Stretching a Single DNA Molecule

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Abstract

Some of DNA's behavior can be modeled as a biopolymer. Understanding the physical force-extension behavior of double-stranded DNA (dsDNA) can reveal more about the biological function of DNA in the cell. With advancements in measurement technologies, this field of study became broadly popular in the 90s Bustamante et al. [2000]. Experiments, including Marko and Siggia [1995], measured this behavior for DNA and fit it to statistical models like the freely-jointed chain (FJC) and worm-like chain (WLC). In the low-force regime, these curves fit very well and show DNA can be very accurately modeled as a biopolymer. We duplicated these results by using the Helmholtz free energy and FJC model to derive the expressions and curves for its force-extension behavior in one and three dimensions. Our results confirm the curve of the FJC model overestimates the mid-range extension regime. These errors can be fixed by using the better and more nuanced WLC model.

Introduction

In the early 1990s, new experimental measurement techniques were developed to probe the polymer behavior of single molecules of double-stranded DNA. These include: optical and magnetic tweezers, micropipettes, and atomic force microscopy Bustamante et al. [2000]. Using these methods, DNA was found to have different regimes of force-extension behavior for different magnitudes of forces applied. The four regimes, from lowest to highest force, include: elasticity due to entropy, intrinsic elasticity, overstretching, and covalent bond breaking. Once you break enough bonds, the molecule snaps.

Each of these regimes has complex behavior and does not inform the others. Some of them have been well-understood for some time, like the entropic elasticity regime, as we will discuss. Others are still not completely understood, and it is important to continue to pursue their research. For example, in the overstretching regime, DNA's helical structure becomes supercoiled. Supercoiling happens to be an important factor in gene expression, so understanding more about this structural property can help us understand more functions DNA has in the body Irobalieva et al. [2015]. However, this concept isn't new. It is a commonly held belief in molecular biology that "sequence determines structure determines function." Petsko et al. [2004] Phillips et al. [2012] This philosophy, applied to the force-extension behavior of DNA, has lead scientists to a better understanding of DNA's role in biological processes in the body. This will continue to be a well-researched field and there is still much to learn about the structure and physical behavior of DNA.

In our project, we look at the entropic elasticity regime, the regime of lowest forces. The behavior can be modeled in three ways: Hooke's law, the freely-jointed chain (FJC), and the worm-like chain (WLC). Hooke's law

only agrees with experimental data at low extensions. The FJC model works much better for both low and high extensions. The WLC model works very well over the entire regime.

In the FJC model, the polymer is a molecule of dsDNA, made up of rigid "Kuhn" segments which exhibit random-walk orientations and are considered independent from each other. The Kuhn length of DNA is about 100nm and represents the length of a section of DNA where the orientation of the monomers at one end is completely decoupled from the orientation of those one Kuhn length away. This decoupling is why the model becomes a random walk. The Kuhn length is a measure of the stiffness of the polymer.

In our project, we analyse the behavior of dsDNA statistically using the FJC model. First, we find the Helmholtz free energy of the polymer-optical-tweezers system. Then, we derive the equilibrium and a one-dimensional relation between the applied force and fractional extension. We use this to determine the spring constant of the system at low extensions. We then redo the calculation in a three-dimensional space. These results are plotted and compared to the experimental results and FJC models from prior research.

Model

Modeling a 1D Polymer

Beginning with a 1D model of a freely jointed chain, the total length of the chain can be defined as Na, where N is the number of segments in the chain, and a is the length of one segment. Each segment can only point in one of the two directions, left or right. The number of right-pointing segments is denoted as N_R and the number of left-pointing segments is $N_L = N - N_R$. The net displacement length L can be defined as $L = a(N_R - N_L)$. An external force is applied to this chain that stretches it and we want to investigate the relation between the applied force and the extension of the chain.

We can begin by writing down the Helmholtz free energy of the chain as a function the applied force and net displacement length.

The Helmholtz free energy is given by

$$F = U - TS \qquad (1)$$

The change in F is

$$dF = dU - TdS - SdT \qquad (2)$$

where $dU = TdS - PdV$. The pressure in dU is proportional to the applied force f, and the volume is proportional to the net displacement length L. Rewriting the last term in dU and substituting it into Eq. (2) gives

$$dF = -SdT - fdL \qquad (3)$$

Integrating Eq. (3) gives

$$F = -fL - TS \qquad (4)$$

Now we have to find an expression for entropy. As the polymer gets stretched close to its total length, the number of configurations of its segments decreases and so does the entropy. Thus, the multiplicity is a function of N and N_R. We can apply Stirling's Approximation to the multiplicity and write the entropy as

$$S = k\left[N\ln N - N_R\ln N_R - (N - N_R)\ln(N - N_R)\right] \qquad (5)$$

Substituting Eq. (5) into Eq. (4) gives the full Helmholtz free energy equation.

$$F = -fL - kT\left[N\ln N - N_R\ln N_R - (N - N_R)\ln(N - N_R)\right] \qquad (6)$$

Now we want to find a relation between the applied force f and the fractional extension of the polymer $\frac{L}{Na}$. We can differentiate Eq. (6) with respect to net displacement length L.

$$\frac{\partial F}{\partial L} = -f + \frac{kT}{2a}\ln\left(\frac{N_R}{N - N_R}\right) \qquad (7)$$

The equilibrium state is achieved when the free energy is at minimum. Setting Eq. (7) to 0 and rearranging it gives

$$f = \frac{kT}{2a}\ln\left(\frac{N_R}{N - N_R}\right) \qquad (8)$$

The ratio of right-pointing segments to left-pointing segments is then

$$\frac{N_R}{N - N_R} = e^{\frac{2fa}{kT}} \qquad (9)$$

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We can rewrite $\frac{N_R}{N-N_R}$ as $\frac{1+\frac{L}{Na}}{1-\frac{L}{Na}}$ to introduce the fractional extension into the equation.

$$\frac{1+\frac{L}{Na}}{1-\frac{L}{Na}} = e^{\frac{2fa}{kT}} \tag{10}$$

Rearrange the previous equation reveals the hyperbolic relation between the fractional extension of the polymer and the applied force.

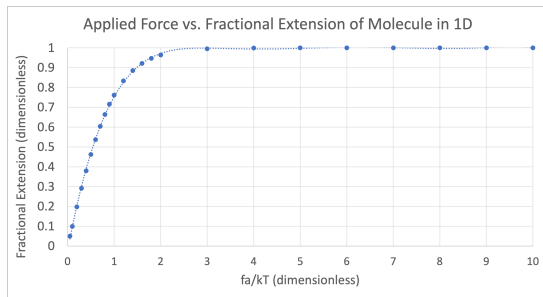$$\boxed{\therefore \frac{L}{Na} = \tanh\left(\frac{fa}{kT}\right)} \tag{11}$$

Figure 1: Applied force vs. fractional extension of the polymer in 1D

Fig. (1) is the graph of Eq. (11). At small forces, the polymer behaves like a spring and stretches linearly. We can find an expression in the form of Hooke's Law to describe this trend.

The natural log in the right-hand side of Eq. (8) can be Taylor expanded to $\frac{2L}{Na}$ under the assumption that $L << Na$. This gives us

$$\boxed{\therefore f = \frac{kT}{Na^2}L} \tag{12}$$

where the spring constant is $\frac{kT}{Na^2}$. However, at large forces, the polymer no longer behaves in a linear fashion, and Eq. (12) breaks down.

Freely Jointed Chain Model for a 3D Polymer

Assumptions for FJC

We are assuming the polymer can be represented by a finite number N of monomers which all have discreet length l (also known as the Kuhn length). We will assume monomers do not have any affect on each other, thus their direction is purely random. This allows us to imagine the shape of the polymer as a result of a random walk.
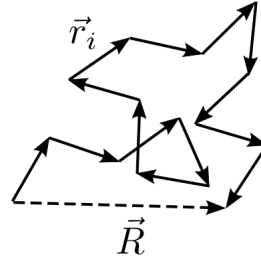

Figure 2: Visualization of monomers pointing in random direction, this is visualized as vectors. Image by Thorinmuglindir [2009]

Derivation for FJC (in 3-D, continuous distribution of all possible directions) Under Constant Force

To begin, let's consider the system to be a single monomer which can rotate freely. The monomer is one of many that makes up a polymer; thus it is tugged by some force f on both sides. By assuming constant force, the energy of this system can be expressed as:

$$U = -W = -\int_C \vec{f} \cdot d\vec{r} = -fa\cos\theta$$

Therefore, we can express the partition function as for this monomer as:

$$Z_1 = \sum_S e^{-E(S)/kT} = \sum_S e^{fa\cos\theta/kT}$$

Recall Moh's rules of evaluating sums: we can approximate this series as an integral.

$$Z_1 \approx \int_{states} e^{fa\cos\theta/kT}$$

Before we can continue any further in the derivation, we must now figure out what are integrating with respect to. Over all possible

state, the only variable changing is the direction of the monomer. I want you to imagine this monomer as a vector from the origin of a 3D graph to the surface of a unit sphere. Each point on the surface is a direction that the monomer could point- they are all possible states. So to summate over all possible points on a sphere, let us invoke something similar to spherical coordinates.

$$Z_1 = \int_0^{2\pi} \int_0^{\pi} e^{fa\cos\theta/kT} \sin\theta \, d\theta \, d\phi$$

$$\therefore Z_1 = 4\pi \frac{kT}{fa} \sinh\left(\frac{fa}{kT}\right)$$

Note, we chose the unit sphere as we only want to sum over the possible directions.

Now that we have Z_1, let's find Z. Recall when comparing distinguishable particles, the partition function is given by: $Z = Z_1^N$. Also recall free energy is given by $G = -kT \ln Z$. Plugging all of this into G, we get:

$$G = -NkT \ln \left[4\pi \frac{kT}{fa} \sinh\left(\frac{fa}{kT}\right) \right]$$

Recall free energy, length and force are related:

$$L = -\frac{\partial G}{\partial f}$$

$$\boxed{\therefore L = Na \left[\coth\left(\frac{fa}{kT}\right) - \frac{kT}{fa} \right]} \quad (13)$$

Now let's check. Will this expression still obey Hook's Law when Taylor expanding at $f \to 0$?

$$L \approx \frac{aN}{3kT} f + O(f^3)$$

$$\boxed{\therefore f \approx \frac{3kT}{Na^2} L} \quad (14)$$

Something interesting to note: the expansion of the 3D relationship (Eq. 14) is exactly 3 times the expansion of the 1D relationship (Eq. 12). Perhaps the 3 comes from the number of dimensions we are modeling.

Model Results

To compare our results to results found by Bustamante et al. [1994], we assumed the following parameters.

$$T = 300\,K$$
$$a = 32.7\,nm$$
$$L_{total} = 32.8\,\mu m$$

T was chosen to be $300\,K$ we expect such an experiment would be conducted at around room temperature. As for a and L_{total} looking at the original paper by Bustamante et al. [1994], we find the original Kuhn length and contour length parameters they used.
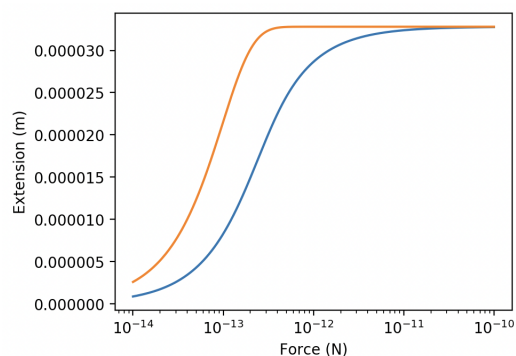

Figure 3: Graph of the force-extension relationship of a 1D Two State Polymer (yellow curve) given by Eq. (12), and a 3D Polymer modeled as a FJC (blue curve) given by Eq. (13).

Discussion

Analysis of our model's performance requires comparison with the experimental elasticity data of dsDNA molecules, which researchers are able to directly measure since the early 1990s through magnetic and hydrodynamic forces Smith et al. [1992]. Though graphs of the respective experimental data are included in our referred previous papers, there is unfortunately no raw data available to us. We thus opted to overlay corresponding curves produced by our one-dimensional and three-dimensional FJC model (Fig. 3) onto the graph from

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Phillips et al. [2012], in order to provide a visual comparison. (Fig. 4)
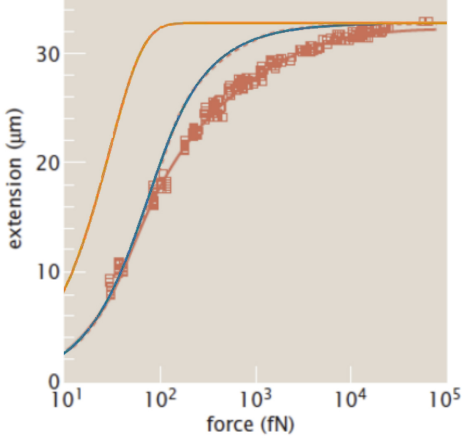

Figure 4: Force-extension curve for double-stranded DNA. Experimental data for force-extension for double-stranded DNA (boxes). Illustrating the distinction between the freely-jointed chain model (orange, blue, dashed), and the worm-like chain model (solid red). Our FJC 3D curve from Fig. 3 (blue) fits well over the original article's FJC curve (dashed red). Bustamante et al. [1994] Phillips et al. [2012]

Our derivation of the three-dimensional FJC model (blue line) perfectly aligns with the original FJC fit (red dashed line underneath) to the small-x data, with a L value of 32.7 μm and a Kuhn length a of 100 nm calculated by Bustamante et al. [1991]. Both predict experimental results accurately below an extension of 10 nm, where the DNA molecule behaves rather similar to a mechanical spring according to Hooke's Law. After overestimating the extension as extension force increases, our model rejoins experimental data at forces higher than 10^4 fN but not exceeding the 31 μm limit. This agreement indicates a full extension of the DNA molecule contour length, Na, and stretching beyond the contour length will likely cause the conventional chain elasticity, upon which our model builds, to breakdown with deformation and enthalpic effects. However, our FJC model is powerful enough to yield rather accurate pre-

dictions of the contour length and the Kuhn segment length of a dsDNA molecule entirely based on the force-extension data within a force range below 10^5 fN.

In the middle range, the force needed to extend the molecule rises higher than the prediction of our FJC model, largely due to its assumption of discrete, inextensible segments that make up the dsDNA molecule. Real DNA molecule segments are neither perfectly straight and rigid, nor strictly independent, which reduces the possibility for extension: depending on the genome-pair sequences, segments of DNA may be intrinsically bent and thus heighten the contractibility of the entire DNA molecule Smith et al. [1992]. A correlation between the directions of neighboring segments also reduces extension by self-avoiding and thus ruling out unlikely alignments. The improved worm-like chain model (red solid line in Fig. 4) simultaneously solves these two imperfections by modeling the dsDNA molecule as a long worm, which gives excellent agreement over the force range in Fig. 4.

This worm-like chain model allows for the flexibility and nuance for the stretching of the DNA molecule which more accurately fits experimental data. Instead of assigning probabilities to definite variables, the WLC model assesses the probabilities of functions to allow for a more continuous analysis of the distribution. Although the WLC model matches extremely well with the experimental data, extrapolating the model to both the high force and low force limits run into even more nuanced issues. In the low force regime, hydrophobic interactions are present as the polymers move past each other. The residues of these polymer chains can attract one another, which would make the chain more resistant against a pulling force. The worm-like chain model does not account for this self-interaction, so the experimental data will deviate from the model at low forces [Fu et al.].

On the other end of the spectrum, the high-force regime, the WLC model does not account for enthalpic effects under large pulling forces. When double stranded DNA is stretched under large pulling forces, an extra enthalpic factor K has to be added to the model to account for

the longitudinal resistance of the chain.

Placed under large stretching forces, the physical attributes of the polymer are changed. It is entirely viable to consider the elasticity of the DNA molecule after it is stretched past its natural length, however, the worm-like chain model does not extend further than the natural length of the polymer chain. This overstretched regime is experimentally significant in discovering more about the denaturation of DNA. When DNA is placed under a stretching force between 60-70 pN, the DNA length increases rapidly and without much extra force. Then after another transition point, the DNA has a larger resistance to stretching and the experimental data's slope increases once more. Punkkinen et al. [2005]

Conclusion

We were able to duplicate the statistical model by Bustamante et al. [1994] of the force-extension behavior of a single molecule of ds-DNA in the entropic elasticity regime using the FJC model. In the 1D model of a polymer, we found that the force-extension relation can be expressed as a hyperbolic tangent function. We found in the limit as L approached 0 or infinity we got valid answer; however, the shape of the curve was wrong. The force-extension via the 3D FJC model improves on the shape of the curve. This equation agrees with experimental data for low and high force regimes, but fails in the middle to find better equations which model force extensions, one must look to the worm-like chain model. This is due mainly due to the FJC model's inability to account for the mechanical forces that arise from monomers interacting with other monomers.

References

Carlos Bustamante, Steven B Smith, Jan Liphardt, and Doug Smith. Single-molecule studies of dna mechanics. *Current Opinion in Structural Biology*, 10(3):279–285, 2000. ISSN 0959-440X. doi: https://doi.org/10.1016/S0959-440X(00)00085-3. URL `https://www.sciencedirect.com/science/article/pii/S0959440X00000853`.

John F. Marko and Eric D. Siggia. Stretching dna. *Macromolecules*, 28(26):8759–8770, Dec 1995. ISSN 0024-9297. doi: 10.1021/ma00130a008. URL `https://doi.org/10.1021/ma00130a008`.

Rossitza N. Irobalieva, Jonathan M. Fogg, Daniel J. Catanese, Thana Sutthibutpong, Muyuan Chen, Anna K. Barker, Steven J. Ludtke, Sarah A. Harris, Michael F. Schmid, Wah Chiu, and Lynn Zechiedrich. Structural diversity of supercoiled dna. *Nature Communications*, 6(1):8440, Oct 2015. ISSN 2041-1723. doi: 10.1038/ncomms9440. URL `https://doi.org/10.1038/ncomms9440`.

G.A. Petsko, G.A.P.D. Ringe, D. Ringe, and Ringe. *Protein Structure and Function*. Primers in biology. New Science Press, 2004. ISBN 9781405119221. URL `https://books.google.com/books?id=2yRDWkHhN9QC`.

Rob Phillips, Jane Kondev, Julie Theriot, and Hernan Garcia. *Physical biology of the cell*. Garland Science, 2012.

Thorinmuglindir. Freely jointed chain random walk, 2009. URL `https://commons.m.wikimedia.org/wiki/File:Ideal_chain_random_walk.svg#mw-jump-to-license`.

C Bustamante, JF Marko, ED Siggia, and S Smith. Entropic elasticity of lambda-phage dna. *Science*, 265(5178):1599–1600, 1994. ISSN 0036-8075. doi: 10.1126/science.8079175. URL `https://science.sciencemag.org/content/265/5178/1599`.

Steven B Smith, Laura Finzi, and Carlos Bustamante. Direct mechanical measurements of the elasticity of single dna molecules by using magnetic beads. *Science*, 258(5085):1122–1126, 1992.

C Bustamante, JF Marko, ED Siggia, and S Smith. Entropic elasticity of lambda-phage dna. *Proc. Natl. Acad. Sci USA*, 88:10009, 1991.

Yi-Ben Fu, Yu-Ru Liu, Peng-Ye Wang, and Ping Xie. A revised worm-like chain model for elasticity of polypeptide chains. *Journal of Polymer Science Part B: Polymer Physics*, 56(4):297–307.

O. Punkkinen, P. Hansen, L. Miao, and I. Vattulainen. Dna overstretching transition: ionic strength effects. *Biophysical journal*, 89 2:967–78, 2005.