

The Structural Properties of DNA Origami



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Abstract

In this thesis, we have investigated the suitability of using oxDNA to model DNA origami nanostructures. We used oxDNA to simulate a variety of DNA origami systems, then analysed the results to determine their key structural properties. Specifically, after introducing the area and outlining the procedure to simulate these systems, we report on three case studies that were based on experimental work; a theoretical project in which we designed an auxetic system based on knowledge gathered from the previous chapters, and a collaborative project with an experimental group looking into novel ‘barrel’ structures.

More specifically, the case studies we looked at were previously experimentally assembled DNA structures including a Möbius strip, six and twelve helix bundle kite structures that were connected with linkers of varying lengths, and ball structures containing six-way Holliday junctions. For these case studies, we found that oxDNA was able to replicate well existing experimental data, and therefore provide reliable insight into the subtleties of these systems that would be hard to elucidate using experimental techniques. As such, we were then able to design an auxetic-style system (in this case meaning a system which expands in two directions simultaneously) and predict its behaviour with confidence. Lastly, we assisted an experimental group by simulating and analysing a series of barrel structures to provide detailed information about these systems.

Looking to the future, we hope that the work outlined here will further encourage other groups, particularly experimental ones, to utilise oxDNA in their own work on DNA origami by both providing evidence of oxDNA's utility, as well as sufficient guidance and assistance to get started.

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Chapter 1

Introduction

1.1 From DNA to DNA nanotechnology

DNA origami is the term used to describe nanostructures that are produced through the folding of DNA.¹ More specifically, a long single-stranded piece of DNA, called a ‘scaffold’, is folded up, pinched together and held in place by shorter strands, called ‘staples’. All together, these form a shape comprised of what appears to be double-stranded DNA helices iterating around a ‘2D’ (appears flat, like a sheet) or 3D structure.

Even though the structure of DNA was not discovered and revealed to the world until 1953, it was already known that it contained nucleotides, called adenine (A), thymine (T), cytosine (C) and guanine (G). In fact, Albrecht Kossel was awarded the Nobel Prize in the Physiology or Medicine category in 1910 for this discovery.² The structure of each of these four bases is shown in Figure 1.1 and the Watson and Crick base pairing rules state that A pairs with T using two hydrogen bonds, and C to G with three.

The helical structure of DNA was confirmed by X-ray crystallography and published at the same time in three papers from the same volume of *Nature* in 1953.

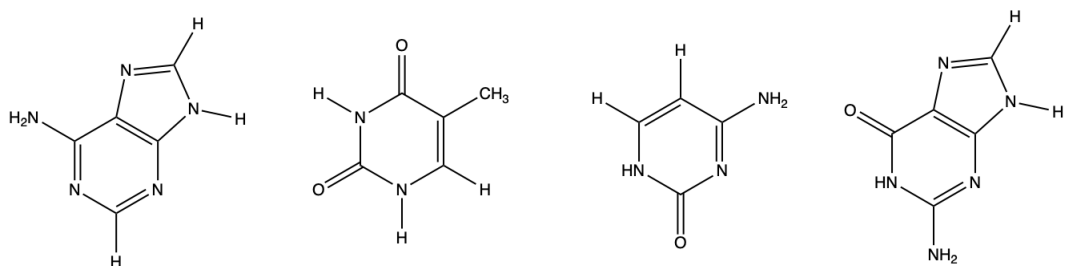


Figure 1.1: The structures of each nucleotide base, shown in the order adenine (A), thymine (T), cytosine (C) and guanine (G).

One of the papers was authored by James Watson and Francis Crick, who presented a model for the DNA double helix in their research entitled ‘Molecular Structure of Nucleic Acids’ reportedly with inspiration from the unpublished work from the authors of the other two papers: Rosalind Franklin and Raymond Gosling (‘Molecular Configuration in Sodium Thymonucleate’), and Maurice Wilkins, Alec Stokes and Herbert Wilson (‘Molecular Structure of Deoxypentose Nucleic Acids’).³⁻⁵ It is worth noting that the Nobel Prize for this discovery was awarded to Watson, Crick and Wilkins. Franklin was omitted from this award despite being a key contributor to the evidence for DNA’s double helical structure,⁶ and the decision to not include her will likely always be a controversial one.

All three papers’ proposed structure suggested that DNA was helical in design and had its (double) phosphate backbone on the outside of the helix, not the tri-stranded helix with phosphate on the inside as proposed by Linus Pauling and Robert Corey the year before.⁷ It was also suggested that there exists major and minor grooving in the structure (these terms are not used although the accompanying diagram in Watson and Crick’s paper depicts it), and that there are 10 base pairs (bps) per helical turn; this value has remained mostly constant but is now

thought to be closer to 10.5 bps per turn.^{3,5,8}

The specificity of Watson-Crick base pairing together with the potential programmability of DNA sequences led to the emergence of a new field called DNA nanotechnology that aimed to use DNA as a material for nanoscale engineering. At the forefront of the emergence of this field was Nadrian ‘Ned’ Seeman,⁹ who outlined his first visions for this field in 1982.¹⁰ Progress was relatively slow at first. Landmarks include Chen and Seeman’s synthesis of a cube-like structure made of DNA, via self-assembly and utilising ‘sticky ends’ to attach monomers together in 1991,¹¹ and Seeman and coworkers’ creation of 2D DNA lattices in 1998.¹² The latter involved the self-assembly of ‘double-crossover tiles’ that could in turn assemble into crystalline arrays via sticky-end interactions.

Seeman’s work paved the way for a scientist named Paul Rothemund to develop a completely new DNA nanotechnology technique that would massively increase the size and complexity of possible DNA nanostructures.¹³ In 2006, the first pioneering paper was published. It detailed a new ‘one-pot’ synthesis technique of using a very long (about 7000 nucleotides) single-stranded piece of viral DNA mixed in with a series of shorter lengths, which then folded without any additional input into a larger ‘sheet’ structure through an annealing process. This new type of DNA nanostructure was termed ‘DNA origami’.

1.2 The Rise of DNA Origami

Figure 1.2 depicts a simple diagram of a staple pinching a scaffold strand in a specific region. The staples are supplied in excess, and the scaffold has comple-

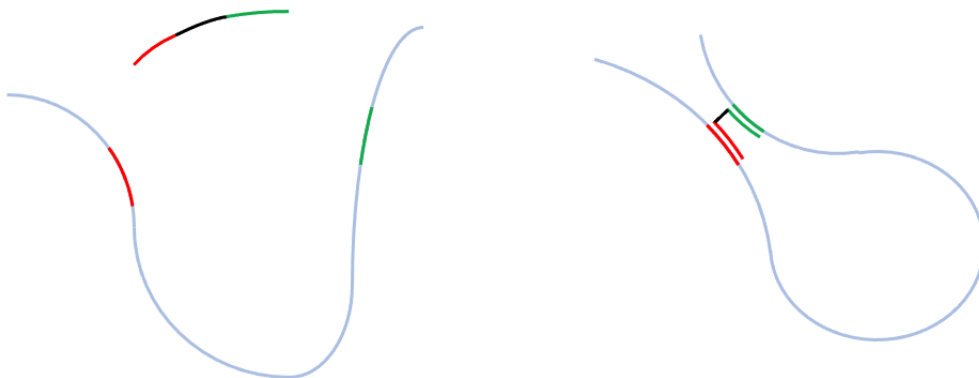


Figure 1.2: A depiction of a staple strand binding to a scaffold to create the start of an origami.

mentary bases to them (shown in red and green) placed in regions which, when formed, will hold the scaffold into the required shape. It is easy to see how scaling this up with a very long scaffold strand and many different staple strands could produce an entire structure. The process itself is fairly straightforward and uses a one-pot technique whereby the solution is heated up and, upon cooling, the strands self assemble and fully-formed products can be obtained in good yields (Rothemund, for example, reported the yields of some structures to be higher than 90%; however, others were lower than 2%).¹³

DNA origami then became almost a nanoscale art form, as a diverse array of structures were creatively designed and synthesised, starting with those produced by Rothemund in his original paper (Figure 1.3). Rothemund's paper was followed by a number of other 'fun' designs including Andersen *et al.* in 2012 designing and assembling dolphins with moving tails;¹⁴ a 3D 'pointer' structure resembling a hand with a pointing finger by Bai *et al.* in 2012,¹⁵ and the popular Stanford

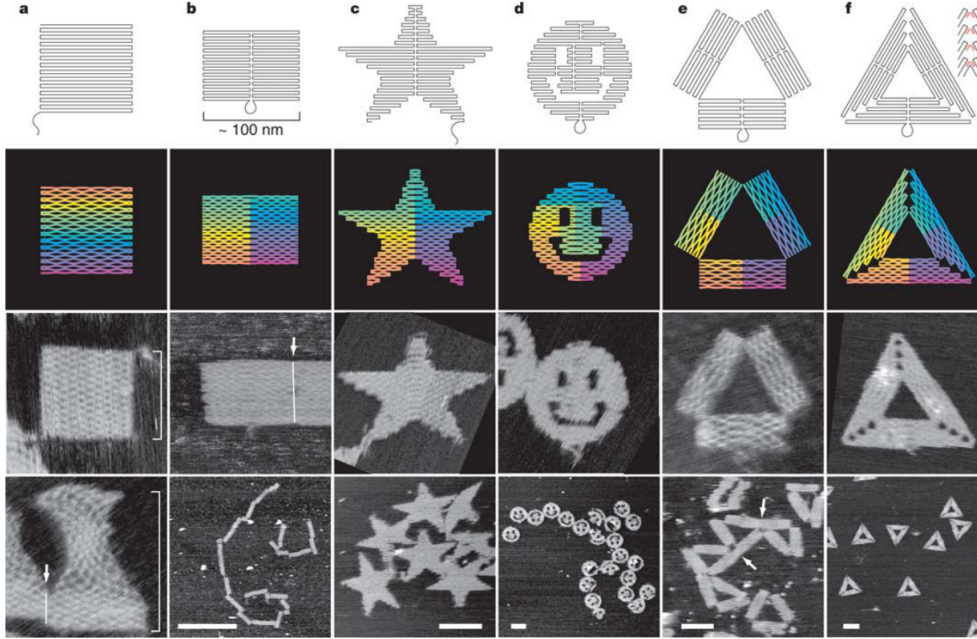


Figure 1.3: A variety of DNA origami structures produced by Paul Rothemund, shown as designs (top two rows) and Atomic Force Microscopy images (bottom two rows). Image reproduced with permission from Springer Nature (Ref. 13).

bunny motif was recreated by Benson *et al.* in 2015.¹⁶

The technique took off, in the sense that it became very popular very quickly, perhaps due to the relative simplicity of the approach and the the high assembly yields that are possible. This popularity is evident from the development of bespoke software specifically to design DNA origami; most influential has been a tool called caDNAno, which was released just three years later in 2009.¹⁷ The development of such computer-aided design tools has further facilitated the widespread usage of DNA origami. For example, the caDNAno program allows the easy design of such nanostructures and provides features such as auto-stapling, which suggests the placement of staple strands according to the layout of the scaffold. It will also generate a base sequence for the entire structure.

Although the initial Rothemund designs were 2D sheets consisting of parallel double helices connected by four-way junctions, the technique was soon extended to 3D arrays of helices on a hexagonal or square lattice by Douglas *et al.* in 2009.¹⁷ By programming mechanical stresses into the individual helices by additional or missing base pairs between junctions these origamis could even be made to be twisted or bent.¹⁸

1.2.1 Wireframe Structures

Starting in 2015 there has been an increasing interest in so called wireframe DNA origamis.¹⁶ These structures are typically 3D polyhedral meshes with edges ranging from single helices to 6-helix bundles. The meshes are often triangulated but can involve other polygons, especially if more stiff edges are used. One of the well-known groups in this area are the Bathe group, currently based at MIT, who have not only have created a wide variety of these structures, but have also released software to allow others to easily design their own, such as DAEDALUS and TALOS.^{19,20} Whilst a number of similar 3D DNA nanostructures had existed before then, the number of papers on this topic rose dramatically in a relatively short period of time, both because of the potential utility of their novel features compared to traditional DNA origami and again because of the release of bespoke software that facilitates their design. These software tools make design questions such as how to route the scaffold through the structure or how to achieve a specific angle at a vertex much easier to accomplish.

1.2.2 Auxetic-style Materials

One recent area particularly facilitated by the development of wireframe structures

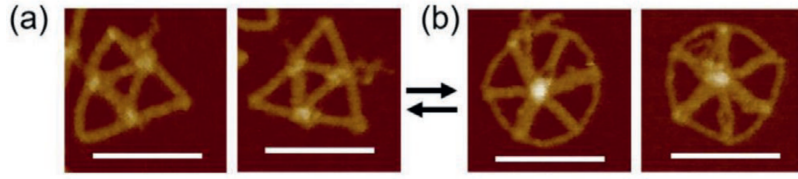


Figure 1.4: A depiction of a double-edged DNA triangle (state a) that can convert into a hexagon shape (state b). Image reproduced with permission from Li *et al.*²²

has been the exploration of using DNA origami to achieve “auxetic” behaviour. Auxetic materials are those which expand in two directions when tension is applied in one. It would be similar to a rubber band expanding in both width and length when pulled upon. For example, two papers by Li *et al.* demonstrated the action of auxetic materials using experimental and simulation techniques (the latter using oxDNA, the same software used in this project).^{21,22} Their designs are relatively straightforward in concept, they use tessellating shapes (often triangles) and ‘jacks’, which are DNA sections whose lengths can be changed, thereby allowing the shapes to expand and contract in size. In their second paper, more sophisticated designs were created using the same ‘jack system’, namely a switchable origami that could interconvert between a triangle (with double edges to provide enough DNA to allow conversion) and a hexagon (Figure 1.4). The flexibility provided by DNA designs thus provides a new avenue to explore auxetic behaviour in the nanoscale regime.

1.2.3 Pharmaceutical Uses

A common question the general public has for any science research is what impact can it have on our everyday life. Initially, DNA origami was investigated mostly

as a new scientific technique, but very quickly researchers began to look at the possibilities for the pharmaceutical industry.

Direct drug delivery is a popular research topic at the moment as it would provide a means to administer medication with more limited, or perhaps even no, negative or severe side effects. A drug that is well-known to have very challenging side effects is cisplatin. In the 1960s it was found to be highly effective in fighting cancers (originally in mice but later in humans) and has remained a very well-known drug in cancer treatments.²³ However, it is an aggressive treatment and, like many cancer treatments, attacks both the healthy and cancerous cells. It is the assault on healthy cells that leads to the unpleasant side-effects. As such, being able to deliver a drug like this directly to cancerous cells, without affecting healthy cells, would be a phenomenal step forward in medical science.

A very important, and early, advance in this area came from Douglas *et al.* with the development of their DNA nanorobot capable of delivering a payload via selective opening when in the presence of proteins. The device opened using a lock-and-key mechanism that was activated by antigens on proteins.²⁴

Another not too dissimilar example was the ‘small box’ capsule that was designed with the aim of being a drug delivery device to specifically target breast cancer cells. Similarly to Douglas *et al.* the concept was that the medication would be placed inside the box, and that the box would only open when receptors on its surface came into contact with antigen markers on the cancer cells’ surfaces.²⁵ Zadegan *et al.* synthesised this box design and demonstrated it was able to open and close three times.

Another important progression in this field was the work of Li *et al.* in 2018 who designed and synthesised a nanorobot that delivered blood clotting compounds to a

tumor site to diminish its blood supply, with the aim of subsequently destroying the mass.²⁶ A notable advance in this work was the *in vivo* testing in mice and Bama miniature pigs which demonstrated the nanorobot to be ‘safe and immunologically inert’ in these animals, two features discussed later in this section that currently remain as two of the largest barriers to using this technology in humans.

Other examples that illustrate the scope of the research in this area include the work of Zhang *et al.* (2014) who used a 2D triangle design also for direct drug delivery against cancer cells;²⁷ Mela *et al.* (2020) who functionalised the previously published design of Yoshidome *et al.* (2012) with the aim of creating an antimicrobial drug delivery device,^{28,29} and the slightly different, but pertinent, work of Wang *et al.* (2018) who visualised the uptake of DNA origami structures by human cells.³⁰

Using DNA origami for pharmaceuticals is often investigated, likely due to the high product yields, the relatively low cost of reagents and the mild conditions needed for assembly. However, it is one challenge to create such structures in a laboratory but another altogether to get them to function as designed in the human body. A recent review covered the practicalities of doing so and covered potential issues such as: the origami being absorbed by cells that have different levels of selectivity; structural stability whilst en route to its target; removal from the body, including processing and excretion through the liver, as well as avoiding recognition by the immune system as a invading species. Although presenting a number of challenges, the author remains quite positive that should these be overcome, there is real potential in the technique for the future.³¹

Researchers have also recently been interested in creating a new generation of

vaccine delivery vectors through the use of DNA origami structures. It is likely that many of the same problems are faced as those of drug delivery devices, in that they need to be accepted by the body, deliver the protein where needed and successfully pass throughout the system, all without being identified as invasive material. The review article of Dobrovolskaia and Bathe discusses the current developments in this field, as well as the remaining challenges.³² They highlight the work of Liu *et al.* (2012) who used an origami as a vaccine vector that was able to provoke an immune response in mice, but without a response to the DNA nanostructure.³³ Another example was Veneziano *et al.* (2020) who aimed to utilise DNA origami designs to space antigens around a structure in order to provoke the largest immune response possible from B-cells.³⁴ Overall, the use of DNA origami in the pharmaceutical industry continues to gain traction and this is likely to continue as the need for more affordable and effective vaccines and pharmaceuticals has always been, and remains, a global need.

1.3 DNA Origami Simulations: from very coarse-grained to all-atom models

We have already discussed how the range of DNA nanostructures investigated experimentally has expanded and developed over time, but so also have the methods to simulate and model them. As mentioned in the previous section, caDNAno was one of the first software design tools that experimentalists and theoreticians alike have used to carry out research in this area.

On the simulation side, the focus of the thesis is on the oxDNA model and

simulation code. First released in 2013 (initially for the modelling of DNA nanotechnology systems, and later being used for DNA origami structures),³⁵ with the second major release following promptly two years later (2015),³⁶ it contained a simple-to-use conversion script from caDNAno form into oxDNA format, and allowed users to simulate DNA structures starting from a caDNAno design. Although the original conversion scripts were part of the oxDNA software release, a dedicated platform (tacoxDNA) was recently developed that allows conversions between a variety of the commonly used file types to represent DNA nanostructures (such as protein data bank (PDB), standard xyz files, and files for programmes such as CanDo).³⁷ Simulations using oxDNA are nucleotide level coarse-grained models and the program remains, to this day, one of the most, if not the most, utilised models in the field at this level of detail. Other coarse-grained level models do exist, such as the 3SPN (3-sites-per-nucleotide) models released in 2007 and 2013.^{38,39} However, they do not appear to be used as routinely as oxDNA. For DNA nanotechnology systems, the number of papers reporting simulation results using oxDNA vastly outnumber any other nucleotide-level model.

If we consider oxDNA, a nucleotide-level coarse-graining molecular dynamics code, to be the middle point in terms of level of detail for modelling a system, then there exist two ends, one being all-atom MD models that include a very high level of detail, and the other being coarser models that use larger units than nucleotides as their fundamental elements. The justification of which method to use is not the aim of this chapter (see Chapter 2 for this discussion), the point here is to provide an overview of those models that are currently available.

Starting with the all-atom models, some well-known and frequently used pro-

grams are AMBER, CHARMM, and GROMACS.^{40–42} Commonly utilised in simulations of (large) biological systems, such as proteins, lipids and carbohydrates, the packages allow the implementation of different force fields. Whilst the newest of these packages was originally released in 2002, they have been developed, modified and have had features added to still keep them as the leaders of all-atom simulations, particularly for biological systems. These packages use simplified force fields rather than explicit calculations of every interaction within the molecules, and are chosen depending on the type of system they were designed to model, for instance, modelling small ionic particles would use different interactions compared to a protein in water.⁴³

The approach described above is often termed molecular mechanics (MM) but the best results for any calculation, given an unlimited, albeit unrealistic, source of time and computing resources, would be obtained using quantum mechanical methods (QM). Quantum mechanics calculations, however, are only suitable for relatively small molecules, as they are such in-depth and time-consuming calculations; therefore, it is not a suitable technique for large biological systems. An approach that aims to incorporate the best features of both type of calculation and that has become increasingly popular is QM/MM, first proposed in 1976 by Warshel and Levitt.⁴⁴ It allows the largely unchanging bulk of the biological system to be modelled using MM, the quicker method suitable for large systems, with the most important areas, where reactions are taking place, modelled using QM. Such a technique would work very well, for example, on enzymes, as they are proteins with an active site; this small area could be modelled using QM, but the bulk of the protein, which changes very little over time, is modelled using MM.⁴⁵ This provides a suitable midway point between efficiency and detail.

QM/MM is generally less utilised for modelling DNA as all DNA nucleotides generally behave in a similar way rather than having a subset that need to be treated significantly differently from the others. Furthermore, the interactions responsible for DNA self-assembly can be relatively well-described using inter-molecular force fields. As such, either a coarse-grained or all-atom approach is generally much more suitable, but, as will later be explained in Chapter 2 in more detail, the latter would be inefficient (in terms of time and resources needed) for DNA origami modelling, due to origami usually being very large structures with an exceedingly large number of particles.

With regards to the level of detail in a model, at the other end of the scale (the least detail included) are the programs that use models that are coarser than a nucleotide-level model, and this is achieved by grouping nucleotides together into larger groups to reduce the number of particles simulated. These provide a quicker way to calculate the overall geometry of a structure, without the need to do intensive calculation. There have been a number of releases of these types of packages in recent years, such as CanDo⁴⁶ and mrDNA.⁴⁷ These two packages work in a not too dissimilar way. CanDo approximates double helices as rods, with two-node beam finite elements representing base pairs to allow for rod elasticity. Crossovers from a caDNAno design are included as rigid connectors. A finite element analysis is run to relax the structure and the final outcome is determined by strains in the structure caused by crossovers.⁴⁶ This model has the benefit of a fast calculation but lacks finer detail that may be obtained through a more detailed model.

mrDNA approximates a DNA helix using a beaded method, where a number of

beads are used to represent the system instead of including all base pairs (initially 1 bead is approximately equal to 5 base pairs). A MD simulation can then be carried out to quickly determine an initial geometry of the system. mrDNA offers the benefit of being able to increase the resolution (fewer base pairs represented by a bead) for additional detail in the model.⁴⁷ It also provides output files to run a simulation in oxDNA, which provides a convenient and quick route to prepare a structure for simulation through this channel as well. It is, however, not straightforward to install and run mrDNA on a standard computer and is currently unsuitable for use on a cluster. In comparison, oxDNA is straightforward to install on most computers and clusters.

The final software packages to discuss here are structural design programs. The very recent release of MagicDNA allows users to more easily design complex DNA origami structures and provides oxDNA output files as default for later simulation.⁴⁸ One benefit of this tool is that the program provides libraries of parts, so structures can be built up straightforwardly in 3D, or they can be built by hand if desired. Another program called oxView also allows easy editing and modification of DNA structures, also with oxDNA output files, and, very conveniently, runs in a web browser so it can be used without needing to download or install any packages.^{49,50}

It is clear that both experiment and simulation have progressed rapidly over the 15 years since the inception of DNA origami and, with the ease with which modelling and simulation can now be carried out, it is not surprising that these approaches have now become widely used in the DNA nanotechnology community. Hopefully we will see this continue, along with the development of further resources.

1.4 Molecular Dynamics: General Principles

This section provides an introduction to molecular dynamics (MD). Details specific to oxDNA, such as interactions included in the model, can be found in Chapter 2; instead the purpose of this section is to briefly outline MD as a whole, how it originated and the general principles behind it.

The first molecular dynamics algorithm was published by Alder and Wainwright in 1957, with a second paper published two years later in 1959.^{51,52} There is one rather prominent line in the discussion of the 1959 paper that says, ‘The size of the system of molecules is limited by the memory capacity and speed of computing machines. With the best presently available computers, it has been possible to treat up to five hundred molecules.’ It is a relief that now we are able to run calculations of tens of thousand of particles in size with relative ease and on much longer time scales, but it is interesting that this limitation is still one that we also face today.

As for the discussion of QM/MM in the previous section, in an ideal world, quantum mechanical methods would be used to carry out all simulations, but this is completely impractical for system of any substantial size due to the computational time scales and memory requirements of such calculations. As such, significant quantities of research and development have gone into making programs that are capable of accurately representing the actions and workings of larger systems, so realistic models of them can be gained on reasonable timescales.

The idea behind MD is to model particle motion according to the classical equations of motion. The particles typically initially move in random directions with equal velocities, but as the simulation continues and collisions occur, their

individual velocities can change. It is easiest to think of this as a box filled with particles that can interact. The particles obey classical motion, so each particle that is moving has kinetic (its movement within the box) and potential (how it interacts with other particles) energy. One of the most well-known algorithms to integrate the equations of motion numerically is the velocity-Verlet (1982) algorithm,⁵³ which was a development on the Verlet algorithm (1967), originally described by Loup Verlet.⁵⁴ If the particles start in a particular position at time t , then the algorithm calculates their new positions a very short time later, $t + \delta t$.

This is carried out by evolving the equations of motion over a timestep split into two halves and two separate calculations:

$$v(t + \frac{1}{2}\delta t) = v(t) + \frac{1}{2}a(t)\delta t \quad (1.1)$$

$$r(t + \delta t) = r(t) + v(t + \frac{1}{2}\delta t)\delta t \quad (1.2)$$

$$v(t + \delta t) = v(t + \frac{1}{2}\delta t) + \frac{1}{2}a(t + \frac{1}{2}\delta t)\delta t \quad (1.3)$$

The first calculation uses a ‘half-kick’ and updates the velocity, the second step then recalculates the new positions of all the particles for a full timestep. Although not an individual equation, the next stage is to calculate the forces between the particles and this appears as the final term of Eqn 1.3, although this could also be done in the final step alongside the other equation components. The velocity for the whole timestep (a second $\frac{1}{2}\delta t$) is then calculated using these new forces and is added to the velocity calculated earlier, as shown in Eqn 1.3. Although this form is different to that originally published by Swope *et al.* in 1982, it is equivalent to that method and is easier to compute, as the forces are calculated in two separate steps, as opposed to both previously appearing in the same line.

This modified method means the previous force result can be overwritten, rather than both needing to be stored simultaneously.⁵⁵

This is the basic premise of molecular dynamic simulations and coding up the above equations will produce a simple microcanonical system for a set of interacting particles in a box. However, the algorithm can be made more sophisticated by including other effects, such as angular momentum for objects that can have position and orientation; any constraints, such as fixing bond lengths or angles, and the use of thermostats to control temperature (this feature is covered in Chapter 2).

1.5 Modelling DNA Origami Nanostructures

When DNA origami are produced experimentally, the current techniques to visualise them are often limited to transmission electron microscopy (TEM) and atomic force microscopy (AFM) methods; however, the resolution of images from using these techniques may not provide a clear and detailed view of the structure. Cryo-electron microscopy (cryo-EM), can give a more detailed picture by constructing a 3D image of a DNA origami from multiple images,¹⁵ but this is currently a more expensive, difficult, and time-consuming process, and so is not routinely used. Therefore, to overcome these issues and gather more information about the systems, it can be useful to use simulation to investigate structures further and in more detail than may be possible to obtain through experimental techniques.

As has been seen above, many software tools exist to model these kinds of systems, but this project aims to demonstrate why oxDNA is one of the most,

if not the most, suitable packages with which to perform these simulations and analysis. By exploring a variety of different DNA origami systems in this thesis we aim to demonstrate that oxDNA is able to capture subtle and diverse effects in DNA origami, and that additional analysis after simulation can be carried out to further investigate their actions and behaviours at equilibrium.

More specifically, we present three case-studies where the experimental work has been previously published (Chapters 3-5); an independent project where a theoretical design for an auxetic material has been simulated (Chapter 6), and collaborative work with an experimental group on their ‘barrel’ designs that are an extension to their work published in 2020 (Chapter 7). Each results chapter has its own introduction to provide an overview of the structure covered, as well as the relevant analysis, results, and conclusions, in addition to the reflective and overall conclusions at the end. Through these projects, we aim to demonstrate oxDNA’s suitability for modelling these systems, but in a wider sense, we hope to encourage those working in this area as well, especially experimentalists, to also utilise this software for their own research.

In addition to these results, we have recently contributed to a textbook chapter for *Methods in Molecular Biology* to help outline how to get started with running oxDNA simulations.⁵⁶ Through a combination of the procedures outlined in this chapter, its examples, and those presented here, along with their additional analysis techniques and the methods to do them, we hope this provides sufficient evidence to demonstrate that oxDNA is a suitable programme for modelling DNA origami, as well as providing a starting point for others to do the same.

Chapter 2

Methods

2.1 General Methods

2.1.1 oxDNA model

The initial oxDNA model was described in the work of Ouldridge *et al.* with further details outlined in Ouldridge’s DPhil thesis the year after.^{57,58} The code was originally released as open-source software in 2013; it was promptly followed by the release of an improved version of the model, which is often referred to as ‘oxDNA2’, in 2015.^{35,36} For the purposes of this thesis, we use the general term ‘oxDNA’ to refer to the oxDNA2 model as here we only use this version of the model. It shares mostly the same interactions with the original, but with three notable developments for the second release: the introduction of major and minor grooving, the introduction of salt dependency (through the screening of electrostatic interactions), so simulations could be run at ‘high’ or ‘low’ salt concentrations, and fine-tuning of the DNA pitch and the twist angle at nicks and junctions so that DNA origami structure could be better modelled.

oxDNA is a nucleotide-level coarse grained model, where each nucleotide is

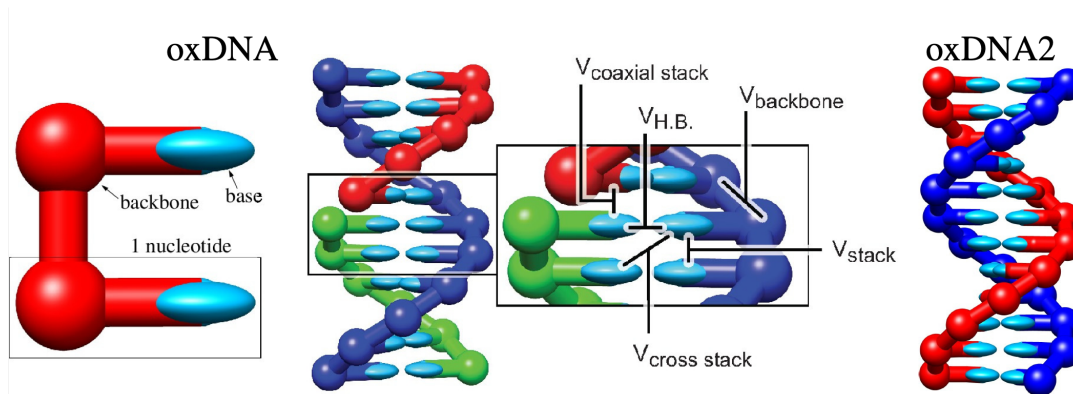


Figure 2.1: The oxDNA model, including an example pair of nucleotides (left); the original oxDNA model (centre, left) and a diagram showing five of the seven main interactions (centre, right). The original model did not have major and minor grooving but this was introduced in oxDNA2 (right) as well as salt dependency. Left-hand 3 images reprinted from Schreck *et al.*, *J. Chem. Phys.*, 2015, **142**, 165101, with the permission of AIP publishing.⁵⁹ Right-hand image reprinted from Snodin *et al.*, *J. Chem. Phys.*, 2015, **142**, 234901, with the permission of AIP publishing.³⁶

considered to be rigid and is modelled as a base attached to a backbone site. There are seven main interactions in the model, five of which are shown in Figure 2.1 and the additional two are an excluded volume and an electrostatics term.

The interactions in more detail are:

1. Excluded volume (not in Figure): This repulsion is described by a cut-and-shifted Lennard-Jones potential. Whereas a Lennard-Jones potential usually has an asymptotic tapering-off attraction potential as $r \rightarrow \infty$, the cut-and-shifted version sets a distance limit between particles for an interaction, and from that distance onward considers there to be no interaction (by moving the potential curve up until the cut-off tail reaches the r axis). One of its effects is to prevent DNA strand backbones from passing through each other.
2. Backbone potential (V_{backbone}): the model uses a finitely extensible nonlinear

elastic (FENE) potential, where the backbone is modelled like a polymer chain of beads. Consequently, there are no distinct sugar and phosphate groups and, instead, they are grouped into one site. However, this potential represents the lengths between backbone sites as springs and allows for backbone extension and rotation, thereby representing the covalent bonds found in DNA backbones.⁵⁸ This approximation has the advantage of reducing computation times compared to models with a more detailed description of the backbone, whilst it was found that most properties of interest, such as the aforementioned backbone extensibility and rotation, could still be accurately modelled.

3. Both the hydrogen bonding (V_{HB}) and stacking between bases (also known as pi-stacking, V_{stack}) on a continuous length of DNA (i.e., no nicks in the backbone) are modelled with a smoothly cut-off Morse potential.⁵⁸ This potential provides a maximum distance for particles to interact, and the smoothing function on either side of the potential allows for continuity.
4. The cross-stacking (interactions between a base and its hydrogen-bonded pair's neighbour, $V_{cross\ stack}$) and coaxial stacking (pi-stacking of bases across a nick in the backbone, $V_{coaxial\ stack}$) interactions are modelled with a harmonic potential.⁵⁸
5. Electrostatics (not in Figure): this term represents the repulsive electrostatic interaction between the phosphates and is modelled using a potential of Debye-Hückel form. This modification introduces a screening term to the electrostatic potentials. This term, which was introduced in the oxDNA2 version of the model, gives rise to an explicit dependence of the model on

the salt concentration.³⁶

The overall potential for the model is given by the following combination of these interactions:

$$V_{oxDNA} = \sum_{\langle ij \rangle} (V_{backbone} + V_{stack} + V'_{exc}) \\ + \sum_{i,j \notin \langle ij \rangle} (V_{HB} + V_{cross\ stack} + V_{exc} + V_{coaxial\ stack}),$$

where V_{exc} and V'_{exc} represents excluded volume, and all others have the same meanings as before. The first term in the equation represents interactions between pairs of nucleotides that are adjacent along the backbone, and the second over all other pairs.^{35,58}

There are many other packages available to model biomolecular/DNA-type systems but mainly at either a higher or lower level of coarse-graining than that presented here. However, at this level of detail, oxDNA remains the dominant software in the field. All-atom models are provided by force-fields such as AMBER or CHARMM; however, for the system sizes considered in this thesis, all-atom simulations would take an unreasonable length of time to perform.^{40,60} A second option would be to use a more coarse-grained model, such as mrDNA, or CanDo, but doing so would require the grouping together of bases to make new, larger sites.^{46,47,61} This would mean that features such as the stacking and unstacking of pairs of bases and the breaking of base pairs would be lost, but these are often a feature of interest in origamis, such as when determining their structural properties. Examples of this might include the stacking/unstacking of Holliday junctions, or the structural changes caused by the breaking of base pairs. For

the system sizes studied in this work, oxDNA offers the best balance between sufficient detail to determine the properties of interest, without requiring excessive time scales for simulation and, as such, is the most appropriate choice of model.

2.1.2 General Protocol: a 2D design into oxDNA form

To begin a simulation of an origami, it is first necessary to take the design, often from experimentalists, and convert it into a form suitable for use in oxDNA. For demonstration purposes, a single barrel structure (the related work is covered in Chapter 7) will be used to illustrate the process of simulating a structure, starting with a 2D design up to the point of simulation. One of the most popular resources for designing origami is software called caDNAno, where the structure is a 2D drawn-out design (Figure 2.2).¹⁷ In this figure, the cylindrical barrel has been ‘cut open’, with the adjacent sides connected by elongated ‘long bonds’ that will need to be reduced to an appropriate size later in the relaxation process. The first step toward a simulation is to convert this into oxDNA form (using inbuilt scripts, or the conversion platform tacoxDNA³⁷) and produce outputs in “oxDNA” format that are viewable in a 3D visualization program such as cogli2 or oxView.^{37,49,50} The output of this conversion creates two files, one topology and one configuration file. The topology file provides information about how nucleotides are topologically arranged with respect to each other (i.e., it can be used to identify neighbours to a base, and also identifies to which base of A, C, G and T it corresponds), and the configuration file gives a variety of information, such as box size, and centre-of-mass coordinates and orientation of each nucleotide, among other information.

Once these files have been obtained, the next step is to carry out a minimisation

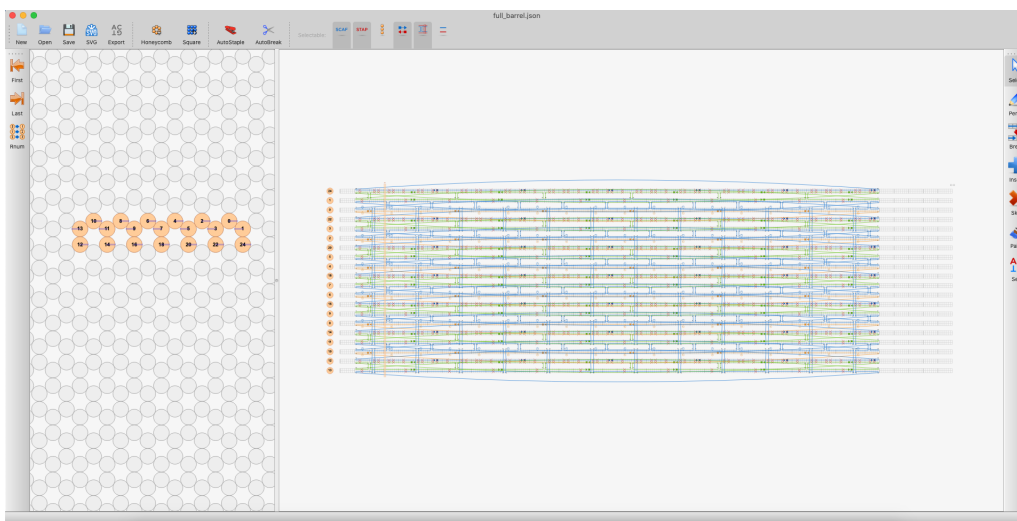


Figure 2.2: The caDNAno design of a single unit barrel structure, originally designed by Wickham *et al.*⁶²

of the configuration's energy. This involves using a modified version of the oxDNA backbone potential that prevents the forces from long bonds from breaking the origami's structure.⁵⁶ The minimisation is done to ensure an excluded volume for each nucleotide is maintained and prevents any two nucleotides from occupying the same region of space (the large forces that result from particle overlaps would otherwise cause a simulation to fail). Visually, it can be difficult to see changes in this step and the result often appears similar to the starting conformation, but monitoring the energy and looking for a plateau is the best way of determining when this has been carried out sufficiently. Additionally, the next relaxation step will often fail in the first few steps if a minimisation is insufficient, so it is important that this step is carried out. Figure 2.3 shows the output from converting and minimising the example structure. It also shows the long bonds that will need to be reduced through the relaxation process.

To reduce the length of these long bonds and create a sensible-looking structure

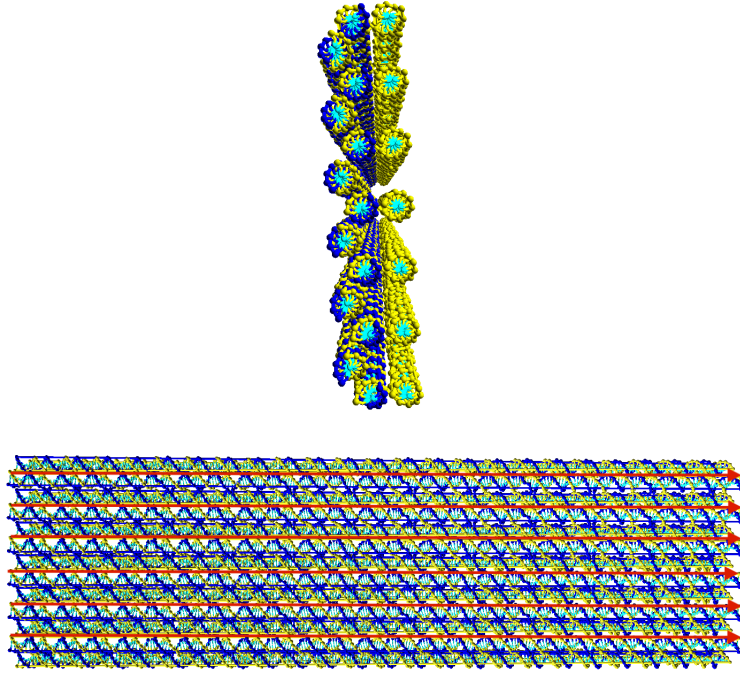


Figure 2.3: The minimised single unit barrel structure, shown from two directions, end-on (left) and side-on (right). Note that red arrows have been overlaid on some of the long bonds to highlight this structural element.

ready to be simulated, a relaxation step is required. This is carried out using a simulation but with a modified backbone potential that prevents the large forces associated with the long bonds from breaking (or ‘exploding’) the origami (as would happen if a standard MD simulation was run with the long bonds present), and therefore allows them to reduce in size.⁵⁶ Carrying out these relaxations (usually requiring around 10^6 steps but, depending on structure, can require more) is a quick process and some of the characteristics of a fully relaxed structure are:

1. Plateaued energy. If the energy has remained constant for a large proportion of the relaxation and continues to the end, this can often be the main indicator of a completed relaxation as further simulation is not noticeably

further relaxing the structure.

2. Backbone distances. Since the relaxation is largely carried out to reduce long bonds, it follows that if these have been removed then this step has been a success.
3. Sensible structure. Given that the designs are often from experimental work, the desired structure is usually known. So if the structure looks similar at the end of relaxation, it is likely the structure has been correctly simulated. Before simulating, however, it is important to ensure there are no topological entanglements present. This can occur during the relaxation of long bonds and can lead to, for example, helices passing through each other and creates an unrealistic representation of the structure. Although areas of entanglement can sometimes be challenging to identify, it is important to check the relaxed structure thoroughly as they can have an impact on the motion and behaviour of an origami. (An interesting question is whether any topological entanglements might be present in experimental DNA origamis due to self-assembly errors. However, this question is beyond the scope of this thesis, and here we always simulate DNA origamis that have the envisaged perfectly-assembled structure.)

Figure 2.4 shows the result from relaxing this example barrel structure partway through (10^5 steps) and at the end after 10^6 steps. The snapshot partway through demonstrates the long-bond problem and also highlights how the structure has moved out of the ‘2D’ shape into a ‘3D’ structure. Once these preparatory steps have been completed, all that is left to do is to simulate the structure.

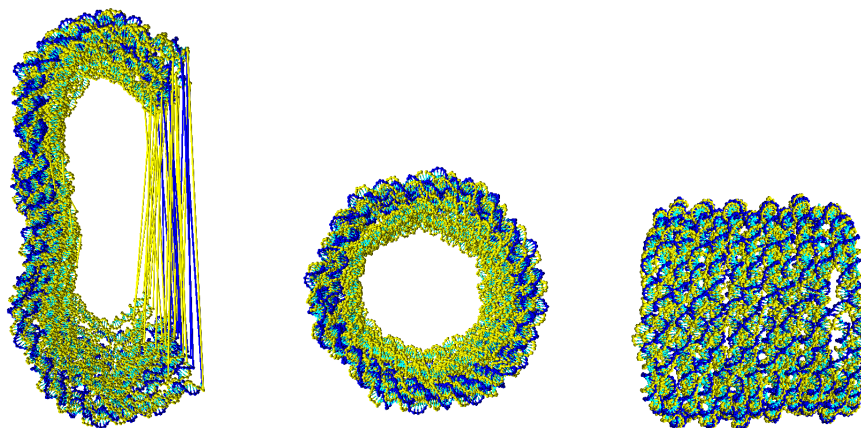


Figure 2.4: Images showing the relaxation of the example barrel structure, partway (10^5 steps) through (left) and the final structure (end and side on views) when relaxation was complete (10^6 steps, centre and right).

2.1.3 Simulations

As minimisations are very short (usually ~ 2000 steps) these can easily be run locally on just a CPU within a few minutes. For the longer processes of relaxation and simulation, it is essential to use GPU capabilities if available, as for large system sizes such as those in this work, running on a CPU would lead to unreasonably long time scales for simulations. This is also the reason why these simulations used Molecular Dynamics (MD) rather than Monte Carlo (MC). It would be feasible to use MC to model these systems, but this approach is currently not available to be run on GPU for oxDNA, whereas MD is — due to the greater ease and efficiency with which MD algorithms can be parallelised.⁶³ As outlined earlier, oxDNA is the most appropriate software to model these systems and for time efficiency, MD needs to be used over MC.

All simulations in this thesis were conducted at 300 K as this is standard oxDNA protocol. Simulations can be carried out at a cooler temperature or standard lab-

oratory conditions if desired, but this minor change in temperature will have minimal impact on the simulation outcome. Salt concentrations for all relaxations was 1.0 M, but some of the work involved using different concentrations for the main simulation stage, either 0.1 M, 0.2 M, or 1.0 M, and will be specified in each chapter where appropriate. The model is parameterised to reproduce the hybridization thermodynamics of DNA in NaCl; however, when using 1.0 M this is approximately equivalent to experimental conditions (12.5 mM MgCl₂).⁵⁶ Simulations were usually run for lengths between 5×10^7 and 1×10^8 steps and, again, will be specified for each structure. Determining an appropriate simulation length depends a lot on the structure, as enough time needs to be provided to ensure all conformations are explored and no energetic or significant structural changes are left unexplored. Generally for the more rigid structures, like the barrels, the times were shorter as there were no significant energetic or structural changes, and for the more flexible structures more time was needed. Generally, if a property can be measured (e.g., an angle) as well as the energy, this could help to guide whether additional time was required. If the property reached a plateau, or the distribution was considered to have been well-sampled, it was decided that the structure was equilibrated.

A brief step-by-step protocol from installation to running an oxDNA simulation can be found in Appendix A. All minimisations were run on local computer CPU, relaxations and simulations were run on a mix of cluster CPU and GPU, but mostly the latter.

2.1.4 Thermostats

Two thermostats were used in this project, a Langevin-type and a strongly-coupled Andersen thermostat, both of which generate diffusive motion of the nucleotides. These are appropriate thermostats for this project as they are both commonly utilised when modelling DNA or biological systems. A brief discussion of solvent effects is initially needed, however, to introduce the thermostat choices. To hydrate an origami would require a staggering number of water molecules. As such, to maintain reasonable calculation times, it is necessary to use an implicit solvent method instead of simulating every individual water molecule. As such, the oxDNA model is parameterised to reproduce the action, behaviour and interaction of DNA in water, thereby negating the need for simulating water.

The thermostats themselves are primarily utilised to allow sampling from the canonical ensemble (whereby a system has a constant number of particles, N , a constant volume, V , and a constant temperature, T) and the idea to achieve this by utilising a thermal bath was first proposed in 1984.⁶⁴ In other words, it acts to maintain a constant temperature. When using an Andersen-type thermostat, particles can randomly have their velocities refreshed by “collisions” with the heat bath, and by choosing the refreshed velocities from a Maxwell-Boltzmann distribution, such a method allows an approximate temperature to be maintained. The Langevin thermostat (usually used for relaxation processes), however, is slightly more sophisticated than thermostats that only resample particle velocities, as it is comprised of two components, a random force that inputs energy into the system and a ‘frictional drag force’ that damps the particles’ motion.⁶⁵ This action is particularly useful for modelling biological molecules, as the velocity and fric-

tional force terms can help to replicate the interaction that large molecules have with water and negates the need to model solvent explicitly.⁵⁵ For oxDNA, this thermostat helps to model the solvent by modelling the dynamics of the particles as though they are interacting with solvent molecules.³⁵

Overall, thermostats are primarily used to maintain temperature of a simulation, but their action in determining velocities of particles lends itself exceedingly well to helping to model implicit solvent, thereby solving two problems at once.

For this project, a Langevin thermostat was normally used for relaxations and a Andersen-type for simulations. These were found to be the most suitable thermostats for each process when the code was originally developed and tested. In some cases, a “Bussi” thermostat was used for relaxations since it dissipates excess kinetic energy more quickly, and has the advantage that it can often relax a structure into a lower potential energy configuration suitable for a simulation more quickly.^{56,66} This option was not routinely available in oxDNA until relatively recently, hence why it was not used from the beginning of the project.

2.1.5 Other relevant parameters

As mentioned, the release of oxDNA2 allowed a choice of salt concentration. Whereas in the original model the salt concentration always has a fixed value of 0.5 M, which was a sensible choice since most experimental work is done in a fairly high salt regime, the newer model allows the simulation of structures at any salt concentration required.³⁵ This term was introduced as an electrostatic potential of Debye-Hückel form and so depends on the ionic strength.³⁶ This is particularly pertinent for any structures with Holliday junctions that are known

to be highly sensitive to changes in salt concentrations.⁶⁷

Also relevant to Holliday junctions (covered in more detail in Chapter 5) are coaxial stacking energies. Holliday junctions can exist in a closed (stacked) or open (unstacked) configuration and engage in coaxial stacking, switching between the two states depending on salt concentration. A coaxial stack, as discussed above, is pi-stacking across a gap in the backbone. It can be useful to measure the magnitude of the stacking energy, particularly when looking at high and low salt concentration regimes. This can be done using an in-built feature in the oxDNA simulation code, and so was not calculated post-simulation. Other parameters can be measured as well, such as cross-stacking, but only coaxial stacking was needed for this project. All other data (angles, distances, etc.) was calculated post-simulation and will be detailed in the results chapters as and when needed.

Chapter 3

Möbius Strip

3.1 Introduction

3.1.1 Experimental Design, and Simulation and Analysis Details

The Möbius strip appears rarely, if ever, in nature and so its creation is credited to two mathematicians who independently discovered it in 1858; however, it is named after only one of them: August Ferdinand Möbius.^{68,69} What makes this shape unique is that, despite being a three-dimensional object, it has only one side. Tracing around a paper strip with a pencil will allow a return to the starting point without any crossing over of the drawn line, or missing any of the ‘sides’ out. Appearing regularly as a staple topic in science (and popular culture), the Möbius strip has appeared in hundreds of journal articles across science and mathematics. Examples include the formation of Möbius strips from a ribbon-style compound of niobium and selenium;⁷⁰ a whole area of organic chemistry called ‘Möbius aromaticity’, which is the investigation into the phenomenon of some antiaromatic structures that can gain energetic stability by twisting their π orbitals to form

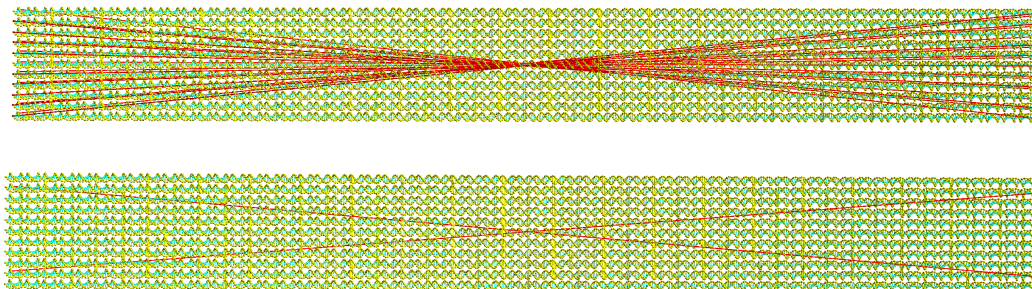


Figure 3.1: The Möbius strip design with all crossovers shown (top) and with only two connections to highlight the nature of the design (bottom). Approximate dimensions of the design are 212 nm in length and 28 nm in width (details of the calculation for these values can be found in section 3.2.2).

a Möbius topology,⁷¹ and Möbius strips have even been created from the manipulation of light beams.⁷² As such, it is unsurprising that researchers in DNA nanotechnology would also be interested in creating an equivalent, and this was done by Han *et al.* in 2010.⁷³

The design of the Rothemund tile, previously discussed in the Introduction, provided the starting point for this experimental work, as the Möbius strip was based on the same design of repeating units with crossovers placed to hold the structure together.¹³ Using the caDNAno design for these experimental structures, provided by the group, it was possible to generate suitable input files in oxDNA format in the form of a flat tile.^{17,73} Figure 3.1 shows the original design of the Möbius strip (yellow) and the connections (red) of how it joins together, alongside a version with only two connections to highlight the nature of the design. It consists of 11 adjacent helices and contains 14 432 nucleotides (where 50% are scaffold nucleotides and the rest correspond to staples). In the caDNAno design there are 18 long bonds that connect the helices from one end of the strip to the other, but these are the same helices with the ends situated opposite each other

about the centreline of the strip. The lower image in Figure 3.1 clearly shows this method of connection, where one corner is connected to its diagonal opposite, and this design was necessary to introduce the 180° twist that generates the classic Möbius strip shape.

The design started flat but would become curved when the long bonds forced the bending of the ends towards each other during the relaxation process. It follows that this design, where either long bond could force closure of one corner before the other (depending on which long bond shrunk sufficiently first), would lead to two potential isomers being formed during simulation. These two isomers are the equivalent of making a paper version by twisting one end of the strip clockwise (we have termed this isomer ‘left-handed’) or anti-clockwise (‘right-handed’) and both versions are likely to also have appeared during the self-assembly process. From this point we will use the terms ‘right-handed’ or ‘left-handed’ to describe the structures. Experimental TEM and AFM images are shown in Figure 3.2 and, although appearing as 2D due to their interaction with the surface, the correct overall shape can be clearly seen. Both isomers were reported as having been produced, determined via the generation of AFM amplitude images from tracing and retracing the structures.⁷³

However, relaxing a structure with such connections can very easily lead to it becoming entangled, which is where helices are pulled through each other leading to an unrealistic structure that is topologically trapped. To negate this issue, the design with only two connections was used as a starting point to allow introduction of the twist, and the other bonds would be restored when entanglement was no longer a concern. To help with the relaxation process, gentle forces were also applied to each corner and provided a small pulling force to help create an initial

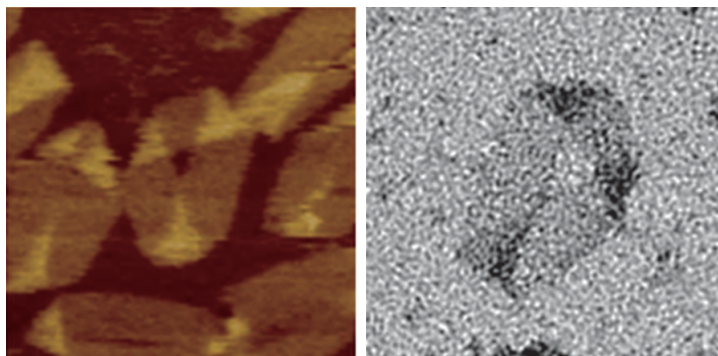


Figure 3.2: AFM and TEM images of the synthesised Möbius strips. For imaging, the structures have been adhered to a surface and it is easy to see the characteristic folds in the ‘corners’ which could be achieved by flattening a paper equivalent. Images reproduced with permission from Springer Nature, Han *et al.*⁷³

curve in the tile. The flat tile was initially aligned within the xy plane and gentle forces (~ 2.4 pN) were added in the z -direction to introduce this bending, the result of which can be seen in Figure 3.3. The forces were only intended to add a bending element to the strip, but it is clear some twisting is already present partway through the relaxation process.

The first isomer produced through this process was the ‘right-handed’ isomer (and an equivalent could be formed with paper by taking a strip of paper, rotating one end anti-clockwise by 180° and joining the ends). During the production of this first isomer, a new software resource became available (mrDNA)⁴⁷ and thus it was possible to produce a more sensible starting structure for the left-handed isomer much more quickly than had been possible for the other. This is because mrDNA is capable of carrying out a rapid geometric analysis of the structure to produce a sensible conformation for an oxDNA simulation. The mrDNA step was carried out by Joakim Bohlin, but the structure underwent the usual relaxation procedure afterwards to prepare it for an oxDNA simulation.

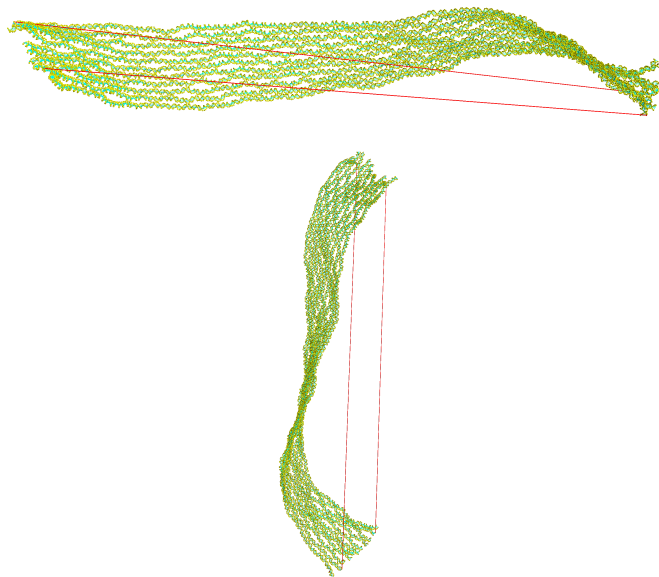


Figure 3.3: The Möbius strip bending due to gentle applied forces during the relaxation process as seen from the side (top) and top-down (bottom). The colour scheme has been maintained as before to highlight the long bonds.

Once both isomers had been fully relaxed, they were simulated for 10^8 steps, at 300 K and 1.0 M salt solution. 10^8 steps were used to ensure each Möbius strip had sufficient time to allow for any thermal fluctuations. Throughout this time, neither structure presented any significant changes in energy or global structure, including a lack of movement of the positions of maximum curvature around the strip (Appendix B). If the Möbius strip did not have a 180° twist in it then a cylinder would have been formed with constant curvature at any point around it. Instead, the twist produces a structure where the curvature varies upon traversing around the Möbius strip. From measuring this across all trajectory frames it was found that, although the curvature value changed depending on position, the measurements at each position did not significantly change as the simulation continued, and this was found to be true for both isomers (Appendix B). Note

that as the strip is uniform in design (i.e. crossovers are in a consistent repeating pattern) the positions of maximum curvature could in principle occur at any point on the strip, however the time scales for the diffusion of the global pattern of twist and curvature along the strip are significantly longer than our simulation times. As such, it was appropriate to generate an average structure for each isomer, and these, along with the visual results and associated analysis, are presented in the results section of this chapter.

3.1.2 Generating Average Structures

An average structure takes all frames of a trajectory into account and generates an average position and orientation for each nucleotide, thereby reducing the structural noise caused by thermal fluctuations. The script used for this was from the oxView package. It works by taking a random frame from the trajectory file and using this as a reference; the associated paper states that the choice of frame has little impact on the mean structure. Each frame in turn is then orientated and superimposed on top of the reference frame in order to minimise the root mean square deviation. When this has been done for all frames, the average positions are taken by summing all positions for particles and dividing them by the number of frames.^{49,50}

Making an average structure is not always a suitable step. If a structure is very flexible and fluctuations cause very different conformations to be adopted over time, then a mean structure likely would not be an accurate representation of the system. For the Möbius strips, the curvature was measured and used to determine how much the structure fluctuated. As will be explained in more detail

in the results section, but was outlined in the previous section, both isomers were found to be sufficiently static that averaging them was an appropriate step.

3.1.3 Details of Analysis Calculations

In order to analyse the structure of each Möbius strip, the curvature, twist, and writhe were calculated. Curvature is, unsurprisingly, a property of a curve and since each Möbius strip was constructed from 11 adjacent helices the central one was used as a centreline. Twist is a property of a ribbon, and using each pair of helices out from the centreline gave five ribbons of increasing width.

Curvature, κ , takes the definition shown below, and is a standard result from the Frenet-Serret relations:⁷⁴

$$\kappa \hat{\mathbf{N}}_{FS} = \frac{d\hat{\mathbf{T}}}{ds} \quad (3.1)$$

$$\kappa = \left| \frac{d\hat{\mathbf{T}}}{ds} \right| \quad (3.2)$$

$$\kappa = \frac{\left| \frac{d\mathbf{r}}{ds} \times \frac{d^2\mathbf{r}}{ds^2} \right|}{\left| \frac{d\mathbf{r}}{ds} \right|^3} \quad (3.3)$$

where $\hat{\mathbf{T}}$ is the tangent to the curve, $\hat{\mathbf{N}}_{FS}$ is the normal and s is the arclength; Eqn. 3.3 is a standard result and a schematic for these quantities can be seen in Figure 3.4. It is routinely used for calculating curvature and is in the pre-existing oxDNA script package that was used to carry out these calculations.^{75a}

$\hat{\mathbf{N}}_{FS}$ can be used to define a Frenet ribbon associated with a curve and hence the twist. More natural in our case is to use the helices either side of the central line of the Möbius strip to define the ribbon and its normal. We first define the

^aThanks to Chak Kui Wong for updating and adapting this script.

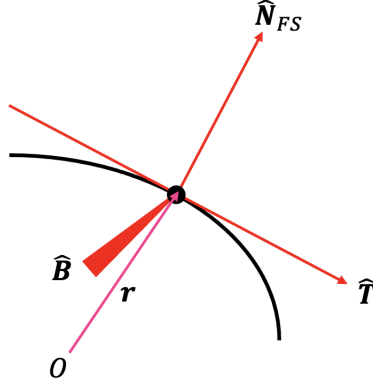


Figure 3.4: A depiction of a curve, with the tangent ($\hat{T}$, in plane of page), the corresponding normal ($\hat{N}_{FS}$, also in plane of page), the binormal ($\hat{B}$, perpendicular to plane of page indicated by a wedge in the schematic), and the vector $\mathbf{r}$ labelled.

binormal as

$$\hat{\mathbf{B}} = \frac{\mathbf{X}_n(s) - \mathbf{X}_{-n}(s)}{|\mathbf{X}_n(s) - \mathbf{X}_{-n}(s)|} \quad (3.4)$$

where $\mathbf{X}_n$ is the centre of the helix n helices out from the central helix and

$$\hat{\mathbf{N}} = \hat{\mathbf{B}} \times \hat{\mathbf{T}}. \quad (3.5)$$

This definition will be used later on in the twist and writhe calculations.

A schematic of the quantities required to calculate the binormal ($\hat{\mathbf{B}}$) are shown in Figure 3.5.

Calculating the curvature had another use in this project: it provided an insight into any movement of points of maximum curvature around each Möbius strip. For a macroscopic equivalent, such as a paper Möbius strip, the curvature would be expected to remain unchanging at all locations around the curve, and therefore not fluctuate in time. However, a DNA origami is subject to significant thermal

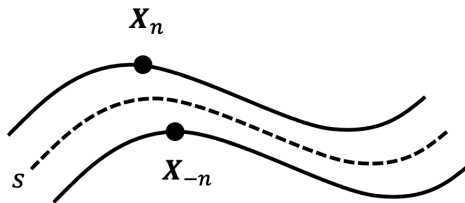


Figure 3.5: A depiction of a ribbon formed of two helices (solid lines) and a central helix (dashed line, also the line depicting s). The points X_n and X_{-n} correspond to points on helices n helices out from the centreline, some distance along s .

motion. As such it could be possible that, for a sufficiently long simulation, the value of curvature at each point around the strip could change. This could be thought of as diffusion of the points of maximum curvature, or ‘twist’, in each isomer. Since the curvature was measured from the same starting location, any change in curvature would have translated directly into a different curvature profile and it was found that neither Möbius strip had a significant change of profile, so the twist was not diffusing around. As such, it was appropriate to calculate an average structure for each isomer. Further details on this finding are provided in the results section.

There were no scripts in the oxDNA package to calculate twist and writhe, but Michael Selby, also in the Doye group, was working on a similar DNA problem so we used his code to carry out similar calculations on the Möbius strip structures. Twist (Tw) and writhe (Wr) are related by the formula $Tw + Wr = Lk$.⁷⁶ Lk is the linking number and in the case of a Möbius strip takes the value of $\pm\frac{1}{2}$, depending on the isomer. As such, provided the Lk remains constant, it is possible for the values of twist and writhe to vary.

Writhe can be quantified as a measure of how much a curve crosses over itself.

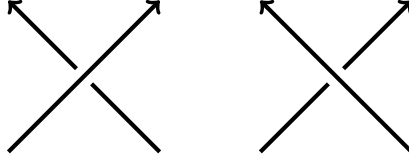


Figure 3.6: The sign of the writhe associated with the crossings of curves, +1 on the left and -1 on the right.

For example, when tying a simple knot it is first necessary to cross one end of the ribbon over itself, thereby introducing writhe to the system. Writhe takes a positive or negative sign, depending on which end of the ribbon crosses the other, and this is defined as shown in Figure 3.6.

Calculating such a quantity for a ribbon, such as the DNA origami Möbius strip, can be carried out using Eqn. 3.6:^{77,78}

$$Wr = \frac{1}{4\pi} \int \int (\hat{\mathbf{T}}_s \times \hat{\mathbf{T}}_{s'}) \cdot \frac{\mathbf{X}(s) - \mathbf{X}(s')}{|\mathbf{X}(s) - \mathbf{X}(s')|^3} ds ds' \quad (3.6)$$

where $\mathbf{X}$ represents a point on the ribbon.

The twist of a ribbon can be calculated using the formula:^{77,79,80}

$$Tw = \frac{1}{2\pi} \int \left(\frac{d\hat{\mathbf{N}}}{ds} \times \hat{\mathbf{N}} \right) \cdot \frac{d\mathbf{X}}{ds} ds \quad (3.7)$$

The process to calculate twist and writhe for these Möbius ribbons involved fitting a cubic spline around each pair of helices forming a Möbius ribbon and then approximating the integrals in Eqns. 3.6 and 3.7 by sums to calculate the twist and writhe, using the definitions in Eqns. 3.4 and 3.5 for the twist calculation.

Namely,

$$Wr = \frac{1}{4\pi} \sum_{i=1}^n \sum_{j=1}^n \left(\hat{\mathbf{T}}_i \times \hat{\mathbf{T}}_j \right) \cdot \frac{\mathbf{X}_i - \mathbf{X}_j}{|\mathbf{X}_i - \mathbf{X}_j|^3} \Delta s^2 \quad (3.8)$$

$$Tw = \frac{1}{2\pi} \sum_{i=1}^n \left(\frac{d\mathbf{N}_i}{ds} \times \mathbf{N}_i \right) \cdot \frac{d\mathbf{X}_i}{ds} \Delta s \quad (3.9)$$

where the fitted spline is split into n points separated by a distance Δs . The values calculated from each equation were then added together to determine the linking number for each Möbius strip.^b

The final analysis technique was to produce centreline projections using the same centreline as for the curvature calculations. This involved removing one of each of the three dimensions (x, y, z) at a time from each centreline and projecting the resultant coordinates onto a 2D plane. The final plots are the coordinates of the centrelines of each isomer with all three dimensions still included and, as such, appears 3D.

3.2 Results and Discussion

3.2.1 General Simulation Outcomes

The first isomer produced was the right-handed one. As mentioned previously, this means that if a paper equivalent were to be made, it would involve an anti-clockwise twist before joining the ends. The left-handed version is, therefore, the opposite and a length of paper would require a 180° clockwise rotation to create a paper equivalent. The simulation was carried out under standard conditions, such

^bThanks to Michael Selby for help and adapting his code for this project.

as 300 K and 1 M NaCl. A resulting representative structure is shown in Figure 3.7 (top). As expected, the Möbius strip takes the classic and well-known form, as would also be produced from a paper equivalent. The edges are not perfectly smooth and instead show fluctuations, but this is to be expected since DNA is an extensible material whereas paper is not, and the effect of thermal fluctuations is negligible on the macroscopic scale.

Whilst the second isomer was expected to be the same shape but opposite in handedness, at the end of the simulation an unexpected result was obtained and is also shown in Figure 3.7 (bottom). Instead of adopting a shape comparable to the first isomer, this one had instead buckled more and formed a structure more similar to a figure of eight. It was postulated that this result was due to a different balance between twist and writhe.

Visually, the left-handed isomer has more writhe than the right-handed version, since the structure could clearly be seen to cross over itself in the middle. This crossing-over is much less defined in the right-handed structure and, instead, the twist is more visible. It was therefore proposed that when the twist and writhe were measured it was likely the left-handed isomer would have more writhe and the right-handed more twist. This was found to be the case and the numerical data is shown in Section 3.2.3, along with further discussion on this topic.

Figure 3.8 shows the result from taking an average structure for each of the Möbius strips. It is not always appropriate to create an average structure, but since the measured curvature every 500 000 steps showed minimal diffusion of the positions of maximum curvature for both isomers, these structures were considered suitably static and an average structure of each would remain as accurate representations of the systems. This process had the added benefit of providing a

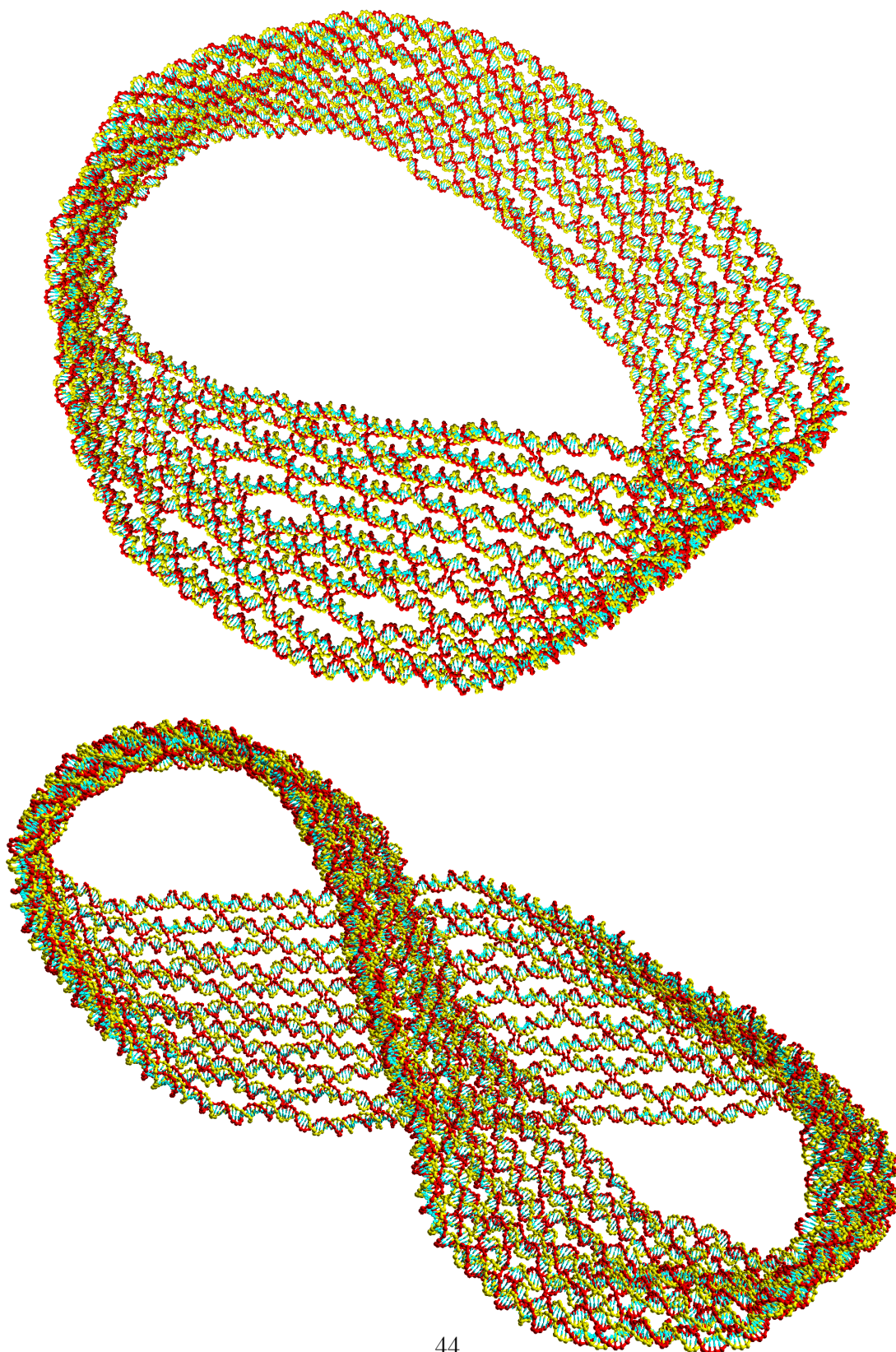


Figure 3.7: A representative equilibrated structure for the right-handed (top) and left-handed (bottom) Möbius strips.

smoother structure with which to measure the other quantities of interest, twist and writhe, and to make the centreline projections. The averaging script used was from the oxView analysis tools package.^{49,50}

A final note on the general structure of each Möbius strip is related to persistence length. The contour length of each structure was calculated to be 215 nm for the right-handed isomer and 209 nm for the left-handed (details of how these lengths were calculated can be found in the next section, 3.2.2 Curvature, where these values are also used). As the persistence length of DNA is reportedly 50 nm,⁸¹ the length of these structures well exceeds the limit where DNA would retain maximum stiffness, allowing each structure to be more flexible and able to conform into these shapes.

3.2.2 Curvature

In 2007, Starostin and Heijden published a paper about the properties of a Möbius strip.⁸² They looked at six Möbius strips all of length 2π and formed of a developable (no Gaussian curvature)⁸² surface, but with varying widths.

A property they measured that is of interest to our work was curvature, and their results are shown in Figure 3.9. Each Möbius strip (plus one corresponding to width = 0) has a line on the profile. Although the profiles are far from identical they share common features, such as:

1. symmetry around the minimum at π ,
2. peaks either side of the minima (regions of highest curvature), and these are in approximately the same locations for the strips with smallest width (magenta, red, green, blue),

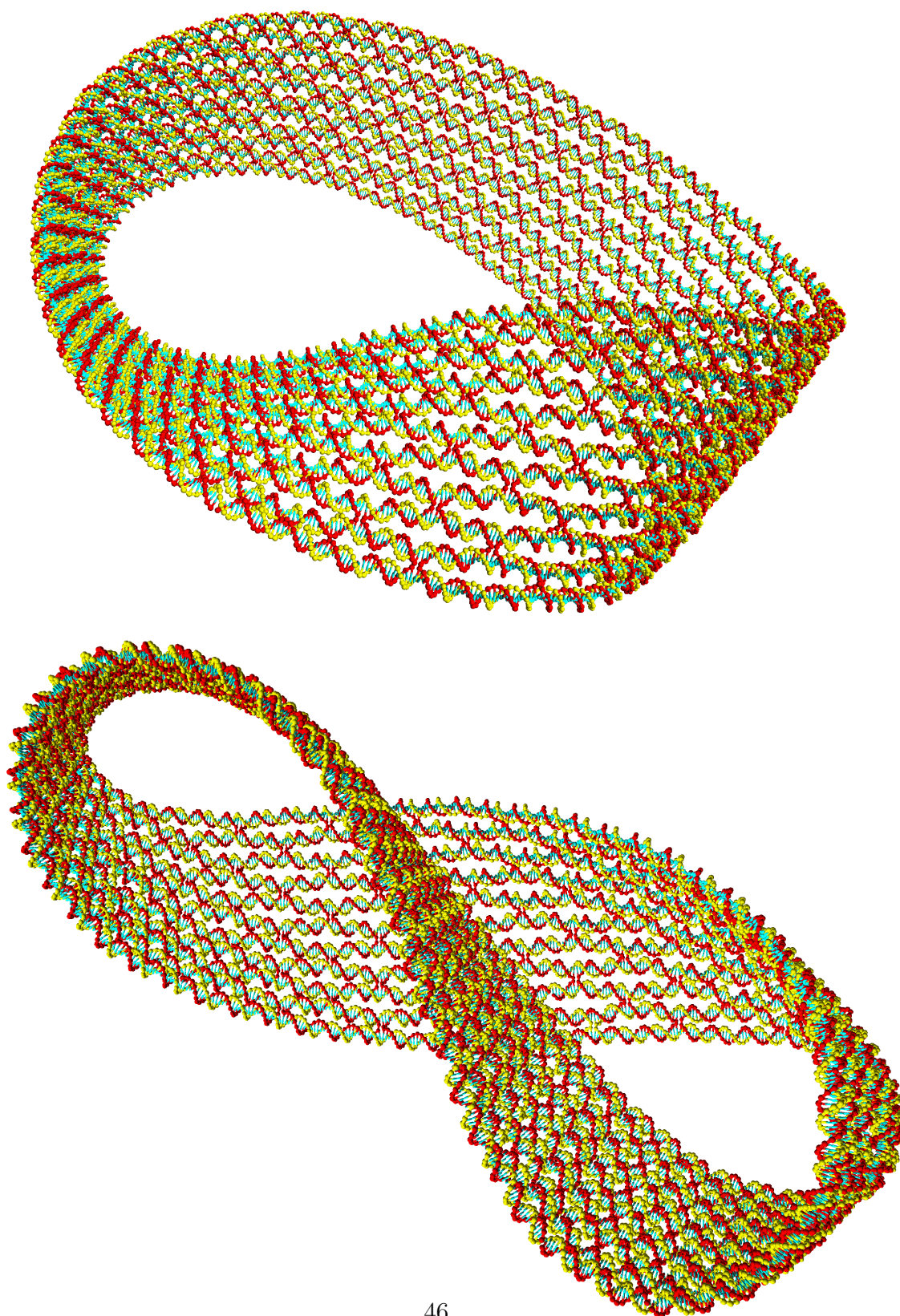


Figure 3.8: The average structure for the right-handed (top) and left-handed (bottom) Möbius strips.

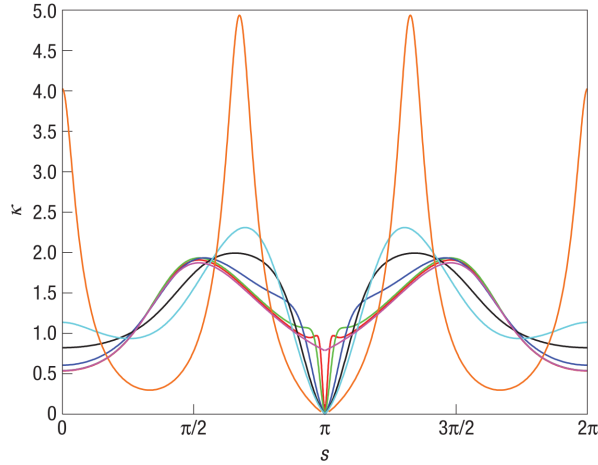


Figure 3.9: Curvature profiles for six Möbius strips of increasing width. The profiles in Möbius strip size order are: magenta (smallest width corresponding to 0), red, green, blue, black, cyan, orange (largest width). Image reproduced with permission from Springer Nature, Starostin and Heijden.⁸²

3. in most cases a second, shallower, minimum on the edge of the plot, corresponding to a second region of low curvature.

In order to compare these curvatures to the DNA versions, it was necessary to determine which Möbius strip from Starostin and Heijden was the closest size match. There were two which visually were the best match, one of width 1.0 and the other 1.6. Table 3.1 shows the aspect ratios for determining the best comparison. To generate the lengths and widths for this comparison, the contour length and width of each DNA Möbius strip was required. The length was calculated for each average structure with a script that determined an average coordinate position of the two nucleotides in each base pair along the central helix, then summing the distances between each adjacent pair upon iterating around the central helix. The width was simply calculated by measuring the distance from one side of the Möbius strip to the other in four positions equally spaced around the strip and

	Length	Width	Aspect Ratio
Comparison Option 1	2π	1.0	6.3
Comparison Option 2	2π	1.6	3.9
DNA right-handed	215	28	7.7
DNA Left-handed	209	28	7.5

Table 3.1: The aspect ratios of two literature strips and the DNA equivalents to find the best size-match for comparative purposes. Length and width units for DNA equivalents are in nm.

averaging the results.

The average aspect ratio for the DNA Möbius strips is therefore 7.6 and so the closest size match is the literature strip with width 1.0 (and length 2π , note properties in strip depend on aspect ratio and not absolute size, reduced units used) and this corresponds to the blue line on the profile in Figure 3.9. Like most of the other lines on the profile, this curve has a minimum in curvature at $s = \pi$, peaks at approximately $s = \frac{\pi}{2}$ and $s = \frac{3\pi}{2}$ and a second shallower minimum at $s = 0$ and $s = 2\pi$ (which are the same location). Here, s is the parameter used by Starostin and Heijden to represent a position along the length of the strip and we have scaled to the equivalent length for the DNA versions. These key locations can be seen in Figure 3.10, which graphically depicts the start, end ($0, 2\pi$) and centre (π) locations for all three Möbius strips, as well as their C_2 rotation axes.

The results from calculating the curvature for the average DNA structures are shown in Figure 3.11, alongside the size-matched Möbius strip curvature profile from Starostin and Heijden. For the left-handed Möbius, the minimum of curvature calculated by the oxDNA script coincidentally occurred at π , the same mapping as for the Starostin and Heijden strip. For the right-handed Möbius strip, the minimum did not happen to occur at π as had been the situation with the left-

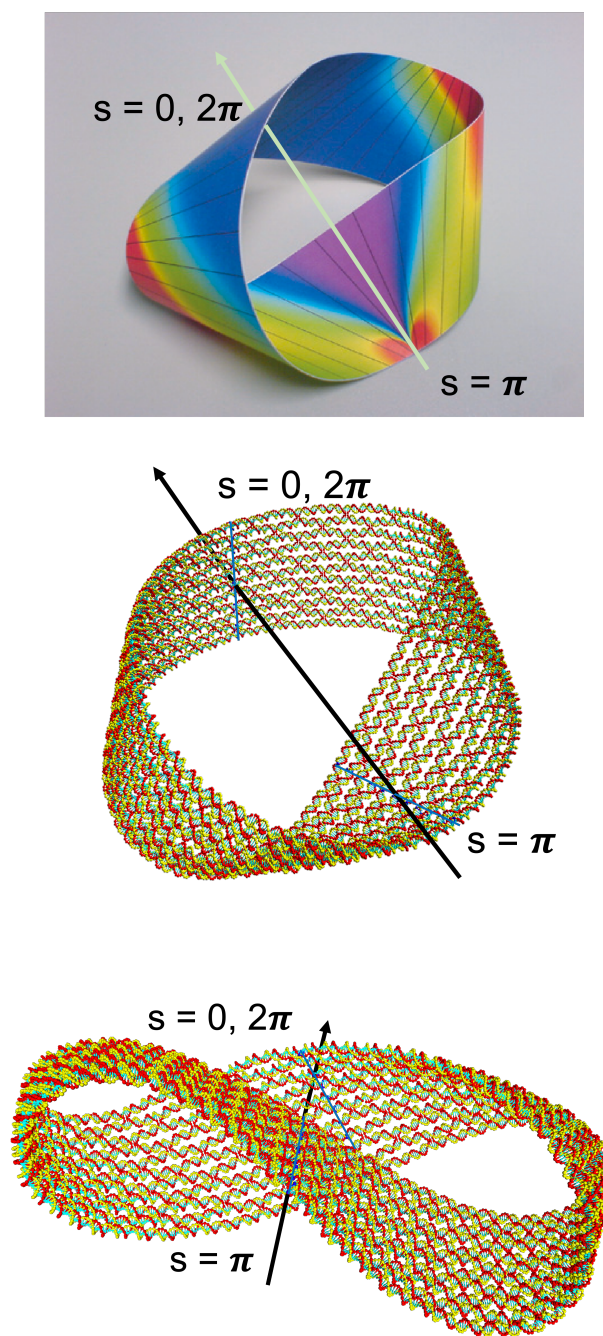


Figure 3.10: An example Möbius strip from Starostin and Heijden (top, width unspecified due to being an example image, but quoted with an aspect ratio of 2π). The right- (middle) and left-handed (bottom) DNA equivalents are shown with the C_2 rotation axes labelled, as well as the points of minimum curvature, $0, \pi$ and 2π . The bases corresponding to these positions have been highlighted in blue for the DNA equivalent. Top image adapted with permission from Springer Nature, Starostin and Heijden.⁸²

handed version. The x -axis was shifted until it did, in order to align this profile with the one from Starostin and Heijden. However, it was found that the point at 0 and 2π is a cusp, and shifting the minimum placed this cusp on the right-hand side of the profile, interrupting its symmetric component. As such, it was decided to displace the minimum slightly from the centre, in order to best maintain the approximate symmetry in the profile that can now be seen.

Whilst neither of the calculated data sets is a direct replication of the literature set, it is important to note that measuring properties of a non-smooth surface like DNA would never produce exactly the same as those for a mathematically ideal Möbius strip. To tidy up the data, it can be useful to fit a smoothing function to the data, and/or a spline to the centreline along which the curvature has been measured (the latter only has been done here). However, even when this technique is implemented, an element of noise in the data is usually inevitable.

Looking beyond these fluctuations, it is possible to see similarities between all three profiles. For instance, a distinct minimum for all three can be seen on each profile. This minimum corresponds to the position of minimum curvature and, as can be seen from Figure 3.10, is found on a ‘flatter’ region of each Möbius strip.

Secondly, the Starostin and Heijden data has a highly symmetric element around the centre minimum, and this is also true for both DNA versions. Also, all three symmetry planes in the profiles align (approximately for the DNA strips) with the C_2 axes for each structure. Although there are interruptions to the main peaks in the DNA data in the form of smaller peaks, both profiles feature two main regions of high curvature, similar to the literature one, thereby maintaining symmetry.

There are very small cusps on the edges of the profiles for the DNA versions,

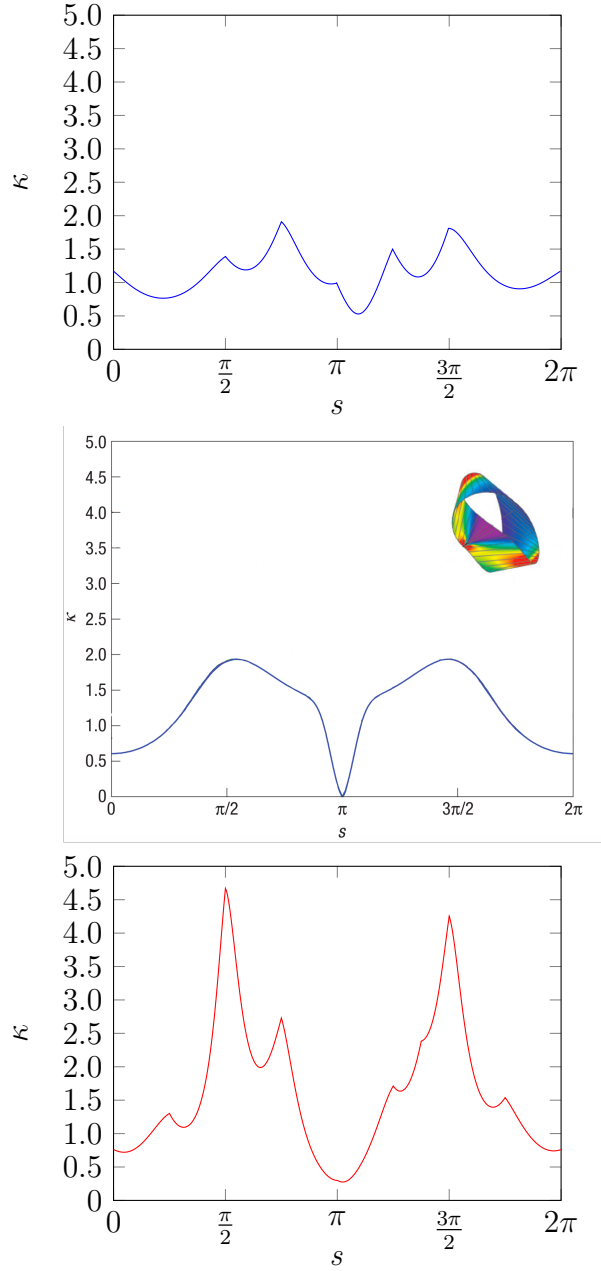


Figure 3.11: Curvature for the right- (top) and left-handed (bottom) DNA Möbius strips, alongside that of Starostin and Heijden (middle). Also shown on the literature profile is an image of the corresponding Möbius that gave rise to the profile (size-matched to the DNA equivalent strips). Note that the y -axis for each DNA Möbius strip has been scaled to match that of the profile from Starostin and Heijden. Centre images adapted with permission from Springer Nature, Starostin and Heijden.⁸²

but these are likely present as DNA is not a smooth surface. However, the major difference is the magnitude of the peaks for each isomer. Whilst the peaks in both the right-handed and Starostin and Heijden strips have a magnitude of about 2, the peaks in the left-handed strip are significantly larger at about 4.5. This indicated that the left-handed isomer had regions of much higher curvature (and visually would appear as sharper and more well-defined curves) than the other two. Looking at the images in Figures 3.8 and 3.10, it is clear to see where they occur and this is most likely due to the different equilibrium structures adopted by the strips. This result itself was due to each isomer exhibiting a different partitioning of the linking number between twist and writhe.

3.2.3 Twist and Writhe

Starostin and Heijden also calculated and plotted a graph of twist as a function of Möbius strip width.⁸² Using their data and the Möbius strip width corresponding to that size-matched previously, the twist of the DNA equivalents should be approximately 0.26, had they both taken the same conformation as the literature version.⁸² Since the left-handed isomer adopted a different conformation, it was expected that the twist and writhe of each structure would be different and, as such, would not have the same twist as for the right-handed.

As discussed when calculating curvature, the central of 11 adjacent helices was used as a centreline, then each helix either side paired up to form five pseudo-ribbons, for which the twist and writhe could be calculated. The smallest width ribbon was made from the two helices directly beside the centreline, then increasing in width until the outermost pair. The results of these calculations are shown in

	Twist	Writhe	Lk
LH (smallest width)	0.0741	-0.584	-0.510
	0.0618	-0.572	-0.510
	0.0821	-0.594	-0.512
	0.0807	-0.592	-0.511
LH (largest width)	-0.0152	-0.495	-0.510
RH (smallest width)	0.293	0.197	0.490
	0.299	0.190	0.489
	0.299	0.191	0.490
	0.317	0.172	0.489
RH (largest width)	0.224	0.267	0.491

Table 3.2: The twist, writhe and their sum, the linking number, for the average structure of each isomer using each ribbon. Note the linking number for each as very close to $\pm\frac{1}{2}$.

Table 3.2, in ascending order for the width of the ribbons.

It is clear that the suggestion that the two isomers have adopted different balances between twist and writhe was correct. The left-handed isomer has significantly more writhe than twist, in all cases it is almost all writhe and no net twist. For the right-handed, the twist is more dominant, approximately 1.5 times larger than writhe, and the values are relatively close to the predicted value of 0.26. As such, the difference between the two isomers has been successfully identified.

The sum of twist and writhe (equalling the linking number) is remarkably close to $\pm\frac{1}{2}$ in all cases, which is the known linking number of a Möbius strip, and the signs are consistent for both right- and left-handed structures. This provides confirmation that the numerical methods being used are behaving correctly. The values of twist and writhe are also fairly consistent across the different definitions of the ribbon with the largest deviations being observed when the outer helices are used. The latter is expected because these helices are least constrained and are

likely to show the most fluctuations and be least representative of the strip.

Since it had been proven that the difference between the isomers was due to the twist and writhe of each, the next sensible step was to determine why this occurred. In order to do so, it was decided to simulate an ‘open’ strip without the ends joined. The output from the original caDNAno tile was used but with all long bonds removed to allow the tile to adopt its preferred structure in the absence of topological constraints. The simulation had the same parameters as for each isomer, and the initial structure and end result of this is shown in Figure 3.12.

It is clear from the result of these simulation images that the tile itself has an inherent right-handed twist, which explained why the right-handed isomer looked as expected. The structure was able to twist in the direction which was most favourable, and the right-handed Möbius strip could adopt the preferred form. For the left-handed isomer, the structure was forced to twist to the left when it would have preferred to adopt a right-handed twist. To reduce the stress of this forced conformation, the twist was partitioned into writhe to try and compensate, and this was allowed since the linking number remained constant.

The reason behind the presence of this intrinsic twist can be explained as a consequence of the original design of the Möbius strip from Han *et al.*⁷³ This design was based on Rothmund’s tile which has since been found to have a right-handed twist. To be flat, the tile would require DNA to have a pitch of $10\frac{2}{3}$ base pairs, as opposed to the ~ 10.5 pitch DNA in solution is expected to have.^{8,13,83} This Möbius tile uses the same design motifs and, as such, leads to a right-handed twist. In the experimental data, it was reported that the left-handed versions were present but, with the techniques available for imaging, such a subtly in structure conformation may have been difficult to elucidate, as none of the strips were reported as having

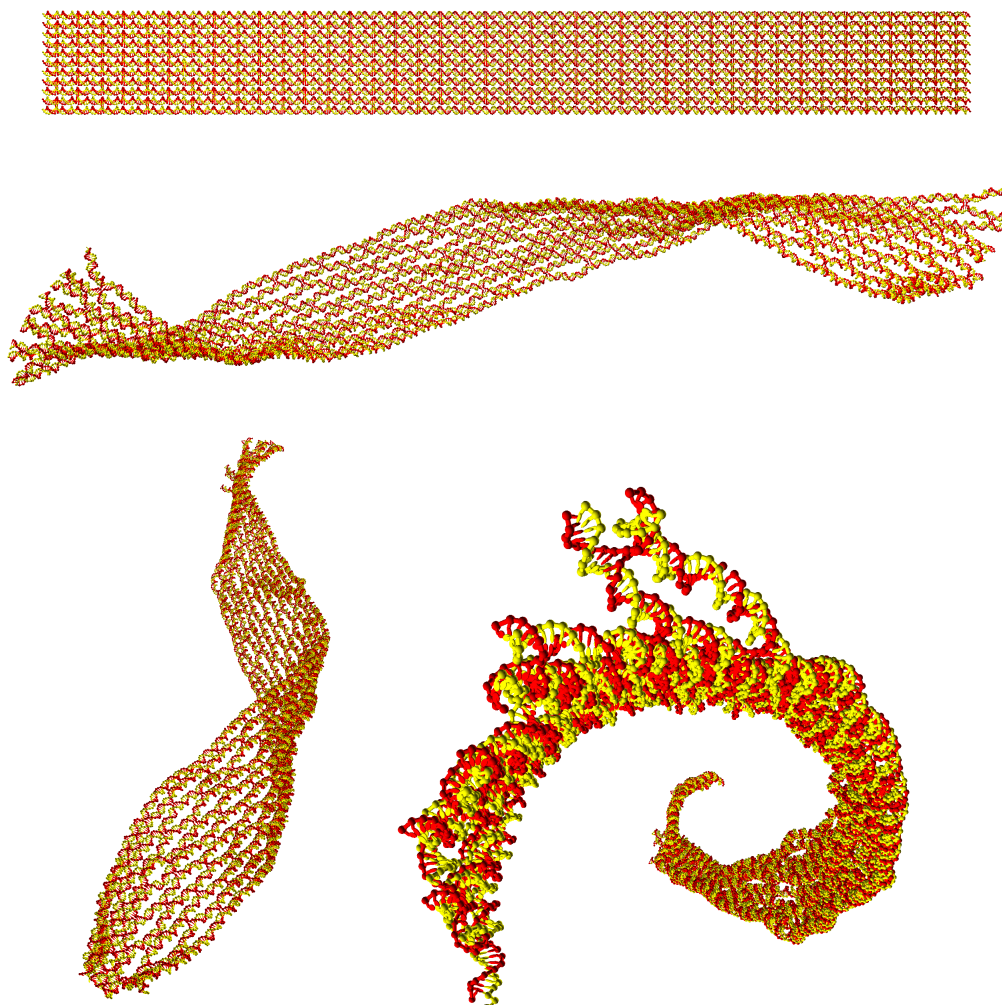


Figure 3.12: Möbius tile initial structure (top) and different perspectives of the end of the simulation: side-on (second down), top-down (bottom left) and end-on (bottom right). Initial provided as a reference image to see changes by the end of simulation.

an unusual conformation. Also the strips are likely to be flattened by adsorption on the surface.

An experimental technique that could potentially be used to determine handedness would be cryo-EM. As mentioned in the Introduction, cryo-EM builds up a 3D image based on a large number of slices taken through the structure. As this

would be a 3D replication of the structure it would likely be able to confirm the handedness predicted here. However it is a technique not routinely used due to it currently being a very time consuming and expensive process.

3.2.4 Centreline Projections

The final analysis was the 2D projections, using the average structures and a centreline produced as an average of base positions along the central helix. Three projections were made per Möbius strip, where one dimension was removed each time. The results for the right-handed isomer all take the expected shape in each plane and are good representatives of those from the literature. These, along with the comparisons from Starostin and Heijden, are shown in Figure 3.13.⁸² The shape of each has been reproduced and matches the trend of the paper equivalents well, except for the orange line that represents the Möbius with very large width. As with the previous data collected, the projections lack the smoothness of a calculated equivalent (this lack of smoothness is one of the reasons for the noise in the plots in Figure 3.11), but overall are a good match to the data available.

Since the left-handed isomer has a different conformation the best matched angle to the literature has been chosen. The most similar projection is that for the xz plane. This makes sense as forming a figure-of-eight conformation from a classic Möbius strip made of paper can be done by pinching the centre of the twist and the ‘edge’ opposite, and this represents a movement mostly in the y -axis direction. As such, the projection not including the y -axis would look the most similar. The other two projections look distinctly different to their right-handed counterparts as they involve the y -axis.

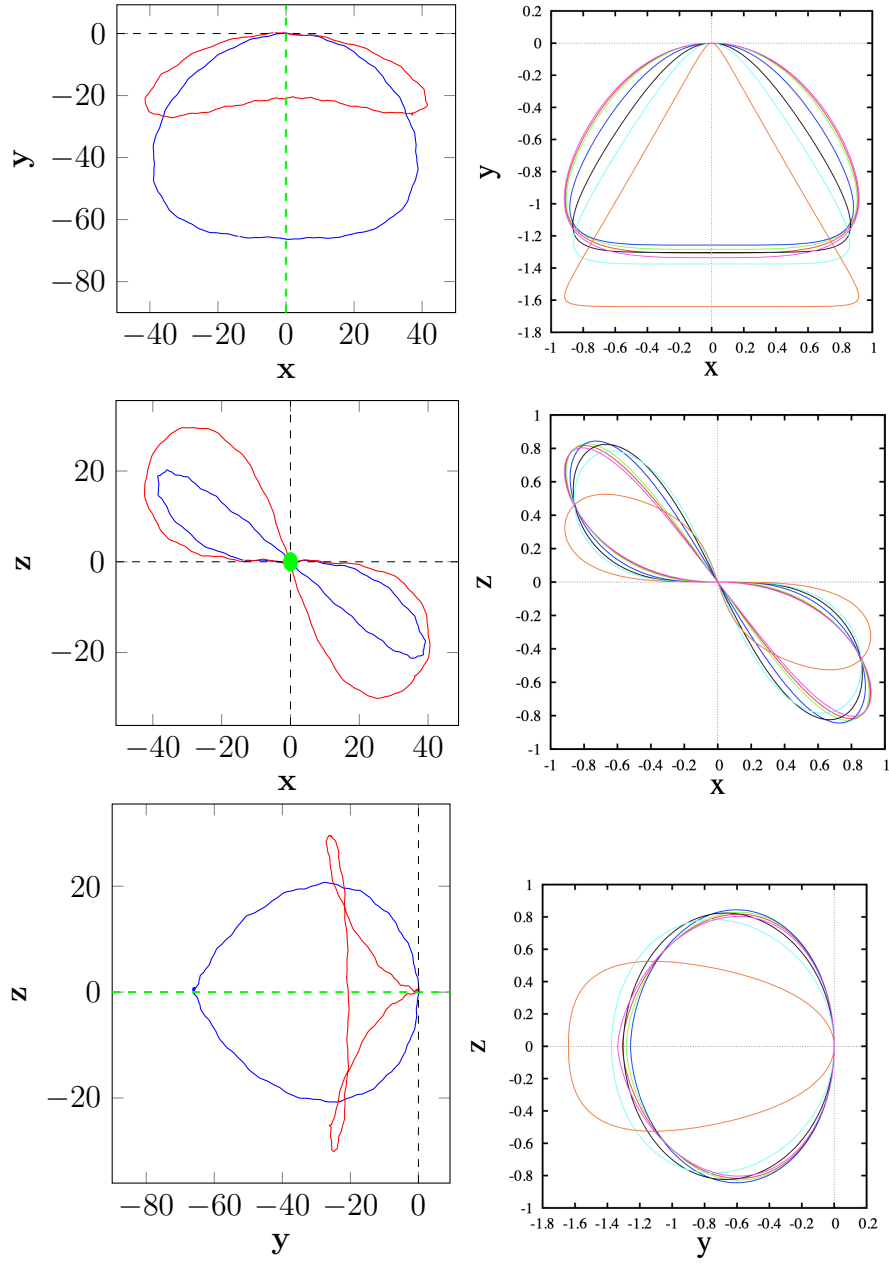


Figure 3.13: Möbius centreline projections for both DNA Möbius strips (right-handed in blue, left-handed in red), and their comparisons from the literature. Right-hand side images reproduced with permission from Springer Nature, Starostin and Heijden.⁸²

Finally, the 3D centrelines are shown in Figure 3.14. Each Möbius strip is shown in the centre in black with its three projections on each surrounding plane. The overall 3D shape matches most closely with the right-handed isomer.

3.3 Conclusions

Two isomeric DNA Möbius strips were simulated and analysed and the results compared to a size-matched ‘ideal’ version from Starostin and Heijden. It was found that the right-handed isomer adopted the same (and expected) conformation as the literature version, but the left-handed structure appeared more strained and instead took a figure-of-eight shape. Average structures for each DNA isomer were generated and the curvature profiles taken. The right-handed structure profile was found to be most similar to the ideal strip. Further analysis of twist and writhe found that the right-handed isomer had more twist, with values similar to that of Starostin and Heijden, whereas the left-handed had more writhe, explaining their different equilibrium conformations.

The ideal Möbius strip would be formed of a developable surface so if it were to be cut open and rolled out, it would form a flat material. For the DNA Möbius strips, the equivalent simulation of the ‘cut open’ Möbius strip was found to have an intrinsic right-handed twist. This was determined to be due to the design being based on Rothmund’s original tile that would require DNA having a pitch of $10\frac{2}{3}$ to be flat, as opposed to the 10.5 expected for DNA double helices in solution. For the right-handed isomer this resulted in a smaller deviation from ideality than for the left-handed strip, which was being folded into a much more unfavourable conformation and led to more of the Linking number being partitioned into writhe.

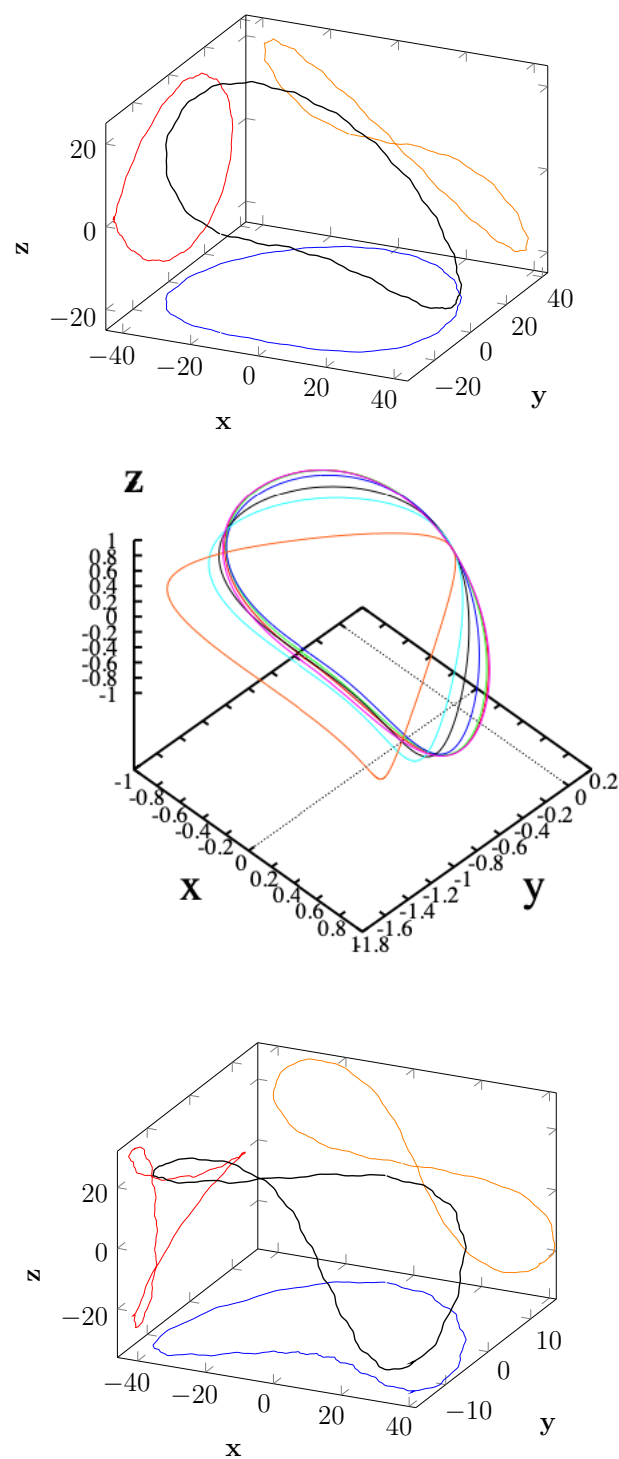


Figure 3.14: 3D projections of the right- (top) and left-handed (bottom) Möbius isomers, alongside the calculated equivalent from Starostin and Heijden (centre). Central image reproduced with permission from Springer Nature, Starostin and Heijden.⁸²

As such, it is unsurprising that the right-handed strip results more closely align with those of the literature Möbius strip.

However, despite this, all three strips shared notable common features. They all had a C_2 rotation axis, meaning their curvature profiles showed symmetry in all cases. They share a minimum at $\sim \pi$, so halfway around the strips from the same starting point (approximately along the C_2 axis), and they show peaks in the same regions as well, so their regions of maximum curvature occur roughly the same distances from the minima. This symmetry occurred again in the centreline profiles. Whilst visually the right-handed profiles appeared most similar to the Starostin and Heijden data, the left-handed one also had planes of symmetry in the same locations.

It was reported in the literature that the left-handed isomer was synthesised during the experimental work, but there was no indication given that a figure-of-eight shape conformation was adopted by any of the strips. It is suggested that, due to limitations in imaging techniques, such a subtle conformational detail may have been difficult to elucidate.

Chapter 4

Tensegrity Kite Structures

4.1 Introduction

4.1.1 Experimental Design, Simulation and Analysis Details

Defining tensegrity is not straightforward as it largely depends on what is being described. The definition in the Collins English Dictionary is in terms of architecture and describes a structure where not all components exist under equal tensions and, instead, a structure is formed of some components that are under tension and some that are not, but together make a rigid unit, such as a bridge. An example of an early large-scale structure of this type is the Munich Olympic Stadium, built for the 1972 Olympics, that has an extensive tensegrity roof held together by a large quantity of steel cables.⁸⁴

Tensegrity structures can be utilised in civil engineering and some recent research has focussed on adding components of this type as load-bearers to buildings in order to, for example, provide resistance against harsh weather conditions and natural disasters. Examples of this type include determining the best load-bearing column structure from a set that varied in the arrangement of cables and struts,

and the theoretical design of earthquake resistant braces, potentially for architectural use (Figure 4.1).^{85,86}

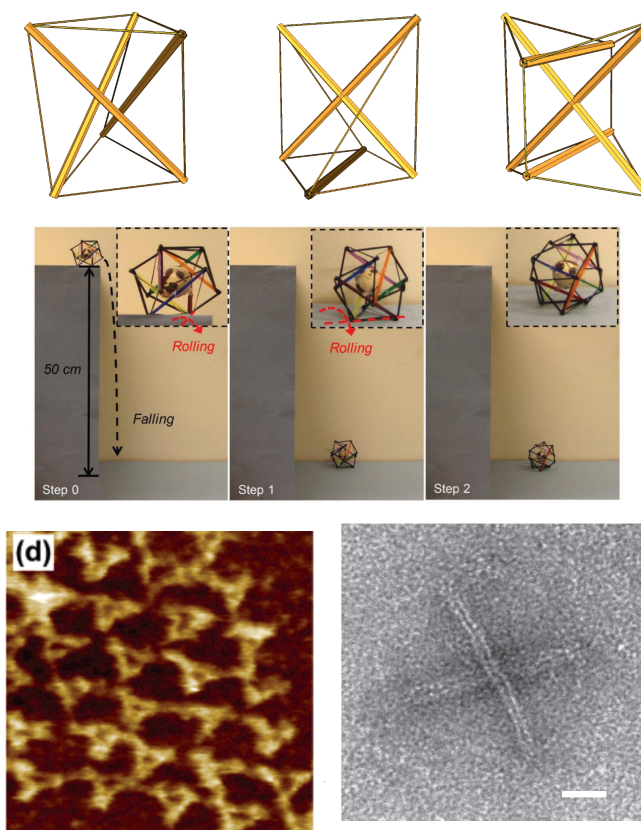


Figure 4.1: Tensegrity structures from the literature. Three different arrangements of theoretical struts for improving the strength of buildings (top), a tensegrity robot that is able to protect a cargo (an egg) even after falling off a tall platform (second from top), AFM images of an array of tensegrity DNA triangles (bottom left) and a TEM image of one of the kite structures focussed on in this case study (bottom right, scale bar quoted as 20 nm). Top image reproduced with permission from Zawadki *et al.* under Creative Commons licence CC BY 4.0, <https://creativecommons.org/licenses/by/4.0/>,⁸⁵ central image reproduced with permission from Wang *et al.*,⁸⁷ bottom left image reprinted (adapted) with permission from Liu *et al.*, *J. Am. Chem. Soc.*, 2004, **126**, 2324-2325, copyright (2004) American Chemical Society,⁸⁸ and bottom right image reproduced with permission from Springer Nature, Liedl *et al.*⁸⁹

In biology, cells are described as having tensile components and these are often

filaments involved in maintaining the shape and outer structure of the cell (cytoskeleton).⁹⁰ These structures can even be found in the robotics world. The work of Wang *et al.* describes the development of their tensegrity robot that is capable of rolling to move around and, due to its design, can resist damage to a cargo and itself after falling off a table (Figure 4.1).⁸⁷

The first DNA tensegrity structures were those from Liu *et al.* who made DNA triangles that were held together by flexible corners and built upon previous attempts in this area.⁸⁸ The project presented in this chapter looks at structures that fall under the same tensegrity umbrella. They are ‘kite’ structures whose movements are dictated by tensions in the system and were originally designed and synthesised by Liedl *et al.*⁸⁹ The kites are formed of two rigid six- or twelve-helix bundles that cross over in the centre and are connected to each other at the ends of the bundles by lengths of single-stranded DNA (ssDNA) that we have termed ‘linkers’. There are no direct connections between the two bundles in the centre (no pivoting action) so their movements are entirely determined by tension in the linkers. We looked at two types of structure. The first was a set of three kites where the bundles were formed of 12 helices (12HB), and the second utilised the same concept but with 6 helix bundles (6HB) as the arms of the structure instead.

Histogram data for the 12HB kites was presented in the experimental work and was produced by measuring the angle between the two bundles for each kite in the TEM images. For the simulated structures, a similar calculation was carried out by taking a ‘ring’ of 12 nucleotides at each end of the bundles and calculating an average position for the bases. A vector along each bundle was then determined

and an angle calculated using Eqn. 4.1:

$$\cos \theta = \frac{\mathbf{a} \cdot \mathbf{b}}{|\mathbf{a}||\mathbf{b}|} \quad (4.1)$$

where $\mathbf{a}$ and $\mathbf{b}$ represent vectors along each bundle. The calculation was carried out as outlined above. Note that the vectors were not projected onto another plane for the angle calculations as without knowing more about the adherence of the DNA origamis to the surface in experiments it would be difficult to identify the correct plane to use, and it is unlikely the TEM images could be used to distinguish which bundle arm was crossing the other, the result of which would also affect the choice of how to project the vectors. All simulations initially started with 10^8 steps, at 300 K and 1 M NaCl. A further simulation for the 6HB 220 nt kite was run under the same conditions for another 10^8 steps as the angle profile had not plateaued. This is covered in more detail in the results section.

4.2 Results and Discussion

4.2.1 12 Helix Bundle Kites

There was ample experimental work to guide this case study as a range of kites had been investigated, differing only by their linker lengths. The results were shown as gel electrophoresis plates and transmission electron microscopy (TEM) images, so it was clear which structures had been successfully assembled from a snapshot of their appearance. From the range of linker lengths that the experimental group used, it was decided to simulate three which represented ‘short’, ‘medium’ and ‘long’ lengths: namely 140 nucleotides (nt), 220 nt and 420 nt, respectively. The

kites all had the same number of nucleotides in the bundles (12980) and only varied in linker length. Using the caDNAno designs and the editing capabilities of oxView, it was possible to quickly produce the three desired structures and prepare them for simulation through an oxDNA relaxation procedure, and the final structures at the end of simulation are shown in Figure 4.2.^{17,49,50}

The 140 nt kite has the most taut linkers and the bundles are in an equal cross shape, indicating that this is the best conformation to balance the tensile forces the bundles exert on the linkers. For the 220 and 420 nt kites, there appears to be a number of hairpin motifs that have formed in the linker. In these systems the tension in the linkers is not sufficient to completely disrupt the tendency of ssDNA to form secondary structure. In the case of the 220 nt kite, the crossing of bundles still looks equal, so the formation of the hairpins may have sufficiently increased the tension in the linkers to hold the bundles in a symmetric cross shape, similar to the 140 nt kite that best balances the forces in the linkers. For the 420 nt kite, however, the bundle positioning is not equal, and their unequal positions suggests that the bundles are more free to diffusively explore a greater distribution of relative angles because of the lower tensions in the linkers. However, these structures were simulated for a relatively short time period and would need a much longer simulation to capture extensive diffusive behaviour. There are also more hairpins in the linkers and the sections with no secondary structure are visibly less extended, both features reflecting the lower tensions in the linkers.

It is also interesting to note that the 12HB arms are always unbent, thus indicating that the tensions in the linkers is always below the Euler buckling force for these rod-like objects (see the discussion in the next section for a full explanation of Euler buckling).

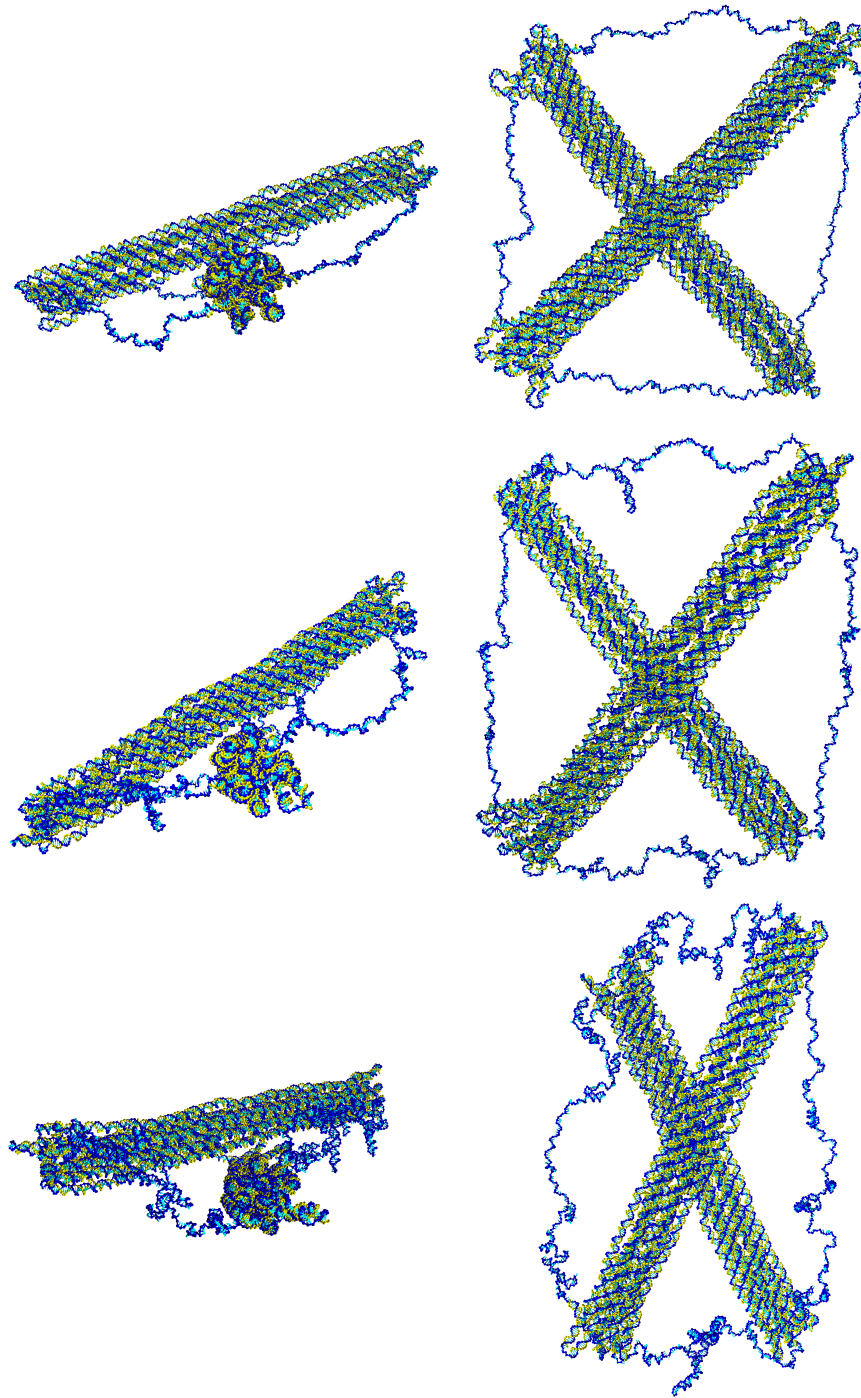


Figure 4.2: The three 12HB kite tensegrity structures at the end of simulation, 140 nt linkers (top), 220 nt (middle) and 420 nt (bottom). The linkers for the 220 nt and 420 nt kites exhibit significant hairpin formation, suggesting lower tensile forces.

Experimental images are available for the 220 nt and 420 nt kites and these are shown in Figure 4.3. There are no available images for the 140 nt kite and it was suggested that the forces in the linkers were too high to allow formation of the bundles, but that for 160 nt linkers and above it was possible to create the correct structure. It is also noted that for all linker lengths (including 140 nt) it was possible to synthesise a parallel bundle arrangement where the arms do not crossover one another, and instead are aligned side-by-side. In the literature, this was attributed to the ‘occasional rupturing’ of one of the linkers and the resultant structures were not the wanted ones as they lacked the cross-over motif of the bundles. That we have been able to simulate the 140 nt kite is a positive result since it provides insight into what this structure would have looked like, had it been able to be assembled. Note that the fact that our simulations suggest that the fully-assembled 140 nt kite is stable is not necessarily in contradiction with experiments since they do not directly address the feasibility of the assembly process. This is because the simulation starts with a complete structure, but that does not mean it would not fall apart during the assembly process if made synthetically. It may also be that the structure falls apart if simulated on a long enough timescale. The simulations address global stability and the experiment kinetic accessibility. It is also clear that the experimental and simulation results are consistent for the other two systems, namely in the sense that the 220 nt kites in both images are a balanced cross of the bundles, whereas the 420 nt kites both are more elongated, due to less tension in the linkers.

The experimental results also measured the angle between the arms of each kite (Figure 4.3) and the results from these compared to our simulation results are shown in Table 4.1. For the two kites with experimentally measured angles, the

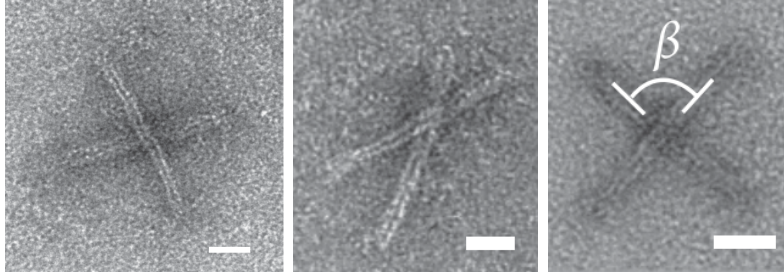


Figure 4.3: TEM images of the 220 nt (left) and 420 nt (middle) and 220 nt again but depicting the angle measured by the experimental group (right). The 140 nt kite was not able to be assembled into the wanted conformation so no TEM image is available. All scale bars are quoted as 20 nm. Images reproduced with permission from Springer Nature, Liedl *et al.*⁸⁹

Kite	Experimental angle (°)	Simulation angle (°)
140 nt	N/A	89
220 nt	90	87
420 nt	90	96

Table 4.1: Experimental and simulation angles for the 12HB kites. Angles calculated as an average from 200 data points over the course of simulation.

results were presented in the literature as a histogram, so the value shown in the table is the most commonly occurring angle. The angles mostly match well and are entirely within the range of the histograms. The simulation results were measured as an average of all angles over the duration of the simulation, and not just taken from the final conformation; the simulations gave the kites the opportunity to diffusively explore the range of conformation accessible to the kites and so the presented results have that taken into account for the angle.

Clearly these results demonstrate that oxDNA is reproducing the experimental results well as the calculated angles are close to the measured ones. Additionally, we have been able to fill in a gap in the experimental work by being able to show the behaviour of one of the structures that could not be assembled in experiment.

It is now possible to see what it would have looked like in terms of conformation and state of the linkers (e.g. no hairpin motifs), the arrangement of the bundles and the angle between them.

Liedl *et al.* had a second kite design that used six helix bundles (6HB). The experimental group only looked at one scenario where the linkers are different lengths that result in a distorted kite, but we did three simulations, again with linker lengths of 140, 220 and 420 nt. This then allowed us to explore the structure of the 6HB kites as well as compare to the 12HB versions.

4.2.2 6 Helix Bundle Designs: Relaxation Outcomes

The output from relaxation of these structures can be seen in Figure 4.4. The first kite with linkers of 140 nt in length was clearly a very strained structure as the relaxation forced the bundles into an arch shape in response to the tensions in the short ssDNA linkers. In a previous relaxation attempt before this result was produced, there were no forces (“mutual traps” to force bases to stay paired) used to maintain the shape and prevent breakage of vulnerable base pairs due to the large forces that may be present in the initially converted structures, and it was found that the structure broke and unravelled significantly. The weak regions leading to this problem were identified and for the second relaxation attempt it was decided to place mutual traps in these areas to keep the structure intact. If the structure later broke in the same way during simulation, when the traps had been removed, then it could be concluded as a consequence of the design, but it was important to ensure the simulation started with the design as intended. It is worth noting here that this bundle bending did not occur in the 12HB kites. An

approximate length calculation for the bundles using OxView yielded ~ 87 nm for the 12HB bundles, and ~ 113 nm for the 6HB kites, so this also contributed to higher strain in the system for the 6HB kite structures.

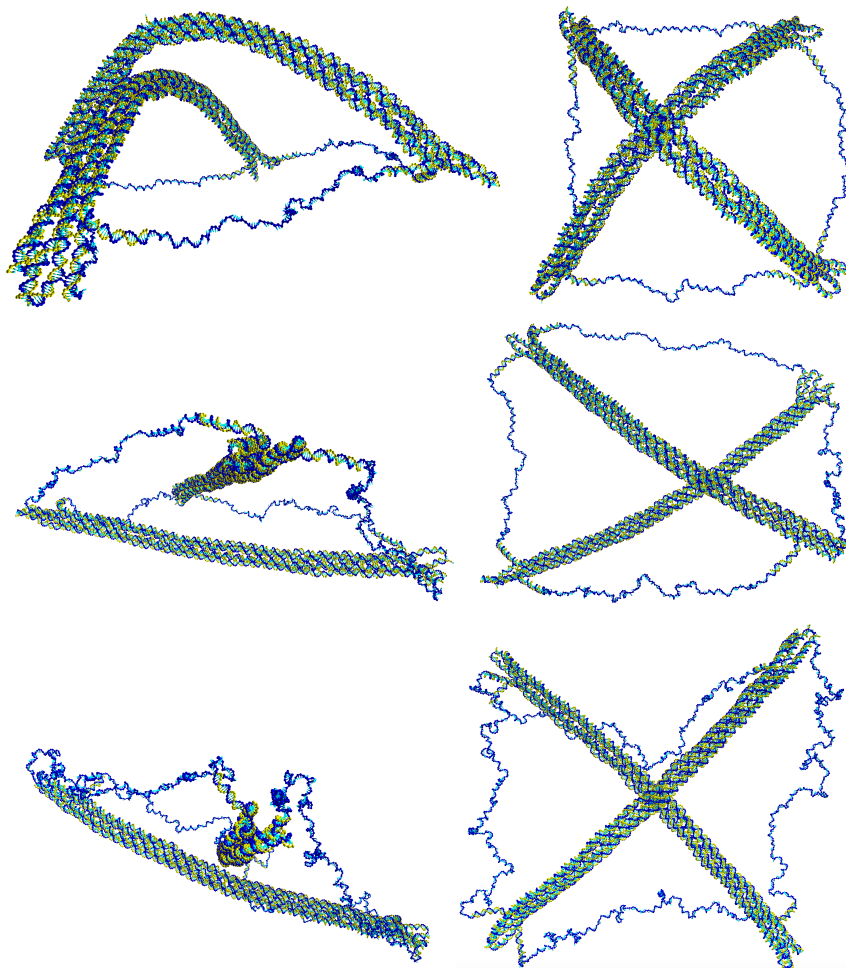


Figure 4.4: The three kite tensegrity structures after relaxation, 140 nt linkers (top), 220 nt (middle) and 420 nt (bottom). The bundles for 220 nt and 420 nt structures start out in a comfortable conformation but the 140 nt kite clearly involves large internal stresses, indicated by the significant arching of the bundles.

With the bundles unable to unravel due to the traps, the tension in the single strand is sufficiently large for the helix bundles to undergo Euler buckling. Euler buckling is when a ‘rod’ (in this case the helix bundles) bends due to the compres-

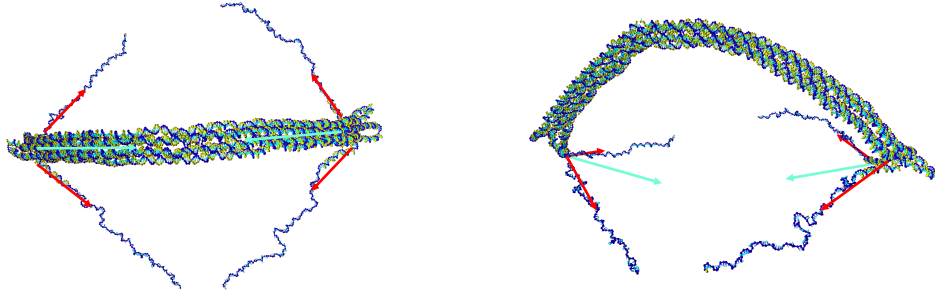


Figure 4.5: The 140 nt kite (top down: left, side view: right) with one bundle removed and arrows added to depict the forces responsible for the Euler buckling. The red arrows along the linkers show the force being exerted on the ends of the bundles, the blue arrow shows the net force. From this annotation, it is clear to see why the bundle has to buckle to accommodate this force as buckling occurs when $F > F_c$ (the force applied, F , is larger than the ‘critical’ force the bundle can withstand, F_c). Arrows are indicative only; they do not represent exact directions or magnitudes of the force.

sive forces applied to both ends of the rod. A modified image of the 140 nt kite to depict this is shown in Figure 4.5. One of the bundles from the 140 nt kite has been removed, along with some linker. The remainder has been annotated with arrows to depict the individual and net forces acting due to the linkers. Euler buckling has previously been observed in experiments on double stranded DNA by Fields *et al.* again using ssDNA linkers to apply the tension.⁹¹ For the 140 nt kite, it is likely the linkers were simply too short and the tension in them too high for the bundles to withstand and so exhibited this buckling. There would exist a critical length for a linker where the bundles could remain straight and, based on the results for the 220 nt linker kite, the tether length required would be between 140 and 220 nucleotides long.

It was possible to estimate the Euler buckling (critical) force (F_c) for these

6HB struts using the formula:

$$F_c^{oxDNA} = \frac{k_B T L_p \pi^2}{L_c^2} \quad (4.2)$$

where k_B is the Boltzmann constant, T is the temperature, L_p is the persistence length for a 6 helix bundle (for oxDNA simulations this has been measured as $6 \mu\text{m}^{92}$), and L_c is the contour length of one of the bundles. The contour length was calculated in the same way as for the Möbius strip in the previous chapter - for one of the helices in one bundle per kite, an average position was calculated for each hydrogen bonded pair along the helix, the distances between neighbouring points were calculated, and then these were summed to calculate the full length. The calculated values are shown in Table 4.2. As the bundles are the same design except for the linker lengths, it would be expected that the F_c for each should be approximately the same, and that is the case as all three values are approximately 14 pN. The estimate for a system like this was approximately 10 pN (calculated as four times the estimated force, 2.51 pN, per linker)⁸⁹, meaning this value is quite similar. For the 140 nt kites, clearly the force exerted by the linkers was larger than the force that each bundle could withstand ($F_{linkers} > F_c$), and was therefore higher than 14 pN. This was not the case for the other two kites, as Euler buckling is clearly not present in the structures as there was little to no bending in the bundle arms, and the linkers appeared much more relaxed and less taut than in the 140 nt kite. There were even small hairpins already formed by the end of each relaxation, consistent with a significantly lower tension in the linkers.

The experimental group looked at one ‘uneven’ kite that had 6HB arms, with one very long linker (2207 nt) and three much shorter (273 nt). Some TEM images

Kite	Euler buckling force (F_c) / pN
140 nt	13.7
220 nt	14.3
420 nt	14.1

Table 4.2: Estimated Euler buckling force (F_c) for each of the kites.

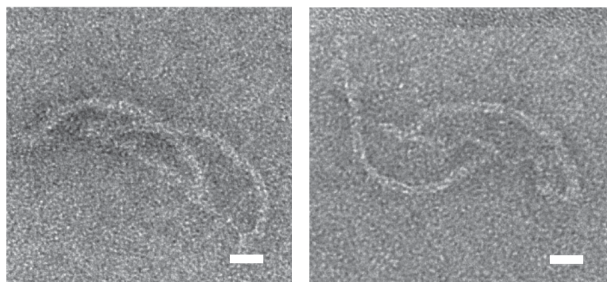


Figure 4.6: Experimental TEM images showing Euler buckling of these length struts but with different linker lengths (one very long, three much shorter). This image shows that whilst the oxDNA relaxation resulted in both bundles buckling in the same direction (like in the left hand image), it is also possible that one strut could have bent in the opposite direction (right). Image reproduced with permission from Springer Nature, Liedl *et al.*⁸⁹ Scale bars quoted as 20 nm.

from the experiments are shown in Figure 4.6. These TEM images appear similar to the structure of the 140 nt oxDNA version, but the main difference is that in the experimental version, some bundles imaged show the Euler buckling occurring in opposite directions. Whilst the oxDNA relaxation outcome is more similar to the first image in the figure (left), it is perhaps also possible the second bundle could have buckled in the opposite direction if the relaxation were repeated.

An additional different, but noticeable, feature in all three structures was the bending of individual double helices out of their respective bundle at the linker attachment points, but since these were reversible bends and not irreversible breaks, it was decided these did not need external forces to keep them within the structures.

4.2.3 6 Helix Bundle Designs: Simulation Outcomes

The structures at the end of simulation are shown in Figure 4.7. The 140 nt kite had the most significant structural change as the six-helix bundles no longer had the very unfavourable arched shape and had, instead, straightened out. As can be seen from the top-down image some bending did remain, but it was significantly reduced compared to the starting conformation. The asymmetric bundle positioning in the 140 nt kite was clear to see as one bundle resided further along the second, as opposed to a cross at the mid-points of each bundle. For both the 220 nt and 420 nt kites the bundles at the start of the simulation were straight and remained so but with some diffusion into an asymmetric position. This feature was less well-defined than for the 140 nt kite but still clear to see.

A second prominent feature across all three kites was the change in linker structure. At the end of each simulation, it was easy to see that some of the linkers were no longer comprised of only single-stranded DNA; instead, some contained a significant quantity of duplex DNA that had originally formed part of the bundle to which the linker was attached. Clearly, some bundle breakage and unravelling had taken place to produce this outcome. For the 140 nt kite, this had been essential in providing the structure with the ability to straighten out the bent bundles into a more suitable and energetically favourable conformation. However, even in the kites with much longer linkers this had occurred as well. The breakage and duplex unravelling had only occurred in two of the four separate linkers in each structure, which explained why the bundle crossing ended up being asymmetric. This was a reflection of the original design and will be covered in more detail in the next section. For the other two kites these breakages still occurred but, since the linkers

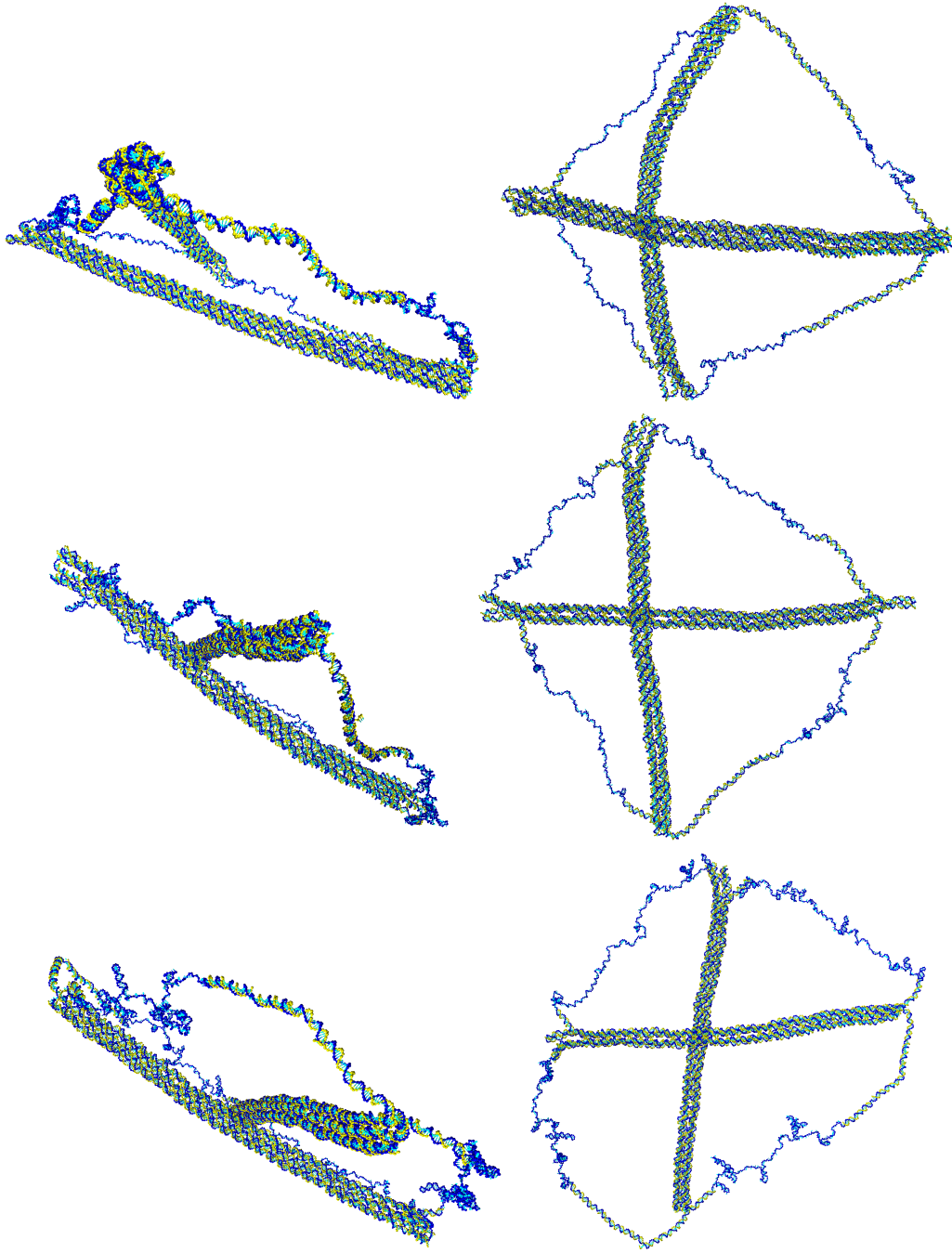


Figure 4.7: The kites at the end of simulation: 140 nt (top), 220 nt (middle) and 420 nt (bottom). Side-on images for all on the left, top-down on the right. Significant features include: straight bundles for the 140 nt kite, the linkers containing significant quantities of double helix for all three, and the asymmetric positioning of bundles relative to each other.

were much longer to begin with, it is likely the tensions in the system were much lower. This suggested that unravelling, and the initial breakage that allowed it, was not difficult to do and required relatively little tension in the system. Additional evidence for this is that some hairpin motifs had already formed by the end of the relaxation procedure in the 220 nt and 420 nt kites suggesting the breakages were not due to force exerted by the linkers, as there was clearly excess present already, and instead occurred due to the little energetic input needed to break them, and the fact that once broken they will not reattach as this would involve the broken duplex folding back into the bundles. The cause of the asymmetry and linker breakage was isolated to motifs in the original design that we have termed ‘weak domains’.

4.2.4 Weak Domains

When these structures were initially designed, it was clearly not the intention that the bundles would locally break and unravel. The breakage in the 140 nt kite was perhaps unsurprising given the high tensions initially in the linkers, but the results for the other two (220 nt and 420 nt) kites shows that actually only a relatively low tension was sufficient to achieve these breakages. The linkers for the 140 nt and 420 nt kites gained the most length, each of the affected linkers gained 145 bases in length, totalling 290 bases for each structure. The extra existed mostly as duplexes, so base pairs were counted as effectively one base increase in length. The 220 nt kite gained 145 bases in one linker that broke entirely, and 88 for the second where not all domains broke. The outcome of straightened bundles (for the smallest kite) and additional linker length providing more entropic freedom was

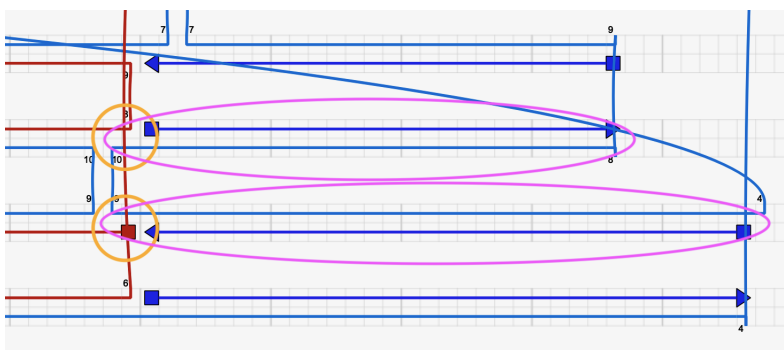


Figure 4.8: caDNAno design showing the weak domains (circled in orange) in the kite designs. Since each kite has the same bundle design, each of them has this same weakness. The bases circled in pink denote the domains that add to linker length.

sufficient to overcome the free-energy cost associated with the loss of base pairs. The breaks occurred in two specific regions of the original caDNAno design and only appeared at two of the four bundle ends, explaining the asymmetric breakage. In both of these regions, there were only four pairs of nucleotides (eight pairs in total across both bundles) holding in a large length of double helix. As there were both energetic benefits and entropic gain to be had from allowing the domain breakage, the benefits outweighed the cost of breaking the eight hydrogen bonds. The caDNAno design for one of the two affected bundle ends, with these weak domains circled, is shown in Figure 4.8. As can be seen from the Figure, the red staple extends across the scaffold by only two hydrogen bonds on each side of the scaffold strand, explaining why the junction was susceptible to breakage. These were the only hydrogen bonds holding the scaffold strand in and it is, therefore, unsurprising that these broke in almost all instances. A second set of weak domains existed on each arm, making a total of eight weak pairs per bundle end and 16 weak pairs per structure that could possibly break during simulation. The first four per bundle that have already been discussed (and circled on the Figure) were classed

as ‘critical’ and the other four on each bundle as ‘non-critical’. The non-critical pairs could only contribute to linker length if the original four pairs had already broken, hence they were not all classed the same. The breakage of all of these pairs was measured and is shown in Table 4.3. As shown in the Table, most of the domains broke. They did occasionally reattach and break again, but the pairs were measured at the end of simulation as this should have represented an equilibrium structure for each kite. The data in this table reiterates what has been concluded

Kite Size	Domains broken (total of 8)	Non-critical domains broken (total of 8)
140 nt	8	8
220 nt	8	5
420 nt	8	8

Table 4.3: Number of domains broken for each of the kite structures, out of a possible 8 for each classification.

previously: the breakages required only a small amount of work, occurred across all the kites and was the cause of the asymmetry and linker lengthening. Three of the 220 nt kite domains were unbroken and this was investigated to determine if there was a specific reason why. When these structures are converted from caDNA_{no} to oxDNA, random base sequences are used and, by chance, the sequence around the only partly broken domain is very high in A and T content. As such, the bases around the domain broke but shifted and re-bonded with other complementary bases in the vicinity. Some have remained bonded to their original pair, but some have bonded to nearby neighbours. In the domain of interest, only one domain broke, but due to this shifting of pairings, the two-base overlap (holding in a large length of duplex that would extend become extended linker) appears to have become a three-base overlap (Figure 4.9) making the junction somewhat more

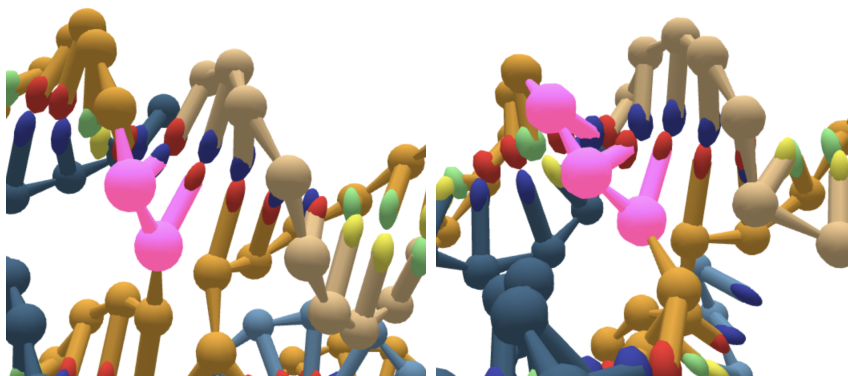


Figure 4.9: The 220 nt kite zoomed in on one part of one of the weak domains. What originally was a two-base overlap (left, highlighted in pink) has become a three-base overlap (right, highlighted in pink) thereby increasing the strength of the junction and explains why the final domains did not break during the initial simulation.

Kite	Simulation angle ($^{\circ}$)
140 nt	96
220 nt	102
420 nt	107

Table 4.4: Simulation angles for 6HB kites. Angles calculated as an average from 200 data points over the course of simulation.

resistant to breakage. The 220 nt kite simulation was continued for another 10^8 steps to see if the final domains would break and by the end the final three domains had also broken.

For completeness, it was also possible to repeat the angle measurement calculations for the 6HB versions and this data is shown in Table 4.4. There was no experimental equivalent as even for the one kite synthesised, there was no angle data reported. The angles are slightly larger than the 12HB kites, but this makes sense when considering these kites gained linker length through breakages, thereby allowing a larger range of accessible angles for the bundles to achieve.

4.3 Conclusions

In this chapter, six tensegrity structures in the form of 12HB and 6HB kites with varying linker lengths (two kites for each length of 140 nt, 220 nt and 420 nt) were successfully simulated. Visually, the 220 nt and 420 nt 12HB simulated kites appeared similar in appearance to the experimental versions imaged through TEM. The 140 nt kite had no equivalent image because the experimental group were unable to assemble this structure in the desired ‘cross-over’ conformation, presumably because the linker forces would have been too strong and prevented assembly. It was only possible to assemble a parallel version, where the bundles did not adopt the desired crossed bundle conformation. As such, they were classified as not having been successfully made. It was determined by the experimental group that kites with linkers shorter than 160nt would be unable to form due to their high tensions and, hence, why no TEM images were available. However, it has been determined from this work that, had a 140 nt kite been assembled, it would have had a stable equilibrium structure and appear not too dissimilar to the other two. The bundles would have been held quite tightly in place by the taut linkers so the cross shape would have been quite well defined, and would likely have had an angle between the bundles of $\sim 89^\circ$.

For the other two kites TEM images were available and, visually, the simulated kites looked similar to the experimental versions. The shape made when the bundles crossed was less regular (more rectangular than the square-like 140 nt kite) but this made sense since the linkers were much less taut (also indicated by hairpin motifs present at the end of simulation). Additionally, the angles between the bundles were measured and found to match well to those determined

experimentally (both around $\sim 90^\circ$).

Overall, for all three 12HB kites, it was possible to simulate the structures to find out more about their structure in terms of physical appearance and angle calculations. Comparisons for both features could be determined for the 220 nt and 420 nt kites and suggestions made for the 140 nt kite structure had it been possible to assemble it. It was therefore concluded that, for all the 12HB kites, oxDNA was capable of well-representing these structures and determining their bundle crossover angle.

For the 6HB kites, it was found that the smallest kite (140 nt) ended the relaxation procedure in a high energy bent conformation (it demonstrated Euler buckling) and, during simulation, broke apart the weak domains at the ends of the bundles to form straighter arms and a more favourable, asymmetric, shape. Even though the 220 nt and 420 nt kites the bundles went into simulation with quite straight bundles, they also broke apart the weak domains at the ends of their bundles, also leading to extended linker lengths. The total number of 2-base domains per kite was 16 pairs (one set of 8 called ‘critical’ and the other 8 ‘non-critical’), and all of these broke for all kites by the end of their simulations (when including the extra run-time for the 220 nt kite). The two primary reasons this breakage of domains occurred was due to (1) straightening out of the bundles pulling against the weak domains (most obviously for the 140 nt kite), and (2) the small amount of work needed to break the domains (in the case of the 220 nt and 420 nt kites).

The 6HB kites broke in an asymmetrical manner as the weak domains only appeared on two of the four bundle ends. The asymmetry is most obvious in the

140 nt kite since the linkers were still relatively taut even with the additional linker length from the broken bundle. This feature was less obvious in the 220 nt and 420 nt kites probably due to the longer linkers constraining the bundle positioning less. The experimental group looked at a distorted kite that had the same fundamental bundle design, but differed in the linker lengths. As the weak domains would also have existed in this structure, it is possible that these kites also had the same breakage issues. As such, this work has been able to provide some subtle details that TEM images might not have been able to capture and emphasizes the importance of the fine details of a design in DNA origamis subject to internal mechanical stress.

When comparing the 12HB to the 6HB versions, there were some main differences:

1. The 12HB kites were much sturdier and did not exhibit any Euler buckling, but this made complete sense as they were much thicker and therefore stronger. Also, there was no bundle breakage so the linkers remained a consistent length throughout, compared to the 6HB versions that broke open their weak domains and allowed for extended linker lengths. It should be noted that an approximate calculation of the bundle lengths (using OxView software) yielded results of ~ 87 nm for the 12HB kite and ~ 113 nm for the 6HB kite. This significantly contributes to the difference in results, as the linkers in the 12HB kites would experience less strain than the 6HB kites, due to the reduced bundle lengths.
2. The angles for the 12HB kites were very similar to those with experimental data available, the 6HB angles were larger likely due to the extended linker

lengths that gave the bundles more access to a wide range of possible angles.

Overall, it has been shown that oxDNA has accurately captured the overall structure and more subtle details for all of these kites. This provides further evidence that oxDNA is a suitable model for origami systems like these, and that the software can help to elucidate further information that experimental techniques cannot always achieve.

Chapter 5

Ball with Holliday Junctions

5.1 Introduction

5.1.1 Experimental Design, and Simulation Preparation and Details

The design for a DNA wire-mesh ‘ball’ was produced by Benson *et al.* in 2015.¹⁶ It was one of a series of designs presented that demonstrated a new method of generating mesh structures with the scaffold iterating around the structure, a feat that would be challenging to do without the accompanying sophisticated algorithm. Other designs included a nicked torus, a stickman and the ‘Stanford bunny’. Although not identical in method of creation or design, these structures bear resemblance, and could be considered something of a precursor, to the wireframe structures that have become increasingly popular in the last few years, the design and synthesis of which is only becoming more complex and sophisticated.^{20,93,94}

The structure of the ball is designed to be an icosahedron (20 sides) where each face has been divided into a further four triangles. Figure 5.1 shows the TEM images from the experimental synthesis of the ball. The group were also able to conduct cryo-electron microscopy analysis that built up a picture of the

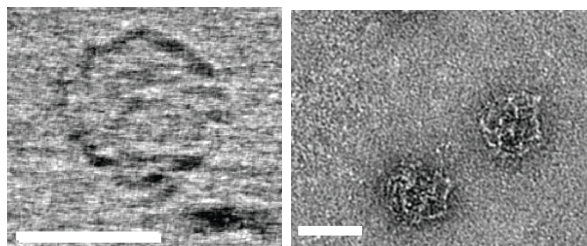


Figure 5.1: The experimental TEM images for the ball structure. Scale bar quoted as 50nm. Images reproduced with permission from Springer Nature, Benson *et al.*¹⁶

structure by taking ‘slices’ through it. These images confirmed the ball was able to fold up into a spherical shape and exist as a hollow structure.

Figure 5.2 depicts the oxDNA pre-relaxation structure, which was converted from a vHelix input file; this initial structure exhibits the intended icosahedral shape. Features of the design can also be mostly clearly seen for this configuration. For example, most edges consist of a single double helix, but a certain fraction consist of two double helices in order to enable the scaffold routing. At the vertices of the icosahedron five DNA struts meet, whereas the junctions at the middle of the icosahedron’s edges involve six DNA struts. Alongside it is the same structure at the end of a relaxation procedure. The design comprises 12 408 nucleotides (a 50/50 split of scaffold and staples) and underwent a standard minimisation and relaxation process.

One feature that makes this structure particularly interesting is the presence of many unique 6-way Holliday-style junctions, the 4-way version of which was first proposed by Robin Holliday in 1964.⁶⁷ The structure of such junctions are very susceptible to the presence of salt and, based on its concentration, can exist in an ‘open’ or ‘closed’ state. The open state is very flexible due to little or no ‘coaxial stacking’ between the duplexes. In an origami with no Holliday junctions,

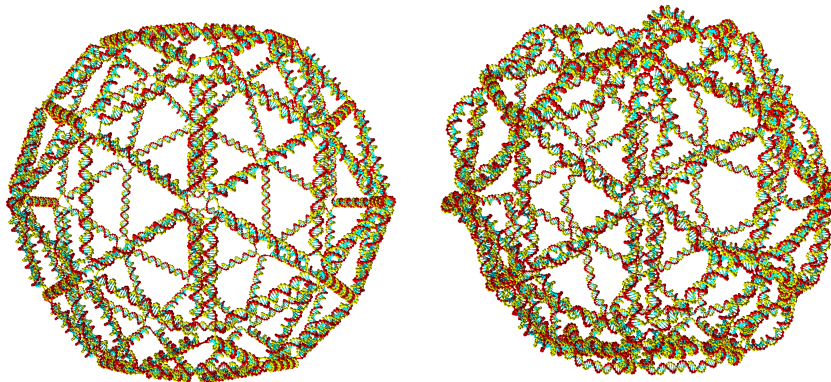


Figure 5.2: The ball mesh structure pre- (left) and post- (right) relaxation.

coaxial stacking occurs most commonly when one staple strand ends and another starts. Whereas a normal duplex would consist of two uninterrupted strands, a coaxial stack is one where stacking between bases remains but they are not all connected by a continuous backbone. The coaxial stacking thus helps to maintain the double-helical structure across the nick. As shown in Figure 5.3, for a closed 4-way Holliday junction, this stacking also appears but across a junction where the direction of two strands changes and both move onto a second strand.

Holliday junctions in a closed state are able to engage in a lot of coaxial stacking compared to those in an open state, and so the junction is more rigid. Although they normally appear as ‘two’ arms, comprised of four components as in Figure 5.3 (bottom image, showing a 4-way junction), the junctions in the ball structures exist as ‘three-stranded’ motifs, so have six components.

Figure 5.4 shows images of individual 6-way Holliday junctions in the open and closed states. The junction in the open state clearly has a gap where there is a lack of stacking, but for the closed conformation it is easy to see the stacking producing 3 mostly ‘closed’ duplexes. The open state exists when the structure is in a ‘low

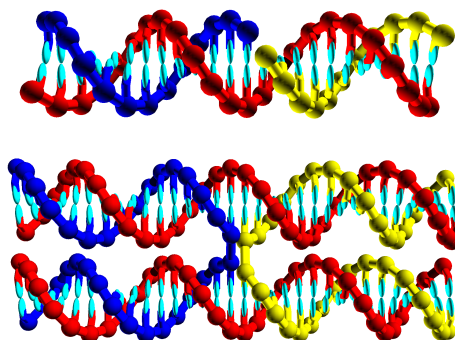


Figure 5.3: Coaxial stacking for a duplex (top), caused by one staple strand ending (yellow) and another beginning (blue), and an example of a closed Holliday Junction (bottom).

salt’ concentration regime, and the closed state when in a ‘high salt’ one. When it is considered that these junctions appear at the end of every edge, it is clear to see that the action of these junctions could have significant consequences on the overall flexibility and conformation of each ball, depending on the salt concentration used.

There are two types of movement the balls can undergo as a result of these junctions opening and closing: local vs global. The local changes are individual junctions opening and closing, as well as the subsequent movement of their neighbours, and global changes can be achieved based on whether there is more or less stacking overall - a more unstacked conformation would look very different to one which engages in high amounts of stacking. To investigate this, we simulated the ball structure at high (1 M) and low (0.1 M) salt concentration. When using the oxDNA software it is possible to monitor the coaxial stacking energy during simulation, where a more negative energy is recorded when more stacking is present. Although the measurement script cannot differentiate between the normal stacking found on edges (due to the scaffold/staple design in all origami) and the Holliday

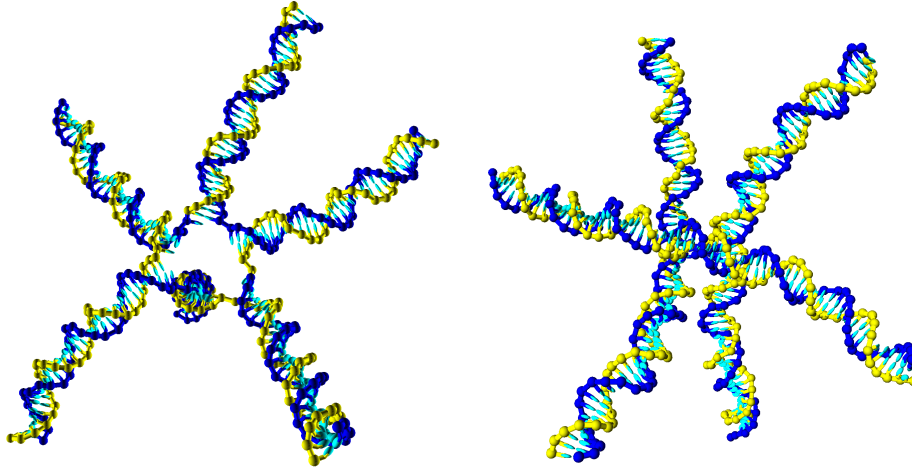


Figure 5.4: The open (left) and mostly closed (right) 6-way junctions.

junctions, the balls were identical in design so any differences can be attributed to the junctions opening and closing.

To monitor and measure changes in these structures, three simulations were run for different timescales (10^5 , 10^6 and 5×10^7 steps), allowing for the observations of local and global effects. Each timestep in the simulation is equivalent to 15.2 fs so, for example, the 10^5 step simulation is approximately 1.52 ns in duration.

5.2 High and Low Salt Ball Results

5.2.1 Simulation Outcomes

Figure 5.5 shows the results from all simulations across all three timescales for both low and high salt concentrations. The most obvious difference between the two outcomes in all cases is that the low salt ball still mostly resembles the original shape of the ball; it is more spherical and recognisable as a ball shape, albeit with some kinks on the surface. Conversely, the high salt ball is far less recognisable.

The spherical shape has been largely lost and the ball appears in a more compact form. In all three high salt ball images, at least one duplex edge has been bent into an arched shape. Such a conformation is energetically uncomfortable and has occurred due to stacking at both junctions either end of the duplex. There are some softer arches visible in the low salt balls, but none as defined or protruding as that in the high salt results.

Additionally, it is clear to see some of the open junctions in the low salt ball, whereas the compact shape of the high salt ball means the junctions are barely visible, largely due to the crowding at junctions where extensive stacking has taken place. It is clear from visual analysis that, as expected, the coaxial stacking at junctions in the low salt ball is minimal, and there is extensive stacking in the high salt ball that has led to a distorted and more strained structure.

5.2.2 Stacking Profiles

The coaxial stacking energy profiles for both salt concentration simulations across each timescale are shown in Figure 5.6. The first major feature of the graphs are the significant differences in stacking energy recorded between high and low salt concentrations. The energies are recorded in the profiles as absolute values as this means a value higher up in the graph corresponds to more stacking, which makes sense intuitively. The trend for all graphs is that the higher the concentration of salt in the simulation, the more stacking the ball undergoes at the Holliday junctions. This matches the visual results, which clearly showed more stacking in the high salt simulations.

The next important feature relates to the local vs global effects for the struc-

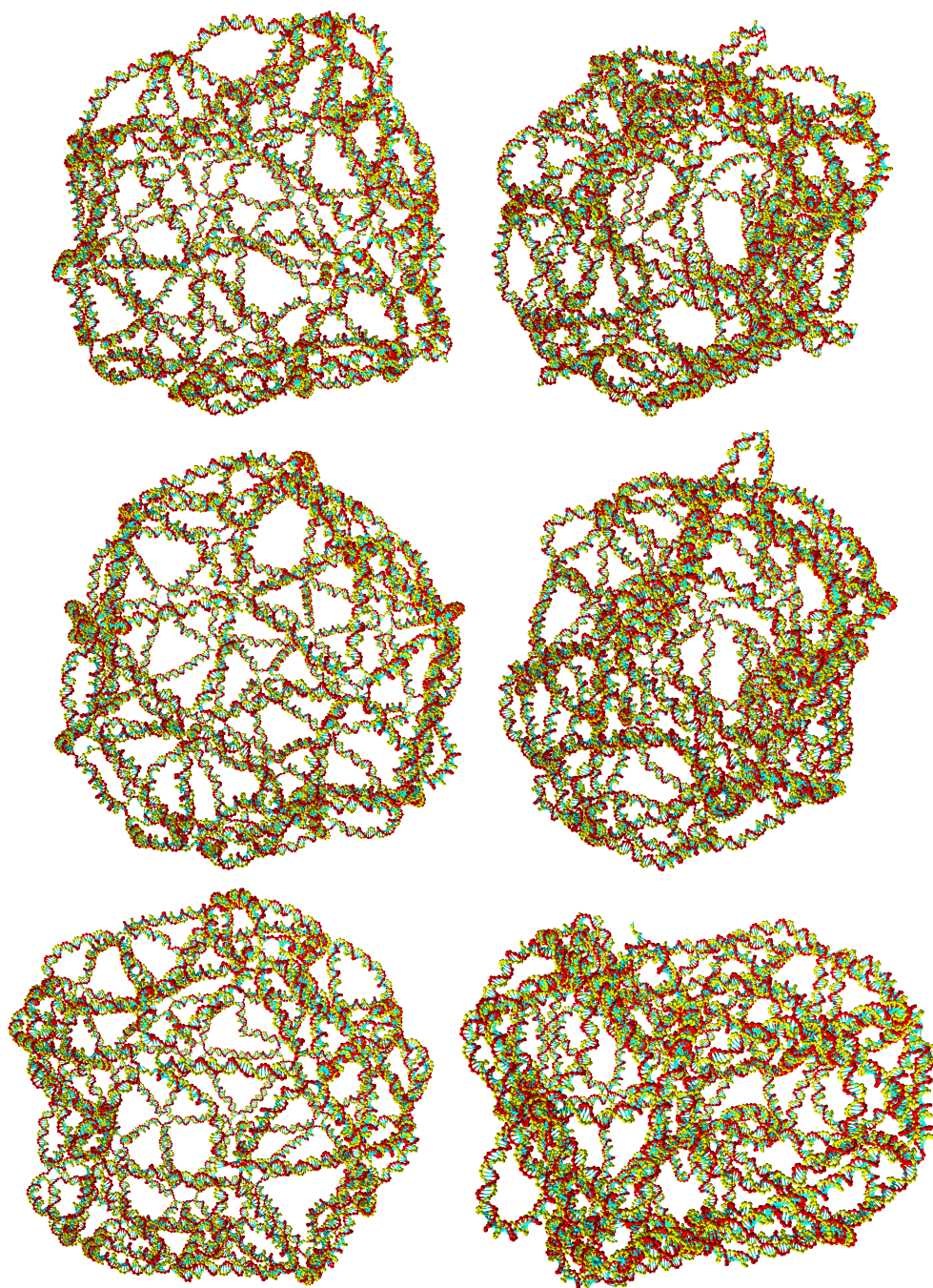


Figure 5.5: The low (left) and high (right) salt balls at the end of all simulations: 10^5 (top), 10^6 (middle) and 5×10^7 (bottom) steps.

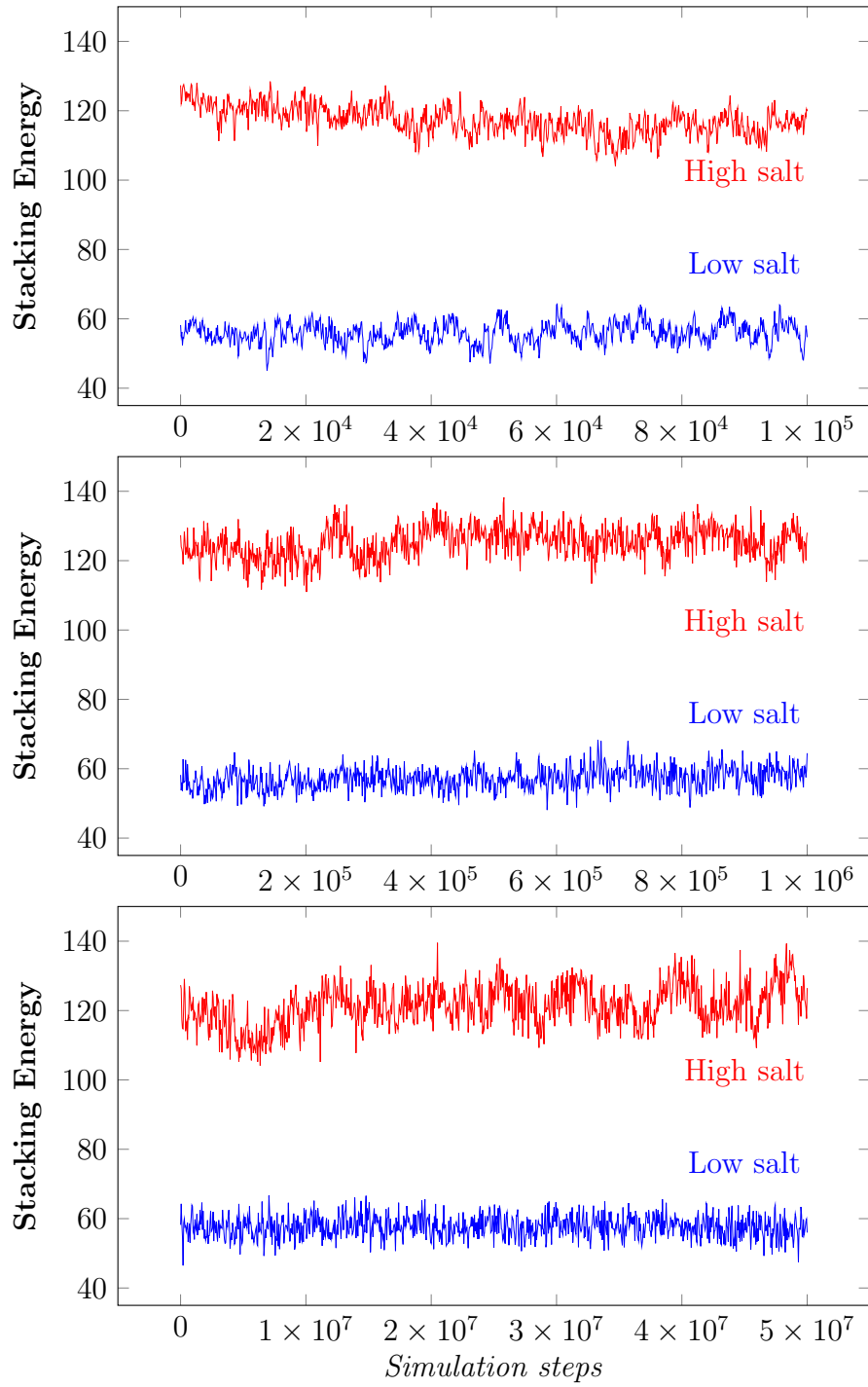


Figure 5.6: Absolute energies for coaxial stacking for high (red) and low (blue) salt concentrations, over short (10^5 steps, top), medium (10^6 steps, middle) and long (5×10^7 steps, bottom) timescales.

tures. The shorter simulations (10^5 and 10^6 steps) provide information as to the changes in extent of stacking at the junctions, as the snapshots were taken on a shorter timescale than for the longer simulations, and could capture quicker changes in the opening and closing of junctions. There is significant fluctuation between the data points, which suggests the junctions are quite dynamic and open/close on a short timescale. However, whilst there is oscillation of individual junctions, on average there are a constant number of junctions open or closed at any time, and this is the case for high and low salt concentration data. This is indicated by the trend line staying approximately constant in all cases.

Overall, for the low salt data over all simulations it is clear most junctions are open, as indicated by the lower recorded energy, but the fluctuations suggest some opening/closing as well. As previously mentioned, some stacking energy will always exist due to staples starting and ending, so a large quantity of this energy can be attributed to that. It is also likely some junctions will randomly close even in low salt concentration conditions, either due to thermal fluctuations or the effect of neighbouring junctions. For the high salt simulations, most junctions are in a closed position at any time, but the fluctuations also show opening/closing as well. It could be the case that having two neighbouring junctions closed is energetically unfavourable (causing the previously highlighted arched edges) and this forces some of the junctions to open and close when ordinarily they would not.

Changes in the overall trend line are more informative about the global structure of the ball at that moment. For the shortest simulation the average trend line stays very constant over both salt concentrations. In the high salt medium

length simulation there is more variation, such as a peak just after 2×10^5 steps, suggesting the ball had a period of significantly more stacking that would lead to a more compact structure. However, variation in the trend line is most noticeable in the longest simulation of 5×10^7 steps. A trough in the data is recorded at approximately 5×10^6 steps, which shows the ball as a whole was in a state of being more unstacked. In other words, a large proportion of the junctions were opened compared to the average. Furthermore, at the end of the simulation there is a peak, which is a time where the ball was undergoing a phase of being highly stacked, where more junctions were closed compared to the average. The low salt ball does not experience the same kind of changes over either of the longer simulations, and the number of junctions open at any time remains largely constant.

Through simulation and the monitoring of these junctions, it has been established that, depending on the salt concentrations the ball structure is exposed to, the junctions can adopt an open or closed form. The conclusion is that an increase in salt concentration promotes the closed conformation, and the reason as to why was determined by Duckett *et al.* in 1988 for 4-way junctions, but is still applicable here for the 6-way junction.⁹⁵ The DNA backbone is very negatively charged, due to the presence of a large number of negatively charged phosphate groups. Each of the junctions are crowded as there are 6 duplexes meeting at one location and this leads to a large build up of electrostatic repulsion in this area. As such, the junctions are unable to stack since this would further increase the repulsion force. However, when in salt solution, the salt can dissociate into ions, where the cations can then ionically bond with the negative charges of the duplex backbones. This decreases the electrostatic repulsion around the junctions and stacking is able to

take place, since the primary factor to prevent it has now been quenched. This explains why in high salt conditions, there is extensive stacking, but in low salt simulations, these necessary charges are unavailable to promote stacking and the junctions remain largely open. As mentioned earlier, not all junctions in the high salt regime are stacked at all times, since this would force edge duplexes into uncomfortable arched shapes, which likely gives rise to the local and global changes observed. The average may be deviated from with troughs due to the structure opening junctions to avoid arched edges, and into peaks due to random or thermal fluctuations that temporarily allow other arches to be sustained.

On closer inspection of some of the high salt junctions at the end of simulation, there are some instances where four of the six duplexes are stacked and two are not. This is perhaps due to steric hindrance rather than charge repulsion since these junctions are still physically crowded.

5.3 Ball with Bulges

The ball with bulges is a modified version of the previous ball structure. It has mostly the same design but with the difference that some helices have an extra base, located at the end, where a junction is. The additional base will make stacking at each junction more difficult due to steric hindrance, so the expected result is that the level of stacking in the ball, particularly for the high salt regime, should decrease. The ball with bulges was run for the same time scales as before, also at 300 K and both low salt (0.1 M NaCl) and high salt (1 M) concentrations. Stacking energy was again measured, with the same assumption that the baseline caused by scaffold/staples starting and ending contributed an equal quantity over

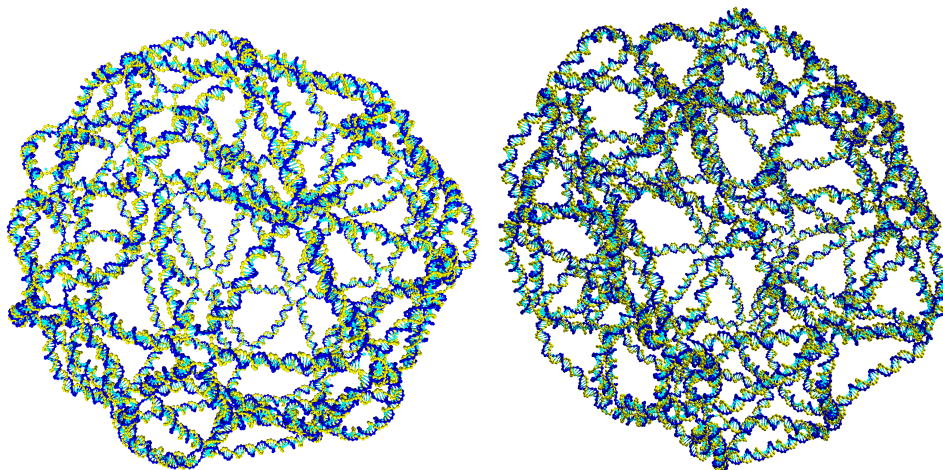


Figure 5.7: The low (left) and high (right) salt balls with bulges at the end of simulation of length 5×10^7 steps.

both salt concentrations.

5.3.1 Simulation Outcomes and Stacking Profiles

Shown in Figure 5.7 are the simulation results for the balls with bulges when simulated in high and low salt concentration regimes. Visually, these two equilibrium structures look quite similar. Both appear to have a significant quantity of opened junctions and there are fewer arched edges. Furthermore, the two structures have similar conformations. Whereas before the high salt ball looked to be very compressed and squashed, these both appear to be less strained.

Stacking profiles for both modified structures for all timescales and salt regimes are shown in Figure 5.8. As there is significant overlap between the data for all the graphs, they have been broken down into their separate components in Figure 5.9. For the low salt concentration simulations, the extent of coaxial stacking is very similar to before - the average was previously ~ 60 and is now ~ 65 . There are, again, some peaks and troughs in the data but overall the stacking is very

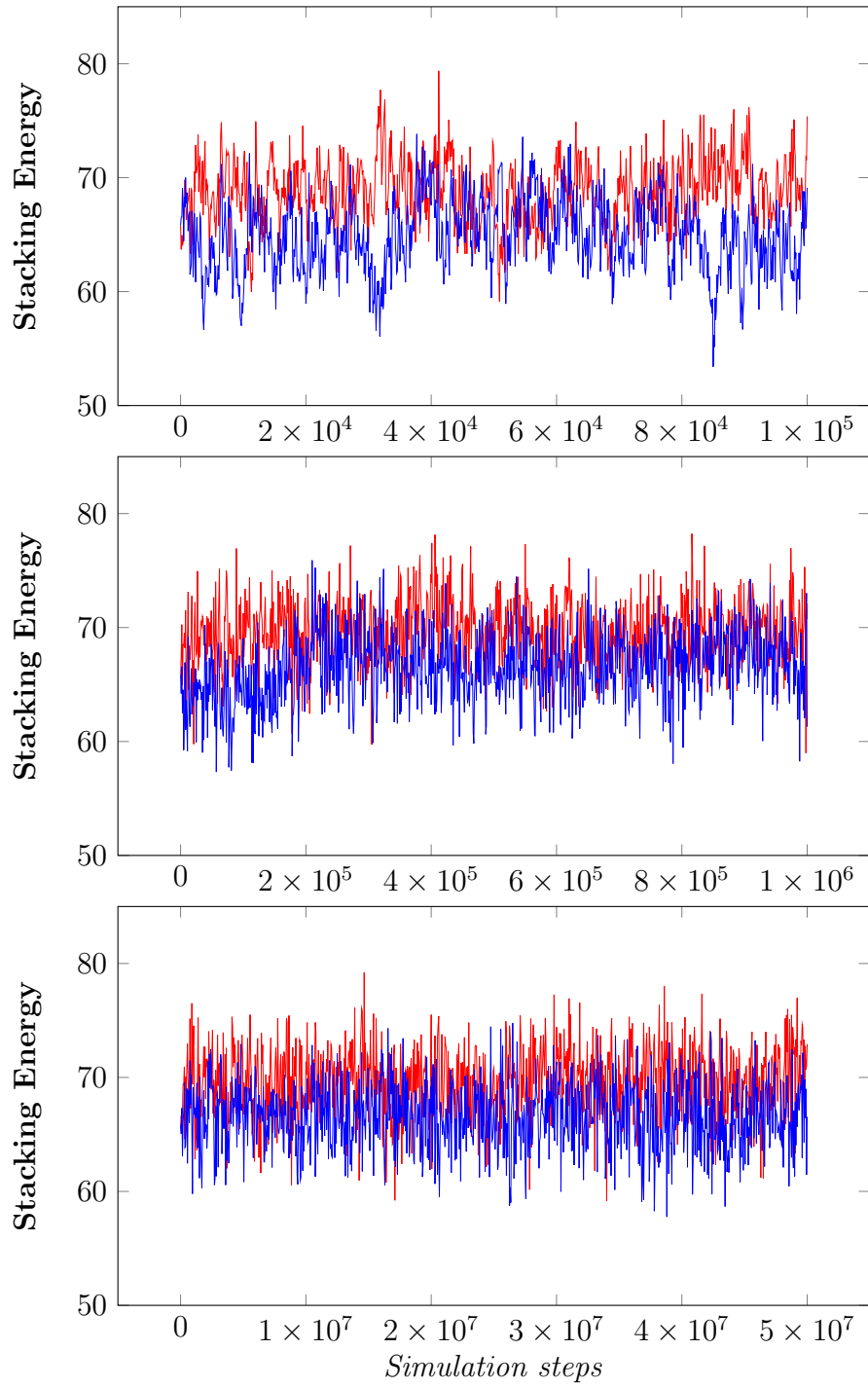


Figure 5.8: Absolute energies for coaxial stacking of the balls with bulges for high (red) and low (blue) salt concentrations, over short (10^5 steps, top), medium (10^6 steps, middle) and long (5×10^7 steps, bottom) timescales.

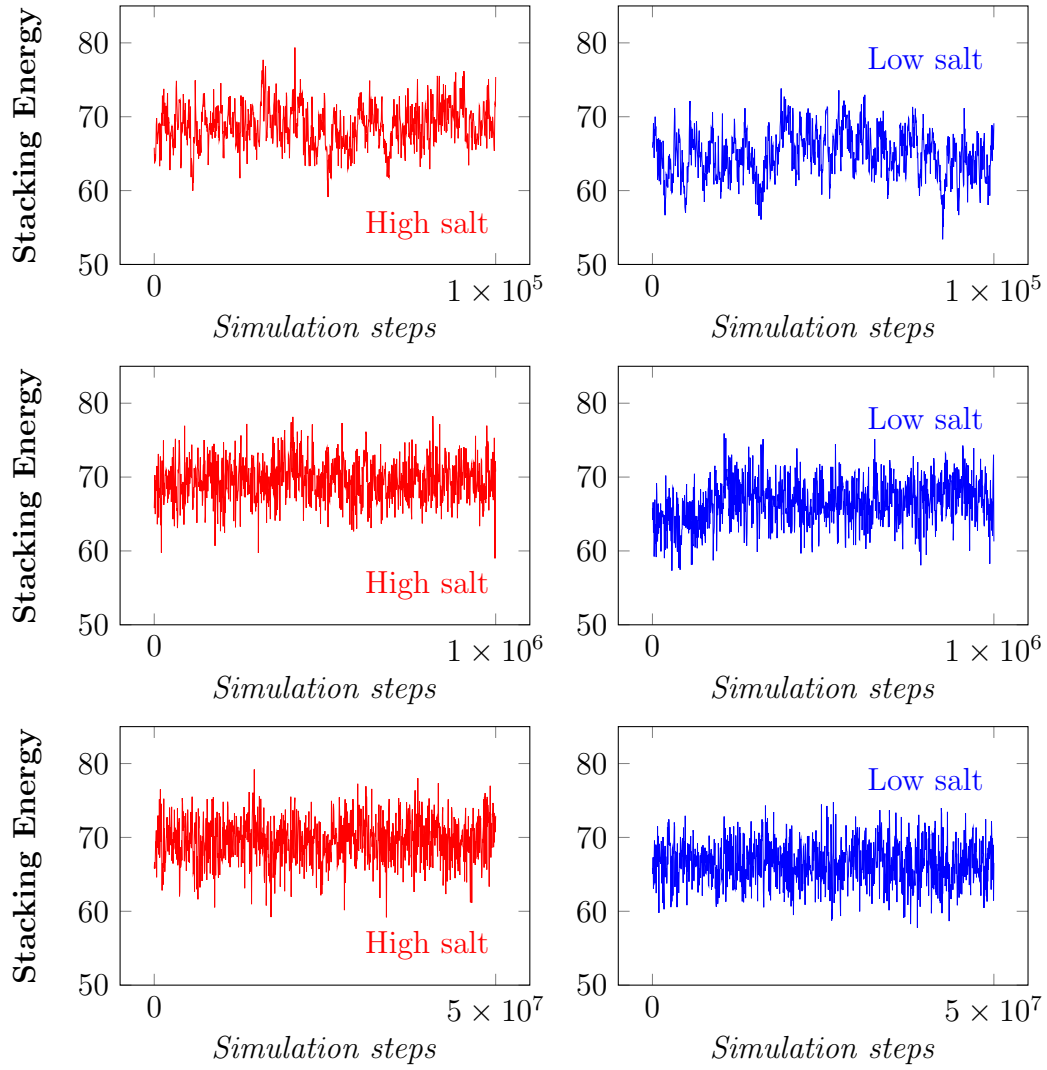


Figure 5.9: Absolute energies for coaxial stacking of the balls with bulges, shown individually, for high (red) and low (blue) salt concentrations, over short (10^5 steps, top), medium (10^6 steps, middle) and long (5×10^7 steps, bottom) timescales.

similar to the previous set.

The most significant change is the decrease in stacking energy for the high salt concentration simulation data. Whereas before the average was around ~ 120 , it has now decreased to around the same value as for the low salt structures, ~ 70 . Local changes, indicated by fluctuations in the data, are still present, but overall the trend line is very consistent.

As predicted, the data for both salt regimes recorded limited stacking due to the additional bases at the junctions. This is particularly applicable in the case for the high salt regime. Whereas the electrostatics were previously the cause for stacking prevention, this time there is steric hindrance. This provided enough disruption to prevent stacking that even a high salt concentration was unable to negate.

5.3.2 Conclusions

In this chapter ball structures with unique 6-way Holliday junctions have been simulated in high (1 M NaCl) and low (0.1 M NaCl) salt concentration regimes, with the effect on coaxial stacking recorded. The junctions can exist in two forms: open and unstacked at low salt concentrations, or closed and stacked at high salt concentrations. To investigate this effect, simulations for both salt regimes across three timescales (10^5 , 10^6 and 5×10^7 steps) were run, and coaxial stacking measured during them. This choice of timescale allowed both the local and global changes of the junctions to be explored.

It was found that, across all simulations, more coaxial stacking was recorded in the high salt regimes and much less for the low salt regimes. Fluctuations in

all data demonstrated that the junctions were dynamic and frequently switching between states across all simulations. Movements in the trendlines demonstrated periods of time where the balls were in overall more stacked or unstacked states, and this was found to happen mostly for the high salt regime structures. Visually, the high salt ball was in a more compressed state, which correlated with the conclusion that more junctions were stacked at any time, making a more constrained shape. It was also possible to observe edges bent into an arch that had been created by stacking occurring at both junctions either side of the edge. Whilst this would have been an unfavourable conformation, it perhaps explained the periods of time where more stacking than average was recorded.

The effect of salt concentration on Holliday junctions was first discovered by Duckett, who determined that this behaviour occurred due to extensive negative repulsion from the DNA phosphate backbone being quenched by the presence of salt cations, thereby reducing the repulsive forces.⁹⁵ At low salt concentration no such prevention existed and the junctions were unable to close due to the high negative repulsion at the junctions.

In addition to these structures, a modified ball was also simulated. It contained additional bases placed at the end of helices and as part of the junctions. After simulation across all three timescales in both salt regimes, two ball structures resulted that were very similar in conformation and extent of coaxial stacking. It was concluded that the presence of the additional nucleotide introduced steric hindrance into the junction which prevent stacking, even in the high salt environment.

We used oxDNA in this chapter to simulate ball structures that had previously been assembled experimentally, and have been able to understand and expand the knowledge into the behaviour of these structures through simulation and subse-

quent analysis.

Chapter 6

A DNA Auxetic Design

6.1 Introduction

6.1.1 The Background of Auxetic Materials

2D auxetic materials have the unusual property of expanding in size in two dimensions when placed under tension in one, in other words, they are said to have a negative Poisson ratio.⁹⁶ It would be like pulling on a rubber band and having it expand in width as well as length. A very well-known example of an auxetic material is the ‘rotating squares’ model, originating from Grima and Evans in 2000.⁹⁷ An equivalent image of this model from Dagdalen *et al.* is shown in Figure 6.1.⁹⁸ As discussed in the Introduction Chapter of this thesis, auxetic materials are quite a new area of research, particularly for DNA Origami and computational chemistry in general; however, there has been a surge of interest in them already on both the nano and macro scale. For example, macro mechanical metamaterials can occur as patterns cut into materials, and an everyday example is the Nike Flyknit running shoe, that has a geometric pattern cut into the sole and expands on impact, reducing the force felt by the runner when the shoe makes contact with the ground.⁹⁹

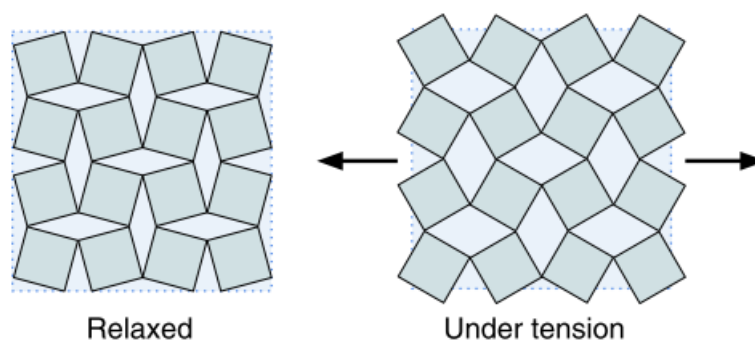


Figure 6.1: The rotating squares auxetic material design, when relaxed the squares are in a closed form but under tension they expand in size in two dimensions. Image reproduced with permission from Dagdelen *et al.* under Creative Commons licence CC BY 4.0.⁹⁸ The link for this licence is <https://creativecommons.org/licenses/by/4.0/>.

There has also been interest in nanoscale auxetic materials, such as the following two examples. Li *et al.* designed and assembled 2D DNA nanostructures that used ‘jacks’ to allow expansion and contraction of structures. The jacks could be shortened or lengthened and this determined the conformation the structure adopted, thereby producing the auxetic effect. Research from Dagdelen *et al.* used computational methods to predict 3D inorganic auxetic structures. Through a targeted screening process the group identified candidate compounds (some are hypothetical) that are predicted to exhibit auxetic behaviour.^{21,98}

In this work, we aim to combine these concepts by using DNA to create auxetic behaviour, and essentially designing nanoscale mechanical metamaterials. In order to do so, we attempted to design a system synonymous to the well-known rotating squares model, which has not been created before with DNA origami, using design techniques learnt from previous projects in this DPhil, and to explore, computationally, the properties of such designs.

6.1.2 Simulation Preparation and Details

The design was based on the rotating squares model, choosing an example which is comprised of 16 individual squares connected together to form the overall structure. It was decided to split this up into four, 4-square ‘subunits’ made of DNA that could then be built up into the larger structure. To maintain the two-dimensional nature of the system it is envisaged that the origami would be adsorbed onto a surface. This is experimentally feasible in a number of ways, e.g. using cholesterol tags to bind an origami to a lipid bilayer.¹⁰⁰ As Holliday junctions had been extensively investigated previously (Chapter 5), these were used as inspiration to use high and low salt concentrations as a method of controlling the movement of the structure. They were also to include a linker across the centre to prevent the structure opening too far. This would not be beyond the rotating squares model design, which only depicts a small gap in the centres of the subunits.

After some trial and error, together with previous knowledge of DNA origami and auxetic systems, a set of guidelines were devised that led to the two developed designs, where the second addressed some problems encountered during the production of the first. The guidelines were:

1. The tiles needed to have crossovers at the end of each helix joining them to both adjacent helices, as crossovers even only a short distance in from the ends were not sufficient to prevent splaying of the helices at the edges of the origami, which in turn affects their ability to coaxially stack (see below). This had implications for the size of the design, and set minimum widths and lengths as a result to ensure crossovers were placed in suitable location to avoid straining the structure.

2. Larger tiles allowed each subunit to be a full-sized origami.
3. As the tiles started in a fully closed position, coaxial stacking occurred between the top and bottom pairs of squares. As such, a low salt concentration could be used to ‘switch off’ this stacking and allow expansion of the structure, and high salt could be used to maintain the closed state, or re-close an open structure.
4. Ideally, one scaffold strand would iterate around an entire four-square design, rather than a multi-strand design, to be in keeping with the idea behind DNA origami design. This criterion was added after the simulation of Design 1 and was the largest change when finalising Design 2.
5. To mimic the effects of adsorption of the origami on a surface the repulsion planes feature that is implemented in oxDNA was used (specifically, two planes equal in force but opposite in direction on each side of the structure) during the relaxation and simulation processes. This had the benefit of flattening out the tiles and allowing easier viewing of their actions.
6. A linker across the centre of the design was useful to limit the extent of opening and, as it was a single-stranded length of DNA, it could either be adhered or not to the ‘surface’ (as it is debatable whether it would be attached or not experimentally). From trials with both linker attached and detached, there was no observable difference, so for the results shown, all designs have a linker constrained within the planes too.

The two designs will be presented individually, so it can be demonstrated how the second improved upon the first. As with all structures, they underwent the usual

minimisation and relaxation procedure (at 1.0 M NaCl), followed by a simulation at 300 K for 10^8 steps for the 4-square units and 5×10^7 for the full 16-square structures. The low salt concentration used was 0.2 M NaCl and the high salt concentration was 1.0 M NaCl.

6.2 Results and Discussion

6.2.1 Design 1

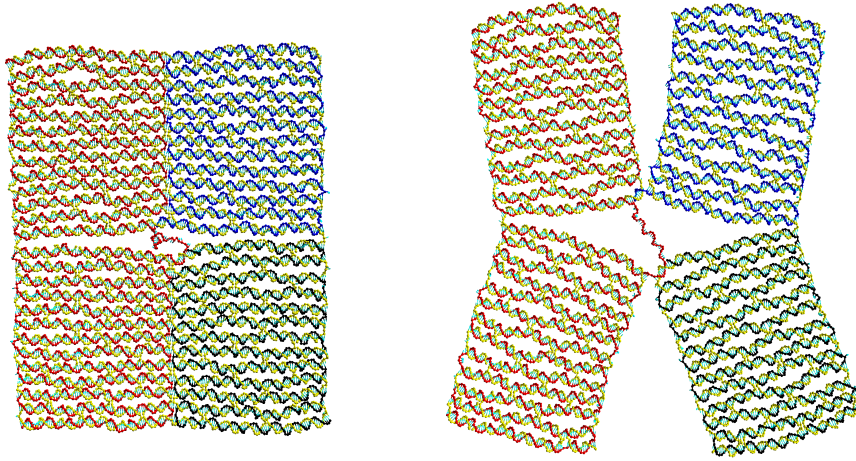


Figure 6.2: The simulation results for a 4-square unit of a rotating squares auxetic material. Closed (high salt, left) vs open (low salt, right) conformation depends on salt concentration. Different coloured scaffold strands used to show the three scaffolds required per structure. The longest (red) is 2112 bases, the other two (blue and black) are 1048 and 1030 bases long, respectively, making an accumulative scaffold length of 4190 bases.

The low and high salt concentration configurations for the first subunit design (8350 nucleotides) is shown in Figure 6.2. As postulated, the low salt concentration disfavours stacking along the inner edges of the squares, so they unzip and open up the structure. The high salt concentration simulation, conversely, maintains

the structure in a closed conformation. As such, the different salt concentrations have controlled the movement of the structure as expected.

This 4-square unit could then be scaled up to make a full 16-square structure, as shown in Figure 6.3. The structure has been made by ‘manually’ adding connections between each of the four 4-square units; ideas about how to allow for self-assembly will be detailed later on. In both figures, colour changes between neighbouring squares have been used to show the different scaffold strands. As mentioned in the guidelines above, ideally, only one scaffold would have been used and this was changed for Design 2 by using a ‘seam’ design that iterated around the whole subunit.

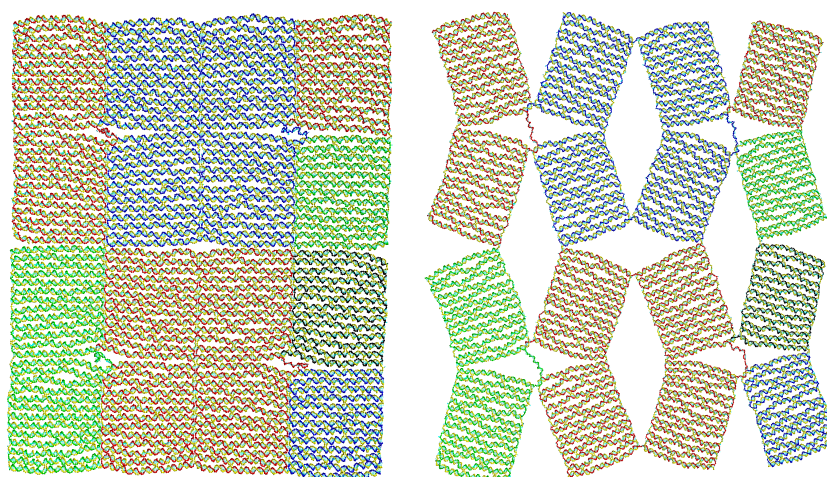


Figure 6.3: The first design scaled up to make a 16-square structure. Multiple scaffold strands were used and is reflected in the colour scheme. Closed (high salt, left) and open (low salt, right) conformations are compared.

6.2.2 Design 2

Images showing parts of both designs are shown in Figure 6.4 to highlight the difference in scaffold routing around the structure. The primary differences are

that this 4-square unit is much larger (11550 nucleotides) and has a ‘staggered seam’ design. Instead of the scaffold running from one side to the other, as with the first design, the second design also involves scaffold crossovers in the body of the origami (such crossover are sometimes called ‘seams’) and allows the entire 4-square unit to be produced from one scaffold strand. In this way one avoids the need for a scaffold end at one end of the outermost helix of the squares, which in turn necessitates multiple scaffolds. This new seam design is much more advantageous since synthesis would be more straightforward. Two other differences

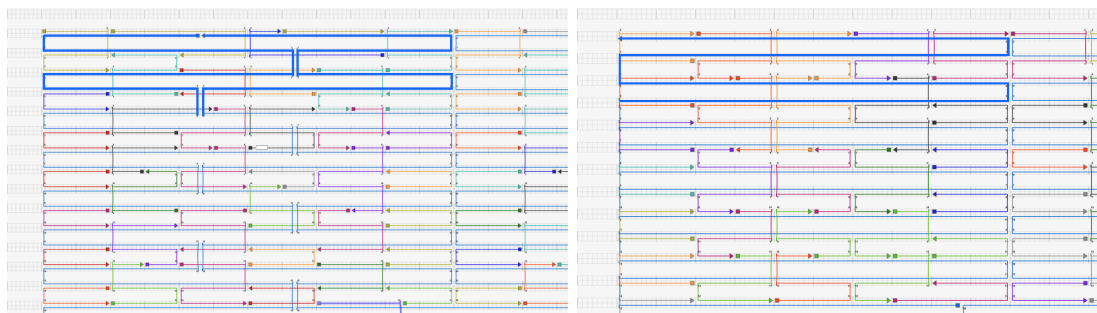


Figure 6.4: caDNAno designs for a staggered seam 4-square unit design (left), compared alongside the previous multi-scaffold design (right). The staggered design allows crossovers in the centre and for one scaffold to be used, rather than a scaffold iterating from left to right that requires multiple. The difference is partly highlighted for demonstrative purposes.

are that the linker in the centre is a long staple strand whereas before it was part of the scaffold, and the connections between 4-square units for the full 16-square structure are staples instead of scaffold strand, this allows each 4-square unit to be a separate scaffold strand, which is more sensible in terms of synthesising a structure like this. The 4-square structure and scaled up 16-square version is shown in Figure 6.5. The scaffold strands have been shown in blue and red; so to make the 16-square assembly, four 4-square units were needed, each with one scaffold strand. As for Design 1, the low salt conditions cause the structure to

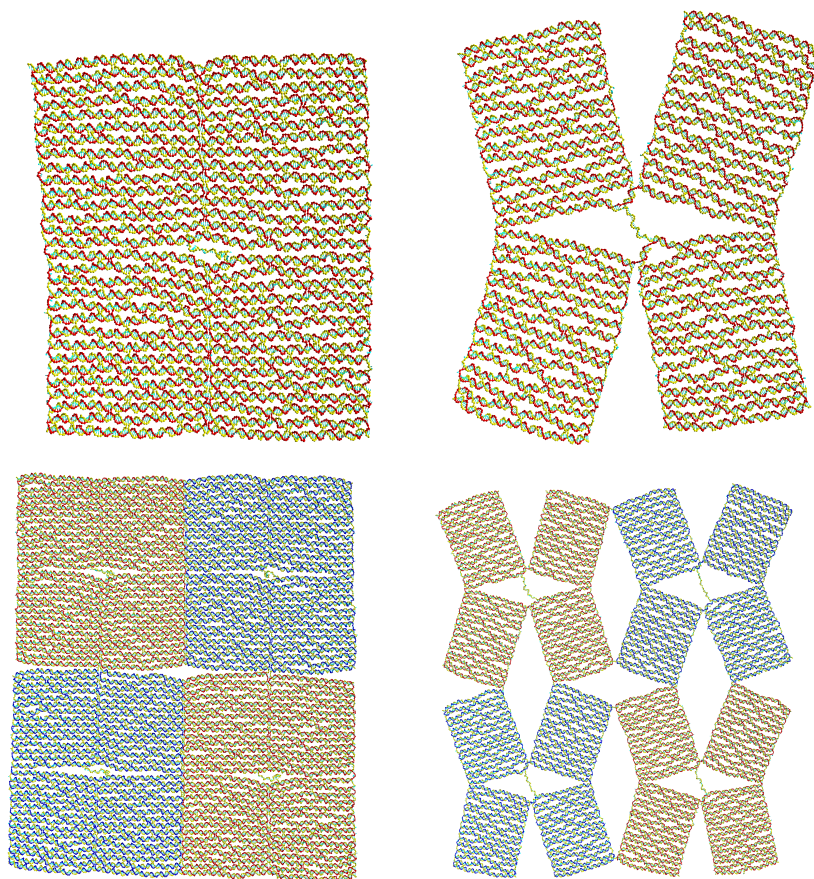


Figure 6.5: Second design with a staggered seam for a 4-square structure (top) and scaled up to make a full 16-square structure (bottom). Colour scheme indicates scaffold strands, one for the 4-square structure and four in total for the 16-square structure assembly.

open up, whereas at high salt it maintains a closed conformation.

From observing every simulation image, it is clear that there are no states where one half of the 16-square unit stays shut and the other opens, it is all either open or closed. This is most likely due to the low salt concentration preventing coaxial stacking along all edges so all are able to open at the same time, there is no need for co-operativity where one side opens and encourages the other to.

To quantify these results, it was decided to estimate the area occupied by

the smallest ‘box’ that could fully contain each structure when open and closed. This would give an approximate indication as to the extent of expansion for each structure. Each structure was rotated to be aligned in the same direction, then the coordinates for the highest and lowest points obtained. The length, width and area for the ‘box’ needed to contain each structure were recorded and the results are shown in Table 6.1. As can be seen from the Table, both the lengths and

	High Salt			Low Salt		
	L (<i>nm</i>)	W (<i>nm</i>)	A (<i>nm</i> ²)	L (<i>nm</i>)	W (<i>nm</i>)	A (<i>nm</i> ²)
4-square structure	77	66	5082	98	82	8036
16-square Structure	155	132	20460	196	176	34496

Table 6.1: Approximate sizes of boxes required to contain the open and closed auxetic materials for both the 4-square structure and full 16-square structure with a staggered seam design. Abbreviations: length (L), width (W) and area (A).

widths of the ‘boxes’ required to hold the structures increased, indicating that auxetic behaviour has been achieved.

Up to this point, we had designed a structure that could reasonably demonstrate auxetic behaviour and was dependent on salt concentration for its expanding and contracting behaviour. Each 4-square unit is a single origami in size, and is comprised of one scaffold strand that, due to a staggered seam design, could iterate around the structure in one piece. To scale the 4-square unit up into an assembly, synthetically, two types of 4-square units would be required where the corners were complementary according to the scheme in Figure 6.6. Two extended linkers and two complementary regions per 4-square unit have been shown here, but it would also be possible to have one 4-square unit with four external linkers

and a second with four complementary regions as well. Either way, two 4-square units with four unique and complementary corners would be required and could reasonably assemble into a larger structure by building up from separate 4-square units. The design outlined previously would require minimal modification for this.

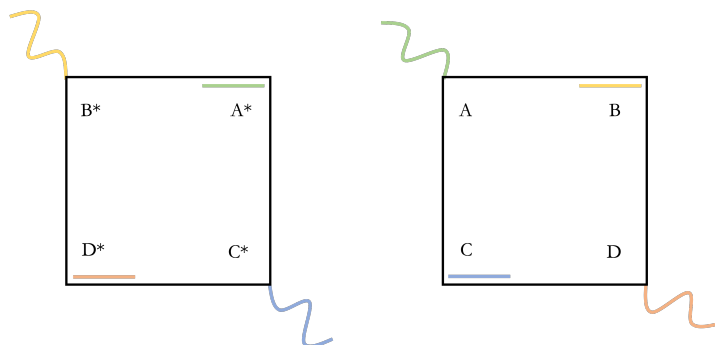


Figure 6.6: Two auxetic 4-square units that have unique but complementary corners that could be used for scaling up into a 16-square (or more) origami.

6.2.3 Cycling through states

Once the 4-square unit was simulated in both high and low salt concentration conditions, it was decided to place the opened structure back into a high salt concentration simulation to determine whether it could, theoretically, cycle through the open and closed states, shown in Figure 6.7. It was found that the 4-square unit cycled around the salt changes, opening and closing quite quickly into each simulation, leading to the conclusion that the salt concentration is a very important driving force behind the auxetic behaviour. It also revealed that the closing of the structure was partly co-operative, once some adjacent duplexes had closed, the other followed quickly, in a ‘zipping up’ manner (visible in the high salt $t = 3,500,000$ step). As such, if the salt concentration could be changed during

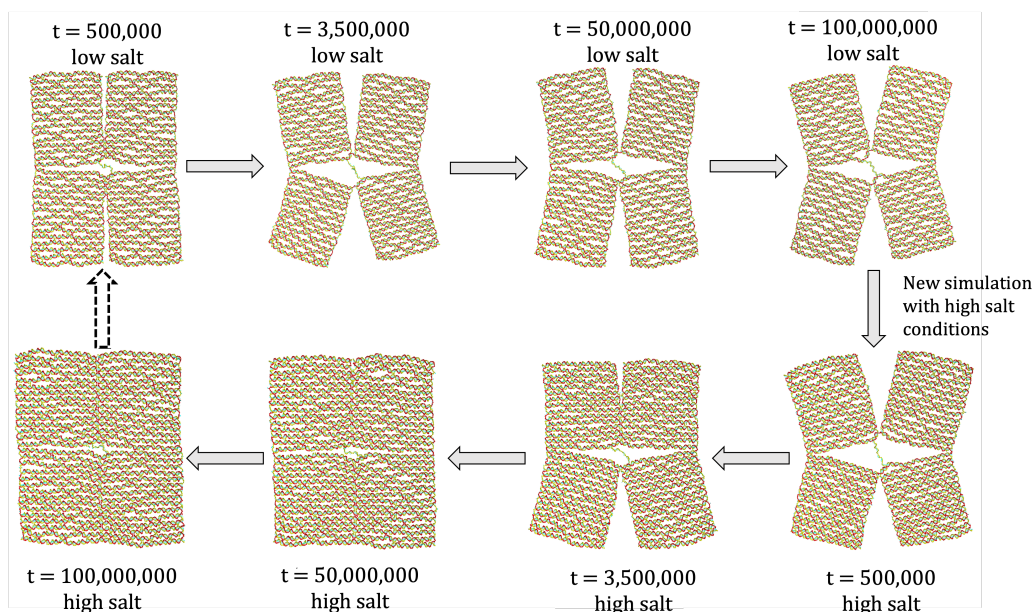


Figure 6.7: Single 4-square unit opening and closing due to two simulations at different salt concentrations: low (0.2 M) and high (1.0 M). Snapshots are taken at times when motifs of interest are present (e.g. the partly ‘zipped’ up structure can be seen at $t = 3,500,000$ steps in the high salt (bottom) images). The final structure from the low salt simulation was placed into a high salt simulation. The final structure from this high salt simulation was not put back into a low salt simulation a second time but it is assumed it would re-open (indicated by a dashed arrow), thereby allowing it to cycle through the opened and closed states, determined by salt concentration.

experiment in a similar manner, then this further demonstrates a suitable method for controlling the auxetic behaviour.

6.2.4 Creating a 3-state design

For all structures in an open state, the linker remained taut, preventing the structure from excessive opening as it was designed to (in order to remain in keeping with the rotating squares model). It was decided to remove the linker for the single 4-square unit to see what happened, and the results are shown in Figure

6.8. The 4-square unit is exactly the same as for Design 2, except for the small modification required to remove the linker and extend a staple to replace where the linker had been connected. As can be seen from the Figure, the low salt

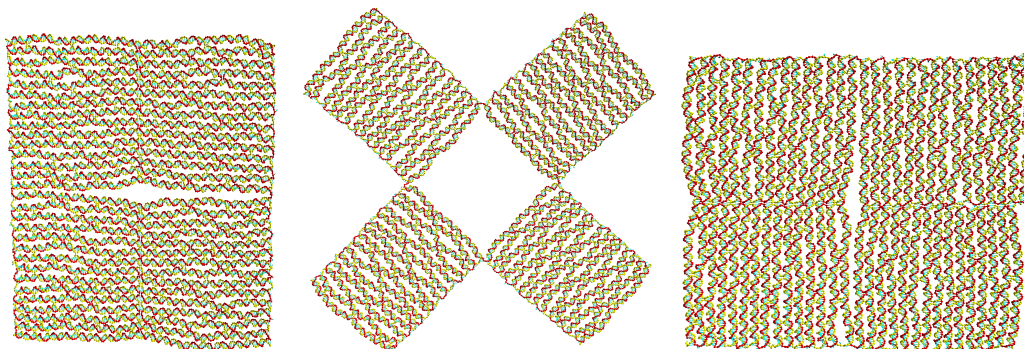


Figure 6.8: A single 4-square unit with the linker removed, high salt (left), low salt (centre), and closed in the opposite direction (right).

4-square unit opened up completely into a structure that could then be closed in two ways. It could either close the same way it opened (vertically), or on the second axis (horizontally), and it was decided to investigate if closing along this one could produce a stable state. As such, we ‘manually’ closed it (using gentle forces during an additional relaxation step) along the horizontal axis, then simulated it to determine if this would be a viable closed structure (right hand side image of Figure 6.8). Whilst the stacking on the right hand side appears to be slightly misaligned, only a small proportion has been disrupted and it is likely due to the small breakage of base pairing for five duplexes on the right hand side that has led to some disrupted coaxial stacking. It is possible that with further simulation, these could repair and correct the misalignment. If this breakage was not there, the bonding would be paired as expected and it would look very similar to the starting conformation, just rotated and stacked along the other edge. The fact this structure has a stable state from closing in the opposite direction suggests

this design has the potential to be an auxetic material that can switch between three states instead of the usual two, again controlled by salt concentration.

6.3 Conclusion

In this chapter, oxDNA has been utilised to design a new auxetic design, equivalent to that of the rotating-squares model, that opens and closes depending on low (open state) or high (closed state) salt concentration. An initial design was simulated and areas of improvement within it identified, with the changes then implemented in a second design. The biggest problem was that the 4-square unit had multiple scaffold strands, so a ‘staggered seam’ design was used to resolve this. The second design was a 4-square unit comprised of only one scaffold strand, and was a full-sized origami. The individual 4-square units and a scaled-up 16-square version were simulated and found to expand in both directions (as is the character of an auxetic material) by changing salt concentration. This change was measured by calculating the smallest sized box that would be able to contain the structure, and in the case for the 4-square unit and 16-square unit, it was found they needed boxes bigger in both length and width, suggesting auxetic behaviour had been achieved.

Also described was a method outlining the potential way to create larger assemblies by modifying the 4-square unit to make two versions with unique, but complementary, corners. This would allow for synthetic scaling up of the structure into larger assemblies.

The 4-square unit was also found to be able to open and close in a cyclic manner, driven by salt concentration during the simulation. It is, therefore, likely

that experimentally this material's auxetic behaviour could be controlled if similar experimental conditions could be produced.

Finally, the linker in the centre, used to prevent the structure from extensive opening, was removed from the 4-square unit design. It was found the 4-square unit fully opened and created a structure that could close in two potential directions, vertically (as has been looked at earlier in the chapter) or horizontally. Using gentle forces, the second closed structure was obtained and simulated, demonstrating it would be a stable state and that the structure could open and then close in different ways, potentially allowing for this auxetic material to exist in three states.

By using caDNAno and oxDNA, we have been able to design and simulate a completely new system and determine its likely behaviour. We have been able to develop a suitable design to replicate the rotating squares model and have done so by applying techniques and principles learnt from previous projects. As such, we have demonstrated oxDNA's utility for designing and testing systems before synthesising them.

Chapter 7

Barrel Structures

7.1 Introduction

7.1.1 Experimental Design, and Simulation and Analysis Details

The barrel design we investigate in this Chapter was developed and assembled by a group currently based at The University of Sydney, Australia, that is led by Dr Shelley Wickham. At the beginning of their 2020 article introducing the barrel structure, the group noted that the original Rothemund tile, discussed numerous times in this thesis already, has been modified over the years to suit the required purpose for which it is wanted; one such example is the Möbius strip in Chapter 3, that was based on this original tile design. The Wickham group drew inspiration from this and wanted to design a ‘3D’ equivalent (which they have termed a ‘3D DNA-barrel pegboard’), that other researchers could then modify for their own needs. The barrel was designed to be strong and rigid, available in multiple sizes and had a cavity that could host, for example, a small organic molecule.⁶² Generally, researchers are very interested in using DNA origami structures as drug

delivery devices, so this barrel could be a potential candidate for such uses, as is also mentioned by the group in their suggested uses section of their article.

Figure 7.1 shows the experimental images from some of these barrel designs.⁶² The gel electrophoresis plates show the different sizes of barrels made, and the overall shape is shown using the DNA-PAINT technique and transmission electron microscopy (TEM).

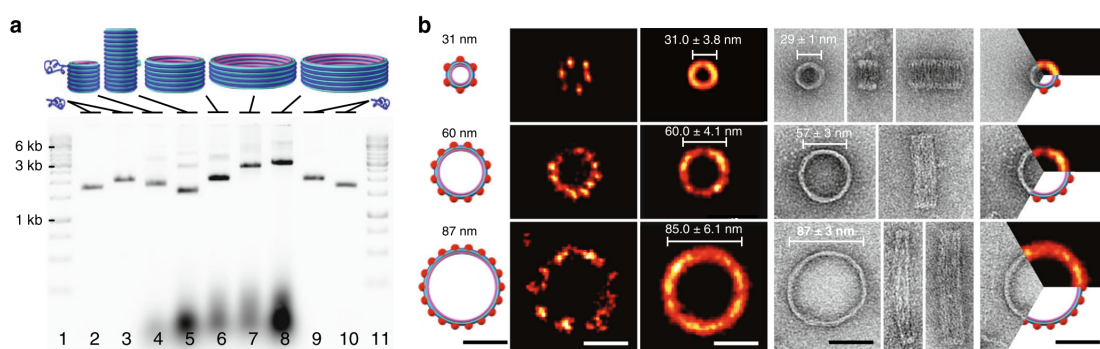


Figure 7.1: Experimental images for DNA-barrel design. The left side shows the gel electrophoresis plates for a variety of barrel sizes, and the right shows both the DNA-PAINT (using biotin markers, depicted in red on the schematic and that fluoresce in the accompanying experimental images) and TEM images for three barrels. Image reproduced with permission under Creative Commons licence CC BY 4.0, <https://creativecommons.org/licenses/by/4.0/>, from Wickham *et al.*⁶²

In collaboration with this group, we have simulated the original barrel design, a variety of new, larger ‘double-barrel’ structures, and finally two (eight-barrel) assemblies. We have helped to reveal some subtle features of these structures that experimental technique perhaps could not, as well as carrying out calculations to take measurements of mechanical properties of the double barrels and assemblies. As the first single barrel had no analysis needing to be carried out, it has been shown as part of the Introduction to illustrate the structure in more detail and to

set the background for the larger structures later on. The simulation outcome for the single barrel is shown in Figure 7.2.

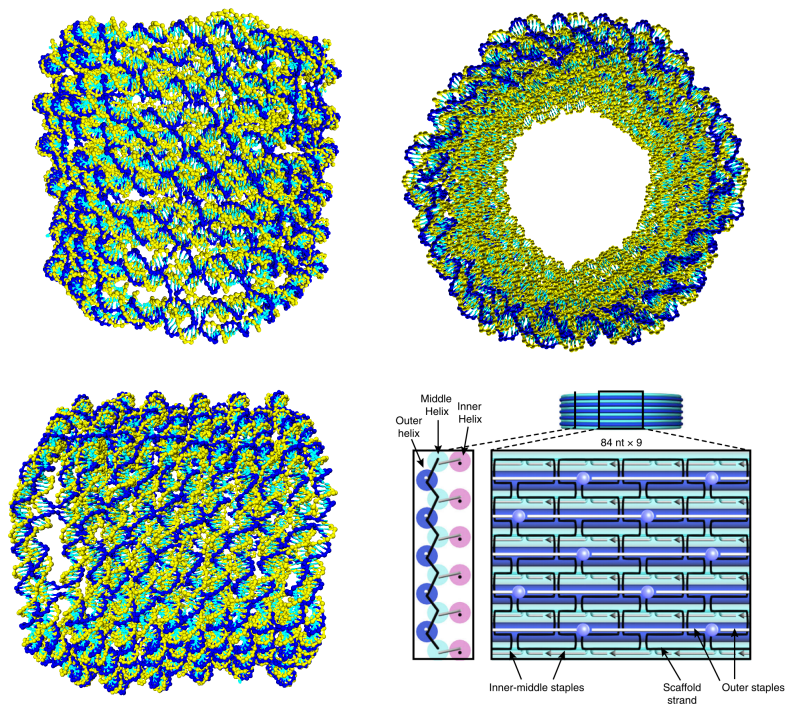


Figure 7.2: The single barrel simulation outcome shown from three positions: side-on vertically (top left), top down to enable viewing of the cavity (top right), and side-on horizontally (bottom left). Also pictured is a schematic of the design for a larger sized barrel, but that has the same design principles and tri-helix-edged structure (bottom right). Bottom right image reproduced with permission from Wickham *et al.* under Creative Commons licence CC BY 4.0, <https://creativecommons.org/licenses/by/4.0/>.⁶²

The simulation confirmed what was already known about the barrel from synthesis and imaging. Due to its three helix edge design (outer, middle and inner), the surface is incredibly rigid and remained largely unchanged throughout the simulation, and the cavity size is well-maintained, with no sign of any helices falling in that could partially block it. The scaffold strand iterates around the outer and middle helices, but the inner layer is staples only.

After simulating this single barrel unit, we simulated three further caDNAno designs, using files provided by the group, of different types of ‘double barrel’ structures, where the method of connecting one barrel to its partner varied for each pair of barrels. The three types of join (direct connection, bridges, and a coaxial version with one barrel on top of the other) will be covered individually in the results section, along with further details of the assemblies, made from using the double-barrel structures as monomers. Figure 7.3 shows an example of one of the types of double barrel, to demonstrate what they look like and to serve as an example for how one pair is connected. A common feature to both pairs of

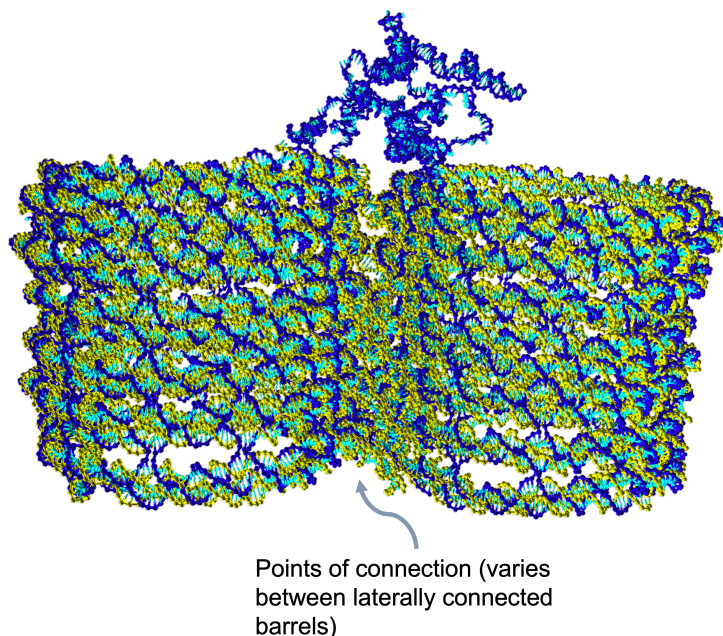


Figure 7.3: An example image showing the bridge-connected double barrel structure. The bridges are the points of connection between these barrels and the means for holding the pair together. Also note the ssDNA linkers protruding from the top of both barrels that was thought to potentially impact the movement of each barrel with respect to the other.

laterally connected barrels are the long linkers attached at the top of both barrels

(as can be seen protruding out at the top of the barrels in Figure 7.3). These are two separate, single-stranded pieces of DNA that are part of the scaffold, and were included as they were thought to potentially have an impact on how the barrels moved with respect to each other.

7.1.2 Analysis Calculations

A schematic of the quantities calculated for the double barrels and assemblies is shown in Figure 7.4. For each measurement in the Figure, the left-hand barrel is taken as the reference, and the movement of the right-hand one away from it is measured. In the Figure, this is represented as the deviation of the red arrow

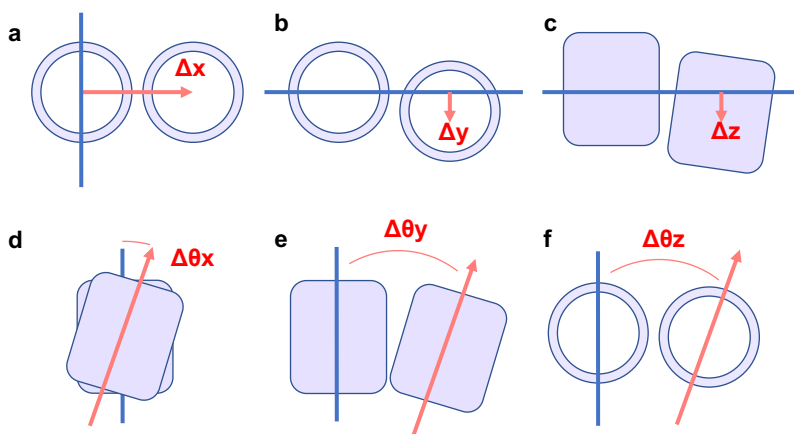


Figure 7.4: A graphical depiction of the measurements for the double barrel and assembly structures. It includes three length (top) and three angle (bottom) calculations.

from the blue line, in the directions indicated in the images. The method used to calculate these values was partly collaborative, with a detailed outline for the calculations suggested by the experimental group, but the final method used and subsequent coding done as part of the work for this thesis. To best outline how

the script worked, the calculation of one length and one angle will be outlined in this section. These quantities will be Δx (Figure 7.4a) and $\Delta\theta y$ (Figure 7.4e).

Initially, ‘top’ and ‘bottom’ centres of masses (CoM) needed to be calculated for both barrels. This was carried out by choosing two rings of nucleotides at the top and at the bottom of each barrel, and simply taking an average of their positions (since all nucleotides in the model would have the same mass). This generated two centres of masses per barrel, so four in total. A vector was then used to form a Z axis for each barrel (Eqn 7.1). From this, the origin of each barrel could be calculated as being the halfway point along this vector (Eqn 7.2).

$$Z_{barrel} = CoM_{top} - CoM_{bottom} \quad (7.1)$$

$$O_{barrel} = CoM_{bottom} + \frac{1}{2}Z_{barrel} \quad (7.2)$$

A second, and distinctly different, centre of mass was then required for each barrel that needed to be approximately halfway down the edge of each barrel. The connection points that were used to hold the barrels together were used as markers and, again, an average position calculated from them. This, along with the origin, could be used to define the X axis for each barrel (Eqn 7.3).

$$X_{barrel} = CoM_{connection\ points} - O_{barrel} \quad (7.3)$$

The ‘x-axis’ was orthogonalised onto the ‘z-axis’ for each barrel, then all four

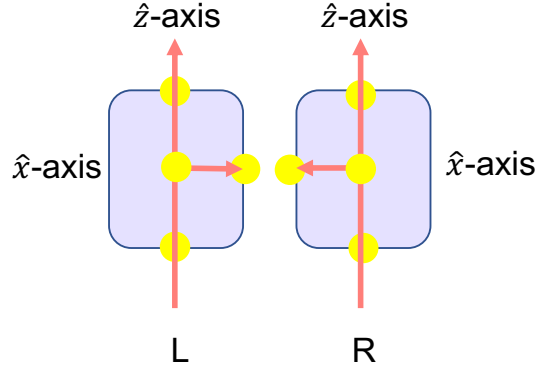


Figure 7.5: Graphical depiction of partway through barrel measurement calculation. The yellow dots at the top and bottom represent nucleotide ring centres of mass, the central ones show the halfway point of the $\hat{z}$ -axis vectors (and approximate centre of each barrel) and the edge dots are the centres of mass of points of connection between each barrel. The horizontal arrows from origin to edge represent the $\hat{x}$ axis for each.

normalised (Eqs 7.4-7.6).

$$X_{barrel\ ortho} = Z_{barrel} - Z_{barrel} \frac{(X_{barrel} \cdot Z_{barrel})}{|Z_{barrel}|^2} \quad (7.4)$$

$$\hat{Z}_{barrel} = \frac{Z_{barrel}}{|Z_{barrel}|} \quad (7.5)$$

$$\hat{X}_{barrel} = \frac{X_{barrel\ ortho}}{|X_{barrel\ ortho}|} \quad (7.6)$$

A graphical depiction of these steps is shown in Figure 7.5.

The cross product of these two vectors for each barrel produces the corresponding ‘y-axis’ in the scheme (Eqn 7.7), which also then needs to be normalised (Eqn 7.8). Repeating this for both barrels provides all vectors needed to define the

system.

$$Y_{barrel} = \hat{Z}_{barrel} \times \hat{X}_{barrel} \quad (7.7)$$

$$\hat{Y}_{barrel} = \frac{Y_{barrel}}{|Y_{barrel}|} \quad (7.8)$$

The distance measurements (Δx , Δy and Δz) can then be calculated using the distance between the two origins of each barrel (Eqn 7.9), and taking the dot product of that distance with the normalised vectors in each direction. Δx is shown as the example (Eqn 7.10), and for Δy and Δz , $\hat{X}$ just needs to be replaced with the corresponding direction.

$$O_{LH \rightarrow RH} = O_{right\ hand\ barrel} - O_{left\ hand\ barrel} \quad (7.9)$$

$$\Delta x = O_{LH \rightarrow RH} \cdot \hat{X}_{LH\ barrel} \quad (7.10)$$

To calculate the three angles, as shown in Figure 7.4**d-f**, the right handed barrel axis shown in each scenario first needed to be projected onto the plane of the page. This was calculated for each pair by projecting the right hand barrel's vector of interest (as shown in Figure 7.4) onto the normal of the plane, and subtracting that from the vector itself, the same as the orthogonalisation step earlier on. Eqn 7.11 is used for calculating $\Delta\theta y$, Figure 7.4**e**, where the axes shown on the screen for both barrels is the $\hat{Z}$ -axis. Due to the orientation of the barrels, the normal to the plane of the screen in this situation is the $\hat{Y}$ -axis of the left hand barrel.

$$Z_{RH\ barrel\ projected} = \hat{Z}_{RH\ barrel} - \hat{Y}_{LH\ barrel} \frac{(\hat{Z}_{RH\ barrel} \cdot \hat{Y}_{LH\ barrel})}{|\hat{Y}_{LH\ barrel}|^2} \quad (7.11)$$

The output could then be normalised, as before, to produce $\hat{Z}_{RH\text{ barrel projected}}$. The angle between the two vectors shown in Figure 7.4e could then be calculated using a simple cosine rule calculation to obtain the magnitude of degree of rotation (Eqn 7.12).

$$|\Delta\theta y| = \arccos \left(\hat{Z}_{LH\text{ barrel}} \cdot \hat{Z}_{RH\text{ barrel projected}} \right) \quad (7.12)$$

However, as the barrel could be rotated ‘forwards’ (towards the plane of the page) or ‘backwards’ (away from the plane of the page), a last step to determine the sign of the angle needed to be carried out and the specific scenario for $\Delta\theta y$ is shown in Eqn 7.14,

$$\Delta\theta y \text{ sign} = \hat{X}_{LH\text{ barrel}} \cdot \hat{Z}_{RH\text{ barrel}} \quad (7.13)$$

The sign of this calculation (positive or negative) would be the same as the sign for $\Delta\theta y$.

7.2 Results and Discussion

7.2.1 Lateral connection 1: ‘Old motif’

The first double barrel structure simulated was called the ‘Old Motif’ structure. It was named so as its synthesis presented challenges to the experimental group, and they soon after decided upon a second design (the ‘New Motif’) to continue their research into double barrel structures. The Old Motif design consisted of two barrels adjacent to each other with single, direct connections between them. This

means the scaffold and staples crossed directly from one barrel to the next with no bridges or spacers between. A simple schematic of the situation is shown in Figure 7.6, and the simulation output is shown in Figure 7.7.

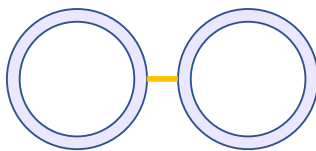


Figure 7.6: A top-down schematic showing how the Old Motif double barrel structure is connected; there are a single column of 6 separate connections between the barrels (the top one only can be seen in this Figure). The orange connection shown separating the barrels is exaggerated to indicate the position of the connections. The DNA iterates directly from one barrel to the other, there are no bridges or spacing between them.

The points of connection for the Old Motif barrel structure can clearly be seen in the top image of Figure 7.7. There are 6 distinct places where the barrels appear to be touching and these are the points of connection. The barrels appear to be quite well aligned visually, with no significant movement or rotation of the barrels away from each other.

Despite the visual appearance of this structure, it was vital to carry out detailed analysis to determine any twists/displacements that are more subtle than that which could be observed. As outlined in the Introduction, 6 measurements (3 displacements and 3 angles) were measured, and the results from these calculations are shown in Figure 7.8. Trajectory frames were recorded 250 times over 5×10^7 steps, so every 2×10^5 steps and for each of those frames, each of the six measurements was calculated. This has been presented in histogram format to show the distribution of data for each of the measurements. The Δx measurement is approximately the width of one barrel unit (as should be expected since this is the deviation of one barrel origin to the other), but both the Δy and Δz show a

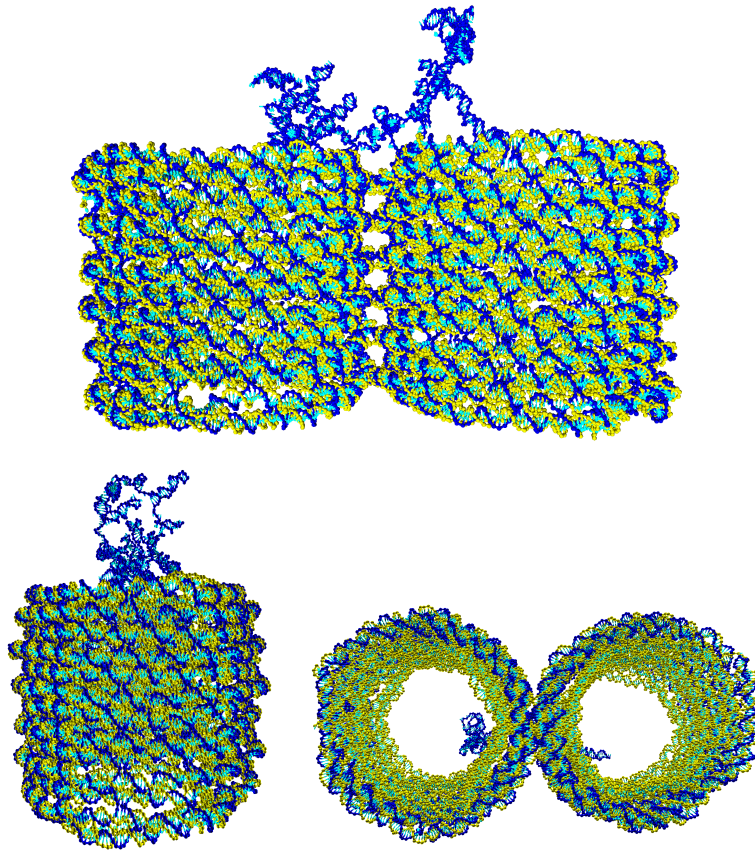


Figure 7.7: Old Motif double barrel simulation outcomes: a side-on view where the points of connection can clearly be seen down the centre (top); an end-on view (of the right-handed barrel) showing the barrels are fairly aligned in this direction (bottom left), and a view down the barrels from the base of the structures, to prevent the linker obscuring the view (bottom right).

distribution centred close to 0, with a narrow range around it. This shows that the Old Motif design did not cause significant movement of the barrels away from each other, but this is not surprising since the barrels are tightly connected by multiple long staples that would prevent diffusion of each unit away from the other.

The $\Delta\theta x$ value shows the most deviation away from two completely aligned barrels, and shows ‘end-on’ rotation (as previously shown in Figure 7.4d). Since 0° is on the peripheral of the data set, it shows that for every frame recorded,

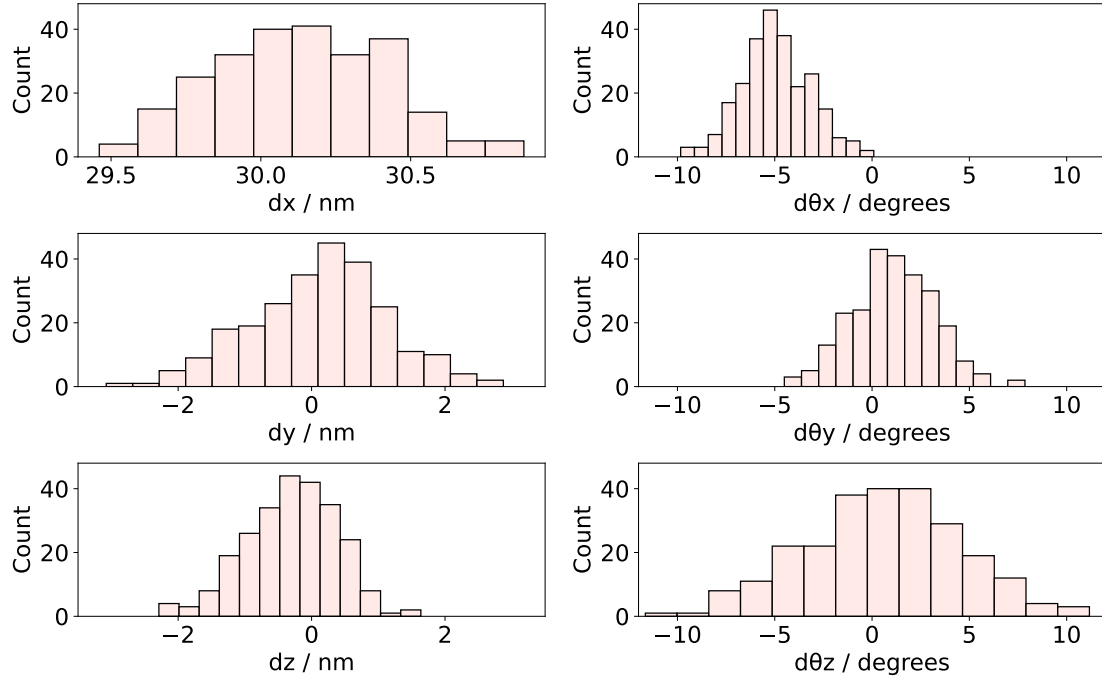


Figure 7.8: Old Motif double barrel simulation data for six measurements: three lengths (Δx , Δy and Δz , left column) and three angles ($\Delta\theta x$, $\Delta\theta y$ and $\Delta\theta z$, right column). Figure 7.4 shows the graphical descriptions of the measurements. Note that the x -axis label for the dx measurement is different to the others in the same column due to covering a vastly different range.

there was almost always a small recorded twist in the structure, with the most commonly occurring angle around -5° , and so in a specific direction. The angles for $\Delta\theta y$ and $\Delta\theta z$ are less skewed, both with 0° close to the centre, demonstrating that twisting in these directions does occur, but can and does happen in both directions ('forwards' and 'backwards'). $\Delta\theta z$ in particular shows quite a wide range of accessible angles ($\sim 20^\circ$ in range).

This data suggests that rotations in the $\Delta\theta y$ and $\Delta\theta z$ directions is quite mobile, in that it is able to rotate in both directions. This is in contrast to $\Delta\theta x$, which only appeared to rotate in one direction. A possible reason for this could be that the barrels get 'stuck' in the $\Delta\theta x$ direction and are unable to access the other direction

of angles. It should be noted, however, that although a twist appears inherent, the value is very small and not that far from 0° , so it is unsurprising it is not particularly visible in any given frame. Additionally, the free energy cost to align the barrels would be minimal, since the rotation is so small, further emphasising how the twist feature is not significant at this size.

The next double barrel structure investigated was the development on the Old Motif design, called the ‘New Motif’, and will be covered in the next section.

7.2.2 Lateral connection 2: ‘New motif’

The New Motif structure was a development on the Old Motif and has ‘bridges’ to hold the barrels together, rather than scaffold and staples iterating directly from one unit to the other. The bridges have a spacer of two T bases in between them to allow them to bend into the shown ‘V-shape’ and schematics of the system is shown in Figure 7.9.

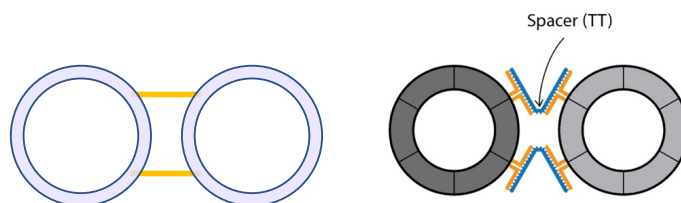


Figure 7.9: Two top-down schematics showing how the New Motif double barrel structure is connected. There are two columns of 6 separate bridges between the barrels (only the top one can be seen in this Figure). The left-hand connection diagram shown is to compare to the Old Motif schematic, the more detailed one (right) was produced by the experimental group and shows how the bridges are connected using staples protruding out of the barrels (orange), and separate ss-DNA staples holding them together (blue). Also shown is a two-base (T) ‘spacer’ allowing these bridges to bend in the centre.

The simulation results from the New Motif barrel are shown in Figure 7.10.

Visually, the simulation outcome appears very similar to the ‘Old Motif’, in that there has been no significant movement of the barrels away from each other (prevented by the bridges) and so the most likely source of any movement will, again, be twisting. The histogram data for this structure is shown in Figure 7.11.

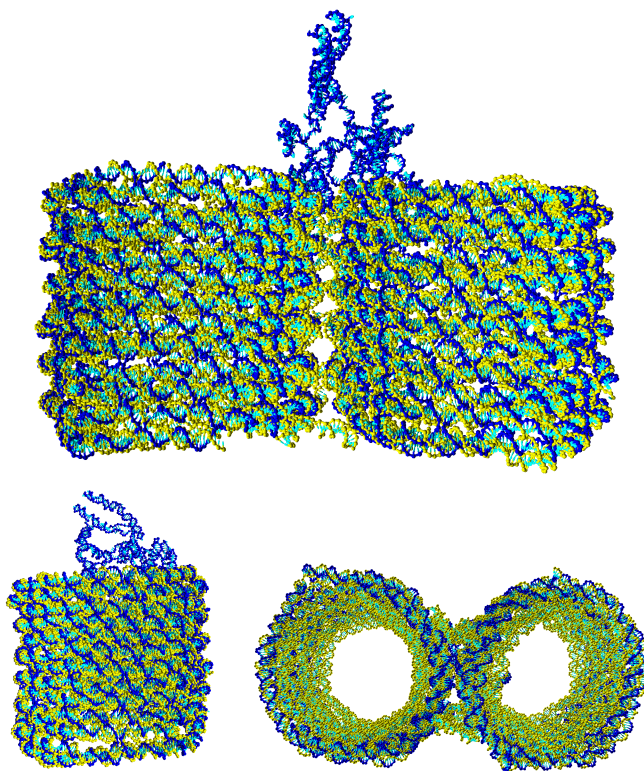


Figure 7.10: New Motif double barrel simulation outcomes: a side-on view where the points of connection (bridges) can clearly be seen down the centre (top); an end-on view (of the right-handed barrel) showing the barrels are fairly aligned in this direction (bottom left), and a view down the barrels from the base of the structures, to prevent the linker obscuring the view (bottom right).

The distance measurements are similar to the Old Motif design, the Δx value is approximately the distance between the two barrels, and the other two lengths are strongly centred around 0° with a narrow range, suggesting there is little movement in these directions. The $\Delta\theta y$ and $\Delta\theta z$ values are both largely centred

around 0° , again suggesting that rotation in these directions is not restricted. The most notable difference, again, is the distribution for $\Delta\theta x$, with a peak rotation angle of $\sim 9^\circ$. Due to the signs of the angles being opposite to the Old Motif, it suggests the barrels are twisted in the opposite direction to that seen previously. As with the Old Motif, however, the free energy cost to align the barrels would be minimal.

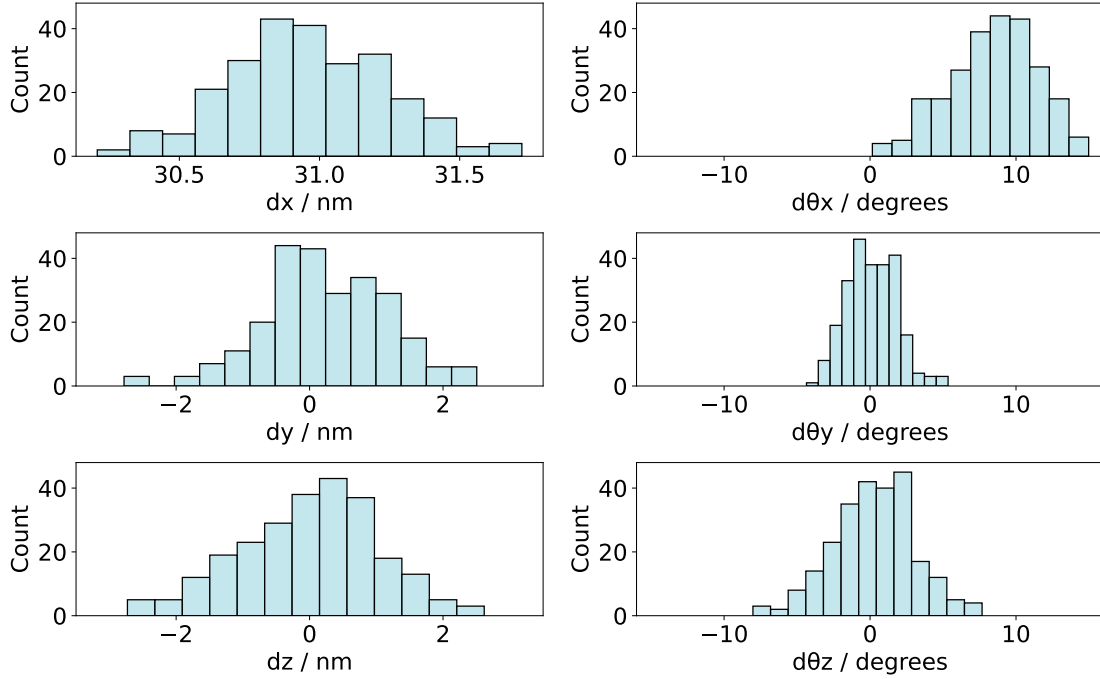


Figure 7.11: New Motif double barrel simulation data for six measurements: three lengths (Δx , Δy and Δz , left column) and three angles ($\Delta\theta x$, $\Delta\theta y$ and $\Delta\theta z$, right column). Figure 7.4 shows the graphical descriptions of the measurements. Note that the x -axis label for the dx measurement is different to the others in the same column due to covering a vastly different range.

A possible reason for this rotation could be the same as for the Old Motif, the barrels rotated away and got stuck in the twisted configuration, unable to realign or move in the other direction. It could also be that a feature in both designs were causing a twist to occur in both structures. To obtain an answer for why this

rotation occurred, the points of connection were investigated in more detail and it was found that both structures were connected to either the other barrel (Old Motif) or the bridges (New Motif) through 4-way Holliday junctions, similar to those in Chapter 5's ball structures. In order to rotate in the opposite direction, this stacking would need to be broken. However, in both cases the measured rotation is minimal, so the free energy cost of aligning the barrels would also be small. It could also be that for a much longer simulation, the barrels would be able to explore all conformations, and that a more even distribution around 0° could be recorded. Figure 7.12 shows close-up images of the presence of these stacked junctions for both structures. This explanation holds for both barrels, since 4-way

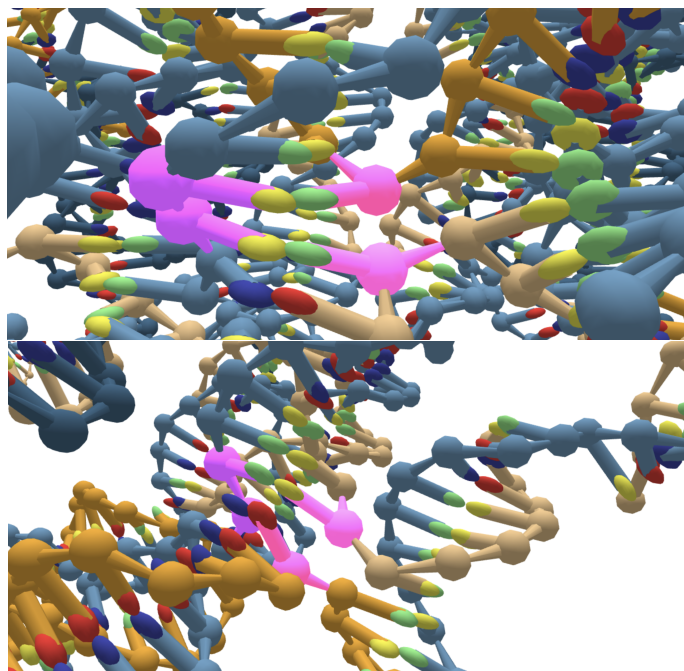


Figure 7.12: 4-way junctions between barrels for both Old (top) and New (bottom) Motif structures. Both exhibit 4-way junctions as a connection point to each barrel, an example of each is highlighted in the images. Stacking across these junctions is the most likely reason for the barrels twisting in the $\Delta\theta x$ direction.

junctions are used for connecting the units together in both situations. However,

the New Motif also often has coaxial stacking across the 2T spacers in the bridges. It appears that this stacking sometimes causes the bridges to be straight rather than bent, and the rotation could well be a combination of both the junctions themselves, and the straight bridges also pushing the barrels in one direction.

The one unexplained outcome is that the rotations for both structures is opposite in direction. Reasons for this could be chance (the barrels in each structure got stuck in opposite directions and they happened to have rotated differently), or it could be that oxDNA has not been able to fully capture the correct directionality of these junctions in these situations. Regardless of the sign of the rotation, it is most important to consider that this twist does remain very small for both structures and is a very subtle feature.

In order to investigate the rotation aspect further (for the New Motif design only), the experimental group changed the length of the spacer in the bridges to 0T, 5T and 10T, to explore any changes in rotation as a result. Before that, the experimental group investigated one final double barrel structure, the coaxially attached version which will be briefly covered next.

7.2.3 Vertical Connection: Coaxial Barrels

The final of the three original double barrel structures is the coaxially connected version. This structure has largely the same design as for the other two, but uses a plug-and-socket method to hold the barrels together vertically, as shown in the simulation outcome images in Figure 7.13.

This structure was presumably more challenging to design as it required three separate scaffold strands to iterate around the whole structure, rather than just the

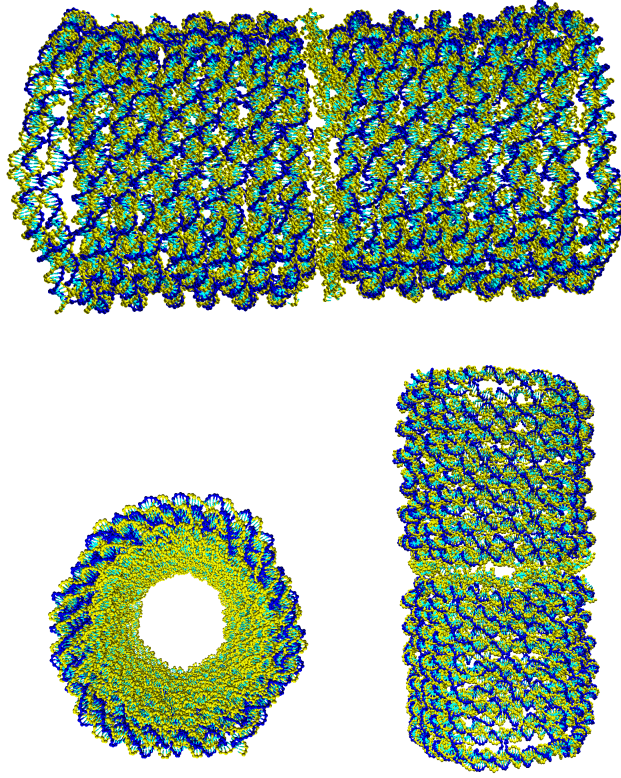


Figure 7.13: Coaxial double barrel simulation outcomes: a side-on view (top) showing the two full barrels with a region of plug-and-socket connections between them, an end-on view (bottom left) and a second side-on view in a vertical orientation (bottom right).

one that had been used for the previous two. Additionally, this structure had no external ssDNA linker or connection points along the edge of the barrels, the latter making calculating the displacement and angle measurements more challenging than previously; we had to manually select equivalent points along the edges as a substitution, so the measurements could be calculated in the same way. The results are shown in Figure 7.14.

Since the barrels were vertically connected, the largest length should be recorded in the Δz direction, which is the case here. The other two lengths show minimal

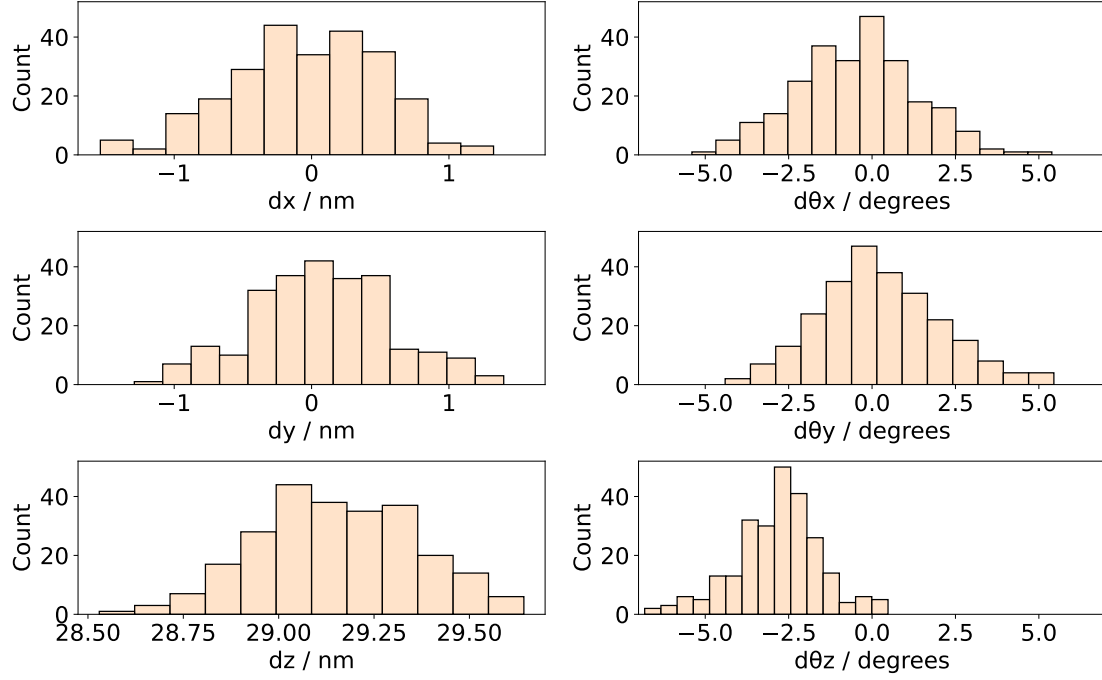


Figure 7.14: Coaxial double barrel simulation data for 6 measurements: 3 lengths (Δx , Δy and Δz , left column) and 3 angles ($\Delta\theta x$, $\Delta\theta y$ and $\Delta\theta z$, right column). The measurements were calculated in the same manner as for the other two double barrel structures. Note that the x -axis label for the dz measurement is different to the others in the same column due to covering a vastly different range.

deviation from 0° , suggesting there is little movement in these directions, and this is similar to the other two structures, which had a strong distribution around 0° for two of the three lengths as well. The angles are quite centred around 0° for $\Delta\theta x$ and $\Delta\theta y$, suggesting little rotation in these directions. There exists a slight preference for rotation for $\Delta\theta z$, but very minimally and less than was seen for $\Delta\theta x$ in the previous two. As shown previously in Figure 7.4f, the $\Delta\theta z$ rotation is as if one barrel were to be unscrewed from the other (like taking a lid off a jar). It makes sense this is the direction of some rotation in this situation since the plug-and-socket design is likely putting the join between the barrels under some tension, and a twist might be relieving some strain within the junctions.

7.2.4 Modified New Motif Designs: 0T, 5T, and 10T

The only difference between these structures and the original New Motif was the number of T bases acting as a spacer in the bridges between the barrels. As suggested by the names, for 0T these were removed entirely and for the others they were increased to 5T bases and 10T bases. Although visual differences between them are difficult to observe, the simulation outcomes are shown in Figure 7.15.

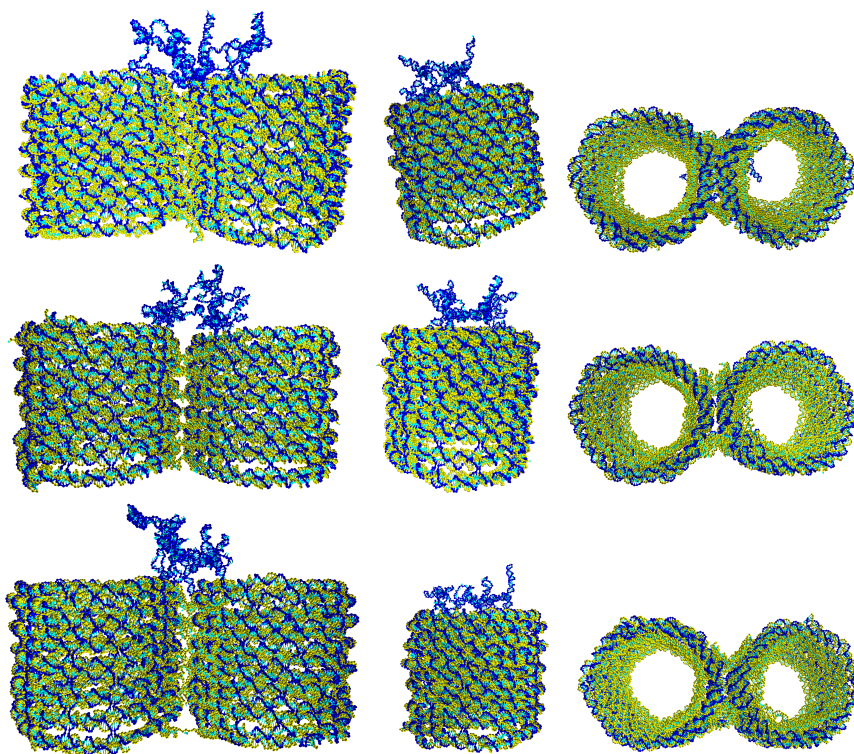


Figure 7.15: Modified double barrel structures that differ by their bridge spacer length: 0T, no spacer (top), 5T (middle) and 10T (bottom). Note that for 0T, the bridges appear straight and at an angle, whereas for 5T and 10T the bridges are harder to see as they lie more along the edges of the barrels. The 0T bridges engage in stacking along their entire length, whereas the 5T and 10T do not, hence why they appear different.

For the 0T structure, the bridges had no spacers in them, resulting in straight and rigid bridges, as can be seen in Figure 7.15. The histogram results for this

structure are shown in Figure 7.16. As with the original New Motif structure, the $\Delta\theta x$ angle has the most deviation from 0° . This is most likely due to the rigid bridges, caused by the lack of spacer T bases, forcing the second barrel to rotate to accommodate their lack of flexibility. Interestingly, both $\Delta\theta y$ and $\Delta\theta z$ appear skewed, but 0° is still part of the distribution and the most common angle is still very small for both, so the twist in these directions remains minimal.

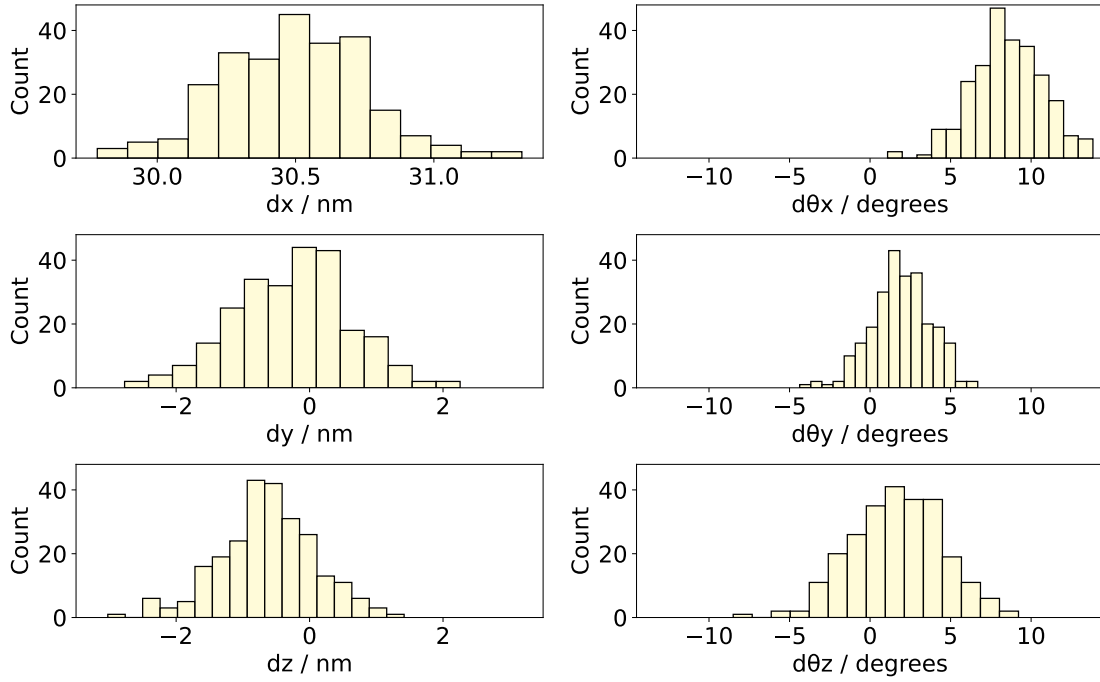


Figure 7.16: 0T modified barrel structure simulation data for six measurements: three lengths (Δx , Δy and Δz , left column) and three angles ($\Delta\theta x$, $\Delta\theta y$ and $\Delta\theta z$, right column). Note that the x -axis label for the dx measurement is different to the others in the same column due to covering a vastly different range.

The 5T and 10T structures had very similar measurement distributions and so the results have been plotted together in Figure 7.17. As with many of the results seen so far, the Δy and $\Delta\theta y$, and Δz and $\Delta\theta z$ show a distribution around, or very close to, 0° , suggesting minimal movement or rotation in these directions.

Interestingly, however, the results for $\Delta\theta x$ has a distribution with 0° more centred than it has been up to this point, but with a wide distribution of accessible angles, ranging from $\sim -10^\circ$ to 20° for both structures. A likely reason for this is that stacking is much harder to do along a multiple base strand of ssDNA. As such, each barrel in each structure is able to move more independently, as the stacking within the bridges caused a large driving force for the rotations and movements of the barrels up to this point. Instead, the distribution of angles is more even as rotation is likely more diffusion controlled and the barrels can move in both directions ('forwards' and 'backwards') instead of being locked in one direction only. The stacking across the bridges or at the points of connection between either of the barrel pairs no longer needs to be broken in order for one barrel to change the direction of its twisting, as it likely cannot stack in the first place. As such, a more even distribution of angles can be achieved, as the barrels can explore a larger range of conformations. It also shows that the barrels can exist mostly aligned when there are no restrictions from the bridge spacers or the 4-way connecting junctions.

Based on this result, a sensible conclusion to draw would be that both the stacking across the bridges and the 4-way junctions where the bridges are connected to the barrels both play a role at the same time, or neither do. When the bridges are stacked and rigid, how they are connected to the barrel likely plays a role in their rotations, as we know the junctions prefer to remain stacked, which will have an impact on barrel positioning. However, when the stacking across the bridge spacers is no longer possible and has been removed (like for 5T and 10T), the 4-way junctions would have no effect on barrel rotation either. We therefore propose that the two features have a partly interconnected relationship in determining

barrel rotation.

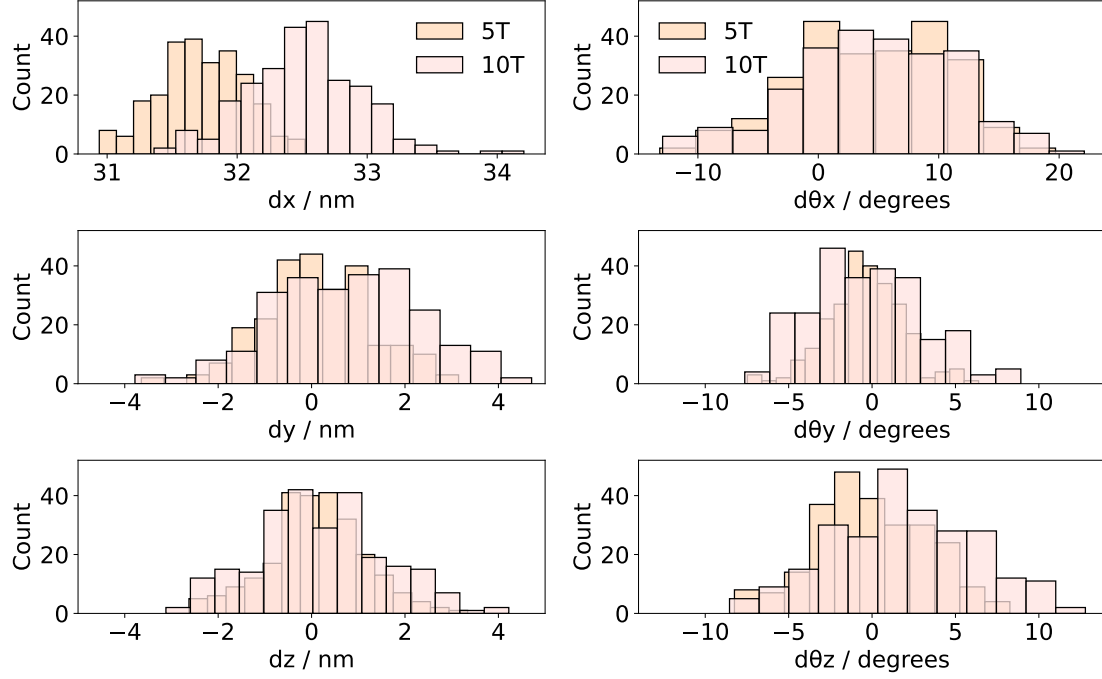


Figure 7.17: 5T and 10T modified barrel structure simulation data for six measurements: three lengths (Δx , Δy and Δz , left column) and three angles ($\Delta \theta x$, $\Delta \theta y$ and $\Delta \theta z$, right column). Note that the x -axis labels for the dx and $d\theta x$ measurements are different to the others in their respective columns due to covering different ranges.

Whilst simulations of DNA origami would normally be conducted with an average sequence model, these simulations were run with a sequence dependence parameter included, so the spacers would be modelled specifically as T bases. In order to keep as much the same as possible for comparison, the New Motif structure was run a second time with this parameter included, with the same overall conclusion and outcome as for the average sequence model (Appendix C). It was found that 0° was further into the centre of the distribution and that there did exist a small rotation of around 5° . As such, the same conclusion stands that

aligning the barrels would also only require a minimal free energy cost.

The final barrel structures we looked into were the larger assemblies. The intention was to determine whether the local twisting effects propagated into larger assemblies or not. As such, two types of assembly were suggested by the experimental group, one ‘linear’ with 8 barrels aligned (made of 4, 2-barrel monomers), and a second with another 8 barrels (also 4, 2-barrel monomers) in a rectangle arrangement. The results from these simulations are outlined in the next section.

7.2.5 Larger Assemblies

Two larger assemblies were simulated, both containing eight barrels, made up of four, double barrel monomers with the New Motif connection style. The first assembly placed all monomers in a straight line and we have termed this the ‘linear’ model. The second arrangement was two rows of two monomers, termed the ‘rectangle’ arrangement. The purpose of these simulations was to determine whether the $\Delta\theta x$ rotation observed in the smaller double barrel units scaled up into the larger assemblies. Whilst we were most interested in the $\Delta\theta x$ angle for different pairs of barrels across the assemblies, we recorded the other 5 measurements as well. The experimental group provided the monomer unit caDNAno designs with protruding single stranded staples that could be used to build bridges and attach the monomers together. When two monomers were positioned nearby, we used oxView to create an additional strand of ssDNA and formed a bridge across the protruding staples. This was repeated as necessary to connect the monomers together to form the larger assemblies. The results for both are outlined in their own sections below.

7.2.6 Assembly 1: Linear arrangement

The simulation outcome is shown in Figure 7.18. The images have been obtained using oxView due to its ability to process very large Origami structures.^{49,50}

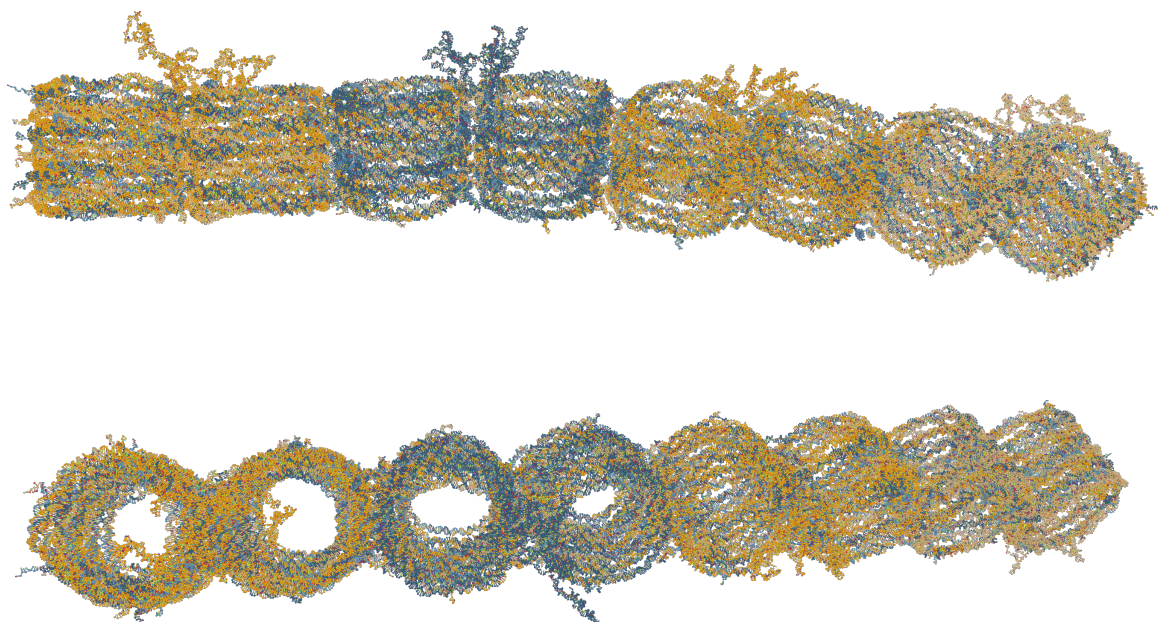


Figure 7.18: Linear assembly comprised of four connected double barrel monomers from a side on (top) and top-down (bottom) view. These images show extensive rotation in the $\Delta\theta x$ direction, suggesting the smaller scale effect is demonstrated on a larger scale as well. The identification method for measurement calculation follows as: Monomer 1, Monomer 2, Monomer 3 and Monomer 4, when proceeding from left to right. The barrel on the left in each monomer is number 1, and the right hand one, number 2.)

From this Figure, it was clear that $\Delta\theta x$ was going to appear as a significant measurement in all histograms, as this was the most obvious visual change upon viewing the simulation images. On the experimental group's suggestion, a total of eight barrel pairs were compared and, as would be expected, the value for $\Delta\theta x$ mostly increased as the barrel pairs compared were further separated. The results

that will be shown here will only be for two pairs: both barrels in monomer 1 (Figure 7.18, far left), and for the left-hand barrel in monomer 1 and the right-hand in monomer 4 (Figure 7.18, the individual barrels that appear on the far left and right). The complete sets of data, also in histogram form for all pairs measured, can be found in Appendix D. The histogram results for the two sets of barrels outlined above are in Figure 7.19.

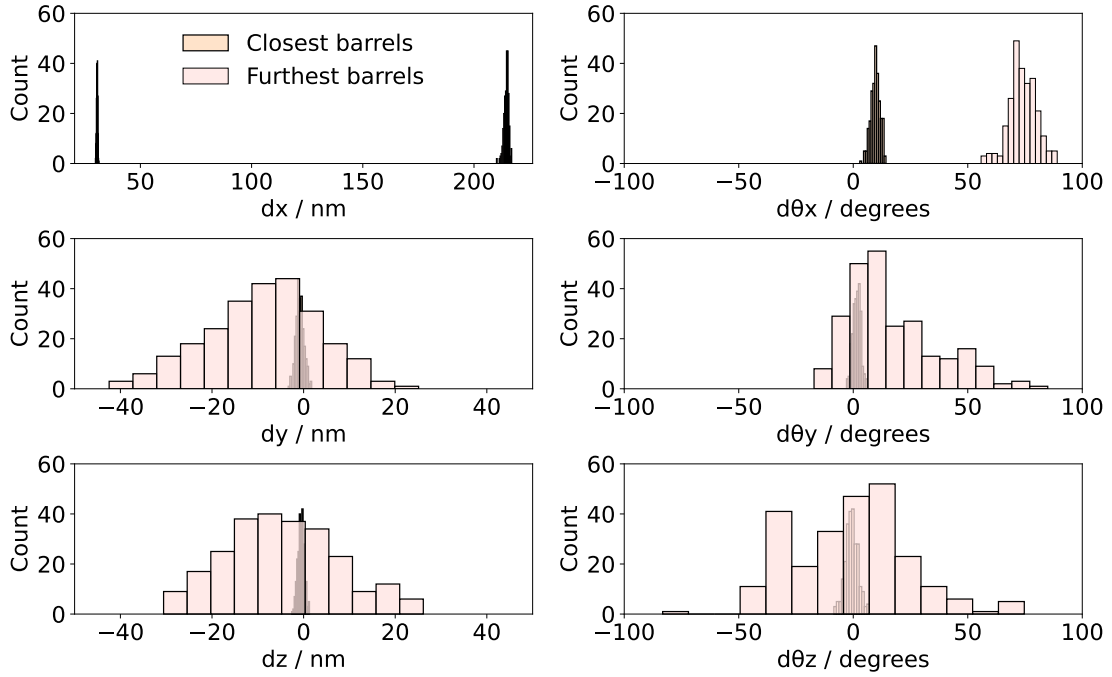


Figure 7.19: Comparison of data for two pairs of barrels in the linear assembly: neighbours from the first monomer (the two closest together), and the first and last barrels along the line of the assembly (the two furthest apart). Note that the x -axis label for the dx measurement is different to the others in the column due to covering a vastly different range. Additionally, due to the data separation the colours are challenging to differentiate in the dx plot. The closest barrel data is on the left, and furthest on the right of this plot.

As was expected, the ranges of angles and displacements are much narrower for the neighbouring barrels than for the separated pair. Whilst there is complete

overlap in the Δy , Δz , $\Delta\theta y$, and $\Delta\theta z$ data, it can be seen that the pair closer together have a far narrower range across these histograms than for the distanced pair. In all these four cases, however, each distribution, regardless of range, contains 0° and three data sets are largely centred around it too (only $\Delta\theta y$ shows a positive skew in the data), suggesting alignment in these directions is possible and was achieved during the simulation. The largest differences are in the Δx and $\Delta\theta x$ data. The large difference for Δx is expected, since the barrels are physically further away, so the measured deviation should be much larger for this measurement for the distanced pair. The data presented reflects that and is also ~ 210 nm, which is approximately $7 \times$ the diameter of one barrel (~ 30 nm), so the right length between the two. The $\Delta\theta x$ data is distinctly different for the two pairs. The neighbouring pair, unsurprisingly, have values similar to that for the original double barrel monomer outlined in a previous section, where the distribution is not around 0° but is quite close to it, so the rotation was relatively minimal. For the furthest pair, the rotation is now very noticeable, not only in the histogram data, but also for the simulation images. This significantly large rotation was clearly present, and the free energy cost of aligning the first and last barrel is likely now significantly higher.

As such, for the linear assembly the rotation in this direction is clearly influenced (and propagated) by the size of the origami, and this particular rotation is exacerbated when the structure is scaled up in this manner. What is unexpected, is that the size of the angles measured are larger than a simple addition for each monomer. The New Motif double barrel with sequence dependence showed a twist of $\sim 5^\circ$, so it would be expected that the furthest spaced pair would be approximately 35° with respect to each other, whereas the recorded angles peak around

70°.

7.2.7 Assembly 2: Rectangle arrangement

The simulation outcome for the rectangle assembly is shown in Figure 7.20. The monomers have been connected in two lines of two monomers (4 barrels), then attached together with bridges in the centre, the same way the linear assembly was made. In contrast to the linear assembly, there appears to be little visible rotation in this structure. Whereas the linear rotating was very obvious, any rotation here is not visually obvious.

As with the linear assembly, multiple pairs of barrels were compared and the results for two pairs of barrels are shown in Figures 7.21 and 7.22. All histogram results for this assembly can be viewed in Appendix E. Although not particularly visible, there was variety in displacements and angles throughout the eight pairs measured. The results of two pairs of barrels will be shown and discussed here, as the main conclusions about the system can be drawn from them. The first data set is shown in Figure 7.21 for one neighbouring pair. In Figure 7.20, it is the pair in the top left corner of the structure, when viewed from the top-down. As with all other double barrel pairs, most of the data is centred around 0° except for $\Delta\theta x$, suggesting that the pair behaves the same in this assembly as it would on its own.

The most unexpected histogram came from the measurements between the second and third barrels along the top row (when viewed from the top-down, neighbouring but not part of the same monomer). The histogram for this pair is shown in Figure 7.22. Instead of there being the usual and expected rotation in the $\Delta\theta x$ direction, instead this measurement is very centred around 0°, with an

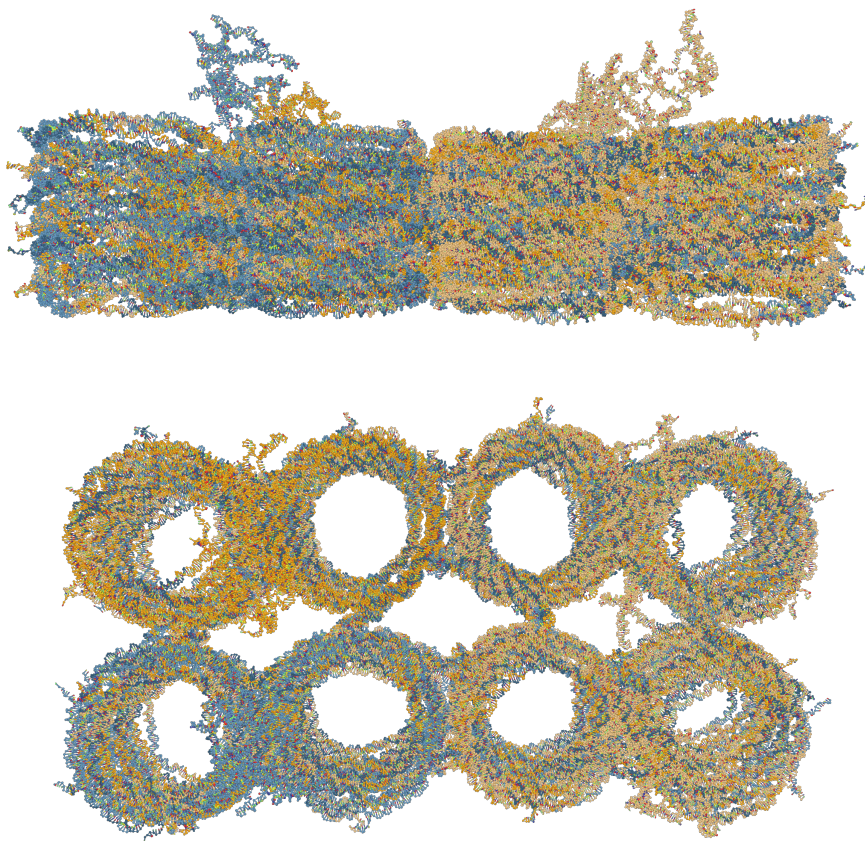


Figure 7.20: Rectangle assembly comprised of four connected double barrel monomers from a side on (top) and top-down (bottom) view. These images show a rigid-looking structure where there does not appear to be significant rotation in any direction. When looking in the top-down image, the identification for measurements is as follows: Monomer 1 top left, Monomer 2 top right, Monomer 3 bottom left, and Monomer 4 bottom right.

even distribution either side of the peak. The rotation most deviated from 0° was actually $\Delta\theta z$. This was surprising, as up to this point this angle had always been measured as close to, and centred around, 0° . Upon viewing the simulation images in detail (Figure 7.23), our theory for this is due to the bridges across the structure existing in different ‘environments’ and therefore adopting different shapes. The two monomers in question are directly connected by two sets of bridges, one which

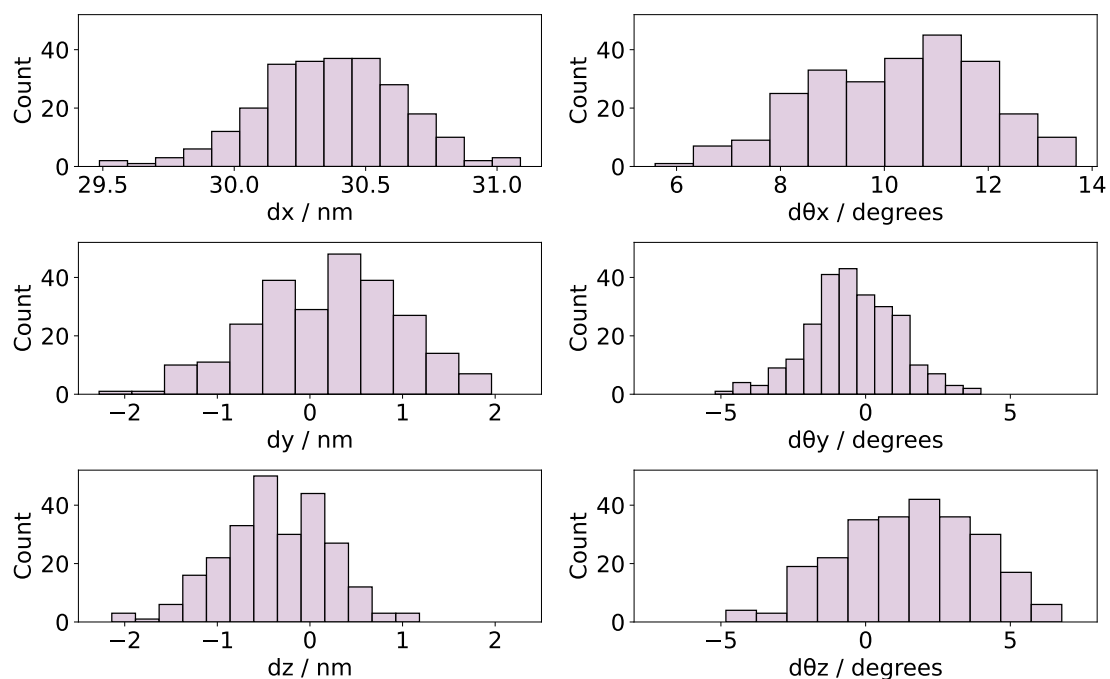


Figure 7.21: Histogram data for one pair of barrels in the rectangle assembly. These barrels are both part of the same monomer, the top left one when looking at the top-down image in Figure 7.20. Note that the x -axis labels for the dx and $d\theta x$ measurements are different to the others in their respective columns due to covering vastly different ranges.

is on the ‘outside’ of the structure, and the other on the ‘inside’. It appears to be the case that the internal bridges are held very tightly in place, likely due to the packed formation and cross-links of other bridges to nearby barrels. This has caused them to adopt a sharp, ‘V-shaped’ geometry, where the bridges have no opportunity to use the 2T spacer linkers to engage in stacking across the bridge, as had been happening previously. Conversely, on the outside of the barrels, the bridges are under no such constraints, due to the lack of any other barrels in the vicinity that could influence their geometry. As such, they are still able to engage in stacking across the 2T spacer and/or, more generally, can adopt a more shallow ‘U-shape’ geometry. Two images of the inside and outside bridges (highlighted)

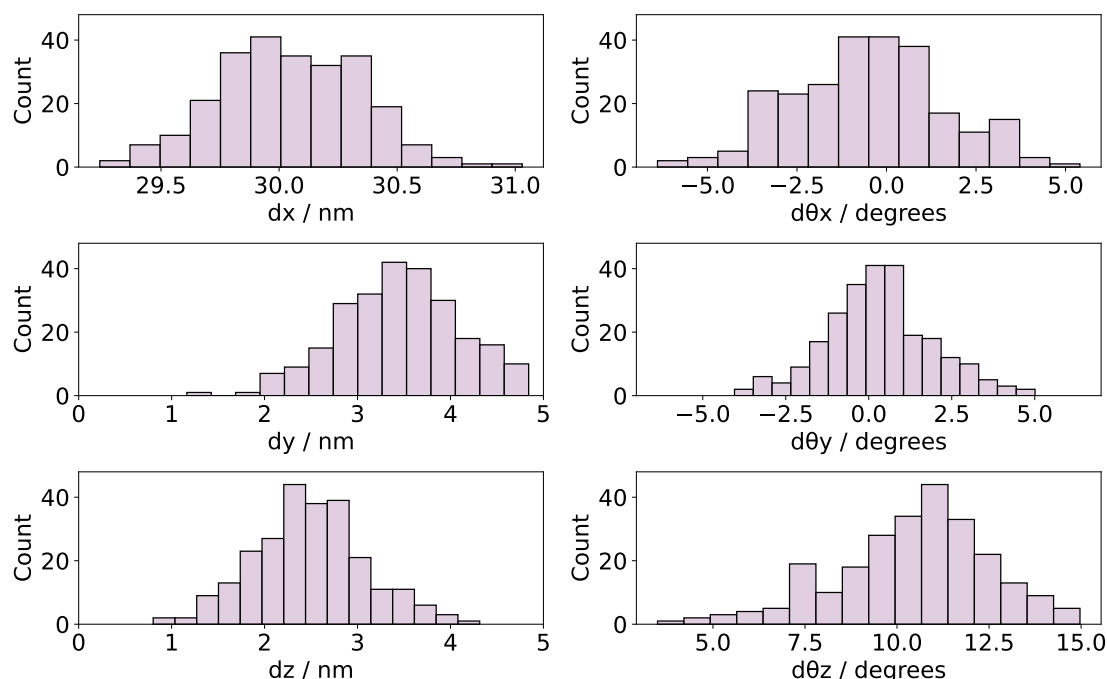


Figure 7.22: A second histogram data for a neighbouring pair of barrels in the rectangle assembly (second and third in the top row). These barrels are neighbours but not part of the same monomer. Note that the x -axis labels for the dx and $d\theta z$ measurements are different to the others in their respective columns due to covering vastly different ranges.

are shown in Figure 7.23 to illustrate this concept.

The bridges can clearly be seen to have adopted different conformations. The shallow bridges are causing a pushing force on the right-hand barrel, but the other set of bridges are unable to contribute a pushing effect (due to lack of stacking). As such, the pushing force is concentrated on only one side of each barrel. The natural result from this motion is the twisting of the barrels away from each other (like removing a lid off a jar) and therefore is shown as a significant change in the $\Delta\theta z$ direction, as has appeared on the histogram.

It should be noted that whilst we can ascertain what has caused the $\Delta\theta z$ angle to change in this particular circumstance, this is unlikely to be a consistent

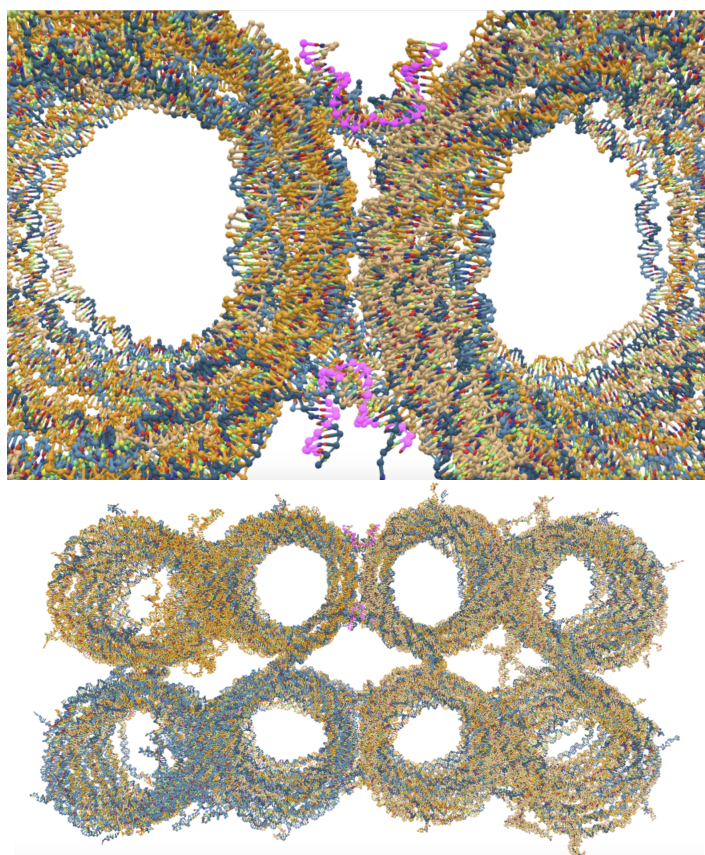


Figure 7.23: The bridges between two adjacent barrels from different monomers. There is clearly a more shallow shape in the top bridge, compared to the more tightly held ‘V-shape’ bridge at the bottom (top). Also shown is a zoomed-out version to show the positions of these bridges (bottom). The top image fits in as the central two barrels from the top row.

feature across all assemblies of this type. In other words, if the structure were to be re-made, relaxed and simulated again, there is no guarantee this exact outcome would occur a second time. For example, the bridges or junctions may not adopt exactly the same conformations, which may have an impact on the overall structure. For the results shown here, the change in the $\Delta\theta_z$ angle is likely due to the structure being unable to explore all conformations for all bridges, due to the cross-linked nature of the assembly that traps the bridges in specific orientations

and geometries.

The conclusion with regards to assemblies is that for the linear version, where there is nothing to prevent the barrels rotating, the systematic rotation appears to scale up. However, the rectangle arrangement has a much more rigid and cross-linked shape, and, in this case, resulted in a rotation in the $\Delta\theta z$ direction, as opposed to the usual $\Delta\theta x$ direction.

7.2.8 Conclusions

In this Chapter, a variety of barrel structures were simulated and displacement and angle measurements calculated. The project started with just a single barrel, then progressed to double barrels with different connections methods, modified versions of those barrels, and finally much larger assemblies using the double barrel structures as monomers.

From the initial single barrel structure, it was found that the barrel was rigid and strong, as had been designed, and maintained a cavity inside that could potentially accommodate a small organic molecule, as was also a suggestion from the experimental group.

There were three types of double barrel that were all connected differently. They were called the Old Motif, New Motif, and Coaxial double barrel structures. The Old Motif structure had six connections points between the two barrels, but these were direct connections from one barrel to the other and there was no space or bridge between them. It was found that the Old Motif had a preferred twist in the $\Delta\theta x$ direction and that the most commonly occurring rotation was around -5° . The other rotations and measurements were mostly around 0° (except for

Δx which was approximately the distance from the centre of one barrel origin to the other, as it should be) and had an even distribution, this made $\Delta\theta x$ the most interesting result.

The New Motif structure had 12 bridges connecting the two barrels, and each bridge had a 2T spacer unit that often partook in stacking across the bridge. Of all the measurements calculated, it was also found that this structure had a preferred twist in the $\Delta\theta x$ direction, but with an average of around $5 - 9^\circ$ (depending on whether sequence specificity was used), so the twist occurred in the opposite direction. As with the Old Motif structure, the other quantities were around 0° and quite even, showing no significant movement or rotations of the barrels in other directions.

The reason for both of these results was the same and attributed to coaxial stacking occurring throughout the points of connection. For the Old Motif, this was at the positions where they were joined together, and the DNA strands that went from one barrel into the next were stacking at the points of connection. For the New Motif, the stacking occurred where the bridges met the barrels, and also across the bridges themselves through the 2T spacer. It appeared that in order to mostly maintain the stacking (in both locations for the New Motif), the barrels were required to rotate.

These rotations for each barrel were opposite in direction and the theory for this is that it was either random, or that perhaps the correct directionality of the junctions was not fully captured by oxDNA.

It does need to be highlighted that the rotation in each of these structures is so small that it is barely visible, and almost certainly not observable experimentally. However, if it were then the technique mostly likely to be able to detect this

subtlety would be cryo-EM. If the barrels were captured with a more significant rotation (perhaps more than 10°) then maybe cryo-EM could show this feature well. It is more difficult to comment on whether it would be possible to observe a rotation of very few degrees (e.g., 1°) in a cryo-EM image, due to the minimal structural difference from an aligned conformation. Whilst the results of both of these structures suggested the presence of a small rotation, the free energy cost to align them remained very low, due to the twist being such a small angle for both.

The coaxially connected barrels were also investigated and found to have a very small rotation in the $\Delta\theta_z$ direction. This was attributed to a small strain produced by the plug-and-socket design used to connect the two halves of the structure.

The next structures investigated were the modified versions of the New Motif, where the spacer across the bridges was changed in length to 0T, 5T and 10T. The 0T structure showed fairly even distributions around 0° for all applicable measurements, except for $\Delta\theta_x$, which had a skewed distribution. This was attributed to the bridges being able to coaxially stack more frequently as the spacer had been removed entirely and allowed the bridges to adopt a more straight, rigid shape. These stacked bridges, like for the New Motif structure previously, likely had a lot of involvement with creating the barrel rotation.

The 5T and 10T results were very similar to each other and the main conclusion was that the distribution of angles for $\Delta\theta_x$ was more varied and overall more centred around 0° (and the same for all other applicable measurements). The most interesting feature was that both had a wider angle distribution, ~ -10 to 20 in both cases. The reasoning for this was thought to be that stacking across the ssDNA spacer would be challenging for these lengths of DNA, so the bridges

were not able to exist in a locked and straight conformation. Therefore, the barrel movements were more independent of each other, and this allowed the structure to access a wider range of angles. It could also be that the barrels were physically slightly further apart (due to the spacers) and this also allowed more free rotation and movement. Finally, it was proposed that both the bridges and their points of connections had a role in producing barrel rotation.

The original three double barrel structures were simulated with an average sequence used, as this is how an origami structure would usually be simulated, but for the modified versions, sequence dependence was used due to the lengths of the spacers. For completeness, the New Motif simulation was repeated with sequence dependence used and the most common angle found to be slightly lower than the previous New Motif structure, but with the same overall conclusion. The measured rotations remained small in both cases and the most common angles were very close together and so, again, the free energy cost to realign the barrels remains very low.

The final structures simulated were the large assemblies, comprised of double barrel monomers with protruding staples that allowed their connection into a larger structure. It was found that when the monomers were all aligned in a straight line to form the linear assembly, there was a visually very noticeable rotation in the $\Delta\theta x$ direction. When the rotation was measured between the first barrel of the first monomer and every other barrel it was generally the case that the further along the pair, the more twist was recorded. The reason for this was theorised to be that the small rotation scaled up into the larger assembly and compounded with each additional monomer. Due to the size of the structure, this effect was

far more noticeable than for the smaller structures. The conclusion from this part was that while the twisted effect is very small in just the double barrel structures, it did scale up when multiple monomers were attached together.

The final simulation was that for the rectangle assembly, which produced histograms with more variety in measured angles and distances. Some pairs had a significant $\Delta\theta x$ and others had a very centred distribution around 0° , in contrast to nearly all of the results that had been seen up to this point. The main unexpected and interesting result from the rectangle was that in a neighbouring pair the $\Delta\theta z$ appeared to be the most dominant angle. After investigation, this was attributed to the bridges connecting this specific pair existing in two types of environment, where they then acted differently on the barrels, causing an unusual twisting mechanism. An important conclusion from the rectangle assembly was that whilst each result could likely be explained for the specific circumstances of this simulation, due to the structure's inability to explore all conformations (as a result of the cross-linked, network nature of the structure), there was no certainty that a similar simulation would produce the same results and that predicting properties for a structure connected in this manner would always be challenging. As such, whilst more generic conclusions could be made (such as the unlikely outcome of ever having visibly/highly rotated barrels in the rectangle arrangement) it would be harder to use just one simulation to make more specific conclusions about its behaviour.

Overall, multiple types of barrel structures have been simulated and analysed. It has been found that they form stable structures and assemblies with a remarkable range of properties with respect to their mechanical movement and behaviour.

Through the use of simulation, we have been able to assist experimentalists in filling in gaps in the knowledge of these structures, as well as helping guide some of their experimental work, which would not have been possible without the use of oxDNA and its capabilities in modelling these types of origami systems.

Chapter 8

Conclusions

Throughout this thesis, we have simulated and analysed a variety of DNA origami nanostructures. We have observed their behaviours, taken measurements of their properties and presented the results over five separate chapters.

In the first chapter, we looked at the design of an experimentally made Möbius strip. Two isomeric strips were simulated, differing only in their handedness, and it was found that they adopted different equilibrium conformations depending on this directionality. We discovered that the structural differences in conformation were due to differing balances between twist and writhe, and was caused by the original design having an inherent, inbuilt twist that preferred one twist direction over the other, thereby producing different conformations when twisted in two, opposite directions (one with the inherent twist and one against it).

No observation about different conformations was noted in the experimental work, this being perhaps due to the subtlety of the feature and the necessity to adhere the structures to a surface for imaging with AFM. Through using oxDNA to simulate these Möbius strips, we have been able to explore these systems in more detail as well as uncovering, and providing an explanation for, a previously

unknown subtlety.

We then moved onto a series of six tensegrity structures, named ‘kites’, that were comprised of arms (or bundles) held together by tensions in their connecting linkers. Again, through the use of simulation we were able to determine their equilibrium structures, take measurements of the angle distributions between the bundles and compare this to experimental data, where available. We found that the rigid 12HB kites appeared very similar to experiment, and also had similar angle distributions, demonstrating that oxDNA is a suitable model for these systems and is able to reproduce known data well.

We then looked at less stiff 6HB kites with the same linker lengths as those for the 12HB kites. The 6HB kite with the shortest linkers demonstrated Euler buckling due to the stresses placed on the system by their connecting linkers when the helix bundles were completely intact. However, our simulations also revealed that, at equilibrium, these domains in these helix bundles would break and partly unravel to relieve tensions in the system. Whilst the experimental work did not look at exactly the same systems, the group also noted the presence of Euler buckling in the 6HB structures they assembled, showing, again, how oxDNA has accurately captured the behaviour of similar systems.

In the third results chapter, we looked at ball structures with 6-way Holliday-style junctions at the vertices and along the edges, which behave differently in high and low salt concentration regimes, causing overall structural changes for the ball. Simulations were run over differing timescales to identify local versus global changes and it was found that a high salt concentration simulation led to a higher level of stacking and a more contracted structure, whereas a low salt concentration

regime led to less stacking at the junctions and a more expanded global structure, albeit with local fluctuations (individual junctions opening and closing) in both regimes. Closed junctions were determined to be a result of the salt quenching electrostatic repulsion around the junctions and enabling stacking. In low salt regimes, the stronger electrostatic repulsions disfavoured junction closing.

Finally, an extra base was introduced within some junctions, leading to steric hindrance in these areas and reduced stacking, even in a high salt regime. This project allowed us to use oxDNA to create an interesting case study of this system, and provide insights to the behaviour of junctions using inbuilt parameters within the code, thereby demonstrating the code's diverse functionality.

The penultimate results chapter looked at designing and simulating an entirely new origami system using principles identified in other chapters. We were able to use a combination of tools and software to create an auxetic-style system resembling the rotating squares model, whose movement was controlled using high or low salt regimes (as had been investigated in the previous chapter). An iterative process was used, where designs were adapted depending on the outcome of a previous trial system until a suitable subunit was designed, the size of a full origami. The subunit opened in low salt concentration, closed in a high salt regime, and could cycle around these two structures. Finally, when the linker controlling the extent of opening was removed, it was found that the origami could potentially exist in three states, closed in one direction, open, and then closed in a second direction. We also proposed a connection method to scale up the monomers into larger assemblies.

oxDNA was a powerful tool in this project, as it enabled us to simulate an entire

system and its expected outcomes using principles covered in earlier chapters. As such, this should provide the evidence that this software would be highly useful to experimental groups looking to supplement their work, or to explore the feasibility of achieving certain design aims computationally, prior to potentially expensive and time intensive experiments. This project is an example of how that could be done.

The final chapter looked at a series of barrel structures where we simulated a large number of origami as part of a collaboration with an experimental group. We started with a single barrel, then looked into double-barrel arrangements with different methods of connections and lengths of those connections, all the way up to full 8-barrel assemblies.

For pairs of barrels in the double and full assemblies, we were able to measure distances and angles between them. For the double barrels, we discovered subtle twists likely due to stacking at connection points between the barrels. When the linking bridges were lengthened, this effect was less pronounced, with angles more centred around zero degrees due to the lower propensities to stack across ssDNA sections, and a subsequent larger range of accessible angles.

For the larger assemblies, we found the linear version had a significant twist as a result of additive effects from rotation within barrel pairs. In the case of the rectangular arrangement, we could see and measure an overall twist, but acknowledged that full exploration of all conformations was restricted compared to the other structures, so could not draw as firm conclusions about its behaviour.

This project provided the opportunity to directly demonstrate the value of using simulation to fill in the gaps of knowledge for experimental groups and how,

together, a more complete picture of the systems studied can be understood.

Throughout these projects, we have been able to achieve the aims we set out in the Introduction. We have used oxDNA to successfully simulate a diverse and varied set of systems and used the resultant measurements and observations to uncover subtleties in these origami to provide a fuller picture of their structural properties. We have demonstrated the accuracy of these models with comparisons to experimental data when available, thereby showing the reliability of oxDNA for these and other similar systems. Finally, we have shown how the combined efforts of experiment and computation using oxDNA can lead to an enhanced understanding of novel systems, and thereby encourage other experimental groups in this area to implement this approach in their own work.

As a final note for this work and looking to the future, we plan to continue to collaborate with Shelley Wickham’s group on the barrel structures work. However, looking at the bigger picture, we hope to encourage other experimental groups to use the oxDNA model and software in their work, by utilising the case studies shown here and the tutorial outlined in *Methods in Molecular Biology* as a starting point.⁵⁶

Bibliography

- [1] S. Dey, C. Fan, K. V. Gothelf, J. Li, C. Lin, L. Liu, N. Liu, M. A. D. Nijenhuis, B. Saccà, F. C. Simmel, H. Yan and P. Zhan, *Nat. Rev. Methods Primers*, 2021, **1**, 1–24.
- [2] *Albrecht Kossel Biographical*, www.nobelprize.org/prizes/medicine/1910/kossel/biographical/.
- [3] J. D. Watson and F. H. D. Crick, *Nature*, 1953, **171**, 737–738.
- [4] M. H. F. Wilkins, A. R. Stokes and H. R. Wilson, *Nature*, 1953, **171**, 738–740.
- [5] R. E. Franklin and R. G. Gosling, *Nature*, 1953, **171**, 740–741.
- [6] B. Maddox, *Nature*, 2003, **421**, 407–408.
- [7] L. Pauling and R. B. Corey, *PNAS*, 1953, **39**, 84–97.
- [8] Z. Li, L. Wang, H. Yan and Y. Liu, *Langmuir*, 2012, **28**, 1959–1965.
- [9] P. Ball, *Nature*, 2021, **600**, 605.
- [10] N. C. Seeman, *J. Theor. Biol.*, 1982, **99**, 237–247.
- [11] J. Chen and N. C. Seeman, *Nature*, 1991, **350**, 631–633.
- [12] E. Winfree, F. Liu, L. A. Wenzler and N. C. Seeman, *Nature*, 1998, **394**, 539–544.
- [13] P. W. Rothemund, *Nature*, 2006, **440**, 297–302.
- [14] E. S. Andersen, M. Dong, M. M. Nielsen, K. Jahn, A. Lind-Thomsen, W. Mamdouh, K. V. Gothelf, F. Besenbacher and J. Kjems, *ACS Nano*, 2008, **2**, 1213–1218.

- [15] X.-c. Bai, T. G. Martin, S. H. W. Scheres and H. Dietz, *PNAS*, 2012, **109**, 20012–20017.
- [16] E. Benson, A. Mohammed, J. Gardell, S. Masich, E. Czeizler, P. Orponen and B. Högberg, *Nature*, 2015, **523**, 441–444.
- [17] S. M. Douglas, A. H. Marblestone, S. Teerapittayanon, A. Vazquez, G. M. Church and W. M. Shih, *Nucleic Acids Res.*, 2009, **37**, 5001–5006.
- [18] H. Dietz, S. M. Douglas and W. M. Shih, *Science*, 2009, **325**, 725–730.
- [19] R. Veneziano, S. Ratanalert, K. Zhang, F. Zhang, H. Yan, W. Chiu and M. Bathe, *Science*, 2016, **352**, 1534.
- [20] H. Jun, T. R. Shepherd, K. Zhang, W. P. Bricker, S. Li, W. Chiu and M. Bathe, *ACS Nano*, 2019, **13**, 2083–2093.
- [21] R. Li, H. Chen and J. H. Choi, *Angew. Chem. Int. Ed.*, 2021, **60**, 7165.
- [22] R. Li, H. Chen and J. H. Choi, *Small*, 2021, **17**, 2007069.
- [23] B. Rosenberg and L. VanCamp, *Cancer Res.*, 1970, **30**, 1799–1802.
- [24] S. M. Douglas, I. Bachelet and G. M. Church, *Science*, 2012, **335**, 831–834.
- [25] R. M. Zadegan, M. D. E. Jepsen, K. E. Thomsen, A. H. Okholm, D. H. Schaffert, E. S. Andersen, V. Birkedal and J. Kjems, *ACS Nano*, 2012, **6**, 10050–10053.
- [26] S. Li, Q. Jiang, S. Liu, Y. Zhang, Y. Tian, C. Song, J. Wang, Y. Zou, G. J. Anderson, J.-Y. Han, Y. Chang, Y. Liu, C. Zhang, L. Chen, G. Zhou, G. Nie, H. Yan, B. Ding and Y. Zhao, *Nature Biotechnology*, 2018, **36**, 258–264.
- [27] Q. Zhang, Q. Jiang, N. Li, L. Dai, Q. Liu, L. Song, J. Wang, Y. Li, J. Tian, B. Ding and D. Yang, *ACS Nano*, 2014, **8**, 6633–6643.
- [28] I. Mela, P. P. Vallejo-Ramirez, S. Makarchuk, G. Christie, D. Bailey, R. M. Henderson, H. Sugiyama, M. Endo and C. F. Kaminski, *Angew. Chem. Int. Ed.*, 2020, **59**, 12698–12702.
- [29] T. Yoshidome, M. Endo, G. Kashiwazaki, K. Hidaka, T. Bando and H. Sugiyama, *J. Am. Chem. Soc.*, 2012, **134**, 4654–4660.
- [30] P. Wang, M. A. Rahman, Z. Zhao, K. Weiss, C. Zhang, Z. Chen, S. J. Hurwitz, Z. G. Chen, D. M. Shin and Y. Ke, *J. Am. Chem. Soc.*, 2018, **140**, 2478–2484.

- [31] D. Balakrishnan, G. D. Wilkens and J. G. Heddle, *Nanomedicine (Lond.)*, 2019, **14**, 911–925.
- [32] M. A. Dobrovolskaia and M. Bathe, *WIREs Nanomed Nanobiotechnol.*, 2021, **13**, e1657.
- [33] X. Liu, Y. Xu, T. Yu, C. Clifford, Y. Liu, H. Yan and Y. Chang, *Nano Lett.*, 2012, **12**, 4254–4259.
- [34] R. Veneziano, T. J. Moyer, M. B. Stone, E.-C. Wamhoff, B. J. Read, S. Mukherjee, T. R. Shepherd, J. Das, W. R. Schief, D. J. Irvine and M. Bathe, *Nat. Nanotechnol.*, 2020, **15**, 716–723.
- [35] J. P. K. Doye, T. E. Ouldrige, A. A. Louis, F. Romano, P. Šulc, C. Matek, B. E. K. Snodin, L. Rovigatti, J. S. Schreck, R. M. Harrison and W. P. J. Smith, *Phys. Chem. Chem. Phys.*, 2013, **15**, 20395–20414.
- [36] B. E. K. Snodin, F. Randisi, M. Mosayebi, P. Šulc, J. S. Schreck, F. Romano, T. E. Ouldrige, R. Tsukanov, E. Nir, A. A. Louis and J. P. K. Doye, *J. Chem. Phys.*, 2015, **142**, 234901.
- [37] A. Suma, E. Poppleton, M. Matthies, P. Šulc, F. Romano, A. A. Louis, J. P. K. Doye, C. Micheletti and L. Rovigatti, *J. Comput. Chem.*, 2019, **40**, 2586–2595.
- [38] T. A. Knotts, N. Rathore, D. C. Schwartz and J. J. de Pablo, *J. Chem. Phys.*, 2007, **126**, 084901.
- [39] D. M. Hinckley, G. S. Freeman, J. K. Whitmer and J. J. de Pablo, *J. Chem. Phys.*, 2013, **139**, 144903.
- [40] D. Case, H. M. Aktulga, K. Belfon, I. Ben-Shalom, J. T. Berryman, S. Brozell, D. Cerutti, T. Cheatham, III, G. A. Cisneros, V. Cruzeiro, T. Darden, R. Duke, G. Giambasu, M. Gilson, H. Gohlke, A. Goetz, R. Harris, S. Izadi, S. Izmailov, K. Kasavajhala, M. Kaymak, E. King, A. Kovalenko, T. Kurtzman, T. Lee, S. LeGrand, P. Li, C. Lin, J. Liu, T. Luchko, R. Luo, M. Machado, V. Man, M. Manathunga, K. Merz, Y. Miao, O. Mikhailovskii, G. Monard, H. Nguyen, K. O’Hearn, A. Onufriev, F. Pan, S. Pantano, R. Qi, A. Rahnamoun, D. R. Roe, A. Roitberg, C. Sagui, S. Schott-Verdugo, A. Shajan, J. Shen, C. Simmerling, N. Skrynnikov, J. Smith, J. Swails, R. Walker, J. Wang, J. Wang, H. Wei, R. Wolf, X. Wu, Y. Xiong, Y. Xue, D. York, S. Zhao and P. Kollman, *Amber 2022*, University of California, San Francisco, 2022.

- [41] B. R. Brooks, R. E. Bruccoleri, B. D. Olafson, D. J. States, S. Swaminathan and M. Karplus, *J. Comput. Chem.*, 1983, **4**, 187–217.
- [42] M. J. Abraham, T. Murtola, R. Schulz, S. Páll, J. C. Smith, B. Hess and E. Lindahl, *SoftwareX*, 2015, **1**, 19–25.
- [43] M. Allen and D. Tildesley, in *Computer Simulations of Liquids*, Oxford University Press, 1987, pp. 29–30.
- [44] A. Warshel and M. Levitt, *J. Mol. Biol.*, 1976, **103**, 227–249.
- [45] M. W. van der Kamp and A. J. Mulholland, *Biochemistry*, 2013, **52**, 2708–2728.
- [46] C. E. Castro, F. Kilchherr, D.-N. Kim, E. L. Shiao, T. Wauer, P. Wortmann, M. Bathe and H. Dietz, *Nat. Methods*, 2011, **8**, 221–229.
- [47] C. Maffeo and A. Aksimentiev, *Nucleic Acids Res.*, 2020, **48**, 5135–5146.
- [48] C. Huang, A. Kucinic, J. A. Johnson, H. Su and C. E. Castro, *Nat. Mater.*, 2021, **20**, 1264–1271.
- [49] E. Poppleton, J. Bohlin, M. Matthies, S. Sharma, F. Zhang and P. Šulc, *Nucleic Acids Res.*, 2020, **48**, e72.
- [50] J. Bohlin, M. Matthies, E. Poppleton, J. Procyk, A. Mallya, H. Yan and P. Šulc, *Nature Protocols*, 2022, **17**, 1762–1788.
- [51] B. Alder and T. Wainwright, *J. Chem. Phys.*, 1957, **27**, 1208–1209.
- [52] B. Alder and T. Wainwright, *J. Chem. Phys.*, 1959, **31**, 459–466.
- [53] W. C. Swope, H. C. Andersen, P. H. Berens and K. R. Wilson, *J. Chem. Phys.*, 1982, **76**, 637–649.
- [54] L. Verlet, *Phys. Rev.*, 1967, **159**, 98–103.
- [55] M. Allen and D. Tildesley, in *Computer Simulations of Liquids*, Oxford University Press, 2nd edn., 1987, pp. 95–146.
- [56] J. P. K. Doye, H. Fowler, D. Prešern, J. Bohlin, L. Rovigatti, F. Romano, P. Šulc, C. K. Wong, A. A. Louis, J. S. Schreck, M. C. Engel, M. Matthies, E. Benson, E. Poppleton and B. E. K. Snodin, *The oxDNA coarse-grained model as a tool to simulate DNA origami*, 2020, <https://arxiv.org/abs/2004.05052>.

- [57] T. E. Ouldridge, A. A. Louis and J. P. K. Doye, *J. Chem. Phys.*, 2011, **134**, 085101.
- [58] T. E. Ouldridge, *Coarse-Grained Modelling of DNA and DNA Self-Assembly*, *DPhil Thesis*, The University of Oxford, 2012.
- [59] J. S. Schreck, T. E. Ouldridge, F. Romano, A. A. Louis and J. P. Doye, *J. Chem. Phys.*, 2015, **142**, 165101.
- [60] S. Jo, T. Kim, V. G. Iyer and W. Im, *J. Comput. Chem.*, 2008, **29**, 1859–1865.
- [61] D.-N. Kim, F. Kilchherr, H. Dietz and M. Bathe, *Nucleic Acids Res.*, 2012, **40**, 2862–2868.
- [62] S. F. J. Wickham, A. Auer, J. Min, N. Ponnuswamy, J. B. Woehrstein, F. Schueder, M. T. Strauss, J. Schnitzbauer, B. Nathwani, Z. Zhao, S. D. Perrault, J. Hahn, S. Lee, M. M. Bastings, S. W. Helmig, A. L. Kodál, P. Yin, R. Jungmann and W. M. Shih, *Nat. Commun.*, 2020, **11**, 5768.
- [63] L. Rovigatti, P. Šulc, I. Z. Reguly and F. Romano, *J. Comput. Chem.*, 2015, **36**, 1–8.
- [64] H. Berendsen, J. Postma, W. van Gunsteren, A. DiNola and J. Haak, *J. Chem. Phys.*, 1984, **81**, 3684–3690.
- [65] M. Allen and D. Tildesley, in *Computer Simulations of Liquids*, Oxford University Press, 2nd edn., 1987, pp. 130–132.
- [66] G. Bussi, D. Donadio and M. Parrinello, *J. Chem. Phys.*, 2007, **126**, 014101.
- [67] R. Holliday, *Genet. Res.*, 1964, **5**, 282–304.
- [68] K. Kornel, *New Scientist*, 2015.
- [69] L. Buckley, *Möbius strip unravelled*, 2007, <https://www.nature.com/news/2007/070709/full/news070709-16.html>.
- [70] S. Tanda, T. Tsuneta, Y. Okajima, K. Inagaki, K. Yamaya and N. Hatakenaka, *Nature*, 2002, **417**, 397–398.
- [71] Z. S. Yoon, A. Osuka and D. Kim, *Nat. Chem.*, 2009, **1**, 113–122.
- [72] T. Bauer, P. Banzer, E. Karimi, S. Orlov, A. Rubano, L. Marrucci, E. Santamato, R. W. Boyd and G. Leuchs, *Science*, 2015, **347**, 964–966.

- [73] D. Han, S. Pal, Y. Liu and H. Yan, *Nat. Nanotechnol.*, 2010, **5**, 712–717.
- [74] J. F. Frenet, *J Math Pures Appl*, 1852, **17**, 24–38.
- [75] A. Gray, in *Modern Differential Geometry of Curves and Surfaces with Mathematica*, CRC Press LLC, Boca Raton, 2nd edn., 1999, ch. 8, p. 192.
- [76] C. C. Adams, *The Knot Book: An Elementary Introduction to the Mathematical Theory of Knots*, American Mathematical Society, Providence, Rhode Island, 2004, p. 187.
- [77] H. K. Moffatt and R. L. Ricca, *Proc. R. Soc. Lond. A*, 1992, **439**, 411–429.
- [78] M. A. Berger and C. Prior, *J. Phys. A: Math. Gen.*, 2006, **39**, 8321–8348.
- [79] A. E. H. Love, *A Treatise on the Mathematical Theory of Elasticity*, Cambridge University Press, 2nd edn., 1944, p. 299.
- [80] F. B. Fuller, *PNAS*, 1971, **68**, 815–819.
- [81] G. S. Manning, *Biophysical Journal*, 2006, **91**, 3607–3616.
- [82] E. L. Starostin and G. H. M. Van Der Heijden, *Nat. Mater.*, 2007, **6**, 563–567.
- [83] M. Levitt, *PNAS*, 1978, **75**, 640–644.
- [84] W. Gilewski, J. Kłosowska and P. Obara, *Procedia Eng.*, 2015, **111**, 242–248.
- [85] A. Zawadzki and A. Al Sabouni-Zawadzka, *Appl. Sci.*, 2020, **10**, 8676.
- [86] F. Fraternali and F. Santos, *Extreme Mech. Lett.*, 2019, **33**, 100578.
- [87] Z. Wang, K. Li, Q. He and S. Cai, *Adv. Mater.*, 2019, **31**, 1806849.
- [88] D. Liu, M. Wang, Z. Deng, R. Walulu and C. Mao, *J. Am. Chem. Soc.*, 2004, **126**, 2324–2325.
- [89] T. Liedl, B. Högberg, J. Tytell, D. E. Ingber and W. M. Shih, *Nat. Nanotechnol.*, 2010, **5**, 520–524.
- [90] E. Boucher and C. A. Mandato, *BBA*, 2015, **1853**, 2649–2661.
- [91] A. P. Fields, E. A. Meyer and A. E. Cohen, *Nucleic Acids Res.*, 2013, **41**, 9881–9890.
- [92] H. Chhabra, G. Mishra, Y. Cao, D. Prešern, E. Skoruppa, M. M. C. Tortora and J. P. K. Doye, *J. Chem. Theory Comput.*, 2020, **16**, 7748–7763.

- [93] W. Wang, S. Chen, B. An, K. Huang, T. Bai, M. Xu, G. Bellot, Y. Ke, Y. Xiang and B. Wei, *Nat. Commun.*, 2019, **10**, 1–8.
- [94] P. Piskunen, S. Nummelin, B. Shen, M. A. Kostianen and V. Linko, *Molecules*, 2020, **25**, 1823.
- [95] D. R. Duckett, A. I. H. Murchie, S. Diekmann, E. von Kitzing, B. Kemper and D. M. J. Lilley, *Cell*, 1988, **55**, 79–89.
- [96] K. Evans, *Chem. Ind.*, 1990, **20**, 654–657.
- [97] J. N. Grima and K. E. Evans, *J. Mater. Sci. Lett.*, 2000, **19**, 1563–1565.
- [98] J. Dagdelen, J. Montoya, M. de Jong and K. Persson, *Nat. Commun.*, 2017, **8**, 1–8.
- [99] E. Jull and H. Gleeson, *New auxetic material stretches the limits*, 2020, <https://physicsworld.com/a/new-auxetic-material-stretches-the-limits/>.
- [100] A. Ohmann, K. Göpfrich, H. Joshi, R. F. Thompson, D. Sobota, N. A. Ranson, A. Aksimentiev and U. F. Keyser, *Nucleic Acids Res.*, 2019, **47**, 11441–11451.

Chapter 9

Appendix

9.1 Appendix A

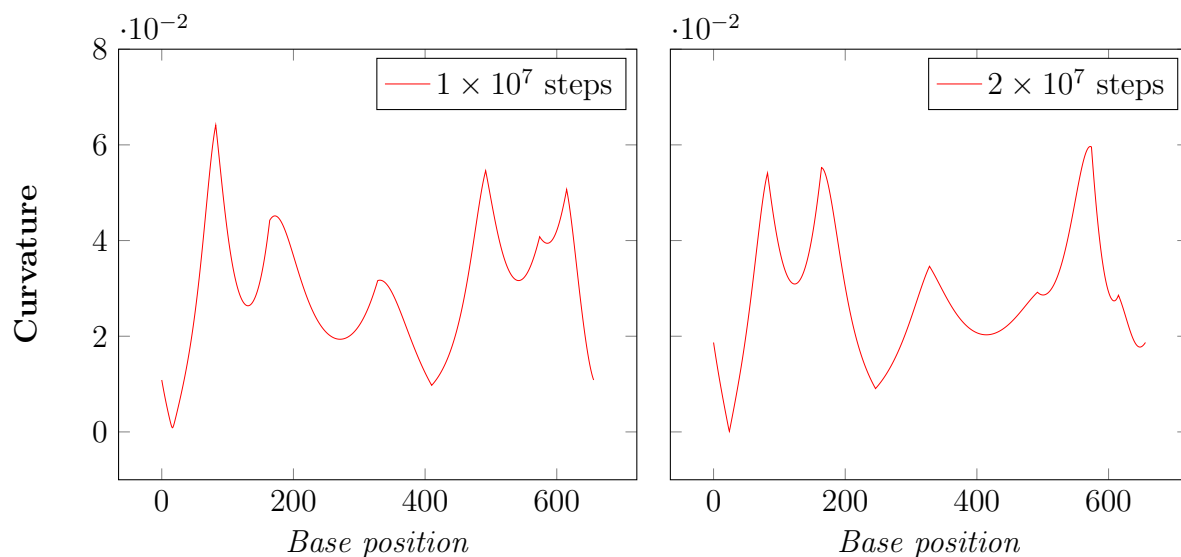
A protocol for running an oxDNA simulation.

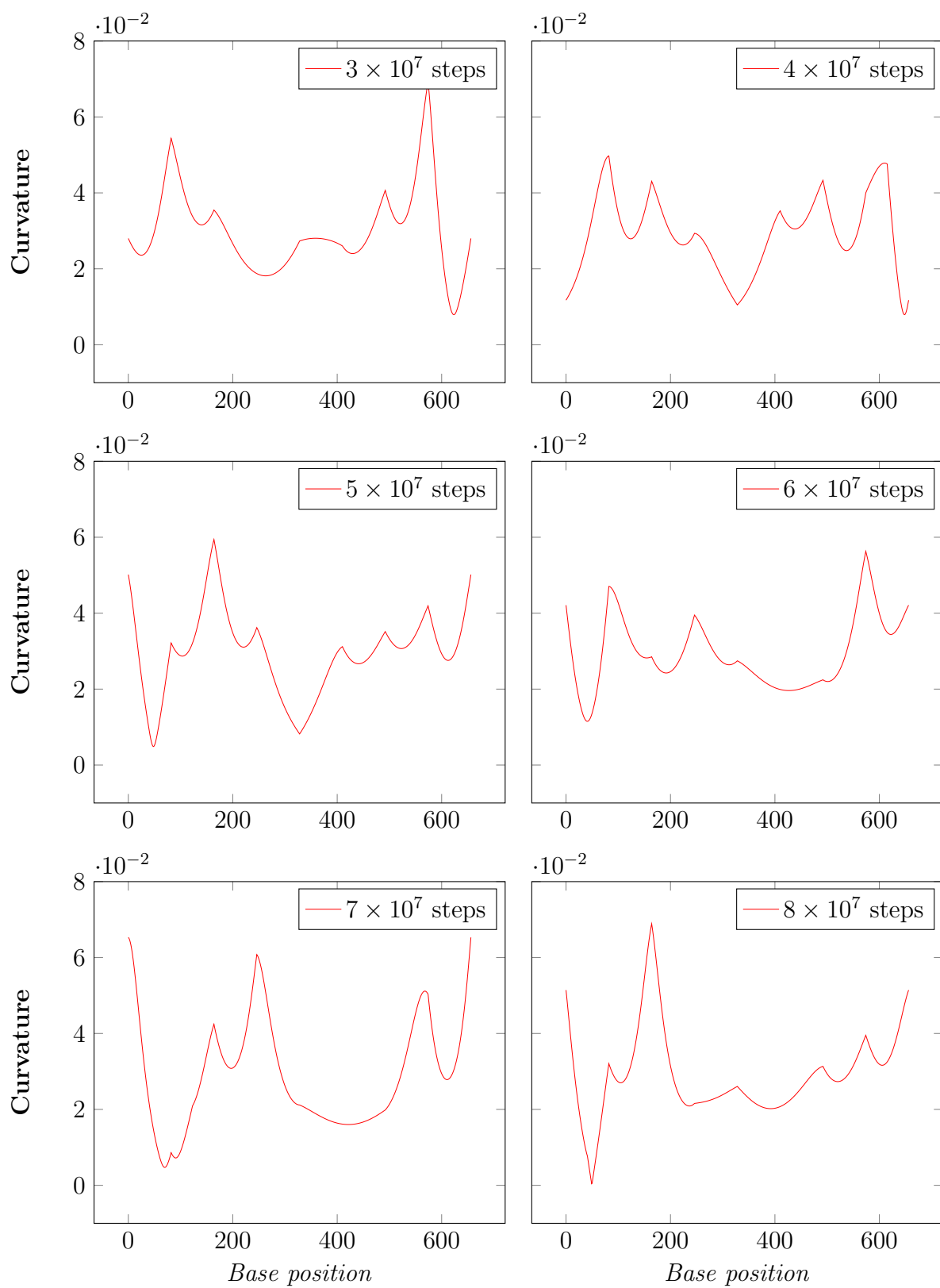
1. Install oxDNA as per the instructions on the Download and Installation information page: https://dna.physics.ox.ac.uk/index.php/Download_and_Installation. oxDNA contains example simulation files which can be used to learn the software before starting simulations on larger structures. It also contains all the required input files for the minimisation, relaxation and simulation processes.
2. Obtain input files. One topology and one configuration file is needed. These can be exported from a caDNAno design, or from using other software such as oxView.^{49,50}
3. Carry out a minimisation process using the topology and configuration files. This can usually be done on a standard computer (using CPU) as there tend to be few steps required. ~ 2000 steps should be sufficient in most cases.
4. The next step is to carry out a relaxation. Again, an input file from the oxDNA package will be required, along with the minimised topology and configuration files from step 3. A relaxation of $\sim 10^6$ steps is usually sufficient but can also be run for longer if needed.
5. If the structure is fully relaxed, the topology and configuration files can then be used for a simulation. At this point, it is possible to include or measure other properties (e.g., hydrogen bonding energy). For more information about what is available and how to include these parameters, please see the relevant webpage: <https://dna.physics.ox.ac.uk/index.php/Documentation>. If using a GPU cluster, it is always worth running a simulation locally first

to ensure it will not crash in the first few steps. This can save some time if using shared resources with queues.

6. When the simulation is complete, it is always useful to then visualise it. A quick and easy way to do this is by using oxView^{49,50} which is an interactive platform accessed through a web browser: <https://github.com/sulcgroup/oxdna-viewer>. To see other visualisation options, please refer to the relevant oxDNA information page: https://dna.physics.ox.ac.uk/index.php/Documentation#Visualisation_of_structures.

9.2 Appendix B





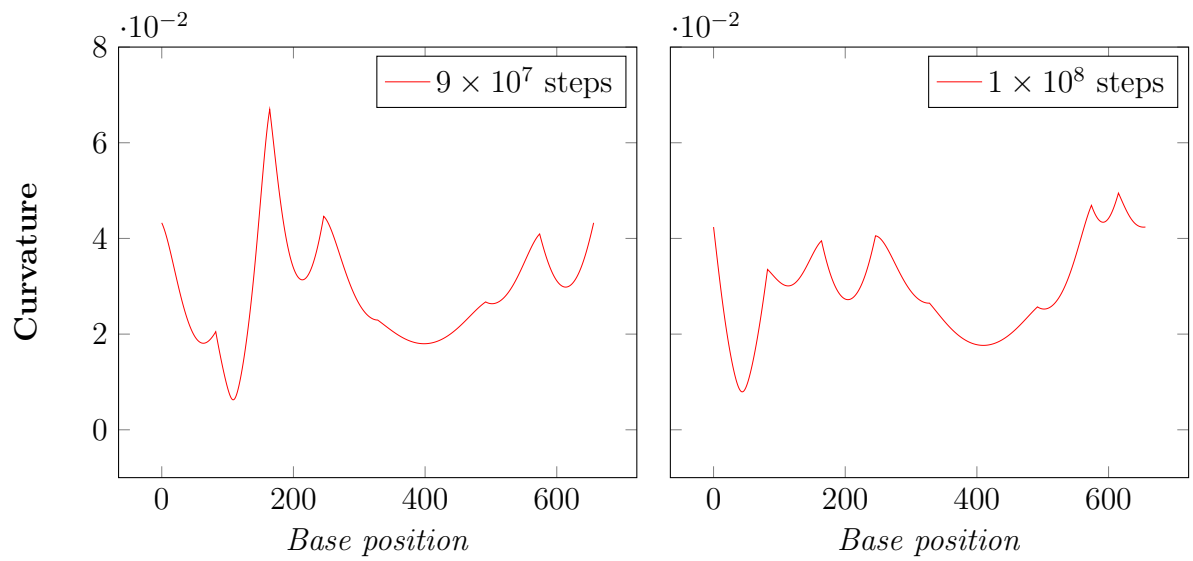
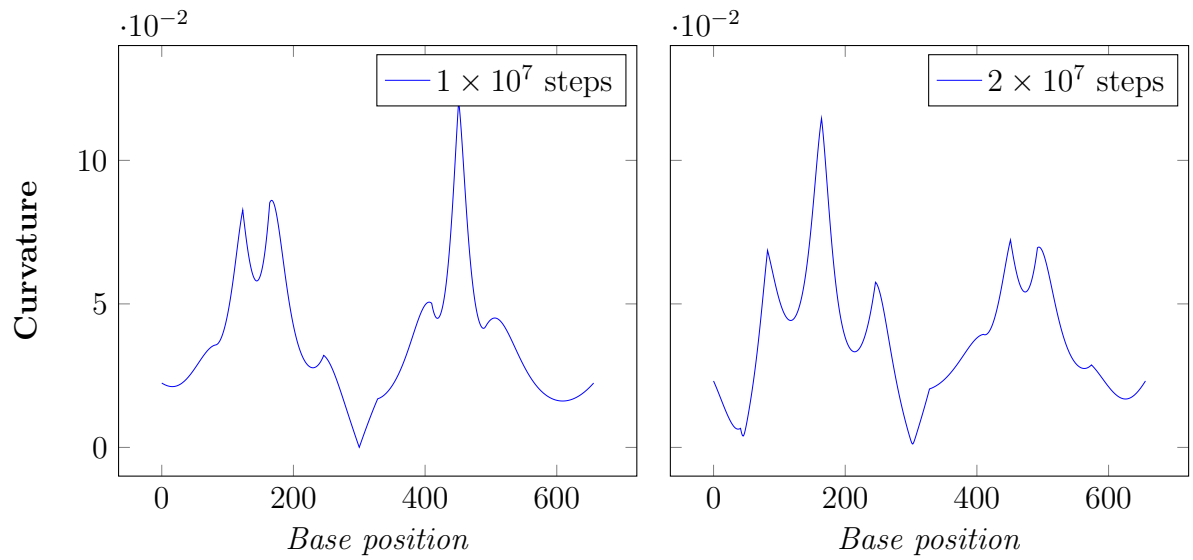
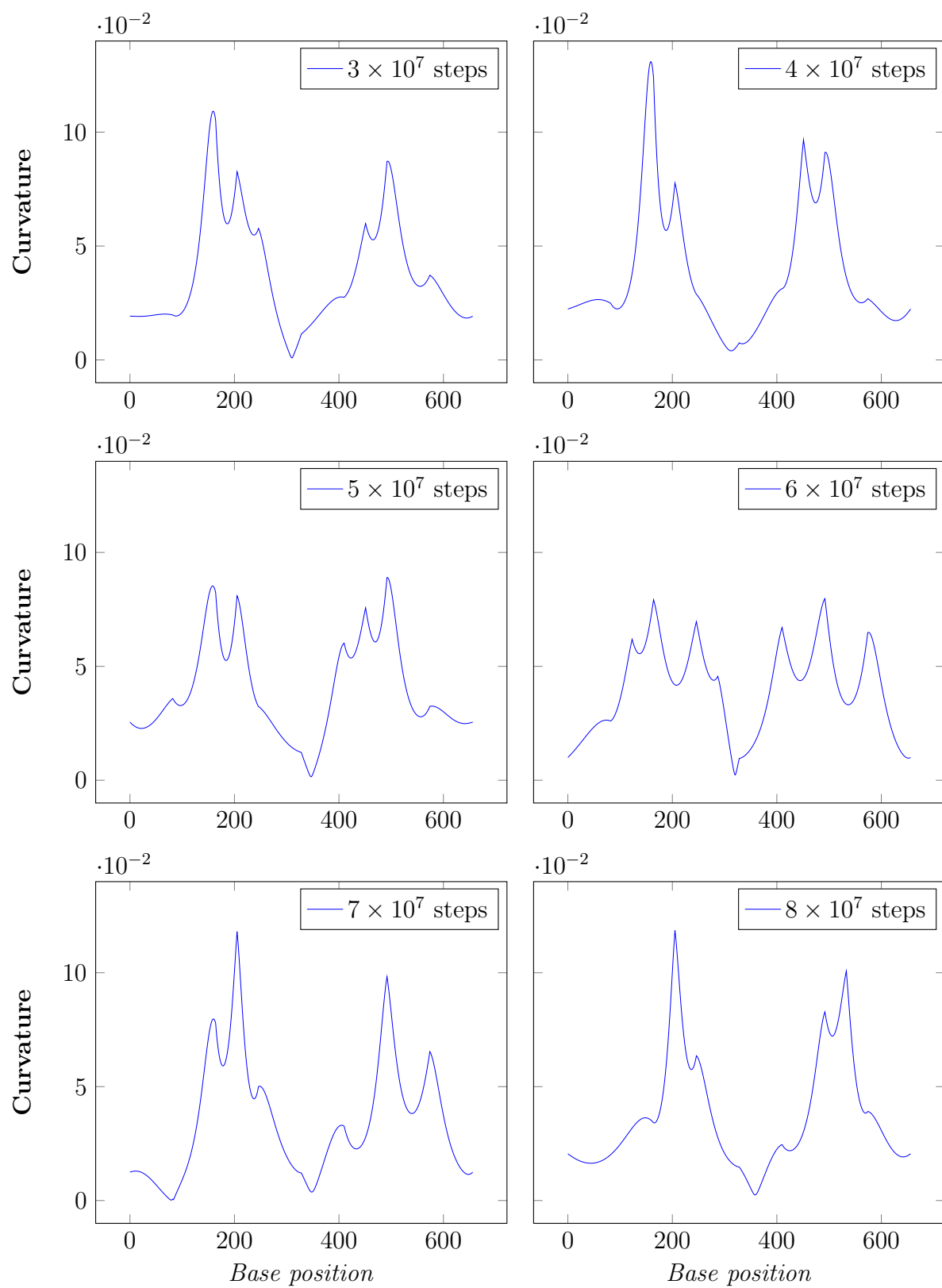


Figure 9.1: Curvature profiles for the right-handed Möbius strip for every 10^7 steps of simulation. The major peaks do not significantly move in position on the profiles.





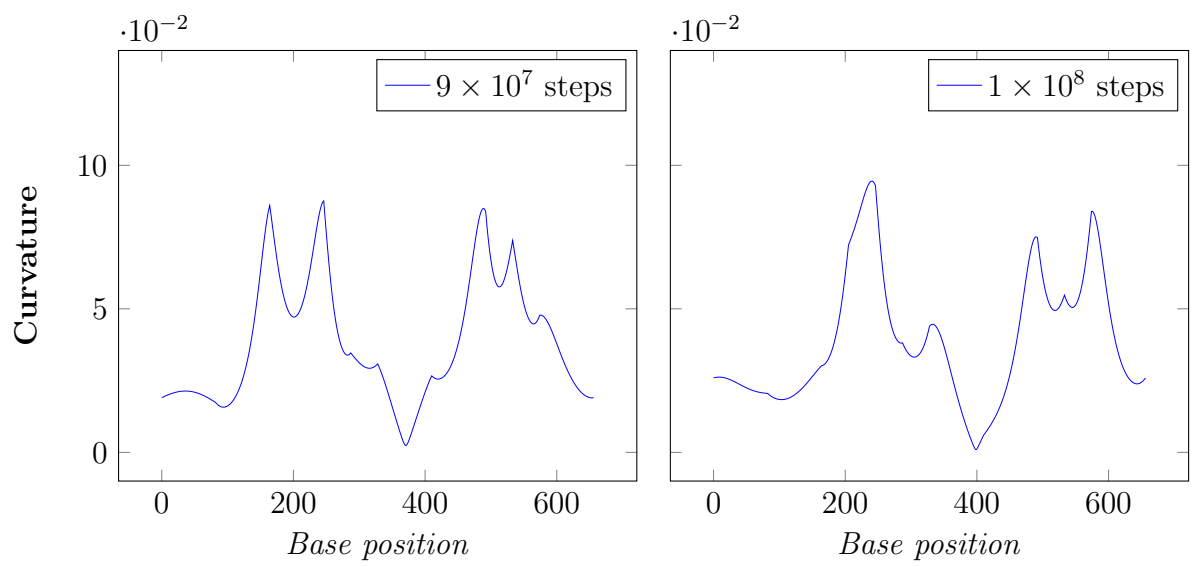


Figure 9.2: Curvature profiles for the left-handed Möbius strip for every 10^7 steps of simulation. The major peaks do not significantly move in position on the profiles.

9.3 Appendix C

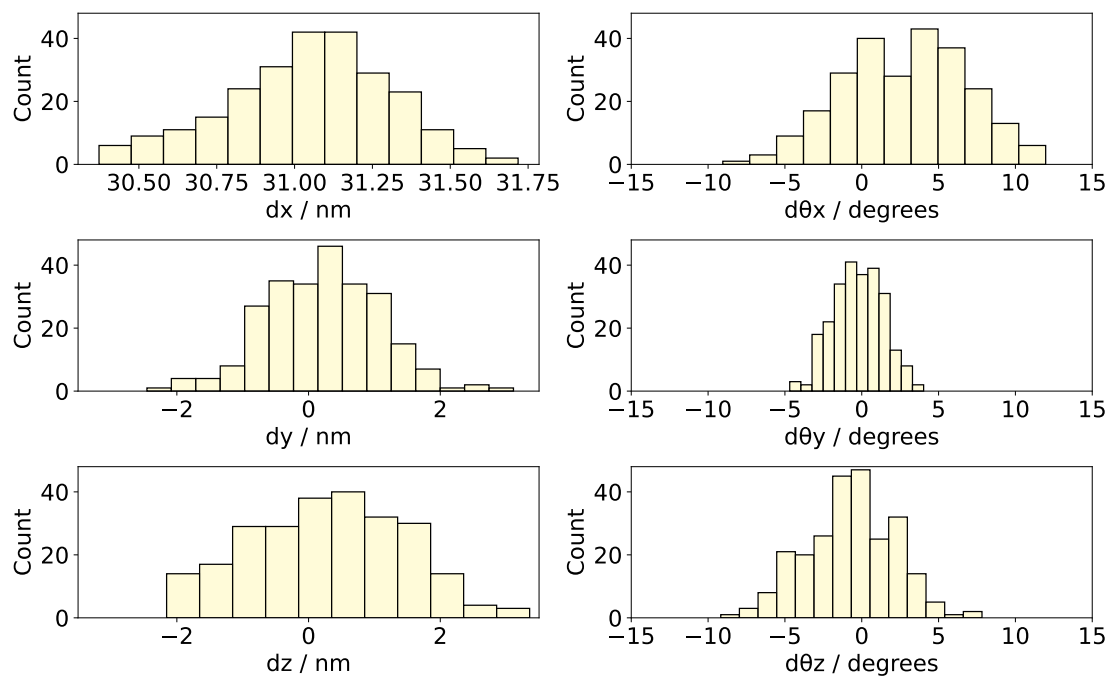


Figure 9.3: Histogram depicting measurements of New Motif barrel structure with 2T spacers. Simulation was run with sequence dependence parameter turned on. Note that the x -axis label for the dx measurement is different to the others in the same column due to covering a vastly different range.

9.4 Appendix D

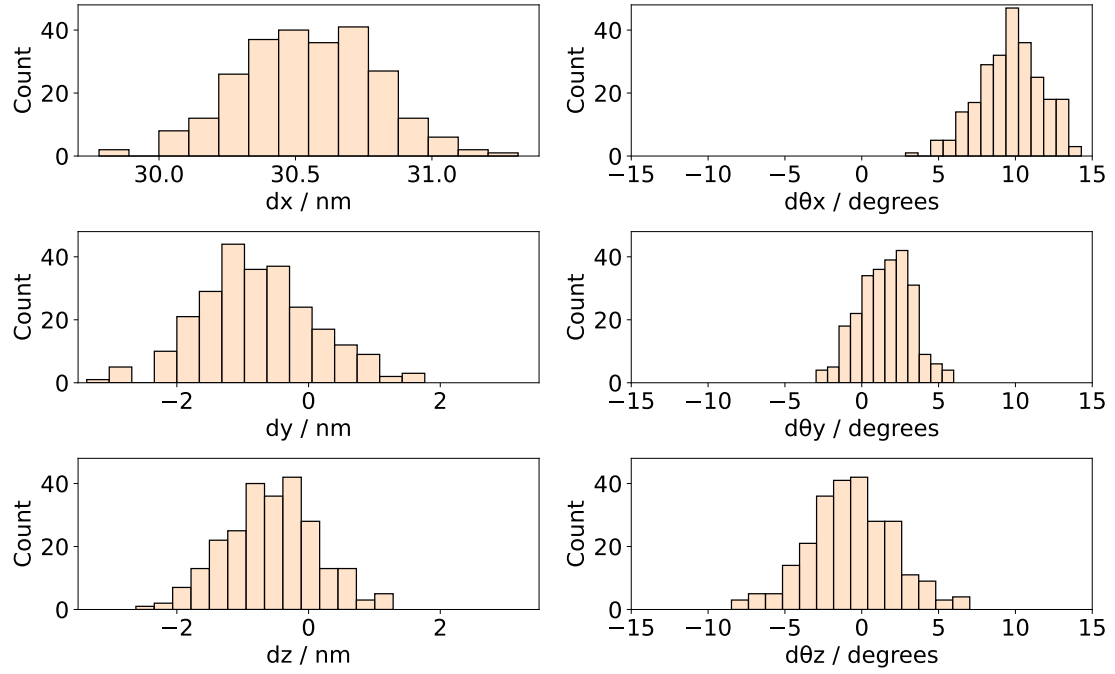


Figure 9.4: Histogram for linear barrel arrangement measurements for Monomer 1 left barrel, and Monomer 1 right barrel. Note that the x -axis label for the dx measurement is different to the others in the same column due to covering a vastly different range.

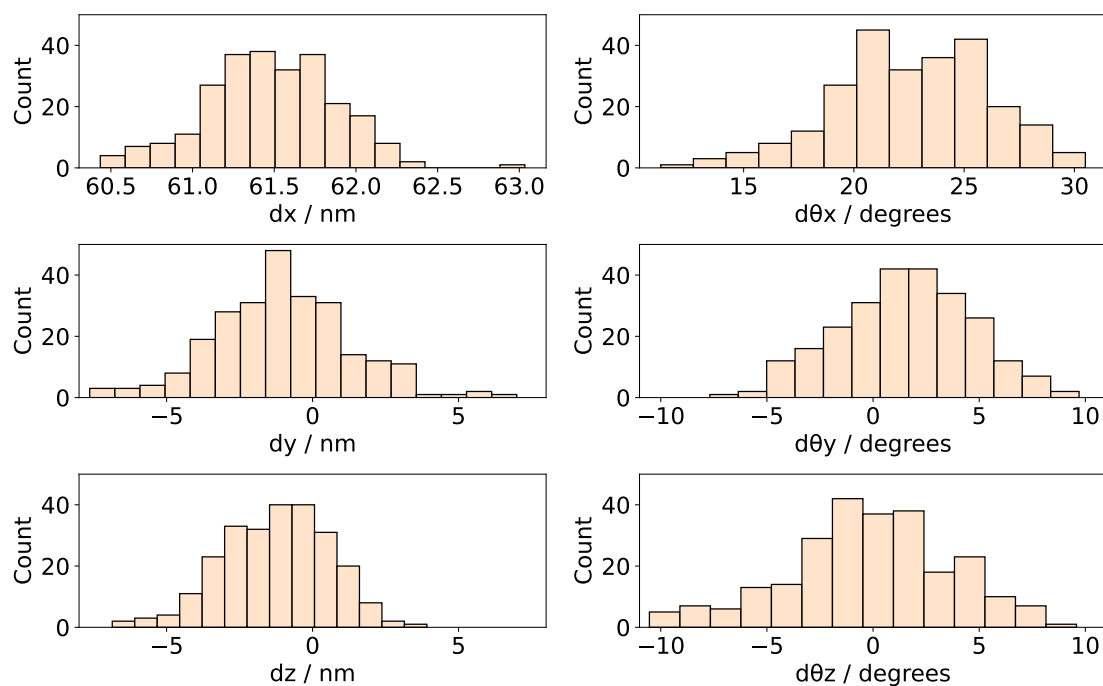


Figure 9.5: Histogram for linear barrel arrangement measurements for Monomer 1 left barrel, and Monomer 2 left barrel. Note that the x -axis labels for the dx and $d\theta x$ measurements are different to the others in their respective columns due to covering vastly different ranges.

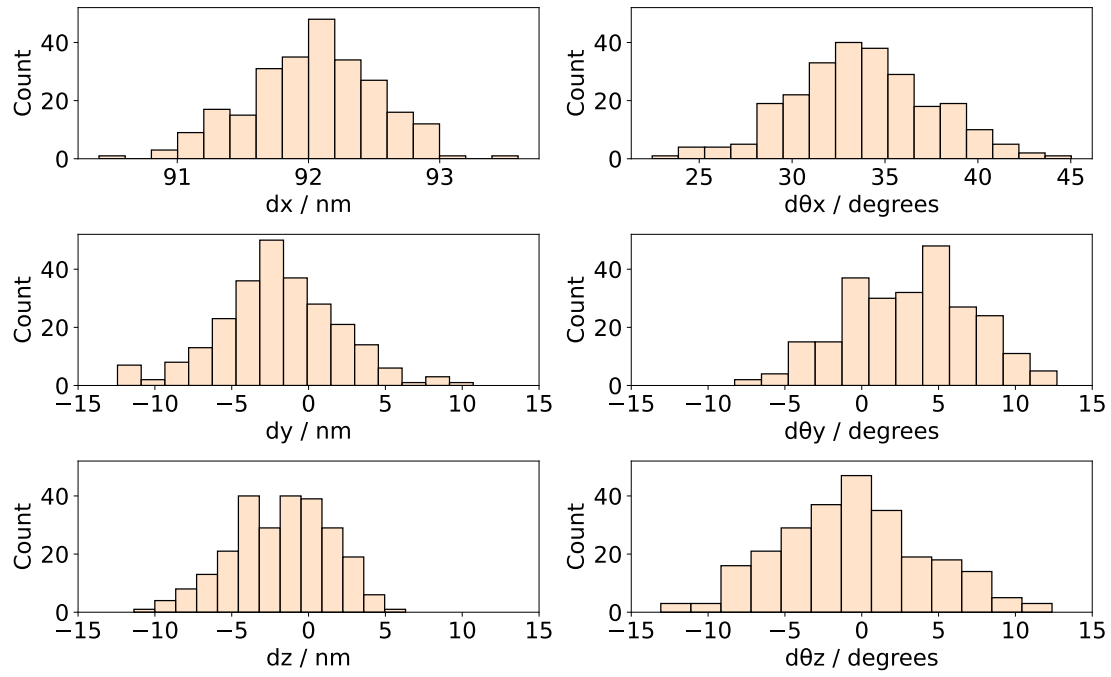


Figure 9.6: Histogram for linear barrel arrangement measurements for Monomer 1 left barrel, and Monomer 2 right barrel. Note that the x -axis labels for the dx and $d\theta x$ measurements are different to the others in their respective columns due to covering vastly different ranges.

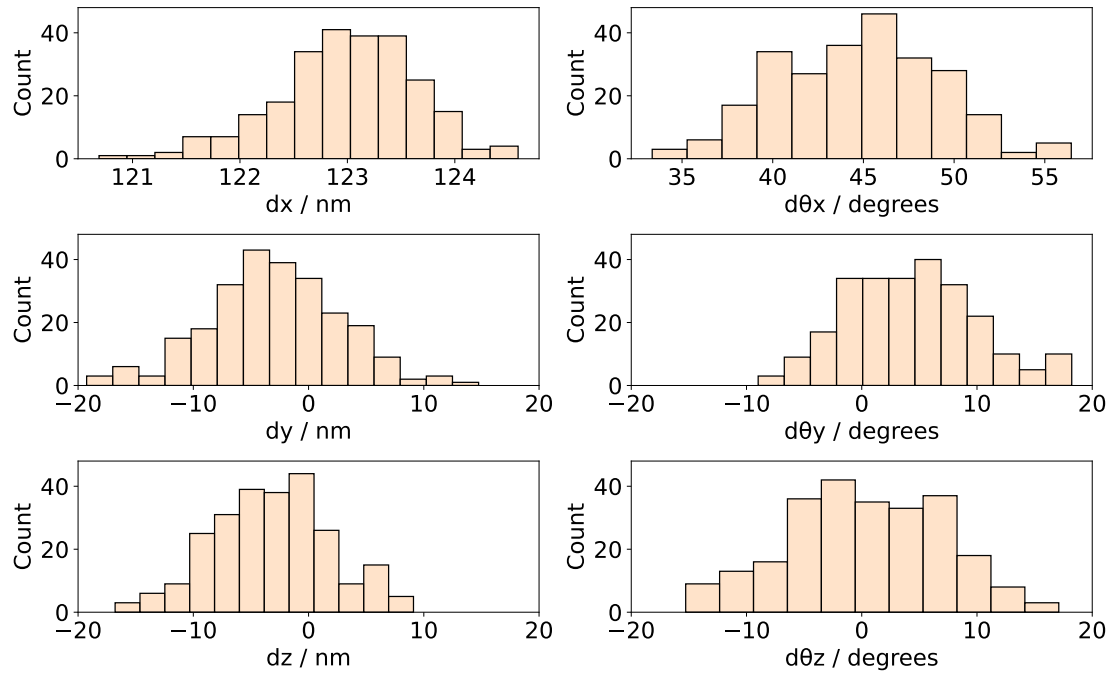


Figure 9.7: Histogram for linear barrel arrangement measurements for Monomer 1 left barrel, and Monomer 3 left barrel. Note that the x -axis labels for the dx and $d\theta x$ measurements are different to the others in their respective columns due to covering vastly different ranges.

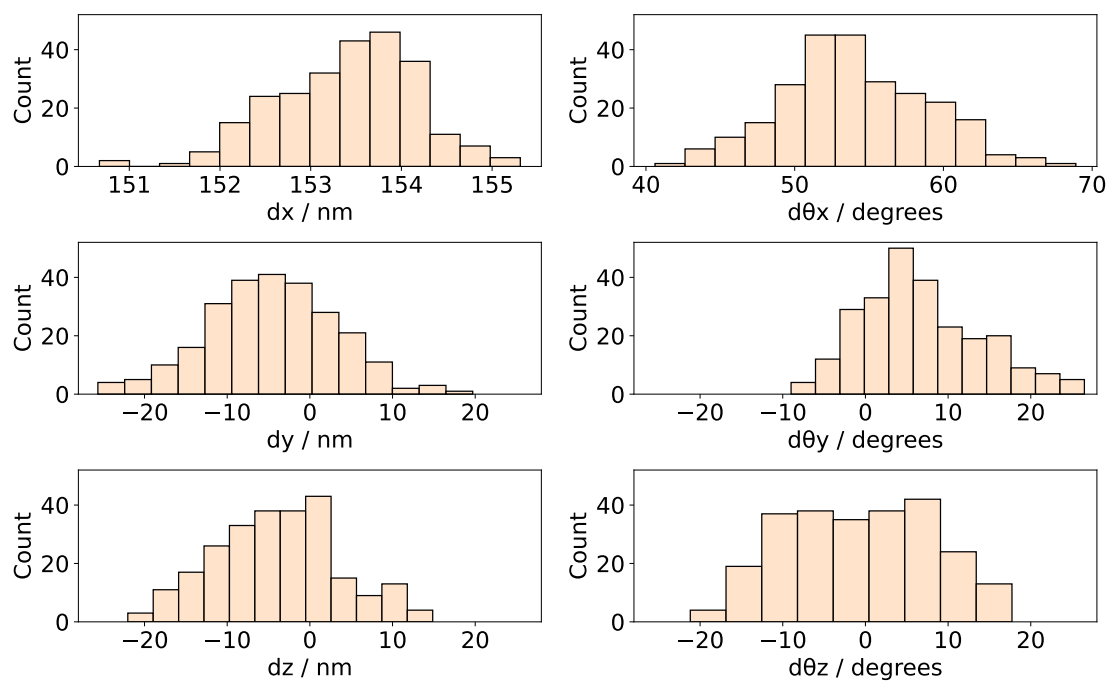


Figure 9.8: Histogram for linear barrel arrangement measurements for Monomer 1 left barrel, and Monomer 3 right barrel. Note that the x -axis labels for the dx and $d\theta x$ measurements are different to the others in their respective columns due to covering vastly different ranges.

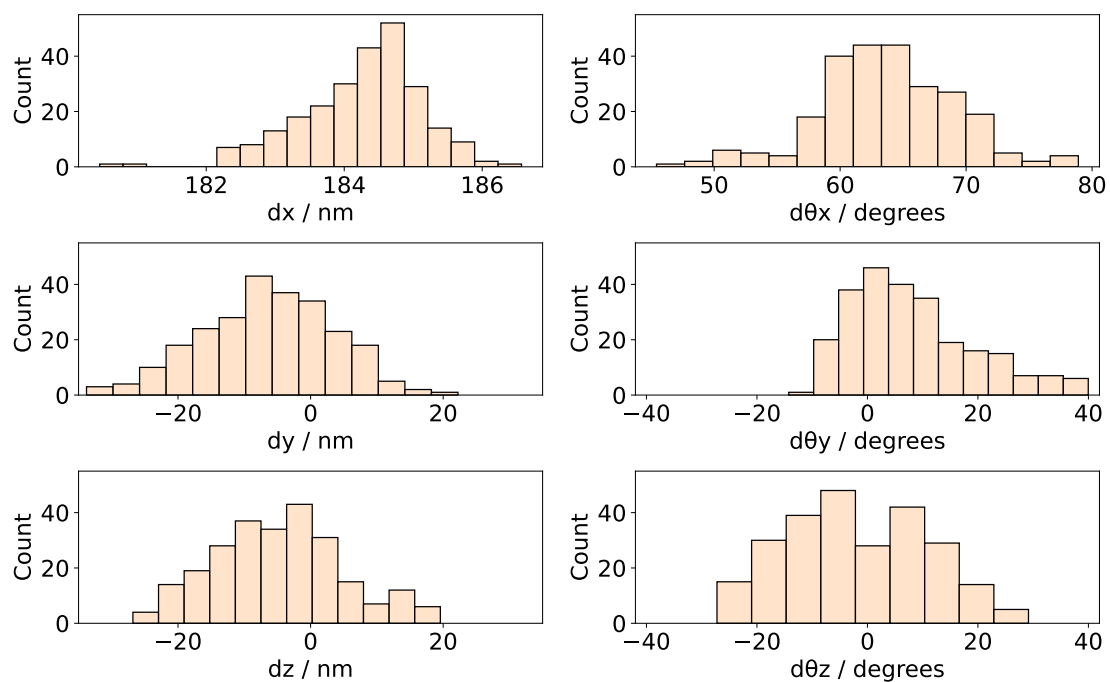


Figure 9.9: Histogram for linear barrel arrangement measurements for Monomer 1 left barrel, and Monomer 4 left barrel. Note that the x -axis labels for the dx and $d\theta_x$ measurements are different to the others in their respective columns due to covering vastly different ranges.

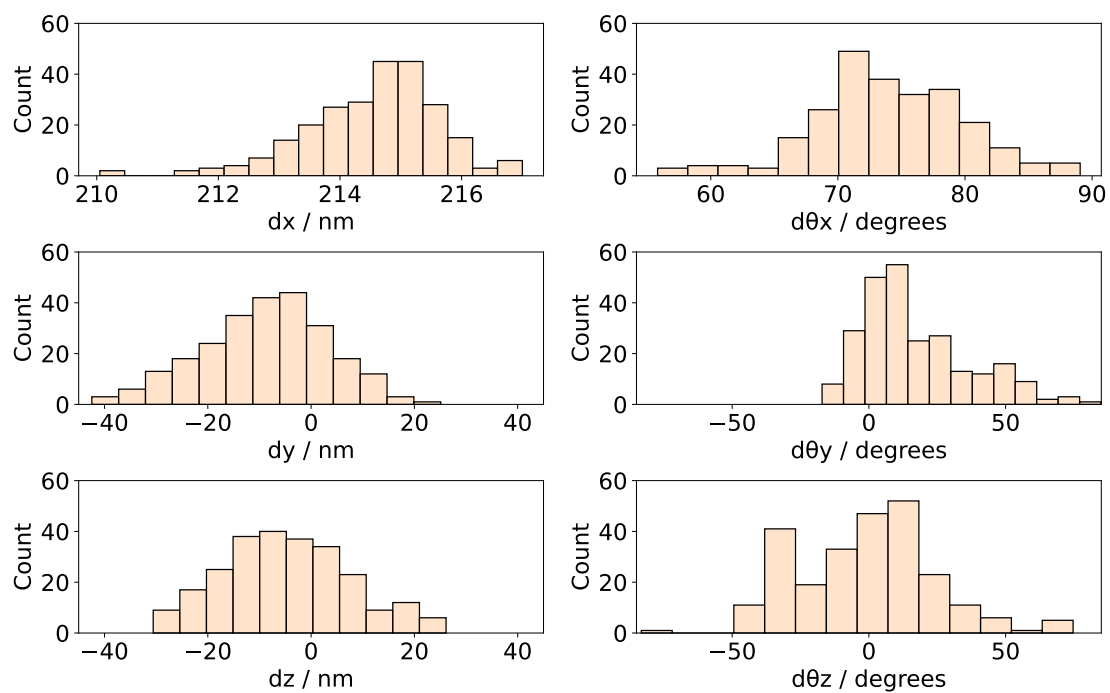


Figure 9.10: Histogram for linear barrel arrangement measurements for Monomer 1 left barrel, and Monomer 4 right barrel. Note that the x -axis labels for the dx and $d\theta x$ measurements are different to the others in their respective columns due to covering vastly different ranges.

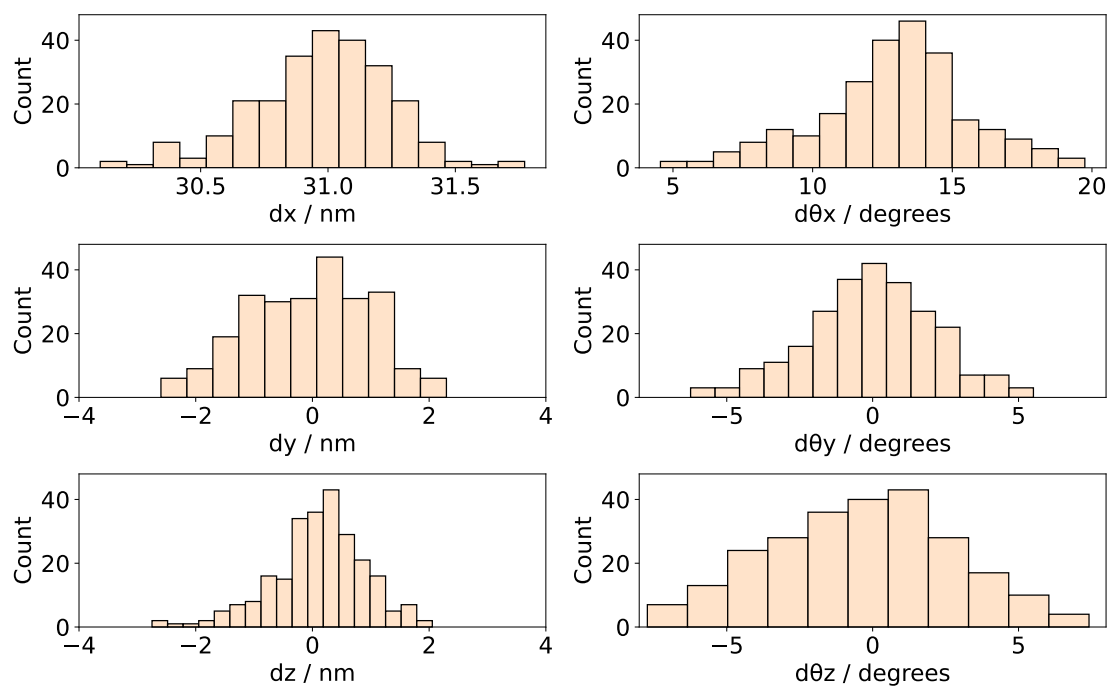


Figure 9.11: Histogram for linear barrel arrangement measurements for Monomer 1 right barrel, and Monomer 2 left barrel. Note that the x -axis labels for the dx and $d\theta x$ measurements are different to the others in their respective columns due to covering vastly different ranges.

9.5 Appendix E

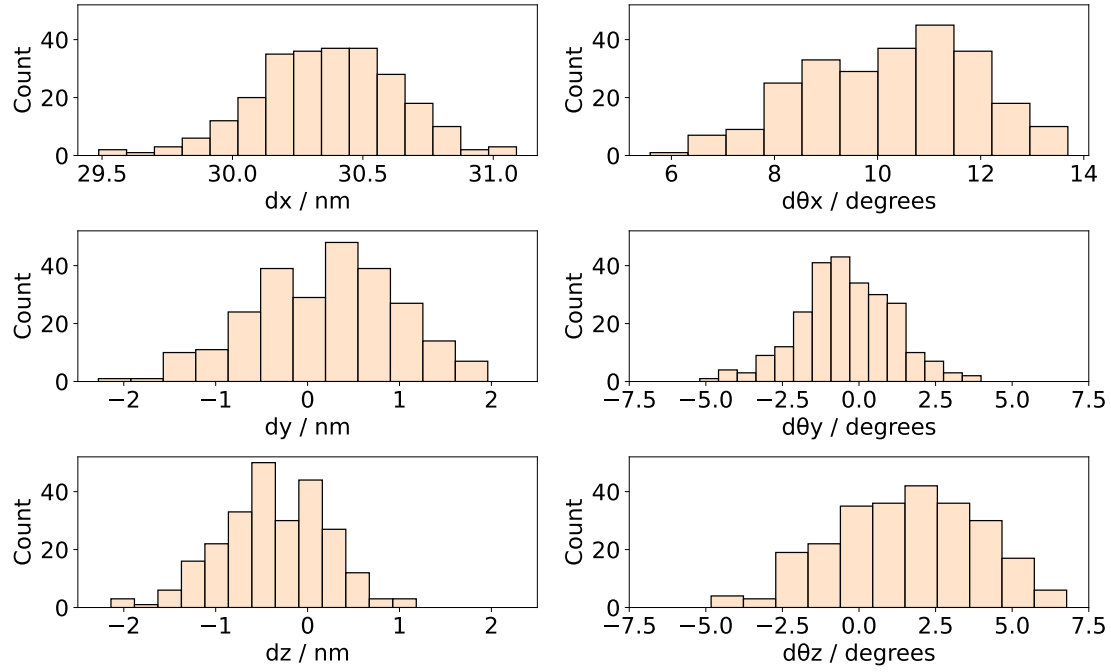


Figure 9.12: Histogram for rectangle barrel arrangement measurements for Monomer 1 left barrel, and Monomer 1 right barrel. Note that the x -axis labels for the dx and $d\theta_x$ measurements are different to the others in their respective columns due to covering vastly different ranges.

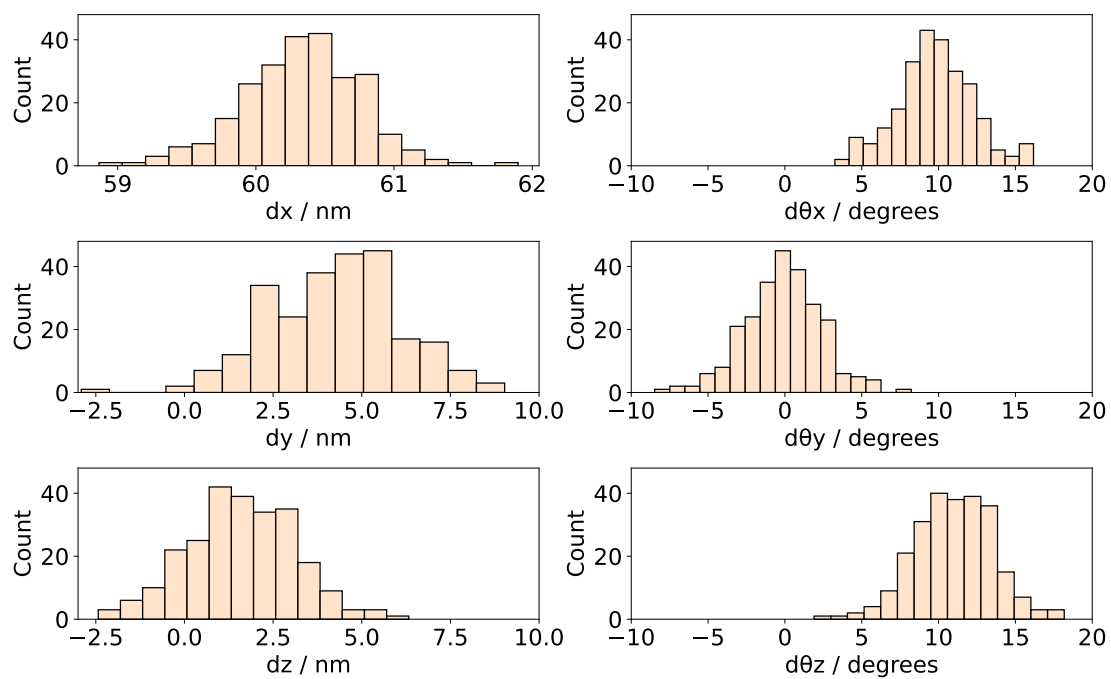


Figure 9.13: Histogram for rectangle barrel arrangement measurements for Monomer 1 left barrel, and Monomer 2 left barrel. Note that the x -axis label for the dx measurement is different to the others in the same column due to covering a vastly different range.

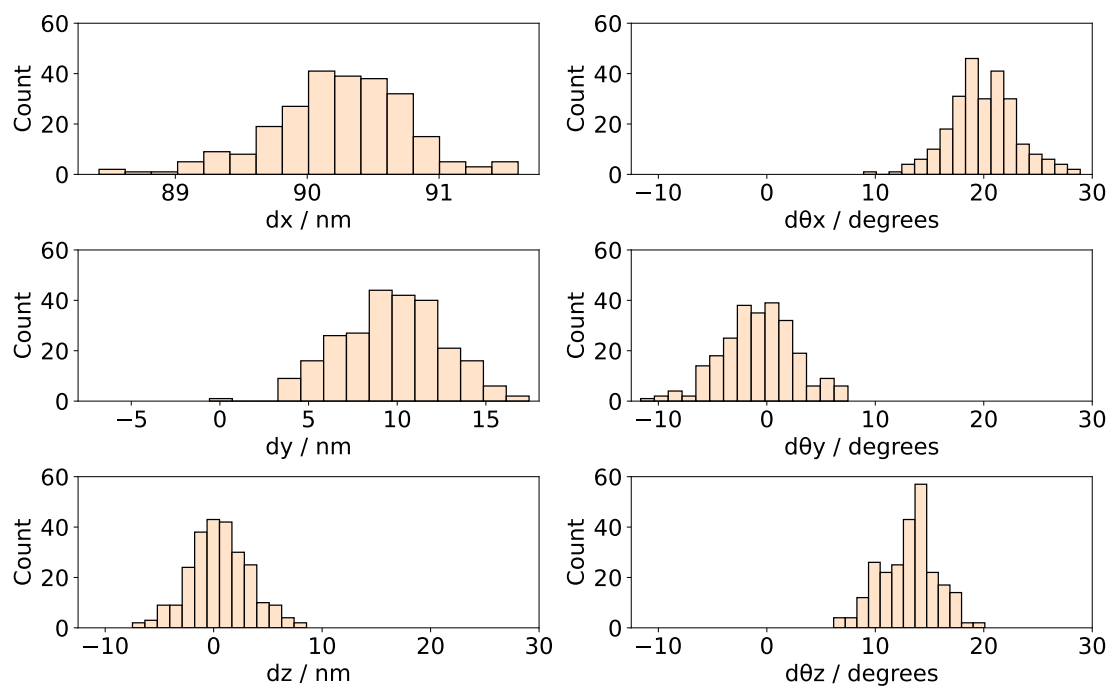


Figure 9.14: Histogram for rectangle barrel arrangement measurements for Monomer 1 left barrel, and Monomer 2 right barrel. Note that the x -axis label for the dx measurement is different to the others in the same column due to covering a vastly different range.

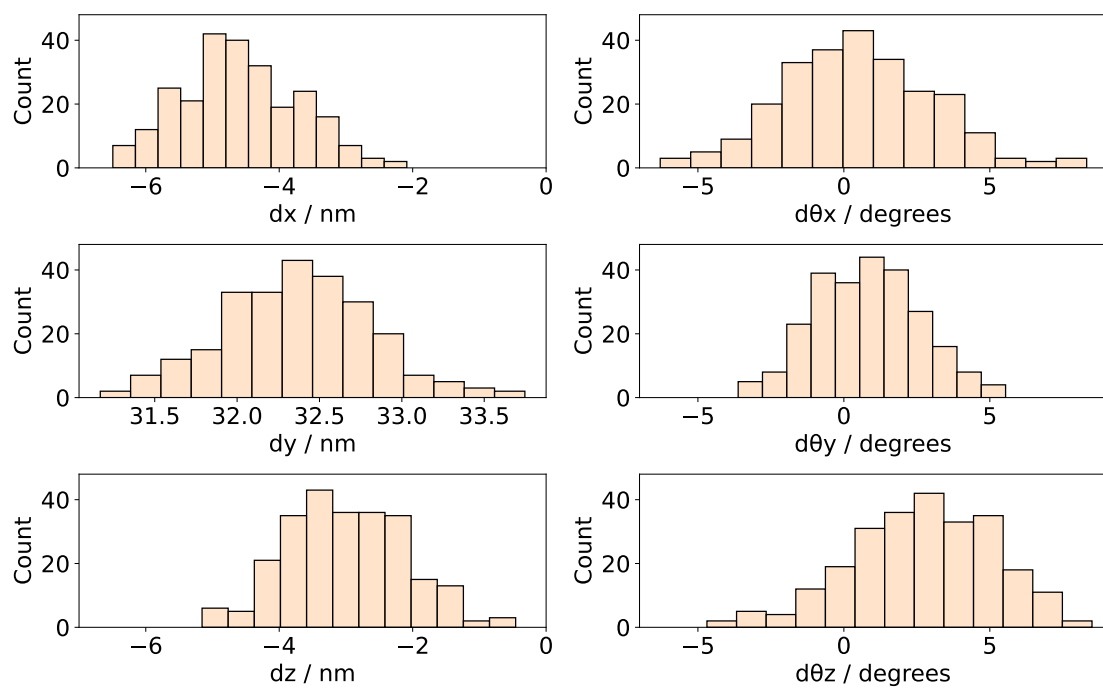


Figure 9.15: Histogram for rectangle barrel arrangement measurements for Monomer 1 left barrel, and Monomer 3 left barrel. Note that the x -axis label for the dy measurement is different to the others in the same column due to covering a vastly different range.

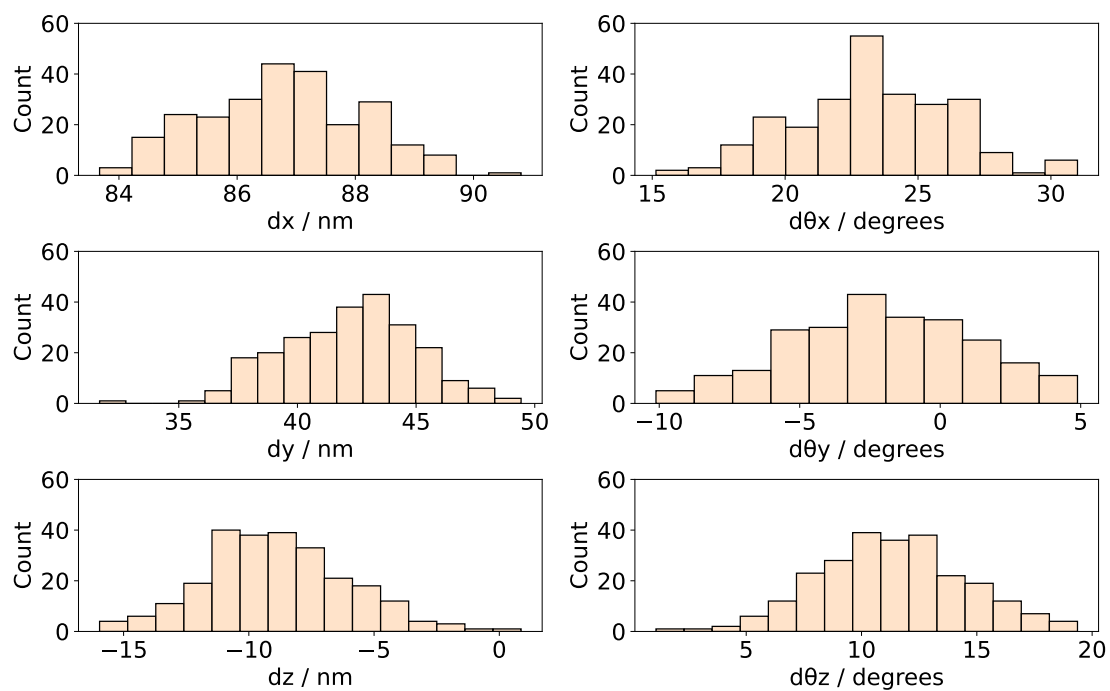


Figure 9.16: Histogram for rectangle barrel arrangement measurements for Monomer 1 left barrel, and Monomer 4 right barrel. Note that the x -axis label for all measurements are different to each other due to each plot covering a vastly different range.

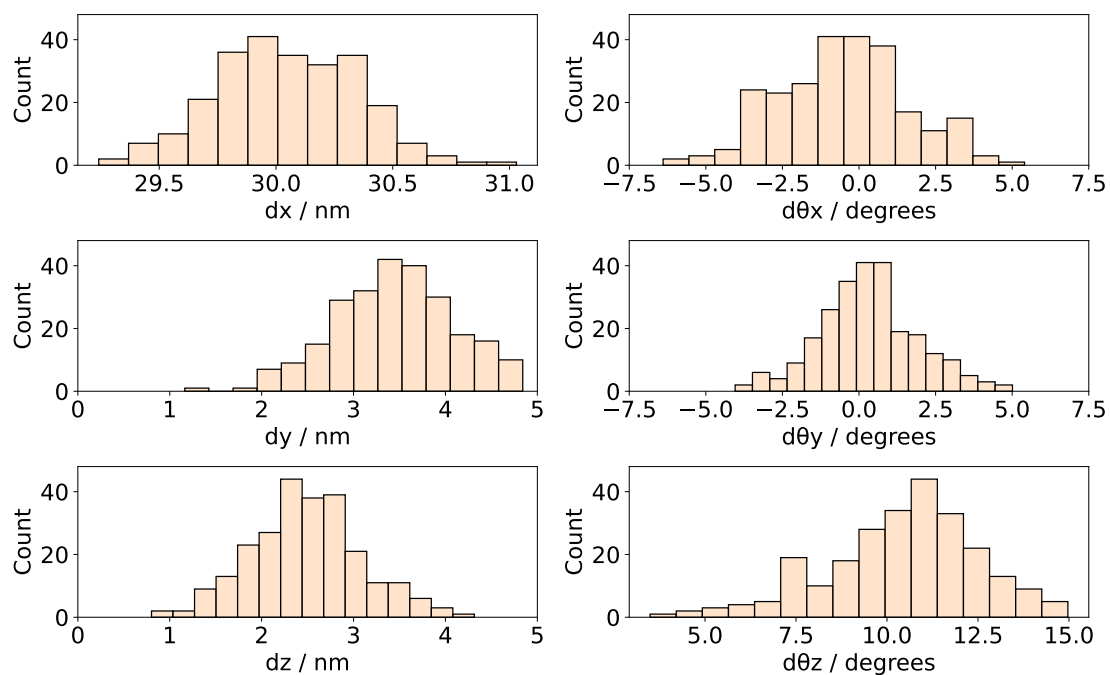


Figure 9.17: Histogram for rectangle barrel arrangement measurements for Monomer 1 right barrel, and Monomer 2 left barrel. Note that the x -axis label for the dx measurement is different to the others in the same column due to covering a vastly different range.

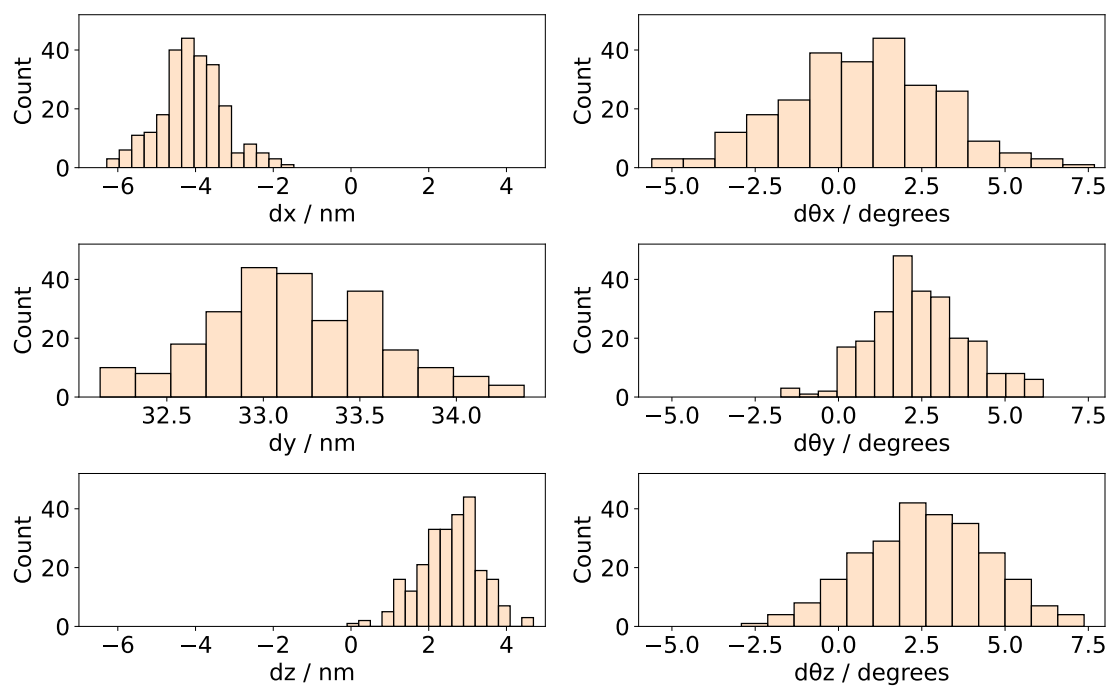


Figure 9.18: Histogram for rectangle barrel arrangement measurements for Monomer 1 right barrel, and Monomer 3 right barrel. Note that the x -axis label for the dy measurement is different to the others in the same column due to covering a vastly different range.

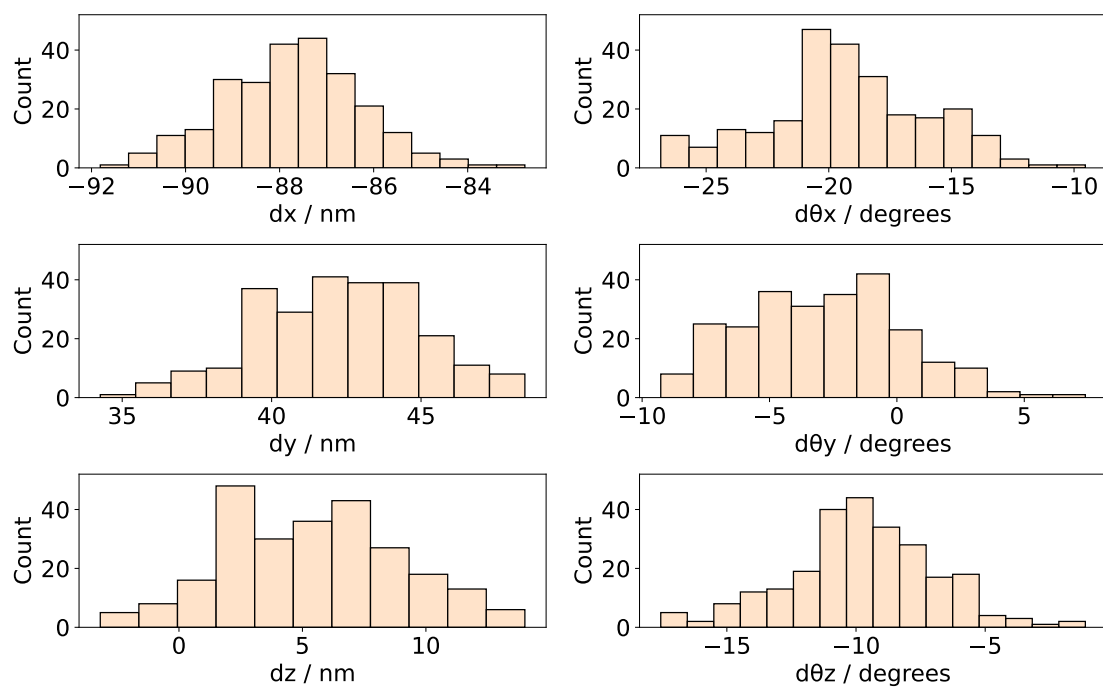


Figure 9.19: Histogram for rectangle barrel arrangement measurements for Monomer 2 right barrel, and Monomer 3 left barrel. Note that the x -axis label for all measurements are different to each other due to each plot covering a vastly different range.