m'^2} \left[m' u - (1+m) E + \operatorname{sn} \left(\frac{\operatorname{dn}}{\operatorname{cn}} + m^2 \frac{\operatorname{cn}}{\operatorname{dn}} \right) \right] \quad (\text{E.8})$$

$$\int \operatorname{sn}^2 \operatorname{nc}^2 \operatorname{nd}^2 = \frac{1}{m'^2} \left[m' (1+m') u - (1+m^2+m'^2) E \right. \quad (\text{E.9})$$

$$\left. + \left(\operatorname{sn}^2 - m'^2 \operatorname{cn}^2 + m^3 \frac{\operatorname{sn}^2 \operatorname{cn}^2}{\operatorname{dn}^2} \right) \frac{\operatorname{dn}}{\operatorname{sncn}} \right], \quad (\text{E.10})$$

where again we note that we have suppressed the arguments of all elliptic integrals and functions for brevity.

Given that $\operatorname{sn}(0) = \operatorname{cn}(K) = 0$, these integrals diverge when evaluated at these limits. Luckily, when multiplied by $\dot{\theta} \propto \operatorname{sncndn}$, these singularities will cancel yielding a finite result. We now need that $\operatorname{sn}(0) = 0$, $\operatorname{cn}(0) = 1$, $\operatorname{dn}(0) = 1$ in order to show that:

$$\left[\operatorname{sncndn} \int \operatorname{nc}^2 \right]_0 = 0 \quad (\text{E.11})$$

$$\left[\operatorname{sncndn} \int \operatorname{nc}^2 \operatorname{nd}^2 \right]_0 = 0 \quad (\text{E.12})$$

$$\left[\operatorname{sncndn} \int \operatorname{sn}^2 \operatorname{nc}^2 \operatorname{nd}^2 \right]_0 = -1. \quad (\text{E.13})$$

Hence $\left[\theta' \int 1/(\theta')^2 dx\right]_0 = -(1-c^2)$ and

$$\left[\dot{\theta} \int 1/\dot{\theta}^2 ds\right]_0 = \lambda^2 \sqrt{1-c^2}/(b-c)(a-c). \quad (\text{E.14})$$

We will also need that $\text{sn}(K) = 1$, $\text{cn}(K) = 0$, $\text{dn}(K) = 1-m$ to show that

$$\left[\text{sncndn} \int \text{nd}^2\right]_K = 0 \quad (\text{E.15})$$

$$\left[\text{sncndn} \int \text{nc}^2 \text{nd}^2\right]_K = \frac{1}{m'} \quad (\text{E.16})$$

$$\left[\text{sncndn} \int \text{sn}^2 \text{nc}^2 \text{nd}^2\right]_K = \frac{1}{m'}. \quad (\text{E.17})$$

This allows us to show that

$$\left[\theta' \int 1/(\theta')^2 dx\right]_{x=K} = \frac{(1-c^2)}{m'} - \frac{(b-c)(b+c)}{m'} \quad (\text{E.18})$$

$$= \frac{1-b^2}{m'}. \quad (\text{E.19})$$

And so,

$$\left[\dot{\theta} \int 1/\dot{\theta}^2 ds\right]_{s=L} = \frac{\lambda^2 \sqrt{1-b^2}}{(b-c)(b-a)}. \quad (\text{E.20})$$

The next integral to compute is

$$\int \frac{\dot{\phi}}{\dot{\theta}^2} ds \quad (\text{E.21})$$

Performing to same variable transformation $s \mapsto x$

$$\int \frac{\dot{\phi}}{\dot{\theta}^2} ds = \frac{1}{q^2} \int \frac{\phi'}{(\theta')^2} dx \quad (\text{E.22})$$

Hence,

$$\int \frac{\dot{\phi}}{\dot{\theta}^2} ds = \frac{1}{4Aq^2} \int \frac{p_\phi - p_\psi (c + (b-c)\text{sn}^2)}{\text{sn}^2 \text{cn}^2 \text{dn}^2} ds. \quad (\text{E.23})$$

Then

$$\int \frac{\dot{\phi}}{\dot{\theta}^2} ds = \frac{1}{4Aq^2} \int \frac{p_\phi - p_\psi c}{\text{sn}^2 \text{cn}^2 \text{dn}^2} - \frac{p_\psi (b-c)}{\text{cn}^2 \text{dn}^2} dx. \quad (\text{E.24})$$

This is similarly just a combination of the integrals of reciprocal elliptic functions as for the previous integral. We can then show that

$$\left[\dot{\theta} \int \frac{\dot{\phi}}{\dot{\theta}^2} ds \right]_0 = \frac{\lambda^2 (p_\phi - p_\psi c)}{A (b - c) (a - c) \sqrt{1 - c^2}} \quad (\text{E.25})$$

$$\left[\dot{\theta} \int \frac{\dot{\phi}}{\dot{\theta}^2} ds \right]_K = \frac{\lambda^2 (p_\phi - p_\psi b)}{A (b - c) (b - a) \sqrt{1 - b^2}}. \quad (\text{E.26})$$

Unfortunately this method of computing our desired integrals does not translate that well when trying to calculate $\dot{\theta} \int \dot{\phi}^2 / \dot{\theta}^2 ds$ as the resultant integrals do not have known closed form solutions. Hence we turn our attention to using a different variable transformation.

E.2 u Integrals

Consider our first integral again but now transformed to the variable $u = \cos \theta$,

$$\dot{\theta} \int \frac{1}{\dot{\theta}^2} ds = - \frac{\dot{u}}{\sqrt{1 - u^2}} \int \frac{1 - u^2}{\dot{u}^3} du \quad (\text{E.27})$$

$$(\text{E.28})$$

$\dot{u} \propto \sqrt{(u - a)(u - b)(u - c)}$ and so similarly to the arc length integrals, when evaluated at the boundary terms $u = c, b$, all terms will vanish unless they have a singularity that will cancel with these square roots. Given all the integrals involve $1/\dot{u}^3$, then from Eq. A.20, we deduce that will need to be able to compute integrals of the form:

$$\int \frac{du}{[(u - a)(u - b)(u - c)]^{3/2}}. \quad (\text{E.29})$$

Byrd does not have closed form solutions for such an integral and the results from Wolfram Mathematica are rather messy and so instead we use partial fractions to show that

$$\begin{aligned} \frac{1}{(u - a)(u - b)(u - c)} &= \frac{1}{(a - b)(a - c)} \frac{1}{u - a} + \frac{1}{(b - a)(b - c)} \frac{1}{u - b} \\ &+ \frac{1}{(c - a)(c - b)} \frac{1}{u - c}. \end{aligned} \quad (\text{E.30})$$

Hence, all the desired integrals be expressed as sums of the general integral

$$I_{n,m}(u) = \int \frac{u^m}{(u-n) \sqrt{(u-a)(u-b)(u-c)}} du. \quad (\text{E.31})$$

From Byrd [161]:

$$I_{n,0}(u) = \frac{2}{(n-c) \sqrt{a-c}} \Pi \left(\frac{b-c}{n-c}, \arcsin \left(\sqrt{\frac{u-c}{b-c}} \right), \frac{b-c}{a-c} \right) \quad n \neq c. \quad (\text{E.32})$$

If $n = c$,

$$I_{c,0}(u) = -\frac{2}{(b-c)} \left[\sqrt{\frac{u-b}{(u-a)(u-c)}} - \frac{i}{\sqrt{a-c}} E \left(\arcsin \left(\sqrt{\frac{a-c}{a-u}} \right), \frac{a-b}{a-c} \right) \right].$$

Similarly if $n = b$

$$I_{b,0}(u) = -\frac{2}{(b-c)} \left[\sqrt{\frac{u-c}{(u-a)(u-b)}} + \frac{i}{\sqrt{a-b}} E \left(\arcsin \left(\sqrt{\frac{a-b}{a-u}} \right), \frac{a-c}{a-b} \right) \right].$$

We now denote the parts of said integrals that do not vanish when multiplied by $\dot{u} \propto \sqrt{(u-b)(u-c)}$ and evaluated at $u = c$ or $u = b$ with a tilde. For example

$$\tilde{I}_{0,c}(c) = -\frac{2}{(b-c)} \sqrt{\frac{u-b}{(u-a)(u-c)}}. \quad (\text{E.33})$$

We can easily derive the reduction formula:

$$I_{n,1}(u) = \int \frac{(u-n) + n}{(u-n) \sqrt{(u-a)(u-b)(u-c)}} du \quad (\text{E.34})$$

$$= \int \frac{du}{\sqrt{(u-a)(u-b)(u-c)}} + n I_{n,0}(u). \quad (\text{E.35})$$

Byrd also gives us that [161]

$$J(u) = \int \frac{du}{\sqrt{(u-a)(u-b)(u-c)}} \quad (\text{E.36})$$

$$= \frac{2}{\sqrt{a-c}} F \left(\arcsin \left(\sqrt{\frac{u-c}{a-c}} \right), \frac{b-c}{a-c} \right). \quad (\text{E.37})$$

Hence,

$$I_{n,1}(u) = \frac{2}{\sqrt{a-c}} F \left(\arcsin \left(\sqrt{\frac{u-c}{a-c}} \right), \frac{b-c}{a-c} \right) + n I_{n,0}(u). \quad (\text{E.38})$$

This first term will vanish when multiplied by $\dot{u}$ and evaluated at the boundary and so

$$\tilde{I}_{n,1}(u) = n\tilde{I}_{n,0}(u). \quad (\text{E.39})$$

Another reduction formula we may derive is:

$$I_{n,2}(u) = \int \frac{(u-n)^2 + 2un - n^2}{(u-n)\sqrt{(u-a)(u-b)(u-c)}} du \quad (\text{E.40})$$

$$= \int \frac{u-n}{\sqrt{(u-a)(u-b)(u-c)}} du + 2nI_{n,1}(u) - n^2I_{n,0}(u) \quad (\text{E.41})$$

$$= \int \frac{u}{\sqrt{(u-a)(u-b)(u-c)}} du + 2nI_{n,1}(u) - n^2I_{n,0}(u) - nJ(u).$$

It can be shown that

$$\int \frac{u}{\sqrt{(u-a)(u-b)(u-c)}} = \frac{2c}{\sqrt{a-c}} \left[\frac{a}{c} \frac{s}{\lambda k} \sqrt{\frac{b-c}{2}} - E \left(\text{am} \left(\frac{s}{\lambda k} \sqrt{\frac{b-c}{2}}, k \right) \right) \right].$$

This will vanish when multiplied by $\dot{u}$ and evaluated at $u=c$ or $u=b$ and so

$$\tilde{I}_{n,2}(u) = 2n\tilde{I}_{n,1}(u) - n^2\tilde{I}_{n,0}(u) \quad (\text{E.42})$$

$$= n^2\tilde{I}_{n,0}(u). \quad (\text{E.43})$$

We now have everything we need to compute our desired integrals.

$$\dot{\theta} \int \frac{1}{\dot{\theta}^2} ds = -\frac{\dot{u}}{\sqrt{1-u^2}} \int \frac{1-u^2}{\dot{u}^3} du \quad (\text{E.44})$$

$$= -\frac{\lambda^2}{2} \frac{\sqrt{(u-a)(u-b)(u-c)}}{\sqrt{1-u^2}} \left[\frac{\tilde{I}_{c,0} - \tilde{I}_{c,2}}{(c-a)(c-b)} \right]. \quad (\text{E.45})$$

When we evaluate these expressions at $s=0$, i.e. $u=c$, we get

$$\left[\dot{\theta} \int \frac{1}{\dot{\theta}^2} ds \right]_{s=0} = -\frac{\lambda^2}{2} \lim_{u \rightarrow c} \frac{\sqrt{(u-a)(u-b)(u-c)}}{\sqrt{1-u^2}} \quad (\text{E.46})$$

$$\times -\frac{(1-c^2)}{(c-a)(c-b)} \frac{2}{(b-c)} \sqrt{\frac{u-b}{(u-a)(u-c)}} \quad (\text{E.47})$$

$$= \frac{\lambda^2 \sqrt{1-c^2}}{(c-a)(b-c)}. \quad (\text{E.48})$$

This agrees with Eq. E.14. Similarly,

$$\dot{\theta} \int \dot{\phi}/\dot{\theta}^2 = -\frac{\dot{u}}{A\sqrt{1-u^2}} \int \frac{p_\phi - p_\psi u}{\dot{u}^3} du \quad (\text{E.49})$$

$$= -\frac{\lambda^2}{2A} \frac{\sqrt{(u-a)(u-b)(u-c)}}{\sqrt{1-u^2}} \left[\frac{p_\phi \tilde{I}_{c,0} - p_\psi \tilde{I}_{c,1}}{(c-a)(c-b)} \right] \quad (\text{E.50})$$

$$= -\frac{\lambda^2}{2A} \frac{\sqrt{(u-a)(u-b)(u-c)}}{\sqrt{1-u^2}} \left[\frac{(p_\phi - p_\psi c) \tilde{I}_{c,0}}{(c-a)(c-b)} \right]. \quad (\text{E.51})$$

Hence,

$$\left[\dot{\theta} \int \dot{\phi} / \dot{\theta}^2 \right]_{s=0} = \frac{\lambda^2}{A} \frac{1}{\sqrt{1-c^2}} \left[\frac{(p_\phi - p_\psi c)}{(c-a)(c-b)} \right]. \quad (\text{E.52})$$

Now we can tackle the integral that was impenetrable to us when using the methods of Section E.1, namely

$$\dot{\theta} \int \dot{\phi}^2 / \dot{\theta}^2 = -\frac{1}{A^2} \frac{\dot{u}}{\sqrt{1-u^2}} \int \frac{(p_\phi - p_\psi u)^2}{(1-u^2) \dot{u}^3} du \quad (\text{E.53})$$

$$= -\frac{\lambda^2}{2A^2} \frac{\sqrt{(u-a)(u-b)(u-c)}}{\sqrt{1-u^2}} \int \frac{(p_\phi - p_\psi u)^2}{(1-u^2)} \quad (\text{E.54})$$

$$\times \frac{1}{(u-a)(u-b)(u-c)} \frac{1}{\sqrt{(u-a)(u-b)(u-c)}} du. \quad (\text{E.55})$$

We need to decompose this integral into our constituent parts. Firstly,

$$\frac{(p_\phi - p_\psi u)^2}{(1-u^2)} = \frac{1}{2} \left(\frac{1}{1-u} + \frac{1}{1+u} \right) (p_\phi - p_\psi u)^2. \quad (\text{E.56})$$

The final decomposition will be that

$$\frac{1}{(1 \pm c)(u-c)} = -\frac{1}{1 \pm c} \left(\frac{1}{1 \pm u} \mp \frac{1}{u-c} \right). \quad (\text{E.57})$$

Hence the only non-zero contributions will be from

$$\frac{(p_\phi - p_\psi u)^2}{2(c-a)(c-b)} \left[\frac{1}{1+c} + \frac{1}{1-c} \right] \frac{1}{u-c} = \frac{(p_\phi - p_\psi u)^2}{(c-a)(c-b)} \frac{1}{1-c^2} \frac{1}{u-c}. \quad (\text{E.58})$$

This means that the non-zero contributions to our final expression are

$$\frac{(p_\phi - p_\psi c)^2}{2(c-a)(c-b)} \left[\frac{1}{1+c} + \frac{1}{1-c} \right] \frac{1}{u-c} = \frac{(p_\phi - p_\psi c)^2}{(c-a)(c-b)} \frac{1}{1-c^2} \tilde{I}_{c,0}. \quad (\text{E.59})$$

Evaluating at $s = 0$

$$\left[\dot{\theta} \int \dot{\phi}^2 / \dot{\theta}^2 \right]_{s=0} = \frac{\lambda^2}{2A^2} \frac{1}{\sqrt{1-c^2}} \frac{(p_\phi - p_\psi c)^2}{(c-a)} \frac{1}{1-c^2} \frac{1}{(b-c)}. \quad (\text{E.60})$$

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